Celebrating Prof. Edwards' Receiving the Nobel Prize

16th World Congress on In Vitro Fertilization

6th World Congress on In Vitro Maturation

September 10-13, 2011
Tokyo, Japan

Final Program and Abstracts

International Society for In Vitro Fertilization
with the cooperation of
The Japan Society of Fertilization and Implantation

Congress President: Osamu Kato (Director, Kato Ladies Clinic)
Congress Vice-President: Hisao Osada (Former Professor, Nihon University)

FINAL PROGRAM

ABSTRACTS

CERTIFICATE

WELCOME MESSAGE FROM THE PRESIDENT OF ISIVF

Dear Colleagues,

The 16th World Congress on In Vitro Fertilization (IVF), which will be held in Tokyo Japan in September 2011, is the main International Meeting of the year focusing on IVF and Assisted Reproductive Technologies (ART). With the rapid advances that continue to be achieved in these fields, this congress will constitute an important and unique multidisciplinary forum for the exchange of knowledge and the development of new ideas between the clinicians, basic and clinical scientists, embryologists and allied health professionals, that it brings together.

The congress is being held in the exciting, dynamic, progressive yet traditional city of Tokyo. Come and enjoy the congress and all of the historic, cultural, artistic and culinary delights that this most welcoming and unique city has to offer.

I would like to warmly thank the Japanese team and especially Dr Hisao Osada who has organized the 16th World Congress on IVF despite great difficulties related to the recent tsunami and its dramatic consequences.

All the speakers have agreed to come and the participants will be numerous both because of the quality of the scientific program and also to show our solidarity.

I wish you a wonderful Congress and a memorable stay in Tokyo.

René FRYDMAN
President, International Society for In Vitro Fertilization

ABOUT ISIVF

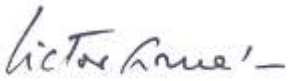
ISIVF's primary mission is to promote research and clinical development of in vitro fertilization (IVF) of human oocytes for the treatment of infertility and as an Assisted Reproductive Technology. Objectives include:

1. Promulgating ethical practice and standards of practice of IVF treatment;
2. Fostering collaboration between the various centers in the world that offer IVF treatment;
3. Organizing symposia for the purposes of presentation, discussion, exchange and publication of research, data, knowledge, theories and standards pertaining to IVF, and specially, a forum under the name "World Congress on In Vitro Fertilization".

WELCOME MESSAGE FROM THE VICE-PRESIDENT OF ISIVF

Dear Colleagues,

It is with great pleasure that I welcome you to this 16th World Congress of In Vitro Fertilization and to one of the world's most interesting and exciting cities: Tokyo.
This congress celebrates Professor Robert G Edwards and his great accomplishments for which he has been awarded the Nobel Prize. This prestigious award honors him; it also honors the whole field of reproductive medicine which has been transformed by him.
Holding the Congress such a short time after the devastating earthquake and tsunami, Japan experienced, is a testimony to the resilience of the Japanese people and the strong commitment and hard work of the local organization headed by the President and Vice president of the Congress Dr. Osamu Kato and Prof. Hisao Osada. I thank them personally and on behalf of the International Society for In Vitro Fertilization.
I trust that you will enjoy the rich and interesting academic and social program the congress offers. I also hope that you will take the opportunity to enjoy Tokyo and other parts of Japan, and its unique and exquisite art and culture.



Victor GOMEL
Vice-President, International Society for In Vitro Fertilization

WELCOME MESSAGE FROM THE PRESIDENT OF IVM

Dear Colleagues,

I am pleased to welcome you to the 6th World Congress on IVM and to Tokyo.
On behalf of the International Society of In-Vitro Maturation (ISIVM), I extend our sincere condolences to the Japanese people for the terrible losses and suffering they have recently incurred. Their bravery and resilience in the face of unspeakable tragedy have been a remarkable testament to human dignity and endurance.
This meeting marks a special event in the history of reproductive medicine: the presentation of the 2010 Nobel Prize in Physiology or Medicine to Professor Robert Edwards. On a personal note, Professor Edwards has played a very special role in my life as a cherished mentor and, along with Howard Jacobs and Stuart Campbell, as a partner in the London Women's Clinic. I am grateful and privileged to have worked with him and deeply appreciate the formative role he played in my career.
This well-deserved honor took more than 30 years in coming, but I believe it reflects a recognition of the growing importance of reproductive medicine. Following the birth of the first IVF baby through the pioneering achievements of Professor Edwards, as well as his early work in in-vitro maturation (IVM), ART has advanced by leaps and bounds over the past three decades.
The advent of IVM heralded a simpler form of treatment without the need for expensive fertility drugs that could also have unpleasant and potentially dangerous side effects. However, because of its initially poor pregnancy rates, it has been slow to gain acceptance. But as clinical pregnancy rates/cycle started approach 50% in the best centres internationally, IVM is rapidly growing in importance as a viable treatment. It is an attractive option for patients at high risk of ovarian hyperstimulation syndrome, those who have had recurrent unexplained IVF failures, and those who have had poor or hyper responses to IVF. IVM, followed by oocyte vitrification, also gives cancer patients, and those with other conditions such as severe SLE, the option of preserving their fertility without the need for contraindicated gonadotropin stimulation before they undergo oocyte retrieval and oocyte vitrification. In Montreal, our team has preserved fertility of over 200 women with various medical conditions and in a medical trial achieved a live-birth rate of 20%/cycle through IVM/oocyte vitrification and reported the birth of the first four healthy babies.
By attending this meeting, you will have the opportunity to learn about the latest advances in IVM and its value as a complementary treatment to IVF.
As more and more centres provide IVM as an option to their patients, the ISIVM believes it is essential to establish an international registry to track success rates, pregnancy outcomes, and the health of newborn babies. This major initiative is important to all ART practitioners and I urge you to join us to make your voice heard. The benefits of membership are fully explained at our website, www.isivm.com.
I hope you enjoy the Congress and your time in the unique and exciting city of Tokyo.

Seang Lin Tan
President, International Society for In Vitro Maturation

WELCOME MESSAGE FROM THE CONGRESS PRESIDENT AND THE CONGRESS VICE-PRESIDENT


Osamu Kato


Hisao Osada

Dear Colleagues, Ladies and Gentlemen,

It is our great pleasure to welcome you all to the 16th World Congress on In Vitro Fertilization.

Over the past decade and a half, the World Congress on In Vitro Fertilization has established itself as one of the most significant international gatherings for all doctors and scientists who are working in the field of reproductive health. Inviting more than 1000 health professionals, including clinicians, scientists, embryologists and professionals working in allied fields, the Congress is a vital forum for the exchange of cutting-edge information in this rapidly advancing field.

We have been preparing to hold this prestigious congress for years and have been really looking forward to welcoming you. In the midst of our preparation, though, the devastating earthquake struck the Tohoku District of Japan on March 11, 2011, and as a result it nearly became impossible for us to hold this congress. However, our friends all over the world have extended their kind support to us and our fellow countrymen and women and thanks to you all, we are now able to hold the congress as planned here in Tokyo. For your unflinching support, we would like to express our deepest gratitude.

Before this trying experience, however, we had been thrilled to receive a wonderful piece of news - the presentation of the 2010 Nobel Prize in Physiology or Medicine to Professor Robert Edwards, the patron of the International Society for In Vitro Fertilization. We ourselves remember well the time he made a visit to our clinic in Tokyo. We are very happy and honoured to have a chance to review and celebrate his achievements in the Opening Ceremony of this Congress.

We have prepared a scientific program for the Congress that focuses on a broad array of fascinating and vital topics. We are confident that it will prove to be an exciting and rewarding experience for professionals of all kind involved in our field.

We hope each and every one of you will have an unforgettable, once-in-a-lifetime intellectual and cultural experience in this great metropolis.

Osamu Kato
Congress President

Hisao Osada
Congress Vice-President

Congress President

Osamu Kato (Japan)

Congress Vice-President

Hisao Osada (Japan)

International Scientific Committee

Michel Abou Abdallah	(Lebanon)	Pak-Chung Ho	(China)	Richard Scott	(USA)
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Gautam Allahbadia	(India)	Robert Jansen	(Australia)	Gamal A. Serour	(Egypt)
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Jacques Cohen	(USA)	Shin Yong Moon	(Korea)	Wolfgang Würfel	Germany)
Aygül Demirol	(Turkey)	Huang Guo Ning	(China)	Cao Yun Xia	(China)
Klaus Diedrich	(Germany)	Hrishikesh D. Pai	(India)	Frank Yelian	(USA)
Jacques Donnez	(Belgium)	Kuang Yan Ping	(China)	Sergey Yakovenko	(Russia)
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(Executive Board members from Japan Society of Fertilization and Implantation)

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Hiroshi Imai	Osamu Kato	Yuji Taketani	Kaoru Yanagida
Minoru Irahara			

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Hirotsune Kaijima
Hirofumi Kamiya
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Yasuhito Michikura
Yasuyuki Mio
Yoshiharu Morimoto
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Masanori Ochi
Hiroshi Okada
Hisao Osada
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Masahide Shiotani
Makio Shozu
Kou Sueoka
Rikikazu Sugiyama
Katsuhiko Takahashi
Yuji Takehara
Atsushi Tanaka
Kenichi Tatsumi
Yukihiro Terada
Shokichi Teramoto
Kiyotaka Toshimori
Takafumi Utsunomiya
Naoki Yamashita
Koji Yano
Tetsu Yano
Atsumi Yoshida
Hiroaki Yoshida

Board Members of International Society for In Vitro Fertilization (as of May 10, 2010)

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René Frydman
Past President
Takahide Mori
Vice-President/Secretary
Victor Gomel
Treasurer
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Educational Officer
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Barry Cappel
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Liaison Tokyo Congress
Osamu Kato &
Hisao Osada
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Anis Feki

Yona Barak
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K.Y. Cha
Ri-Cheng Chian
Aygul Demirol
Klaus Diedrich
Joshua Dor
Jean-Bernard Dubuisson
Alan Handyside
Roy Homburg
Robert Jansen
Qiao Jie
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Milton Leong
T.C. Li
Basak Balaban
Svend Lindenberg
Shlomo Mashiach
Lisolette Mettler
Luciano Nardo
Zev Rosenwaks
Gerald Schatten
Joseph Schenker
Lynette Scott
Gamal Serour
Ilan Tur-Kaspa
Weon Young Son
Jerome Straus
Erol Tavmergen
Anna Veiga
Sergey Yakovenko

CONGRESS INFORMATION

Dates and Venue

Dates: September 10-13, 2011
Venue: Keio Plaza Hotel
2-2-1, Nishi-Shinjuku, Shinjuku-ku, Tokyo 160-8330
Phone: +81-3-3344-0111
www.keioplaza.co.jp

Contacts

Secretariat of the 16th World Congress on IVF

Secretariat General: Fumihito Aono
c/o Kato Laidies Clinic
7-20-3, Nishi-Shinjuku, Shinjuku-ku, Tokyo 160-0023, Japan
Fax: +81-3-3366-3908
E-mail: office@isivf2011.org

Registration Office

JTB Global Marketing & Travel Inc., (JTB GMT) has been appointed as the official registration office for the congress and will handle registration and hotel accommodation.

JTB Global Marketing & Travel Inc.
Convention Center (CD102079-041)
2-3-11, Higashi-Shinagawa, Shinagawa-ku, Tokyo 140-8604, Japan
Phone: +81-3-5796-5445
Fax: +81-3-5495-0685
E-mail: ISIVF2011@gmt.jtb.jp

Registration

Registration (Reception) Desk: 4th floor

Opening Hours:

Saturday	September 10	08:00-18:00
Sunday	September 11	08:00-18:00
Monday	September 12	08:00-18:00
Tuesday	September 13	08:00-14:00

Delegate registration includes:

Opening & Closing Ceremony
Welcome Reception
Access to the Exhibit Hall
Access to all sessions
Delegate Bag
Final Programme & Abstracts
Certificate of Attendance*
*A Certificate of Attendance is included in the Final Program & Abstracts (see last page.)

Accompanying person's registration includes:

Opening & Closing Ceremony
Welcome Reception
Access to the Exhibit Hall
Tokyo City Tour (10th - 12th)

A 3-hour sightseeing tour to visit Meiji Shrine and Tsukiji Fish Market will be organized for guests free or charge (up to 40 participants per tour):

Sat. Sept. 10: 13:30
Sun. Sept. 11: 9:00 and 13:30
Mon. Sept. 12: 9:00

Please reserve your seat at the "Tokyo City Tour desk" located in the Reception area on the forth floor. Reservation will be made on a first-come, first-served basis.

Name Plates

Official Congress name plates will be required for all Congress functions, scientific sessions and the exhibit area. Individuals who lose their name plates will be required to pay a fee to obtain a personalized replacement.

Message Board

A message board will be located near the Registration Desk. Announcements from the secretariat or any personal messages will be placed on the board.

Poster Area (Hana BCD, 4F)

The poster area is open at all times on the 4th floor. Delegates can visit posters at their convenience. Poster viewing time is as follows (poster presenters are requested to be at their posters at those times):

Sunday September 11 10:00-10:30; 13:00-13:20
Monday September 12 10:00-10:30; 13:00-13:20

Commercial Exhibition (Hana BCD, 4F)

Saturday	September 10	08:00-17:00
Sunday	September 11	08:00-17:00
Monday	September 12	08:00-17:00
Tuesday	September 13	08:00-14:30

* Coffee and other refreshments are available at the Exhibition area.

Lunch

Lunch boxes will be provided free of charge for luncheon seminars (you can eat while attending the lecture during the lunch break). Vouchers for the lunch boxes will be provided at the dedicated desk located near the Registration Desk on the 4th floor on a first come first served basis.

Luncheon Seminars are arranged for 12:00-13:00 on Sunday, September 11, and Monday, September 12 at Concord Ballroom, A, B, and C on the 5th floor. For topics and speakers, please see the program.

Coffee and Refreshments

Served in the Commercial Exhibition area at all times.

Instructions for Speakers

1. TIME ALLOWANCE FOR PRESENTATION

Plenary Lecture: 30 minutes (25 minutes presentation and 5 minutes discussion)
Symposium: 20 minutes (15 minutes presentation and 5 minutes discussion)
Pre-Congress Workshop: 30 minutes
Free Communication: 15 minutes (10 minutes presentation and 5 minutes discussion)

2. AT THE TIME OF YOUR PRESENTATION

- Meet with the session Chairpersons in the session room 15 minutes before the beginning of the session.
- Please be certain that the length of your oral presentation does not exceed the allotted time.
- Chairpersons have been instructed to terminate lectures which exceed their allotted time.

3. EQUIPMENT FOR PRESENTATION

PowerPoint Presentation
Session rooms are provided with one Windows laptop computer on which Microsoft PowerPoint is installed (OS: Windows XP English version; Software: Microsoft PowerPoint 2003/2007/2010) and an LCD projector. The podium is equipped with a monitor, a mouse and a keyboard. You are requested to bring the data for your presentation on a CD-ROM or a USB memory stick, or your own laptop computer.
*Macintosh is not available. If you wish to use a Macintosh PC, please bring your own PC.

1) If you bring data:
You are requested to bring the data for your presentation on a CD-ROM or a USB memory stick to the Speaker Ready Room (“Kaede” room on the 4th floor) and upload your presentation at least 30 minutes before your session begins. Only a CD-ROM in Windows format or USB memory stick is acceptable. The name of the file should be labeled with your name. Please also see the <NOTES>.

2) If you bring your own laptop computer:
Windows machines with Windows 98/98SE/2000/ME/XP/VISTA/7 or Macintosh machines with any Mac OS (8.6/9.04-9.2.2/X.1-X.5 and subsequent versions) are acceptable. Please note that Japan operates on 100 volts for electrical appliances, with a cycle of 50 Hz in Tokyo.

The plug type in Japan is **A with two flat blades**. The computer to be used for presentation must be equipped with a **D-sub-15 pin video output**. Please make sure that you prepare the necessary converters for your computer so that they meet the requirements stated above. Also, please bring your own AC adaptor.

You are requested to bring your computer and check its connection at the Speaker Ready Room (the “Kaede” room on the 4th floor) at least 30 minutes before your session begins.

For all computers, check the following settings:
-Cancellation of power-saving features: Cancellation of Sleep, Screen Saver, etc.;
-Resolution of screen: 1024x768 pixels (XGA) or less.

In the session room, please have your computer powered on when the presentation prior to your own begins and hand it over to the operator at the PC desk in the session room, with your presentation file opened. (You may be required to restart your computer if the connection to the projector is not successful.) Since there will be only one connection cable to the projector, the operator will connect it at the beginning of your presentation. The projector has a standard mini-D-sub 15 pin (three sequences) connector. If your computer has a different connector, you should bring your own conversion cable.

<NOTES>
* When you make a PowerPoint file for your presentation, please make sure that all graphics are embedded in the presentation file. Fonts should be standard fonts, such as Times New Roman, Arial, or Courier. If non-standard fonts must be used, they should be embedded in the presentation files. Also, please set up the slide size for “On-screen show”.
* If your presentation includes video/animation, only ones that can be played by “Media Player” are acceptable.
*The name of the file should be labeled with your name.

4. SPEAKER READY ROOM (for slide preview and data check)

The Speaker Ready Room is “Kaede” on the 4th floor. It is open during the following hours:

Saturday	September 10	08:00-17:30
Sunday	September 11	08:00-17:30
Monday	September 12	08:00-17:30
Tuesday	September 13	08:00-13:00

Please make sure to visit the room with your data/PC at least 30 minutes before your session begins.

5. NEXT SPEAKER’S SEAT

You are requested to be seated at the Next Speaker’s seat, located in the left front row 10 minutes before your presentation starts. If you are bringing your own PC, please hand it over to the operator at the PC Desk in the session room.

Instructions for Chairpersons

All session chairpersons are requested to meet with their speakers in the session room 15 minutes before the session begins.

* Please welcome delegates to the session and introduce each speaker by title, name of all authors, and affiliation (if any) as listed.
* Any changes to the program should be announced at the beginning of the session.
* An attendant will be present in the session room to assist you with any last minute details.
* Please keep each presentation on schedule. If a presentation is cancelled, do not change the timing of the other presentations.

Instructions for Poster Presenters

Venue: Hana B, C, and D, 4th floor

To provide ample time for all participants to view and discuss poster presentations, each poster will be on display for the entire duration of the Congress.

Poster presenters are requested to be at their posters during the Poster Viewing Time as follows:

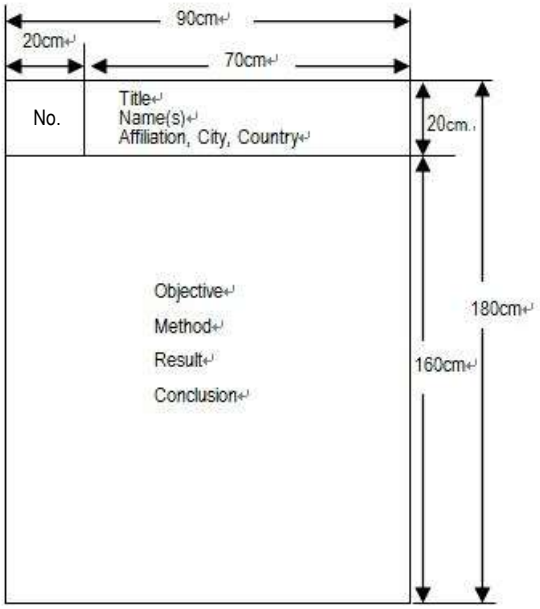
Sunday September 11: 10:00-10:30 and 13:00-13:20
Monday September 12: 10:00-10:30 and 13:00-13:20

Poster presenters are responsible for setting up and removing their posters according to the following schedule:

Setting up: Saturday, September 10, 18:00-20:00 or Sunday, September 11, 8:00-9:00
Removal: Tuesday, September 13, 12:00-15:00

<Notes for Poster Presentation>

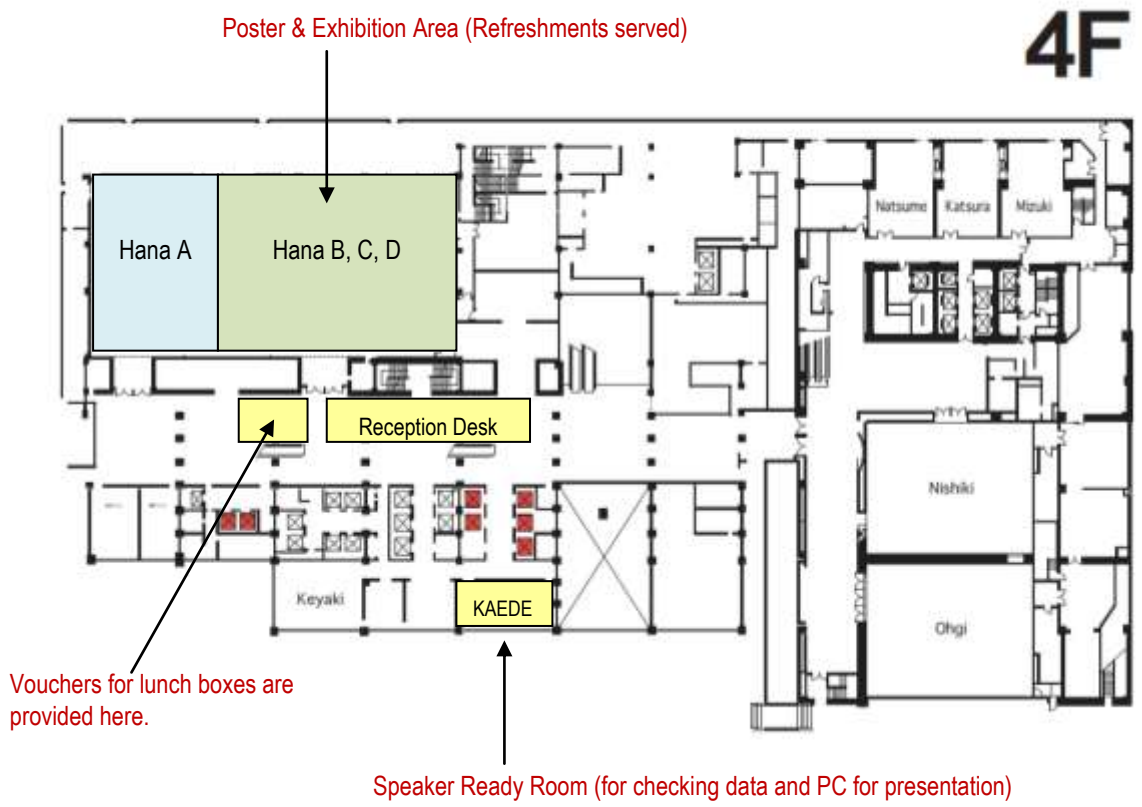
1. All posters are to be written entirely in English.
2. A presentation number to be placed at the top left of the poster will be provided by the Secretariat. Each author is requested to indicate the “title”, the “authors’ names” and the “authors’ affiliations”, at the top right of the panel within an area measuring 90cm wide by 20 cm high.
3. The usable area for the contents is a space 90cm wide and 160cm high. The layout of the presentation contents is at the authors’ discretion.
4. Posters are to be attached to the boards with drawing pins, which will be provided at the Poster Area. No paste, glue, staples or nails are permitted.
6. Please stay in front of your poster during the Poster Viewing times.
7. The best poster award will be presented in the Closing Ceremony on Tuesday, September 13 (14:30-15:00).

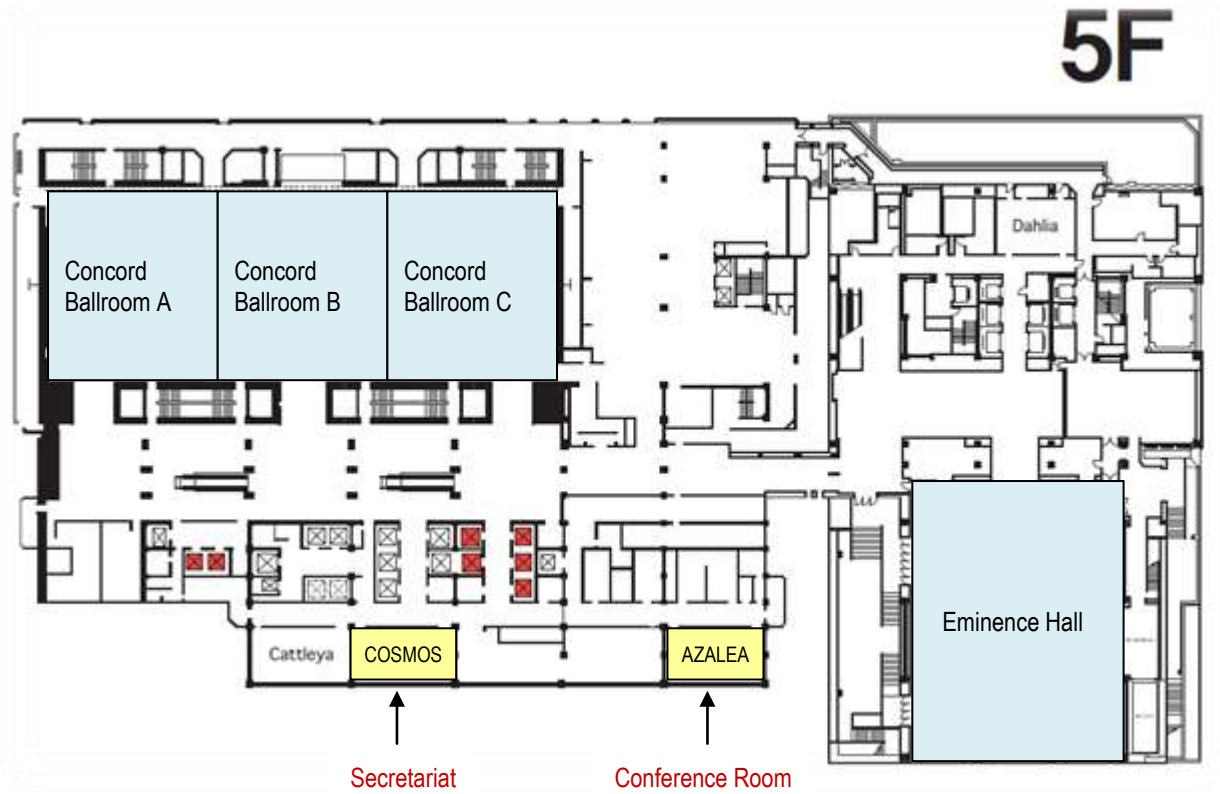


Best Abstract Awards

Two abstract awards winners, one for poster and one for oral presentation, will be selected and provided by the International Society for In Vitro Fertilization. The Winners will be announced in the Closing Ceremony.

Floor Plan of the Congress Venue





Social Program

♦ **Official Opening Ceremony and Lecture**
(Included in registration fee for delegates and registered accompanying persons)
Venue: Eminence Hall, 5th floor
Date: Saturday, September 10
Time: 18:30-19:30
Dress Code: Business (e.g. tie and jacket)

♦ **Welcome Reception**
(Included in registration fee for delegates and registered accompanying persons)
Venue: Concord Ballroom ABC, 5th floor
Date: Saturday, September 10
Time: 19:30-21:30
Dress Code: Business (e.g. tie and jacket)

♦ **Congress President's Dinner**
(Invited guests only)
Venue: Eminence Hall, 5th floor
Date: Sunday, September 11
Time: 18:30-20:30
Dress Code: Business (e.g. tie and jacket)
(An invitation card is included in the delegate bag for the invited guests.)

♦ **Gala Dinner (Buffet)**
(Requires ticket)
Venue: Concord Ballroom AB, 5th floor
Date: Monday, September 12
Time: 18:30-20:30
Those who wish to take part in the Gala Dinner can purchase tickets at the Registration Desk for 10,000 yen. (An invitation card is included in the delegate bag for the invited guests.)
Dress code: Business/Informal

♦ **ISIVF General Assembly & Closing Ceremony**
(Included in registration fee for delegates and registered accompanying persons)
Venue: Concord Ballroom ABC, 5th floor
Date: Tuesday, September 13
Time: 14:20-15:00
Dress Code: Business/Informal

Travel Desks

♦ **At Narita Airport**

A Travel Desk for the Congress will be set up at the Narita Airport to assist your journey to the Congress venue on September 9 and 10, from 8:00 to 20:00.

♦ At the Congress Venue (Keio Plaza Hotel)

A Travel Desk operated by Tokyo Metropolitan Government will be opned in the reception area during the Congress. Please contact Travel Desk personnel should you have any questions about your travel arrangements, sightseeing, etc.

TOKYO CITY TOUR

This tour is specially organized for overseas guests by Tokyo Metropolitan Government. It is intended for accompanying persons but any delegates can take part in the tour if seats are available.

Dates:

September 10th (Sat)	13:30-16:30
September 11th (Sun)	9:00-12:00 & 13:30-16:30
September 12th (Mon)	9:00-12:00

Tour fare: free

Booking:

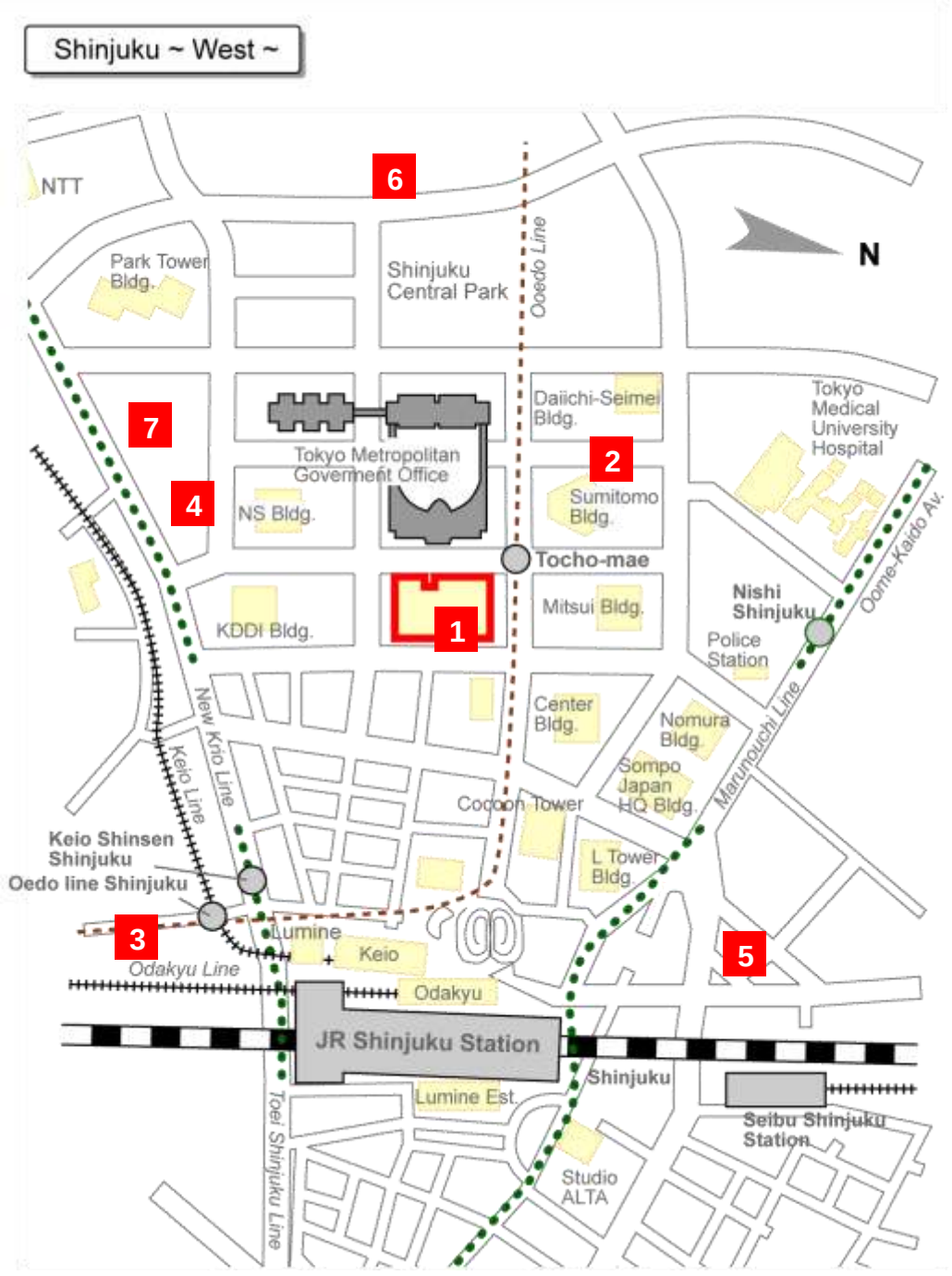
Please sign up for the tour at “Tokyo City Tour desk” located on the 4th floor. Max number of participants for each tour is limited up to 40. Reservation will be made on a first-come, first-served basis.

Tour course:

First, visit the tranquil Meiji Shrine, dedicated to the Emperor Meiji, the first emperor of modern Japan. Then visit Tsukiji Outer Fish Market, where you will have a chance to try sushi. En route to the hotel, you will enjoy a panoramic view of the Imperial Palace from the coach.

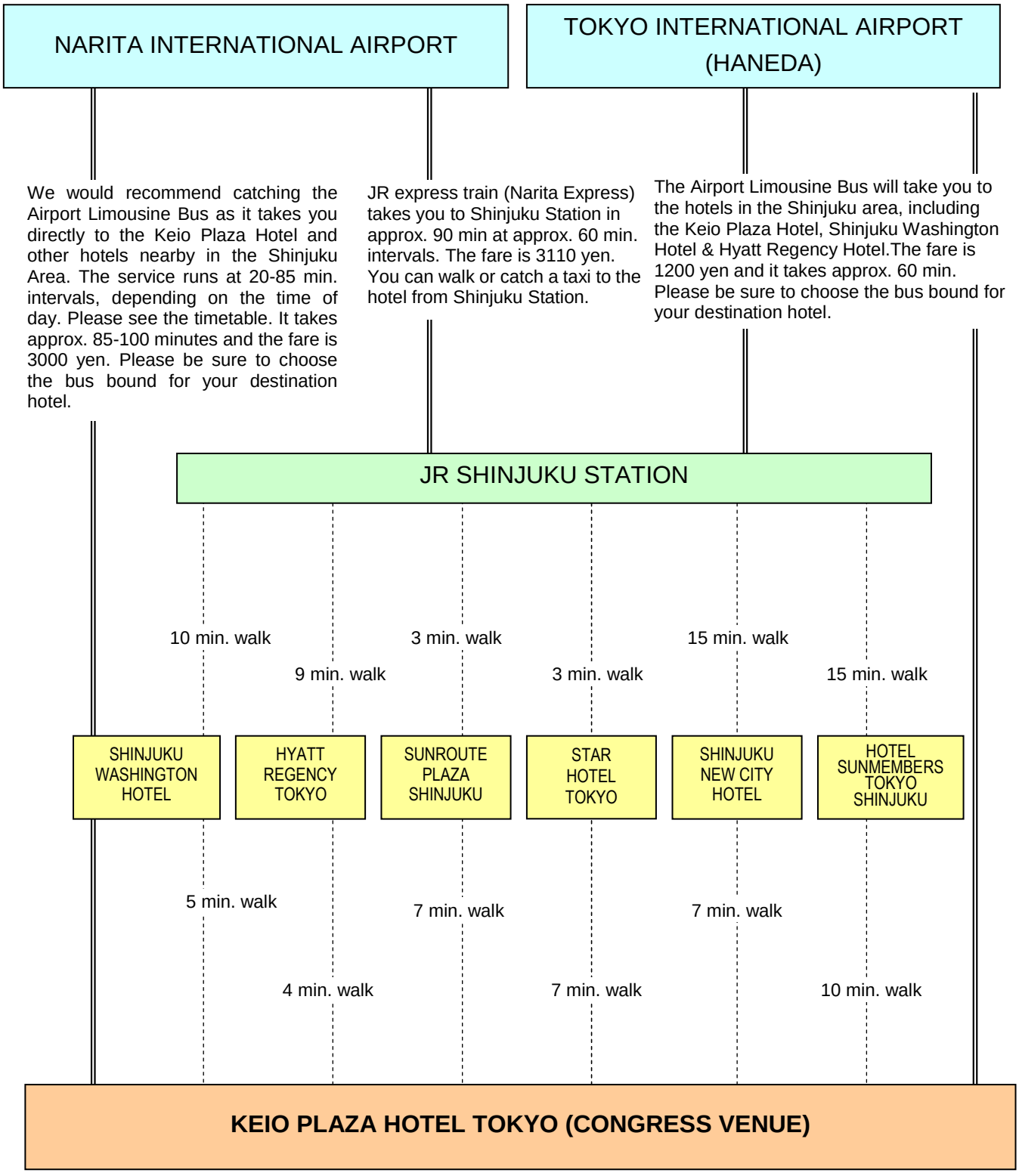
Please note that the tour course will be subject to change with or without notice.

Map of the Congress Venue (Keio Plaza Hotel) and other hotels



1. Keio Plaza Hotel Tokyo (Congress Venue)
2. Hyatt Regency Tokyo
3. Hotel Sunroute Plaza Shinjuku
4. Shinjuku Washington Hotel
5. Star Hotel Tokyo
6. Shinjuku New City Hotel
7. Hotel Sunmembers Tokyo Shinjuku

Access to the Congress Venue (Keio Plaza Hotel) and other hotels



For details of the Airport Limousine Bus service from Narita Airport and Haneda Airport to Keio Plaza Hotel, please see http://www.limousinebus.co.jp/en/platform_searches/index/2/18

Airport Limousine Bus Timetable: from Narita Airport to Keio Plaza Hotel

Narita Airport Terminal 2 Dep.	07:00	07:40	08:30	09:20	10:20	11:00	11:40	12:20	13:00	13:25
Narita Airport Terminal 1 South Wing Dep.	07:05	07:45	08:35	09:25	10:25	11:05	11:45	12:25	13:05	13:30
Narita Airport Terminal 1 North Wing Dep.	07:10	07:50	08:40	09:30	10:30	11:10	11:50	12:30	13:10	13:35
Keio Plaza Hotel Arr.	09:05	09:45	10:35	11:25	12:25	13:05	13:45	14:25	15:05	15:30

Narita Airport Terminal 2 Dep.	13:45	14:30	15:00	15:45	16:30	17:00	17:45	18:15	18:45	19:40
Narita Airport Terminal 1 South Wing Dep.	13:50	14:35	15:05	15:50	16:35	17:05	17:50	18:20	18:50	19:45
Narita Airport Terminal 1 North Wing Dep.	13:55	14:40	15:10	15:55	16:40	17:10	17:55	18:25	18:55	19:50
Keio Plaza Hotel Arr.	15:50	16:35	17:05	17:50	18:35	19:05	19:45	20:15	20:40	21:30

Narita Airport Terminal 2 Dep.	20:10	21:00	21:40	22:35
Narita Airport Terminal 1 South Wing Dep.	20:15	21:05	21:45	22:40
Narita Airport Terminal 1 North Wing Dep.	20:20	21:10	21:50	22:45
Keio Plaza Hotel Arr.	22:00	22:50	23:25	00:15

Airport Limousine Bus Timetable: from Keio Plaza Hotel to Narita Airport

Keio Plaza Hotel Dep.	05:50	06:50	07:50	08:45	09:50	10:50	11:50	12:50	13:50	14:50
Narita Airport Terminal 2 Arr.	07:25	08:25	09:40	10:50	11:50	12:50	13:45	14:40	15:40	16:40
Narita Airport Terminal 1 Arr.	07:30	08:30	09:45	10:55	11:55	12:55	13:50	14:45	15:45	16:45

Keio Plaza Hotel Dep.	15:50	16:45	17:45
Narita Airport Terminal 2 Arr.	17:50	18:50	19:50
Narita Airport Terminal 1 Arr.	17:55	18:55	19:55




General information about Japan

Population: Around 128 million

Capital: Tokyo

Currency: Yen (usually indicated as ¥)
The yen comes in the following denominations: Coins: 1, 5, 10, 50, 100, 500 yen
Bank notes: 1000, 2000 (very rare), 5000, 10000 yen.

Climate: Average temperature in September: 23 degrees C.

Units of measure: Metric system

Electricity: 100V, with 50 Hz in east Japan and 60 Hz in west Japan

Time Zone: GMT + 9

Passports & Visa: If you are a citizen of one of the over 50 countries with which Japan has a "general visa exemption arrangement", you need only a valid passport to enter Japan as a "temporary visitor". Otherwise, you need to obtain a visa before entering the country. Temporary visitors from most countries are allowed to stay for up to 90 days.

Consumption Tax: Paid by consumers when they purchase goods and services. The current rate is 5%. Shops and other service providers are required to include the consumption tax in the prices shown.

Tipping: Basically, it is not a custom to give tips, except in high class ryokan (traditional Japanese inn.)

About Tokyo

Tokyo, a sprawling metropolis with a population of nearly 13 million people, is one of the world's great cities. Originally a small castle town in the 16th century, by the 18th century it was the centre of the Shogun's power and already one of the largest cities in the world. Since the middle of the 19th century, it has been the capital and the financial and cultural hub of modern Japan.

With this history behind it, Tokyo today is a fascinating blend of the traditional, the modern and the hyper-modern, a fascinating blend of East and West, and yet at the same time a uniquely Japanese city. Tokyo really is a place that has something for everyone – a place traditional temples and shrines stand cheek by jowl with towering skyscrapers, and small traditional bars and shops can be found just behind some of the most exciting and innovative architecture to be found anywhere in the globe.

With its super-efficient transport system and its extremely low rates of crime, Tokyo is a city where one can enjoy the best that the world can offer, whether it is in terms of art and culture, cuisine, the latest hi-tech gadgetry, financial wizardry, or human warmth and friendliness. Whatever your interests, Tokyo can offer you an experience that you will never forget.

For more information, please visit: http://www.japan-guide.com

Accompanying Person's Tour

Why not join the 3-hour, free sightseeing tour to visit Meiji Shrine and Tsukiji Fish Market? The tour starts in front of the Keio Plaza Hotel at the following hours: 9:00 for September 11 and 12; 13:30 for September 10 and 11. Please reserve your seat at the Accompanying Person's Tour Desk located next to the Reception Desk on the 4th floor of Keio Plaza Hotel. Seats are provided on a first come first served basis.

Traditional Sightseeing Spots in Tokyo

The Imperial Palace
The current Imperial Palace (Kokyo) is located on the former site of Edo Castle, a large park area surrounded by moats and massive stone walls in the center of Tokyo, a short walk from Tokyo Station. It is the residence of Japan's Imperial Family. The Imperial Palace East Gardens are open to the public throughout the year except on Mondays, Fridays and special occasions.

Asakusa
Asakusa is the center of Tokyo's “shitamachi”, lit. "low city", one of the districts of Tokyo which have preserved a certain atmosphere of the old city. Asakusa‘s main attraction is Sensoji, a very popular Buddhist temple, built in the 7th century.
The temple is approached via the Nakamise, a shopping street that has been providing temple visitors with a variety of traditional, local snacks and tourist souvenirs for centuries.

Sightseeing Spots in Shinjuku

Shinjuku is one of the 23 wards of Tokyo, but the name commonly refers just to the large entertainment, business and shopping area around Shinjuku Station. Handling more than two million passengers each day, Shinjuku Station is Japan‘s busiest railway station, served by six railway companies and about a dozen railway and subway lines, including the JR Yamanote Line.

Tokyo Metropolitan Government Office (Tocho)
The 243 meter tall twin towers and surrounding buildings contain the offices and the assembly hall of the metropolitan government of Tokyo, as well as observatories on the 45th floor of each tower. The view from the southern tower is considered the best.
Open daily 9:30 to 23:00 (south observatory until 17:30), except December 29-31, January 2-3 and occasional inspection days. Furthermore, the north observatory is closed on the 2nd and 4th Monday and the south observatory on the 1st and 3rd Tuesday of each month, except if a public holiday falls on the closure day, in which case the observatory is closed the following day. Admission is free.

Shinjuku Gyoen

Shinjuku Gyoen is one of Tokyo's largest and most pleasant parks and best cherry blossom viewing spots. It was opened to the public in 1949, after it had served as a garden for the Imperial Family since 1903. Open from 9:00 to 16:30. Closed on Mondays (Tuesday when Monday is a national holiday) from December 29 to January 3. There are no closure days during the cherry blossom season (late March to late April) and the Chrysanthemum Exhibition (first half of November). Admission is 200 Yen.

Museums (Please note that entries are permitted until 30 minutes before the closing.)

Tokyo National Museum / Ueno

The Tokyo National Museum collects, houses, and displays a comprehensive collection of art works and antiquities from Japan as well as other Asian countries.
Access: 10 mins from JR Ueno Station
Opening Hours: Tue.-Thu.: 9:30-17:00, Fri.: 9:30-20:00
Sat. & Sun.: 9:30-18:00 Closed on Mondays except Sept. 15

National Museum of Nature and Science / Ueno

The Museum boasts one of the richest histories of any museum in Japan. It is Japan's only nationally administered museum, and one of the world's central institutes for research in natural history and the history of science and technology.
Access: 5 mins walk from JR Ueno Station
Opening Hours: 9:00-17:00, Closed on Sept. 5, 12, 20 & 26.

National Museum of Western Art / Ueno

The NMWA was established in 1959 around the core Matsukata Collection as Japan's museum specializing in Western art. The galleries feature pre-18th century paintings including those by Ritzos, Van Cleve, Veronese, Rubens, Van Ruysdael, and Ribera, 19th to early 20th century French paintings including works by Delacroix, Courbet, Manet, Renoir, Monet, Van Gogh, Gauguin, and Moreau and works by the next generation of artists, such as Marquet, Picasso, Soutin, Ernst, Miro, Dubuffet and Pollock.
Access: 1 min. walk from JR Ueno Station
Opening Hours: Normally 9:30-17:30, Closed on Mondays

Mori Art Museum / Roppongi

ROPPONGI HILLS MORI TOWER (53F)
The Musium takes the lead in introducing the newest art from Asia and other regions of the world. Key enphasis is placed on the concepts of being “Contemporary” and “International”.
Access (To Roppongi Hills): 4 min. walk from Exit 3, Roppongi Station on Toei Oedo Subway Line
Opening Hours: Everyday except Tuesdays: 10:00-22:00 (Tuesday until 17:00)

The National Art Center, Tokyo / Roppongi

The National Art Center, Tokyo is a unique and innovative art exhibition facility: Instead of maintaining a permanent collection, it makes the most of a total of 14,000 square meters of exhibition space, one of the largest in Japan, and focuses on serving as a venue for various art exhibitions.
Access: 4-minute walk from Exit 7, Roppongi Station on Toei Oedo Subway line
Opening Hours: Mon, Wed, Thu, Sat, and Sundays 10:00-18:00,
Friday 10:00-20:00, Closed on Tuesdays

The Edo-Tokyo Museum / Ryogoku

The Edo-Tokyo Museum was founded in 1993, as a facility to preserve the historical heritage of Edo -Tokyo. In the Permanent Exhibition area, there can be found original and replicated exhibits, as well as large-scale models, faithful representations of their originals. Also at t the Special exhibition gallery, visitors can enjoy selected exhibits on subjects related to Tokyo's history and culture, scheduled several times a year. The building was modeled after an elevated-floor type warehouse, the height of which is approximately the same as that of Edo Castle tower.

Access: 3-minute walk from Ryogoku Station (West Exit) on JR Sobu Line
Opening Hours: 9:30-17:30 (Saturday until 19:30), Closed on Mondays

Away from Tokyo

Hakone (2 hours from Shinjuku)
Hakone is part of the Fuji-Hakone-Izu National Park, less than 100 kilometers from Tokyo. Famous for hot springs, outdoor activities, natural beauty and the view of nearby Mt. Fuji, Hakone is one of the most popular destinations among Japanese and international tourists looking for a break from Tokyo.

Mount Fuji (2.5 hours from Shinjuku)
Mount Fuji (Fujisan) is the Japan's highest mountain. It is not surprising that the nearly perfectly shaped volcano has been worshipped as a sacred mountain and loved by both artists and common people.

The Hakone Open Air Museum (2.5 hours from Shinjuku)
Access: JR Odawara Stn. (or Odakyu line Odawara Stn.) →Hakone Tozan Railway Chokoku-no-mori Stn. (2min walk)
Open Hours: 9:00-17:00, Open year-round
Admission Fee: Adults 1600 yen, Senior citizens(65 and above) and university and high school students 1100yen, middle and elementary school students 800yen

Kamakura (1 hour from Shinjuku)
Kamakura is a very popular tourist destination. Sometimes called the Kyoto of Eastern Japan, Kamakura offers numerous temples, shrines and other historical monuments. In addition, Kamakura's sand beaches attract large crowds during the summer months.

Nikko (2 hours from Shinjuku)
Nikko is a small city at the entrance to Nikko National Park. It is most famous for the Toshogu, Japan's most lavishly decorated shrine complex and mausoleum of Tokugawa Ieyasu, the founder of the Tokugawa shogunate.
Nikko has been a center of Shinto and Buddhist mountain worship for many centuries, and Nikko National Park continues to offer scenic, mountainous landscapes, lakes, waterfalls, hot springs, wild monkeys and hiking trails.

Kyoto (2 hours and 40 minutes from Tokyo Station by Bullet Train)
Kyoto was Japan's capital and the Emperor's residence from 794 until 1868. It is now the country's seventh largest city with a population of 1.4 million people and a modern face. Over the centuries, Kyoto was destroyed by many wars and fires, but due to its historic value, the city was dropped from the list of target cities for the atomic bomb and spared from air raids during World War II. Countless temples, shrines and other historically priceless structures survive in the city today.

Nara (1 hour from Kyoto)
Japan's first permanent capital was established in the year 710 at Heijo, the city now known as Nara. As the influence and political ambitions of the city's powerful Buddhist monasteries grew to become a serious threat to the government, the capital was moved to Nagaoka in 784. Nara is located in the Kinai plain, less than one hour from Kyoto and Osaka. Due to its past as the first permanent capital, it remains full of historic treasures, including some of Japan's oldest Buddhist temples.

Taxi

Taxi fares typically start from 710 yen for the first two kilometers and increase by roughly 90 yen for every additional 288 meters traveled. The cost also increases when the taxi is not moving for a prolonged time. Late in the evening, rates are raised by 20-30 percent. A plate on the dashboard in the lower corner of the windshield indicates whether a taxi is vacant or not. A red plate indicates that the taxi is vacant, while a green plate indicates the opposite. When you board a taxi, note that the vehicle's left rear door is opened and closed remotely by the driver. You are not supposed to open or close it by yourself. Furthermore, you are not supposed to tip taxi drivers, as the service is included in the price.
If you do not speak Japanese, or your destination is not a well known place, it is recommended that you give your driver the precise address of your destination on a piece of paper or, even better, point it out on a map, since the Japanese address system can be confusing even to local taxi drivers.

Food

Japanese cuisine offers a great variety of dishes and regional specialties. Some of the most popular Japanese and Japanized dishes are listed below.

Sushi
Sushi can be defined as a dish which contains sushi rice, cooked rice that is prepared with sushi vinegar. There are various kinds of sushi dishes.

Tempura
Tempura is seafood, vegetables, mushrooms and other pieces of food coated with tempura batter and deep fried. Tempura was introduced to Japan by the Portuguese in the 16th century, but has become one of Japan's most famous dishes internationally.

Sukiyaki
A nabe dish prepared with thinly sliced meat, vegetables, mushrooms, tofu and shirataki (konyaku noodles). The pieces of food are dipped into a raw egg before eaten.

Shabu-Shabu

Shabu-shabu is Japanese style meat fondue. Thinly sliced meat, along with vegetables, mushrooms and tofu is dipped into a hot soup and then into ponzu vinegar or a sesame sauce before being eaten.

Soba

Soba noodles are native Japanese noodles made of buckwheat flour or a mixture of buckwheat and wheat flour. Soba are about as thick as spaghetti. They can be served cold or hot and with various toppings.

Ramen

Ramen are Chinese style noodles prepared in a soup with various toppings. Ramen is one of the many popular dishes that were originally introduced from China but have become completely Japanized over time.

Yakitori

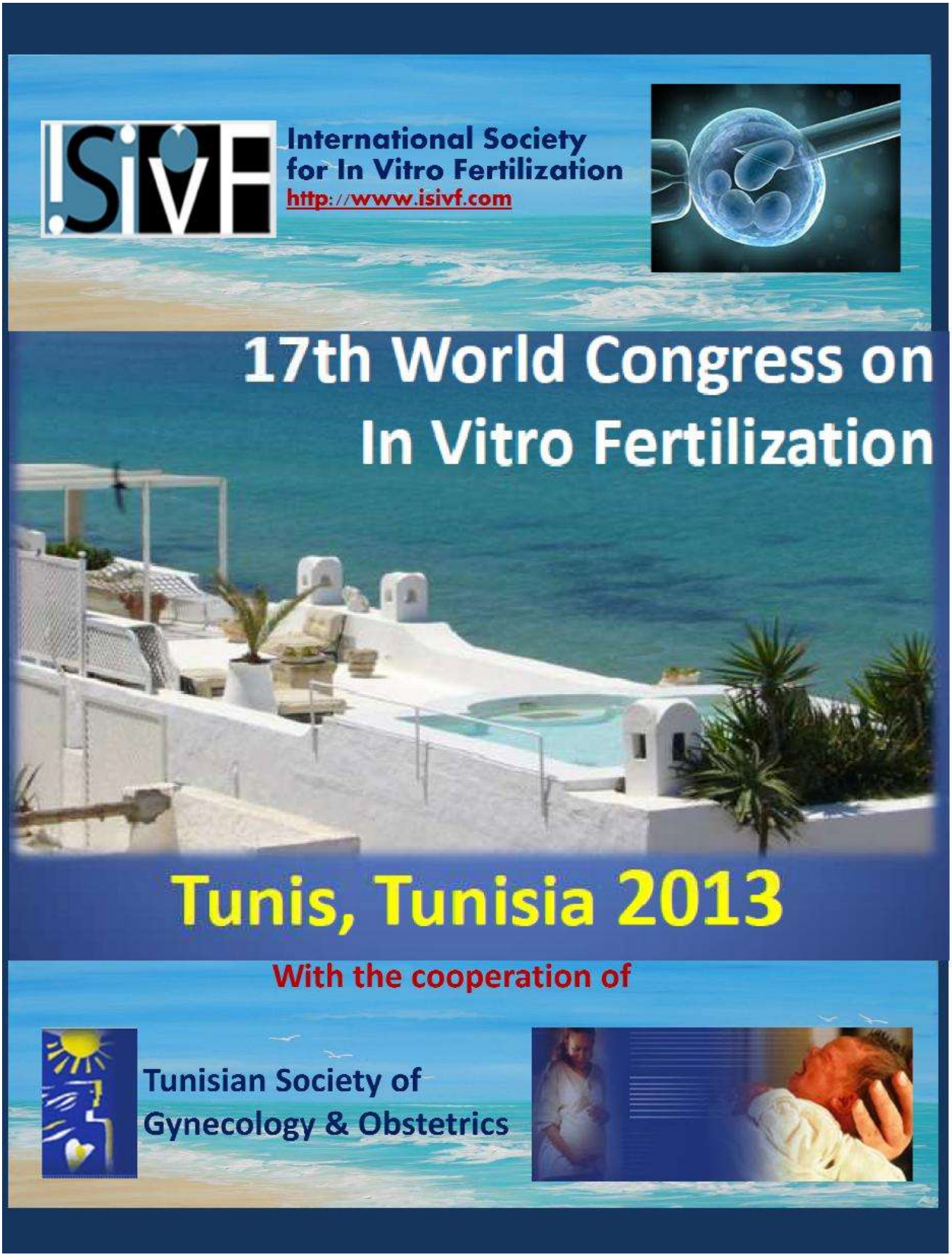
Yakitori are grilled chicken pieces on skewers. Most parts of the chicken can be used for yakitori.

	Concord A (5F)	Concord B (5F)	Concord C (5F)	Hana A (4F)	HANA BCD (4F)
9:00	the29th Annual Meeting of Japan Society of Fertilization and Implantation			9:00-12:00 Pre-Congress Workshop [Vitrification] Chair: Stanley Leibo (USA) Sherman Silber (USA) (1) Lectures Stanley Leibo (USA) Noriko Kagawa (Japan) Sherman Silber (USA) (2) Live demonstration Masashige Kuwayama (Japan)	8:30-17:30 Exhibits
10:00					
11:00					
12:00					
13:00					
14:00				14:00-17:00 Pre-Congress Workshop [PGD] Chair: Naoki Aoyama (Japan) Ilan Tur-Kaspa (USA) (1) Lectures Kou Sueoka (Japan) Suguru Sato (Japan) Ilan Tur-Kaspa (USA) (2) Live demonstration Naoki Aoyama (Japan)	
15:00					
16:00					
17:00					
18:00	18:30-19:30 Opening Ceremony and Lecture (Venue: Eminence Hall・5F) Speaker: Victor Gomel (Canada) 19:30-21:30 Welcome Reception (Venue: Concord ABC・5F)				

	Concord A (5F)	Concord B (5F)	Concord C (5F)	HANA A (4F)	HANA BCD (4F)
8:30	8:20-8:30 Opening Remarks				
9:00	8:30-10:00 Plenary Lectures 1 Chair: Victor Gomel (Canada) Speaker: Osamu Kato (Japan) Speaker: John Zhang (USA) 2 Chair: Sherman Silber (USA) Speaker: Zsolt Peter Nagy (USA) 3 Chair: Takahide Mori (Japan) Speaker: Shin Yong Moon (Korea)				8:30-18:00 Exhibits and Poster Viewing
10:00	10:00-10:30 Coffee Break & Poster Viewing				
11:00	10:30-11:50 C-1 Management of PCO patients (1) Chair: Timur Gürgan (Turkey) Chair: Makio Shozu (Japan) 1 Takahide Mori (Japan) 2 Rogerio A. Lobo (USA) 3 Muchsin Jaffar (Indonesia) 4 Khaled Mahmoud (Tunisia)	10:30-11:50 C-4 Treatment of benign tumor prior to IVF-ET (1) Chair: Zion Ben-Rafael (Israel) Chair: Hassan Sallam (Egypt) 1 Hassan Sallam (Egypt) 2 Issam Lebbi (Tunisia) 3 Hiroshi Nabeshima (Japan) 4 Victor Gomel (Canada)	10:30-11:50 C-8 ART-cryopreservation: Is oocyte cryopreservation a revolution in IVF? Chair: Stanley Leibo (USA) Chair: Masashige Kuwayama (Japan) 1 Luis Arturo Ruvalcaba Castellón (Mexico) 2 Masashige Kuwayama (Japan) 3 Safaa Al Hasani (Germany) 4 Stanley Leibo (USA)	10:30-12:00 Oral Communications	
12:00	12:00-13:00 Luncheon Seminar 1 GnRH antagonists in ovarian stimulation for IVF and ICSI from practical perspective Chair: Takafumi Utsunomiya (Japan) Speaker: Atsushi Tanaka (Japan) Sponsored by MSD K.K.	12:00-13:00 Luncheon Seminar 2 Clinical aspects of GnRH analogue use in Assisted reproduction Chair: Osamu Ishihara (Japan) Speaker: Naoaki Kuji (Japan) Sponsored by Mochida Pharmaceutical Co., Ltd.	12:00-13:00 Luncheon Seminar 3 Potential of Piezo-Assisted Micromanipulation Chair: Fumihito Aono (Japan) Speaker: Teruhiko Wakayama (Japan) Hiroshi Morita (Japan) Akiko Yabuuchi (Japan) Sponsored by Prime Tech Ltd.		
13:00	13:00-13:20 Coffee Break & Poster Viewing				
14:00	13:20-15:00 C-2 Management of PCO Patients (2) Chair: Rogerio A. Lobo (USA) Chair: Khaled Mahmoud (Tunisia) 1 Makio Shozu (Japan) 2 Chii-Ruey Tzeng (Taiwan) 3 Toshiro Kubota (Japan) 4 Timur Gürgan (Turkey) 5 Hiroaki Shibahara (Japan)	13:20-15:00 C-5 Treatment of uterine myoma or adenomyosis prior to IVF-ET (2) Chair: Victor Gomel (Canada) Chair: Issam Lebbi (Tunisia) 1 Jean-Bernard Dubuisson (Switzerland) 2 Yuji Hiramatsu (Japan) 3 Zion Ben-Rafael (Israel) 4 Hisao Osada (Japan) 5 Michael DeRosa (USA) Sponsored by Mochida Pharmaceutical Co., Ltd.	13:20-14:40 C-9 Children follow-up in ART (1) Chair: Fethi Zhioua (Tunisia) Chair: Osamu Ishihara (Japan) 1 Jia-Yin Liu (China) 2 Ilan Tur-Kaspa (USA) 3 Yukiko Katagiri (Japan) 4 Dov Feldberg (Israel)	13:20-14:40 C-12 Laboratory advances in IVF (2) Chair: Yoshihiko Hosoi (Japan) Chair: Zsolt Peter Nagy (USA) 1 Tsunehisa Makino (Japan) 2 Tae-Ki Yoon (Korea) 3 Pierre Vanderzwalmen (Belgium) 4 Gábor Vajta (Australia)	
15:00	15:00-15:20 Coffee Break	15:00-15:20 Coffee Break	14:40-15:00 Coffee Break	14:40-15:00 Coffee Break	
16:00	15:20-16:40 C-3 Controlled ovarian stimulation (1) Chair: Bruno Lunenfeld (Israel) Chair: Milton Leong (Hong Kong) 1 Bruno Lunenfeld (Israel) 2 Baris Ata (Canada) 3 Maximilian Murtinger (Austria) 4 Pak-Chung Ho (Hong Kong) Sponsored by MSD K.K.	15:20-16:40 C-6 Oncofertility - Fertility preservation in women with malignant disease (1) Chair: Claus Yding Andersen (Denmark) Chair: Takeshi Maruo (Japan) 1 Rufino Garcia-Otero Reina (Spain) 2 Werner Lichtenegger (Germany) 3 Takuma Fujii (Japan) 4 Masaaki Ando (Japan)	15:00-16:20 C-10 Children follow-up in ART (2) Chair: Hideharu Kanzaki (Japan) Chair: Dov Feldberg (Israel) 1 Peter Brockerhoff (Germany) 2 Fethi Zhioua (Tunisia) 3 Ri-Cheng Chian (Canada) 4 Tetsunori Mukaida (Japan) 16:20-17:40 C-11 Laboratory advances in IVF (1) Chair: Pierre Vanderzwalmen (Belgium) Chair: Gábor Vajta (Australia) 1 Artur Bernard (Hungary) 2 Guoning Huang (China) 3 Nicolas Zech (Austria) 4 Zsolt Peter Nagy (USA)	15:00-18:00 Oral Communications	
17:00	16:40-18:00 C-3 Controlled ovarian stimulation (2) Chair: Svend Lindenberg (Denmark) Chair: Minoru Irahara (Japan) 5 John Zhang (USA) 6 Svend Lindenberg (Denmark) 7 Hassan Sallam (Egypt) 8 Zion Ben-Rafael (Israel) Sponsored by MSD K.K.	16:40-18:00 C-7 Oncofertility - Fertility preservation in women with malignant disease (2) Chair: Klaus Diedrich (Germany) Chair: Kazunori Ochiai (Japan) 1 Shokichi Teramoto (Japan) 2 Claus Yding Andersen (Denmark) 3 Liong Tulusan (Germany) 4 Bruno Lunenfeld (Israel)			
18:00	18:30-20:30 President's Dinner (Venue: Eminence Hall・5F)				

	Concord A (5F)	Concord B (5F)	Concord C (5F)	HANA A (4F)	HANA BCD (4F)
8:30					
9:00	8:30-10:00 Plenary Lectures 4 Chair: Atsushi Tanaka (Japan) Speaker: Alan Handyside (UK) 5 Chair: Chii-Ruey Tzeng (Taiwan) Speaker: Sherman Silber (USA) 6 Chair: Shuetsu Suzuki (Japan) Speaker: Victor Gomel (Canada)				8:30-18:00 Exhibits and Poster Viewing
10:00	10:00-10:30 Coffee Break & Poster Viewing				
11:00	10:30-11:50 C-13 Controlled ovarian stimulation (3) Chair: Herbert Zech (Austria) Chair: Osamu Tsutsumi (Japan) 1 Bill Yee (USA) 2 Milton Leong (Hong Kong) 3 Johan Smitz (Belgium) 4 Herbert Zech (Austria)	10:30-12:10 C-16 Embryo development and competency from activation to implantation Chair: Jin-Ho Lim (Korea) Chair: Atsushi Azumaguchi (Japan) 1 Nelly A. Frydman (France) 2 Moncef Benkhalifa (France) 3 Atsushi Azumaguchi (Japan) 4 Svend Lindenberg (Denmark) 5 Peter Kovacs (Hungary)	10:30-11:50 C-19 AMH: Is it essential for assessing ovarian reserve Chair: Bunpei Ishizuka (Japan) Chair: Hsin-Fu Chen (Taiwan) 1 Yoshimasa Asada (Japan) 2 Budi Wiweko (Indonesia) 3 Bunpei Ishizuka (Japan) 4 Hsin-Fu Chen (Taiwan)	10:30-12:00 Oral Communications	
12:00	12:00-13:00 Luncheon Seminar 4 The role of AMH in female reproduction Chair: Yoshiharu Morimoto (Japan) Speaker: Budi Wiweko (Indonesia) Sponsored by Medical & Biological Laboratories Co.,Ltd.		12:00-13:00 Luncheon Seminar 5 Fertility tourism in the U.S. Chair: Yuji Takehara (Japan) Speaker: John Zhang (USA) Sponsored by Tosoh Corporation		
13:00	13:00-13:20 Coffee Break & Poster Viewing				
14:00	13:20-14:20 C-14 Advances in basic research and laboratory techniques in IVM (1) Chair: Yoshiharu Morimoto (Japan) Chair: Ri-Cheng Chian (Canada) 1 Nelly A. Frydman (France) 2 Hiroaki Yoshida (Japan) 3 Johan Smitz (Belgium)	13:20-14:20 C-17 ART-Gamete manipulation Chair: Moncef Benkhalifa (France) Chair: Atsushi Tanaka (Japan) 1 Akiko Yabuuchi (Japan) 2 Moncef Benkhalifa (France) 3 Atsushi Tanaka (Japan)	13:20-14:40 C-20 The role of ART in international development (1) Chair: Willem Ombelet (Belgium) Chair: Sheryl Vanderpoel (Switzerland) 1 Shangwei Li (China) 2 Gautam Allahbadia (India) 3 Daniel S. Seidman (Israel) 4 Oriol Coll (Spain)	13:20-15:00 APART Session: MSCs - From basic research to clinical applications Chair: Yuji Takehara (Japan) Chair: Byung-Rok Do (Korea) 1 Byung-Rok Do (Korea) 2 Ken Nakama (Japan) 3 Noriko Kagawa (Japan) 4 Rie Yamadera (Japan) 5 Yuji Takehara (Japan)	
15:00	14:20-15:20 C-14 Advances in basic research and laboratory techniques in IVM (2) Chair: Jie Qiao (China) Chair: Hiroaki Yoshida (Japan) 4 Ri-Cheng Chian (Canada) 5 Yoshiharu Morimoto (Japan) 6 Hai Ying Chen (Canada)	14:20-15:20 C-18 Update on male infertility - IMSI/ ICSI/MD-TESE/PGD Chair: Sherman Silber (USA) Chair: Hiroshi Okada (Japan) 1 Sherman Silber (USA) 2 Atsumi Yoshida (Japan) 3 Pierre Vanderzwalmen (Belgium)	14:40-15:40 C-20 The role of ART in international development (2) Chair: Daniel Seidman (Israel) Chair: Tetsunori Mukaida (Japan) 5 Claus Peter Janisch (Germany) 6 Sheryl Vanderpoel (Switzerland) 7 Willem Ombelet (Belgium)	15:00-15:20 Coffee Break	
	15:20-15:40 Coffee Break	15:20-15:40 Coffee Break		15:20-17:50 Oral Communications	
16:00	15:40-17:20 C-15 Clinical outcomes; toward a more successful IVM Chair: Johan Smitz (Belgium) Chair: Aisaku Fukuda (Japan) 1 Jie Qiao (China) 2 Shu Hashimoto (Japan) 3 Jin-Ho Lim (Korea) 4 Aisaku Fukuda (Japan) 5 Svend Lindenberg (Denmark)	15:40-17:40 ISF Session by the Israeli Society of Fertility Chair: Arye Hourwitz (Israel) Chair: Martha Dirnfeld (Israel) 1 Yaron Rabinovici (Israel) 2 Arye Hourwitz (Israel) 3 Martha Dirnfeld (Israel) 4 Daniel Seidman (Israel) 5 Ariel Revel (Israel) 6 Shevach Friedler (Israel)	15:40-16:00 Coffee Break 16:00-18:00 STGO Session by the Tunisian Society of Gynecology and Obstetrics Challenging issues in ART (1) Chair: Hedi Khairi Chair: Anis Feki Chair: Said Lazrak 1 Youssef Boutaleb (Morocco) 2 René Frydman (France) 3 Amina Oumziane (Algeria) Challenging issue in ART (2) Chair: Mahmoud Kharouf Chair: Moise Fiadjoe Chair: Nabil Ben Zineb 1 Khaled Terras (Tunisia) 2 Moncef Benkhalifa (France) 3 Merdassi Ghaya (Tunisia)		
17:00					
18:00					
	18:30-20:30 Gala Dinner (Venue: Concord AB・5F)				

	Concord A (5F)	Concord B (5F)	Concord C (5F)	HANA A (4F)	HANA BCD (4F)
8:30					
9:00	8:30-10:00 Plenary Lectures 7 Chair: Yoon-Seok Chang (Korea) Speaker: Klaus Diedrich (Germany) 8 Chair: Hiroshi Okada (Japan) Speaker: Gianpiero D. Palermo (USA) 9 Chair: Yoshiharu Morimoto (Japan) Speaker: Seang Lin Tan (Canada)				8:00-14:30 Exhibits and Poster Viewing
10:00	10:00-10:30 Coffee Break	10:00-10:30 Coffee Break	10:00-10:30 Coffee Break		
11:00	10:30-11:50 C-21 Repeated implantation failure: how to improve implantation in ART Chair: Kouichi Takakuwa (Japan) Chair: Yael Gonen (Israel) 1 Yael Gonen (Israel) 2 Kouichi Takakuwa (Japan) 3 Hiroshi Fujiwara (Japan) 4 Moritoshi Seki (Japan)	10:30-11:50 C-22 Pre-implantation genetic screening: an update Chair: Hidekazu Saito (Japan) Chair: Jiaen Liu (China) 1 Atsushi Tanaka (Japan) 2 Wilfried Feichtinger (Austria) 3 Kou Sueoka (Japan) 4 Alan Handyside (UK)	10:30-12:10 C-23 Stem cell and fertility preservation: Where are we at with stem cells? Chair: Kwang Yul Cha (Korea) Chair: Oktay Kadayifci (Turkey) 1 Hidenori Akutsu (Japan) 2 Anis Feki (Switzerland) 3 Hsin-Fu Chen (Taiwan) 4 Kwang Yul Cha (Korea) 5 Takehiko Ogawa (Japan)	10:30-12:00 Oral Communications	
12:00			12:10-13:10 Luncheon Seminar 6 Global inequity: Access to Assisted Reproductive Technologies Chair: Osamu Ishihara (Japan) Speaker: Sheryl Vanderpoel (Switzerland)		
13:00	13:00-13:30 Coffee Break	13:00-13:30 Coffee Break	13:10-13:30 Coffee Break		
14:00	13:30-14:30 Special Guest Lecture Chair: Akira Iritani (Japan) Speaker Shinya Yamanaka (Japan)				
15:00	14:30-15:00 ISIVF General Assembly & Closing Ceremony				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In Vitro Fertilization
Tunis, Tunisia 2013
With the cooperation of
Tunisian Society of
Gynecology & Obstetrics

SCIENTIFIC PROGRAM

1. Special Guest Lecture:

Tuesday, September 13 13:30～14:30 (Concord Ballroom ABC)

Chairperson: Akira Iritani (Japan)

13:30～14:30 **Induction of pluripotency by defined factors**
- Shinya Yamanaka (Japan)

2. Opening Ceremony, Opening Lecture and Welcome Reception:

Opening Ceremony: Saturday, September 10 18:30～19:30 (Eminence Hall, 5F)

18:30～19:00 Opening Ceremony

19:00～19:30 Opening Lecture A tribute to Robert Edwards - the pioneer of human IVF
-Victor Gomel (Canada)

Welcome Reception: Saturday, September 10 19:30～21:30 (Concord Ballroom ABC, 5F)

3. Plenary Lectures:

Sunday, September 11 8:30～10:00(Concord Ballroom ABC)

8:30～8:50 Plenary Lecture -1 (1) **Chairperson: Victor Gomel (Canada)**
Advances in natural and mild stimulation cycle IVF
- progress over the last 15 years
-Osamu Kato (Japan)

8:50～9:00 Plenary Lecture -1 (2) **Chairperson: Victor Gomel (Canada)**
Clomid® ovarian stimulation for IVF and frozen ET
-John Zhang (USA)

9:00～9:30 Plenary Lecture-2 **Chairperson: Sherman Silber (USA)**
Non-invasive assessment of embryo quality
-Zsolt Peter Nagy (USA)

9:30～10:00 Plenary Lecture -3 **Chairperson: Takahide Mori (Japan)**
The prospect of embryonic stem cell research
-Shin Yong Moon (Korea)

Monday, September 12 8:30～10:00 (Concord Ballroom ABC)

8:30～9:00 Plenary Lecture-4 **Chairperson: Atsushi Tanaka (Japan)**
Pre-implantation genetic diagnosis: an update
-Alan Handyside (UK)

9:00～9:30 Plenary Lecture -5 **Chairperson: Chii-Ruey Tzeng (Taiwan)**
Preservation of fertility
-Sherman Silber (USA)

9:30～10:00 Plenary Lecture -6 **Chairperson: Shuetsu Suzuki (Japan)**
The role of reproductive surgery in the era of IVF&ART
-Victor Gomel (Canada)

Tuesday, September 13 8:30～10:00 (Concord Ballroom ABC)

8:30～9:00	Plenary Lecture -7	**Chairperson: Yoon-Seok Chang (Korea)** Recent advance of controlled ovarian stimulation *-Klaus Diedrich (Germany)*
9:00～9:30	Plenary Lecture-8	**Chairperson: Hiroshi Okada (Japan)** Tending to male factor couples *-Gianpiero D. Palermo (USA)*
9:30～10:00	Plenary Lecture-9	**Chairperson: Yoshiharu Morimoto (Japan)** IVM for fertility treatment and preservation of fertility *-Seang Lin Tan (Canada)*

4. Pre-Congress Workshops:

1) Vitrification

Saturday, September 10 9:00～12:00 (Hana A)

Chairpersons: Stanley Leibo (USA)
Sherman Silber (USA)

(1) Lectures	
1. Basic science of vitrification	Stanley Leibo (USA)
2. Ovarian reserving (basic research)	Noriko Kagawa (Japan)
3. Ovary transplant: Ovary allotransplantation between	Sherman Silber (USA)
(2) Live demonstration	
4. The Cryotop® method	Masashige Kuwayama (Japan)

2) Pre-implantation genetic diagnosis (PGD)

Saturday, September 10 14:00～17:00 (Hana A)

Chairpersons: Naoki Aoyama (Japan)
Ilan Tur-Kaspa (USA)

(1) Lectures	
1. Overview	Kou Sueoka (Japan)
2. Whole genome amplification is a new basic technology for next-generation preimplantation genetic diagnosis on mendelian inheritance disease	Suguru Sato (Japan)
3. To PGD or not to PGD? How many oocytes are needed to PGD with?	Ilan Tur-Kaspa (USA)
(2) Live demonstration	
4. Efficient techniques of PGD for recurrent pregnancy loss (Live)	Naoki Aoyama (Japan)

5. Concurrent Symposia:

C-1 Management of PCO patients (1)

Sunday, September 11 10:30～11:50 (Concord Ballroom A)
Chairperson: Timur Gürgan (Turkey)
Makio Shozu (Japan)

10:30～10:50	C-1. 1	Androgenic spectrum concept of PCO morphogenesis *-Takahide Mori (Japan)*
10:50～11:10	C-1. 2	Do phenotypic differences in PCOS affect fertility treatment and outcomes? *-Rogerio A. Lobo (USA)*
11:10～11:30	C-1. 3	Laser assisted ICSI for oocytes matured in vitro from PCO patients *-Muchsin Jaffar (Indonesia)*
11:30～11:50	C-1. 4	The use of GnRH antagonist in the controlled ovarian stimulation for IVF in PCOS patients *-Khaled Mahmoud (Tunisia)*

C-2 Management of PCO Patients (2)

Sunday, September 11 13:20～15:00 (Concord Ballroom A)
Chairpersons: Rogerio A. Lobo (USA)
Khaled Mahmoud (Tunisia)

13:20～13:40	C-2. 1	Polycystic ovary syndrome is associated with metabolic abnormalities in the purine mononucleotide pathway and tricarboxylic acid cycle *-Makio Shozu (Japan)*
13:40～14:00	C-2. 2	How to get pregnancy in women with PCOS *-Chii-Ruey Tzeng (Taiwan)*
14:00～14:20	C-2. 3	ART Management in PCO *-Toshiro Kubota (Japan)*
14:20～14:40	C-2. 4	PCO and ovarian drilling *-Timur Gürgan (Turkey)*
14:40～15:00	C-2. 5	PCO and transvaginal hydrolaparoscopic ovarian drilling (THLOD) *-Hiroaki Shibahara (Japan)*

C-3 Controlled ovarian stimulation (1)

Sunday, September 11 15:20～16:40 (Concord Ballroom A)
Chairpersons: Bruno Lunenfeld (Israel)
Milton Leong (Hong Kong)

15:20～15:40	C-3. 1	Available tools to personalize ovarian stimulation - Dose prediction/protocol selection *-Bruno Lunenfeld (Israel)*

15:40～16:00	C-3. 2	Advances in ultrasound monitoring of IVF cycles by automated volume measurement *- Baris Ata (Canada)*
16:00～16:20	C-3. 3	Volmetric measurements of the follicular development *-Maximilian Murtinger (Austria)*
16:20～16:40	C-3. 4	Ovarian stimulation and endometrial receptivity *-Pak-Chung Ho (Hong Kong)*

C-3 Controlled ovarian stimulation (2)

Sunday, September 11 16:40～18:00 (Concord Ballroom A)

Chairpersons: Svend Lindenberg (Denmark)
Minoru Irahara (Japan)

16:40～17:00	C-3. 5	Minimal ovarian stimulation (mini-IVF) for IVF utilizing vitrification and cryopreserved embryo transfer *-John Zhang (USA)*
17:00～17:20	C-3. 6	Poor responder patients undergoing IVF treatment *-Svend Lindenberg (Denmark)*
17:20～17:40	C-3. 7	Prediction and prevention of the ovarian hyperstimulation syndrome (OHSS) – an evidence-based approach *-Hassan Sallam (Egypt)*
17:40～18:00	C-3. 8	What should we prefer for IVF – the agonist or antagonist? *-Zion Ben-Rafael (Israel)*

C-4 Treatment of benign tumor prior to IVF-ET (1)

Sunday, September 11 10:30～11:50 (Concord Ballroom B)

Chairpersons: Zion Ben-Rafael (Israel)
Hassan Sallam (Egypt)

10:30～10:50	C-4. 1	Evidence-based management of endometriosis-associated infertility *-Hassan Sallam (Egypt)*
10:50～11:10	C-4. 2	Surgery of endometriosis in infertility: state of the art! *-Issam Lebbi (Tunisia)*
11:10～11:30	C-4. 3	Surgical management of recurrent endometrioma: prior to IVF or not *-Hiroshi Nabeshima (Japan)*
11:30～11:50	C-4. 4	Management of hydrosalpinges prior to IVF-ET *-Victor Gomel (Canada)*

C-5 Treatment of uterine myoma or adenomyosis prior to IVF-ET (2)

Sunday, September 11 13:20～15:00 (Concord Ballroom B)

Chairpersons: Victor Gomel (Canada)
Issam Lebbi (Tunisia)

13:20～13:40	C-5. 1	Surgery of intra-mural leiomyomas in infertile patients *-Jean-Bernard Dubuisson (Switzerland)*
13:40～14:00	C-5. 2	Myomectomy during pregnancy *-Yuji Hiramatsu (Japan)*
14:00～14:20	C-5. 3	Uterine fibroids and IVF- what is the controversy? *-Zion Ben-Rafael (Israel)*
14:20～14:40	C-5. 4	A novel operative treatment of severe adenomyosis prior to embryo transfer *-Hisao Osada (Japan)*
14:40～15:00	C-5. 5	Different surgical approaches for myomectomy and for adenomyosis *-Michael DeRosa (USA)*

C-6 Oncofertility - Fertility preservation in women with malignant disease (1)
Sunday, September 11 15:20～16:40 (Concord Ballroom B)
Chairpersons: Claus Yding Andersen (Denmark)
Takeshi Maruo (Japan)

15:20～15:40	C-6. 1	The oncofertility: consequences of a previous oncological treatments and possibilities *-Rufino García-Otero Reina (Spain)*
15:40～16:00	C-6. 2	Fertility Sparing in Stage Ia2 to Ib1-Cervix Carcinoma *-Werner Lichtenegger (Germany)*
16:00～16:20	C-6. 3	Abdominal radical trachelectomy in our experience of 126 cases *-Takuma Fujii (Japan)*
16:20～16:40	C-6. 4	Laparoscopic radical trachelectomy *-Masaaki Ando (Japan)*

C-7 Oncofertility - Fertility preservation in women with malignant disease (2)
Sunday, September 11 16:40～18:00 (Concord Ballroom B)
Chairpersons: Klaus Diedrich (Germany)
Kazunori Ochiai (Japan)

16:40～17:00	C-7. 1	Cryopreservation of human oocytes for cancer patients in Japan *-Shokichi Teramoto (Japan)*
17:00～17:20	C-7. 2	Cryopreservation of human ovarian tissue, clinical data and scientific considerations *-Claus Yding Andersen (Denmark)*
17:20～17:40	C-7. 3	Consevative therapy for borderline ovarian cancer *-Liong Tulusan (Germany)*
17:40～18:00	C-7. 4	Infertility, ovulation induction treatments and the incidence of breast,

ovarian and endometrial cancers- thirty years of follow up
- *Bruno Lunenfeld (Israel)*

C-8 ART-cryopreservation: Is oocyte cryopreservation a revolution in IVF?

Sunday, September 11 10:30～11:50 (Concord Ballroom C)
Chairpersons: Stanley Leibo (USA)
Masashige Kuwayama (Japan)

10:30～10:50	C-8. 1	Highly efficient and safe vitrification using hydroxypropyl cellulose as a macromolecular supplement for cryopreservation of oocytes and blastocysts *-Luis Arturo Ruvalcaba Castellón (Mexico)*
10:50～11:10	C-8. 2	Role and possibility of vitrification in human oocyte *-Masashige Kuwayama (Japan)*
11:10～11:30	C-8. 3	Blastulation and pregnancy rates after vitrified human zygote culture for 4 days: Preliminary results *-Safaa Al Hasani (Germany)*
11:30～11:50	C-8. 4	Cryopreservation in human ART *-Stanley Leibo (USA)*

C-9 Children follow-up in ART(1)

Sunday, September 11 13:20～14:40 (Concord Ballroom C)
Chairpersons: Fethi Zhioua (Tunisia)
Osamu Ishihara (Japan)

13:20～13:40	C-9. 1	Evaluate the IVF success rates of accumulated pregnancy rates per oocyte retrieval *-Jia-Yin Liu (China)*
13:40～14:00	C-9. 2	Genetics and health of children born from cryopreserved oocytes *-Ilan Tur-Kaspa (USA)*
14:00～14:20	C-9. 3	What has happened on epigenetics in ART children? *-Yukiko Katagiri (Japan)*
14:20～14:40	C-9. 4	Pregnancy and child outcome after assisted reproductive technologies *-Dov Feldberg(Israel)*

C-10 Children follow-up in ART(2)

Sunday, September 11 15:00～16:20 (Concord Ballroom C)
Chairpersons: Hideaki Kanzaki (Japan)
Dov Feldberg (Israel)

15:00～15:20	C-10. 1	Just Twins? Perinatal data from multiple pregnancies *-Peter Brockerhoff (Germany)*
15:20～15:40	C-10. 2	Perinatal outcomes of ART pregnancies *-Fethi Zhioua (Tunisia)*

15:40～16:00	C-10. 3	Outcome of IVM babies *-Ri-Cheng Chian (Canada)*
16:00～16:20	C-10. 4	Perinatal outcome of vitrified human blastocysts in 11 years experience (5434 attempted cycles) including the rate of monozygotic twinning (MZT) *-Tetsunori Mukaida (Japan)*

C-11 Laboratory advances in IVF (1)

Sunday, September 11 16:20～17:40 (Concord Ballroom C)

Chairpersons: Pierre Vanderzwalmen (Belgium)
Gábor Vajta (Australia)

16:20～16:40	C-11. 1	Novel embryo culturing and monitoring systems *-Artur Bernard (Hungary)*
16:40～17:00	C-11. 2	Effects of cumulus cells removal after 6 h co-incubation of gametes on the outcomes of human IVF *-Guoning Huang (China)*
17:00～17:20	C-11. 3	An integral view on therapeutic options and lab-techniques for individualized therapy in IVF *-Nicolas Zech (Austria)*
17:20～17:40	C-11. 4	How to manage the modern IVF laboratory *-Zsolt Peter Nagy (USA)*

C-12 Laboratory advances in IVF (2)

Sunday, September 11 13:20～14:40 (Hana A)

Chairpersons: Yoshihiko Hosoi (Japan)
Zsolt Peter Nagy (USA)

13:20～13:40	C-12. 1	Chemical substances exposure on IVF-ET environment *-Tsunehisa Makino (Japan)*
13:40～14:00	C-12. 2	Recent insights into the oocyte maturation in ART *-Tae-Ki Yoon(Korea)*
14:00～14:20	C-12. 3	Use of high concentrations of cryoprotectants: is it a justified argument to prefer slow freezing instead of vitrification? *-Pierre Vanderzwalmen (Belgium)*
14:20～14:40	C-12. 4	Embryo culture: Can we perform better than Nature? *-Gábor Vajta (Australia)*

C-13 Controlled ovarian stimulation (3)

Monday, September 12 10:30～11:50 (Concord Ballroom A)

Chairpersons: Herbert Zech (Austria)
Osamu Tsutsumi (Japan)

10:30～10:50	C-13. 1	Superovulation/IUI - An Alternative to IVF *-Bill Yee (USA)*

10:50～11:10	C-13. 2	Treatment of patients with high FSH with delayed stimulation using low dose gonadotrophins *-Milton Leong (Hong Kong)*
11:10～11:30	C-13. 3	Use of hCG in ovarian stimulation *-Johan Smitz (Belgium)*
11:30～11:50	C-13. 4	Follicle stimulation by transdermal application of FSH *-Herbert Zech (Austria)*

C-14 Advances in basic research and laboratory techniques in IVM (1)

Monday, September 12 13:20～14:20 (Concord Ballroom A)

Chairpersons: Yoshiharu Morimoto (Japan)
Ri-Cheng Chian (Canada)

13:20～13:40	C-14. 1	IVM: learning from IVM ? *-Nelly A. Frydman (France)*
13:40～14:00	C-14. 2	Quality evaluation of human & mouse IVM embryo and epigenetic evaluation of human born IVM babies *-Hiroaki Yoshida (Japan)*
14:00～14:20	C-14. 3	Lessons from the mouse follicle culture and in vitro maturation models *-Johan Smitz (Belgium)*

C-14 Advances in basic research and laboratory techniques in IVM (2)

Monday, September 12 14:20～15:20 (Concord Ballroom A)

Chairpersons: Jie Qiao (China)
Hiroaki Yoshida (Japan)

14:20～14:40	C-14. 4	Laboratory aspect of natural cycle IVF/IVM treatment *-Ri-Cheng Chian (Canada)*
14:40～15:00	C-14. 5	Cytoplasmic maturation and mitochondrial activity in human IVM *-Yoshiharu Morimoto (Japan)*
15:00～15:20	C-14. 6	Correct embryo selection improves embryo implantation rates in IVM cycles *- Hai Ying Chen (Canada)*

C-15 Clinical outcomes; toward a more successful IVM

Monday, September 12 15:40～17:20 (Concord Ballroom A)

Chairpersons: Johan Smitz (Belgium)
Aisaku Fukuda (Japan)

15:40～16:00	C-15. 1	IVM application in PCOS patients - Jie Qiao *(China)*
16:00～16:20	C-15. 2	Oocyte maturation from tiny follicles in human ovarian tissue *-Shu Hashimoto (Japan)*

16:20～16:40	C-15. 3	Natural cycle combined with IVM *-Jin-Ho Lim (Korea)*
16:40～17:00	C-15. 4	The effective approach for IVM using Metformin *-Aisaku Fukuda (Japan)*
17:00～17:20	C-15.5	Clincal implications of IVM *-Svend Lindenberg (Denmark)*

C-16 Embryo development and competency from activation to implantation

Monday, September 12 10:30～12:10 (Concord Ballroom B)

Chairpersons: Jin-Ho Lim (Korea)
Atsushi Azumaguchi (Japan)

10:30～10:50	C-16. 1	Morphological aspect *-Nelly A. Frydman (France)*
10:50～11:10	C-16. 2	Early embryo development: what's critical *-Moncef Benkhalifa (France)*
11:10～11:30	C-16. 3	Implantation window *-Atsushi Azumaguchi (Japan)*
11:30～11:50	C-16. 4	Luteal phase support *-Svend Lindenberg (Denmark)*
11:50～12:10	C-16. 5	Predictors of IVF outcome with a particular interest in the amount of gonadotropin administered *-Peter Kovacs (Hungary)*

C-17 ART-Gamete manipulation

Monday, September 12 13:20～14:20 (Concord Ballroom B)

Chairpersons: Moncef Benkhalifa (France)
Atsushi Tanaka (Japan)

13:20～13:40	C-17. 1	Whole ooplasmic replacement in Germinal Vesicle oocytes *-Akiko Yabuuchi (Japan)*
13:40～14:00	C-17. 2	Dynamic genome function and chromosomes abnormalities *-Moncef Benkhalifa (France)*
14:00～14:20	C-17. 3	A novel trial of nuclear transfer for repairing an aged oocyte *-Atsushi Tanaka (Japan)*

C-18 Update on male infertility - IMSI/ ICSI/MD-TESE/PGD

Monday, September 12 14:20～15:20 (Concord Ballroom B)

Chairpersons: Sherman Silber (USA)
Hiroshi Okada (Japan)

14:20～14:40	C-18. 1	Male infertility *-Sherman Silber (USA)*

14:40～15:00	C-18. 2	Microdissection Testicular Sperm Extraction (MD-TESE) *-Atsumi Yoshida (Japan)*
15:00～15:20	C-18. 3	IMSI, already 8 years in ART practice. Where do we stand? *-Pierre Vanderzwalmen (Belgium)*

C-19 AMH: Is it essential for assessing ovarian reserve

Monday, September 12 10:30～11:50 (Concord Ballroom C)

Chairpersons: Bunpei Ishizuka (Japan)
Hsin-Fu Chen (Taiwan)

10:30～10:50	C-19. 1	Clinical application of AMH in the reproductive medicine *-Yoshimasa Asada (Japan)*
10:50～11:10	C-19. 2	Chronological aging vs biological aging: AMH as an early marker of biological aging *-Budi Wiweko (Indonesia)*
11:10～11:30	C-19. 3	POF and AMH *-Bunpei Ishizuka (Japan)*
11:30～11:50	C-19. 4	Dynamic serum AMH levels and AMH-related gene expression in human ovarian follicles *-Hsin-Fu Chen (Taiwan)*

C-20 The role of ART in international development (1)

Monday, September 12 13:20～14:40 (Concord Ballroom C)

Chairpersons: Willem Ombelet (Belgium)
Sheryl Vanderpoel (Switzerland)

13:20～13:40	C-20. 1	Implementation of the fertility assistance program after the Wenchuan earthquake in China *-Shangwei Li (China)*
13:40～14:00	C-20. 2	Cross-border cryo-shipping of vitrified embryos (CCVE): the newest ART success story *-Gautam Allahbadia(India)*
14:00～14:20	C-20. 3	Cross-border reproductive care: exploitation or opportunity in the global quest for a baby? *-Daniel S. Seidman (Israel)*
14:20～14:40	C-20. 4	Oocyte donors: prevalence of genetic, infectious diseases and others *-Oriol Coll (Spain)*

C-20 The role of ART in international development (2)

Monday, September 12 14:40～15:40 (Concord Ballroom C)

Chairpersons: Daniel Seidman (Israel)
Tetsunori Mukaida (Japan)

14:40～15:00	C-20. 5	Vouchers for health: A demand side output-based aid approach toreproductive health services. A model to pay for infertility services?

-*Claus Peter Janisch (Germany)*

15:00～15:20	C-20. 6	Global challenges and perspectives -*Sheryl Vanderpoel (Switzerland)*
15:20～15:40	C-20. 7	Affordable IVF: low cost IVF – The Arusha project -*Willem Ombelet (Belgium)*

C-21 Repeated implantation failure: how to improve implantation in ART

Tuesday, September 13 10:30～11:50 (Concord Ballroom A)

Chairpersons: Kouichi Takakuwa (Japan)
Yael Gonen (Israel)

10:30～10:50	C-21. 1	How to improve implantation in ART: novel approaches -*Yael Gonen (Israel)*
10:50～11:10	C-21. 2	Studies on the efficacy of immunotherapy using paternal mononuclear cells for patients with infertility -*Kouichi Takakuwa (Japan)*
11:10～11:30	C-21. 3	Improvement of implantation rates using autologous peripheral blood mononuclear cells -*Hiroshi Fujiwara(Japan)*
11:30～11:50	C-21.4	Prevention of infertility in patients for the antiphospholipid syndrome and deficiencies of natural anticoagulants -*Moritoshi Seki (Japan)*

C-22 Pre-implantation genetic screening: an update

Tuesday, September 13 10:30～11:50 (Concord Ballroom B)

Chairpersons: Hidekazu Saito (Japan)
Jiaen Liu (China)

10:30～10:50	C-22. 1	PGD for recurrent pregnancy loss -*Atsushi Tanaka (Japan)*
10:50～11:10	C-22. 2	Polar body analysis in clinical practice as compared to pre-embryo morphology for selection of the best for transfer -*Wilfried Feichtinger (Austria)*
11:10～11:30	C-22. 3	PGD outcome and alternatives for single gene disorder -*Kou Sueoka (Japan)*
11:30～11:50	C-22.4	Preimplantation genetic diagnosis for aneuploidy using whole genome amplification and microarray comparative genomic hybridization -*Alan Handyside (UK)*

C-23 Stem cell and fertility preservation: Where are we at with stem cells?

Tuesday, September 13 10:30～12:10 (Concord Ballroom C)

Chairpersons: Kwang Yul Cha (Korea)
Oktay Kadayifci (Turkey)

10:30～10:50	C-23. 1	Human ES cell and iPS cell derivation: Clinical applications and biological characterization *-Hidenori Akutsu (Japan)*
10:50～11:10	C-23. 2	Pluripotent stem cell as a source of germ cells - Is it still a fiction? *-Anis Feki (Switzerland)*
11:10～11:30	C-23. 3	Development of pluripotent stem cells to female germ cells *-Hsin-Fu Chen (Taiwan)*
11:30～11:50	C-23. 4	Testicular stem cells as sources of fertility preservation and cell therapy *-Kwang Yul Cha (Korea)*
11:50～12:10	C-23. 5	In vitro production of functional sperm in cultured neonatal mouse testes *-Takehiko Ogawa (Japan)*

6. STGO Session (Tunisian Society of Gynecology and Obstetrics)

(Coordinators: Khaled Mahmoud, Fethi Zhioua, Issam Lebbi)

Challenging issues in ART (1)

Monday, September 12 16:00～17:00 (Concord Ballroom C)

Chairpersons: Hedi Khairi
Anis Feki
Said Lazrak

16:00～16:20	STGO (1) 1	How to manage hyper-response to COS in IVF *-Youssef Boutaleb (Morocco)*
16:20～16:40	STGO (1) 2	How to manage poor-responders? *-René Frydman (France)*
16:40～17:00	STGO (1) 3	The thin endometrium in ART: What to do? *-Amina Oumziane (Algeria)*

Challenging issues in ART (2)

Monday, September 12 17:00～18:00 (Concord Ballroom C)

Chairpersons: Mahmoud Kharouf
Moise Fiadjoe
Nabil Ben Zineb

17:00～17:20	STGO (2) 1	How to treat the female causes of repeated implantation failure? *-Khaled Terras (Tunisia)*
17:20～17:40	STGO (2) 2	How to manage sperm investigation after ICSI Failure? *-Moncef Benkhalifa (France)*
17:40～18:00	STGO (2) 3	How to improve laboratory procedures to enhance implantation? *-Ghaya Merdassi (Tunisia)*

7. ISF Session (Israeli Society of Fertility) (Coordinator: Daniel Seidman)

Monday, September 12 15:40～17:40 (Concord Ballroom B)

Chairpersons: Arye Hurwitz
Martha Dirnfeld

15:40～16:00	ISF 1.	Fertility enhancement in patients with adenomyosis using MRI guided HIFUS *-Yaron Rabinovici (Israel)*
16:00～16:20	ISF 2.	Gonadotropin-releasing hormone agonist trigger: the way to eliminate OHSS *-Arye Hurwitz (Israel)*
16:20～16:40	ISF 3.	From endometrial injury to IMSI: New approaches for repeated IVF failure *-Martha Dirnfeld (Israel)*
16:40～17:00	ISF 4.	High ART success rates when financial constraints are completely lifted *-Daniel Seidman (Israel)*
17:00～17:20	ISF 5.	Autologous transplantation of very thin ovarian fragments which preserve the ovary's main cortex structure lead to successful pregnancy *-Ariel Revel (Israel)*
17:20～17:40	ISF 6.	How should we consult candidates for fertility preservation due to a malignant disease regarding their expected ovarian response to COH? *-Shevach Friedler (Israel)*

8. APART Session: MSCs - From basic research to clinical applications

Monday, September 12 13:20～15:00 (Hana A)

Chairpersons: Yuji Takehara (Japan)
Byung-Rok Do (Korea)

13:20～13:40	APART 1.	Clinical efficacy of adipose MSCs in human *−Byung-Rok Do (Korea)*
13:40～14:00	APART 2.	Human lipoaspiration *−Ken Nakama (Japan)*
14:00～14:20	APART 3.	Vitrification of adipose MSCs *−Noriko Kagawa (Japan)*
14:20～14:40	APART 4.	Preparation of adipose-derived stem cells *−Rie Yamadera (Japan)*
14:40～15:00	APART 5.	Discussion about clinical applications of adipose MSCs *−Yuji Takehara (Japan)*

9. Oral Communications

Sunday, September 11 10:30～12:00 (Hana A)

Chairpersons: Daniel Bodri (Hungary)
Atsumi Yoshida (Japan)

O-001 Does male reproductive tract CD52 (mrt-CD52) prevent complement activation via binding C1q?

○L. Hardiyanto, A. Hasegawa, S. Komori

Hyogo College of Medicine, Kobe, Japan

O-002 Technology Research of Prostatic Urethral Irrigation and Drainage Catheter Injection

○Weidong Huang

Xinjiang Jiayin Hospital Center for Reproductive Medicine, Urumqi, China

O-003 Measurement of reactive oxygen species in neat and washed sperm, is there any difference?

○Mohammadreza Moein, Nasim Tabibnejad, Jalal Ghasemzadeh

Yazd Research and Clinical Center for Infertility, Iran

Chairpersons: Naoki Takeshita (Japan)
Naoki Aoyama (Japan)

O-004 Whole Genome Amplification is efficient for CTG repeats length detection of PGD for DM1

○Tomoyoshi Sakurai[1,2], Kou Sueoka[2], Kaori Takahashi[2], Suguru Sato[2], Kenji Sato[2],Yasunori Yoshimura[2]

[1]Saitama municipal Hospital Department of Obstetrics and Gynecology, Department of Obstetrics and Gynecology, Saitama, Japan,

[2]Keio University School of Medicine, Tokyo, Japan

O-005 Reducing multiple pregnancies by selection of a single chromosomally normal blastocyst for transfer using array CGH (aCGH) analysis of 24 chromosomes within 24 hours

○Zhihong Yang[1], Xiaohong Liu[2], Shala Salem[1], Jiaen Liu[2], Rifaat Salem[1]

[1]Pacific Reproductive Center, Torrance, California USA, [2]Beijing Jiaen Hospital, 29 Zhichun lu, Beijing 10083, China

O-006 Preimplantation genetic screening of eggs and blastocysts with CGH-microarray: clinical experience with the first 51 cases

○Stuart Lavery[1], Ben Lavender[1], Paul Knaggs[1], Anastasia Mania[1], Geoffrey Trew[1], Dagan Wells[2]

[1]IVF Hammersmith, Hammersmith Hospital, London UK, [2]Reprogenetics Oxford UK

Sunday, September 11 15:00～18:00 (Hana A)

Chairpersons: Budi Wiweko (Indonesia)
Shu Hashimoto (Japan)

O-007 Most Motile Sperm Can Be Separated at the Bottom of the Microfluidic Channel of a Plastic Microfluidic Sperm Sorter

○Koji Matsuura[1], Keiji Naruse[2]

[1]Research Core for Interdisciplinary Sciences, Okayama University, Okayama, Japan, [2]Cardiovascular Physiology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

O-008 Polymerization of Insulin-Like Growth Factor-Binding Protein-1 (IGFBP-1) Potentiates IGF-I Actions in Placenta

○M Kabir-Salmani[1], H Shibuya[2], K Sakai[2], Y Wachi[2], M Iwashita[2]
[1]National Institute of Genetic Engineering and Biotechnology,Tehran, Iran, [2]Department of Obstetrics and Gynecology, Kyorin University School of Medicine, Tokyo, Japan

O-009 MicroRNAs associated with human embryo implantation defects

○Ariel Revel[1], Hanna Achache[2], Juliet Stevens[3], Smith Yoav[4]
[1] Hadassah University hospital. Jerusalem, Israel, [2]Institute for Drug Research, School of Pharmacy, Faculty of medicine, The Hebrew University of Jerusalem, Jerusalem, Israel, [3]University of Oxford, Oxford, United Kingdom, [4]Genomic Data Analysis Unit, The Hebrew University of Jerusalem, Jerusalem, Israel

O-010 Pre-ovulation leptin serum as a marker for endometrial receptivity

○Andon Hestiantoro[1], Marly Susanti[1], Atikah Barasila[2]
[1]Division Reproductive Immuno-endocrinology, Department of Obstetrics and Gynecology, Faculty of Medicine University of Indonesia, Dr. Cipto Mangunkusumo., [2]Departmen of Hystology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia

Chairpersons: Daniel Bodri (Hungary)
Akira Kuwahara (Japan)

O-011 Application of Mechanical Stimuli Using a Microfluidic Air Actuation System: Dynamic Embryo Culture

○Koji Matsuura[1], Yuka Kuroda[1], Keiji Naruse[2]
[1]Research Core for Interdisciplinary Sciences, Okayama University, Okayama, Japan, [2]Cardiovascular Physiology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

O-012 Caffeine treatment influences in vivo and in vitro fertilization, embryo development and morphology in mice

○Yoshimasa Yokota[1], Mikako Yokota[1], Hidemi Yokota[1], Setsuko Sato[1], Yasuhisa Araki[2]
[1]Yokota Maternity Hospital, [2]The Institute for Advanced Reproductive Medical Technology, Gunma, Japan

O-013 Dynamic analysis of compaction initiation in human embryos using time-lapse cinematography

○Kyoko Iwata, Keitaro Yumoto, Akifumi Imajo, Yumiko Iba, Yasuyuki Mio
Mio Fertility Clinic, Yonago, Japan

O-014 Chromosomal analysis of cleavage embryos and blastocysts derived from tripronuclear zygotes after ICSI.

○Shimpei Mizuta, Nobuhiko Kataoka, Hiromi Hashimoto, Yasushi Kuroda, Shoji Kokeguchi, Masahide Shiotani
Hanabusa Women's Clinic, Hyogo, Japan

Chairpersons: Muchsin Jaffar (Indonesia)
Fumihito Aono (Japan)

O-015 THE DYNAMIC PROCESS OF SPERM PENETRATION OF THE HUMAN OOCYTE ANALYZED USING TIME-LAPSE CINEMATOGRAPHY

○Yoshiteru Kai, Kyoko Iwata, Keitaro Yumoto, Akifumi Imajo, Yumiko Iba, Yasuyuki Mio
Mio Fertility Clinic, Yonago, Japan

O-016 Depletion of Plk1 delays cell cycle progression in endometrial cells derived from endometriosis

Li Tang[1,2], Tian-Hua Zhou[3], Jian-Zhong Sheng[4], Yan-Ting Wu[2], Ming-Yue Dong[2], ○He-Feng Huang[2]
[1]The First People's Hospital of Yunnan Province, [2]Women's Hospital, Zhejiang University, School of Medicine, [3]Zhejiang University, School of Medicine, [4]University of Calgary, Canada

O-017 Male fetal DNA detection in maternal serum from pregnant cynomolgus monkeys (Macaca fascicularis) in an established breeding colony

○Lubna YASMIN[1], Jun-ichiro TAKANO[2], Yasushi NAGAI[3], Junko OTSUKI[3], Tadashi SANKAI[1]

[1]Tsukuba Primate Research Center, National Institute of Biomedical Innovation , [2]Department of Research Resource Developments, The Corporation for Production and Research of Laboratory Primates, [3]Nagai Clinic

O-018 Clinical outcome of 852 tubal factor infertility patients without ART after Falloposcopic tuboplasty

○Shoji Kokeguchi, Nobuhiko Kataoka, Seiji Ogata, Satoshi Yamada, Yukiko Matsumoto, Masahide Shiotani

Hanabusa Women's Clinic, Hyogo, Japan

Monday, September 12 10:30～12:15 (Hana A)

Chairpersons: Saffa Al Hassani (Germany)
Tesunori Mukaida (Japan)

O-019 Outcomes of vitrified-warmed embryo transfer (FET) cycles: single blastocyst transfer versus double blastocyst transfer

○Maria Milyutina, Elena S. Miadova

Perinatal Medical Center, Moscow, Russia

O-020 Are Mild ART-derived blastocysts more favorable than conventional COH-derived ones?

○Yasushi Takai[1], Ken Ohara[1], Shigetaka Matsunaga[1], Masahiro Saito[1], Osamu Ishihara[2], Hiroyuki Seki[1]

[1]Saitama Medical Center/Saitama Medical University, Saitama, Japan, [2]Saitama Medical University, Saitama, Japan

O-021 Progress towards a universal warming method after vitrification

○Aniko Reichart[1], Gabriella Uhereczky[1,2], Miklos Sipos[1], Vince Forgacs[1], Gabor Vajta[2]

[1]Forgacs Institute, Budapest, Hungary, [2]BGI Shenzhen, Yatian District, Shenzhen, China

Chairpersons: Nicolas Zech (Austria)
Masao Jinno (Japan)

O-022 Failure of pronucleus formation in in vitro matured and fertilized oocytes from SOD1-deficient mice

○Naoko Kimura[1], Yasuko Sato[1], Manami Suenaga[1], Satoshi Tsunoda[2], Junichi Fujii[2]

[1]Laboratory of Animal Reproduction, Graduate School of Agricultural Sciences, Yamagata University, Tsuruoka, Japan, [2]Department of Biochemistry and Molecular Biology, Graduate School of Medical Science, Yamagata University, Yamagata, Japan

O-023 A randomized double-blind comparative study between two different laser assisted hatching in the frozen-thawed embryo transfer at the cleavage stage

○Lei Ao

Yunnan First People Hospital, Kunming, China

O-024 A trial for observation of chromosome dynamics in human embryos using a live-cell imaging system

○Yoshiharu Nakaoka[1], Shu Hashimoto[1], Ami Amo[1], Keijiro Ito[1], Yoshiharu Morimoto[1], Kazuo Yamagata[2]

[1]IVF Namba Clinic, Osaka, Japan, [2]Research Institute for Microbial Diseases, Osaka University

O-025 Autologous transplantation of very thin ovarian fragments which preserve the ovary's main cortex structure lead to successful pregnancy
○Ariel Revel[1], Meital Lebovich[1], Alex Simon[1], Neri Laufer[1], Einat Eizenmann[1], Eduardo Mitrani[2]
[1]Hadassah University hospital. Jerusalem, Israel, [2]The Alexander Silberman Institute of Life Sciences, hebrew university, Jerusalem, Israel

Sunday, September 12 15:20～18:05 (Hana A)
Chairpersons: Yealin Frank (USA)
Yumi Nagata (Japan)

O-026 Important implications of advanced glycation end-products (AGE) in poor ART outcomes and a novel successful therapy for very severe ART patients with sitagliptin possibly by decreasing AGE.
○Masao Jinno[1], Masayoshi Takeuchi[2], Aiko Watanabe[1], Jun Hirohama[1], Naohisa Hatakeyama[1], Rie Hiura[1]
[1]Women's Clinic Jinno, Tokyo, Japan, [2]Kanazawa Medical College, Ishikawa, Japan

O-027 A novel long protocol of GnRH agonist and hMG regimen: a dramatical increase in pregnancy rate by induction of diminished but significant mid-cycle LH surge.
○Masao Jinno, Aiko Watanabe, Jun Hirohama, Naohisa Hatakeyama, Rie Hiura, Rika Nishiyama
Women's Clinic Jinno

O-028 GnRHa: a promising alternative of HCG to trigger ovum maturation in minimal stimulation cycle
Ze Wu, Bo Deng, Yonggang Li, Yunxiu Li, ○Yanping li
Department of Reproduction and Genetics; Reproductive Medicine Centre, The First Peoples Hospital of Yunnan Province, China

O-029 The observation of effect of two different COH protocols on the IVF-ET outcome of aged patients
Bo Deng, Ze Wu, Yanping Ma, ○Yonggang Li
Department of Reproduction and Genetics; Reproductive Medicine Centre, The First Peoples Hospital of Yunnan Province, China

Chairpersons: Maximlian Murtinger (Austria)
Moritoshi Seki (Japan)

O-030 EFFICACY OF SEQUENTIAL TREATMENT PROTOCOL WITH HIGHLY PURIFIED URINARY FSH AND RECOMBINANT FSH FOR CONTROLLED OVARIAN STIMULATION
○Hong Ye, Guoning Huang, Li Pei, Pinghong Zeng, Xiu Luo
[1]Chongqing Genetic and Reproductive Institute, Chongqing Obstetrics and Gynecology Hospital, China

O-031 Application of elective single embryo transfer(e-SET)
○Yonggang Li, Yanping Ma, Ze Wu, Lian Deng, Bo Deng, Mengying Gao
Dep of Reproduction and Genetics, Yunnan first people hospital, Kunming, China

O-032 A Novel function of Human Pinopodes by Expressing L-Selectin Ligand during Window of Implantation
○M Kabir-Salmani [1], R Nejatbakhsh[2], H Hosseini[3], E Dimitriadis[4], M Iwashita[5]
[1]National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran., [2]Dept. Anatomical Sciences, Shaheed Beheshti Medical University, Tehran, Iran, [3]Molecular and Cellular Biology Research Center, Shaheed Beheshti Medical University, Tehran, Iran, [4]Prince Henry's Institute of Medical Research, Australia., [5]Dept. Ob/Gyn, Kyorin Medical University, Tokyo, Japan

O-033 Obstetric outcomes of ART pregnancies in women over 35 years old
○Xiaohui Tang, Tomoko Adachi, Setsuko Nakayama, Yoshiharu Takeda, Hideki Sakamoto, Masao Nakabayashi
Department of Obstetrics and Gynecology, Aiiku Maternal and Child Health Center, Aiiku Hospital, Tokyo, Japan

Chairpersons: Yan Ping Kuwan (China)
Yasuyuki Mio (Japan)

O-034 The age of 35 years is the critical age for successful TESE-ICSI in nonobstructive azoospermic patients with normal karyotype and nonmosaic Klinefelter syndrome.
○Hiroshi Okada[1], Yoshitomo Kobori[1], Mitsunobu Koshida[2,3], Ken-Ichi Tatsumi[2,3], Kazutaka Terai[3,4], Osamu Maruyama[5]
[1]Department of Urology, Dokkyo Medical University Koshigaya Hospital, Saitama, Japan, [2]Koshida Clinic, [3]Umegaoka Women's Clinic, [4]Department of Urology, Shakaihoken Kamata General Hospital, [5]Department of Urology, Juntendo University Faculty of Medicine

O-035 Comparing study of effectiveness of nifedipine and magnesium sulfate for acute tocolysis of preterm labor and threatened preterm labor
○Fariba Nanbakhsh[1], Farzaneh Broomand[2], Zahra Yekta[3], Rita Doosti[4], Pooya Mazloomi[5]
[1]Urmia University of Medical Sciences,Urmia,IRAN, [2]Urmia University of Medical Sciences,Urmia,IRAN, [3]Urmia University of Medical Sciences,Urmia,IRAN, [4]Urmia University of Medical Sciences,Urmia,IRAN, [5]Urmia University of Medical Sciences, Urmia, IRAN

O-036 The Change of Serum Anti-Mullerian Hormone (AMH) Level by Chemotherapy and Operation in Premenopausal Women with Gynecological Cancer
○Masaru Hayashi, Akiko Shoda, Nobuaki Kosaka, Yoshiko Moshiduki, Ichio Fuykasawa
Department of Obstetrics and Gynecology, Dokkyo Medical University, Tochigi, Japan

Sunday, September 13 10:30～11:45 (Hana A)

Chairpersons: Mette Munk (Denmark)
Shoji Kokeguchi (Japan)

O-037 Alternative treatment options for patients with ovarian insufficiency based on Natural Cycle IVF
○Markus Nitzschke
Milagro Kinderwunschzentrum Bodensee AG, Kreuzlingen, Switzerland

O-038 CORRELATION OF BODY MASS INDEX WITH OUTCOME OF IN VITRO FERTILIZATION IN A DEVELOPING COUNTRY
○Neeta Singh, Prerna Gupta, Suneeta Mittal, Neena Malhotra, Anupama Bahadur
All India Institute of Medical Sciences, New Delhi, India

O-039 Bilateral ovarian endometriomas removal does not affect the IVF outcome
○Andrej Vogler, Martina Ribic Pucelj, Irma Virant Klun
Department of Obstetrics and Gynaecology, University Medical Centre, Ljubljana, Slovenia

O-040 The new stripping technique for low protrusion rate uterine myoma in TCR
○Toshimichi Oki, Chie Oki, Toshihiko Kawamura, Akiko Gibo, Hideki Yamasaki, Tsutomu Douchi
Wemen's Medical Center, Kagoshima Univesity Hospital, Kagoshima, Japan

O-041 Evaluation of short-term and long-term outcome of tubal conservation in the treatment of tubal ectopic pregnancy
○Toshimichi Oki, Yukiko Nakajou, Chie Oki, Akiko Gibo, Toshihiko Kawamura, Tsutomu Douchi
Wemen's Medical Center, Kagoshima Univesity Hospital, Kagoshima, Japan

O-042 2000IU hCG in high responders does not affect the outcomes of IVF

○YanPing Kuang[1], Yun Wang[1], QiFeng Lyv[1], John Zhang[2]

[1]Department of Assisted Reproductive, Shanghai Ninth People's Hospital Affiliated Shanghai JiaoTong University School of Medicine, Shanghai, China; [2] New Hope Fertility Center, New York, U.S.

10. Poster Presentaions

Poster viewing time (Hana B C D)

Sunday, September 11 10:00～10:30, 13:00～13:20

Monday, September 12 10:00～10:30, 13:00～13:20

P-001 The influence of crowding on Leydig cell, weight gain, testosterone and cortisole levels in mice

○Maryam Ghasemi, Farzad Rajaei

Department of Anatomy, Qazvin University of Medical Sciences, Qazvin, Iran

P-002 Identification of basic reprogramming factors associated with a pluripotent potential of *in vitro* cultured spermatic stem cells in domestic animals.

Sung-Min Kim[1], Mayako Fujihara[1,2], Sadeep Goel[3], Mahesh Sahare[1], Naojiro Minami[1], Masayasu Yamada[1], ○Hiroshi Imai[1]

[1]Kyoto University, Kyoto, Japan, [2]Smithsonian Coservation Biology Institute, Front Royal, Virginia, USA, [3]Center for Cellular and Molecular Biology, Hyderabad, India

P-003 Effect of diet contain sesame seed on the rat testis

Javad Amini mohabadi[1], ○Hassan Hassani Bafrani[2], Morad Pasha Eskandari Nasab[1], Mohammad Hossein Shahir[1], Hossein Nikzad [3], Aliakbar Taherian[4]

[1]Zanjan University, [2]Anatomical Research Center, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran, [3]Department of Anatomy & Embryology, Scientific Director of the IVF Lab, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran, [4]Department of Anatomy & Embryology, Scientific Director of the IVF Lab, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran

P-004 Analysis of Y chromosome microdeletions in Kashanian infertile males

○Mahnaz Torfeh[1], Hassan Hassani Bafrani[2], Ebrahim Sakhinia[1], Mahdi Rohani[3]

[1]Department of Biochemistry and genetics, Faculty of Medicine, Tabriz University of Medical Sciences, [2]Anatomical Research Center, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran. , [3]Department of Bacteriology, Pasture Institute of Iran

P-005 The usefulness of testicular sperm extraction with the Trucut biopsy needle

○Syuichi Iida, Masakuni Suzuki, Ikuo Tachibana, Sigetomo Takahashi, Takahiro Noda, Osamu Fuzii

M, Suzuki's Memorial Hospital, Miyagi, Japan

P-006 An evaluation of the confounding effect of sperm abnormalities on pregnancy and implantation rates and miscarriage rates following in vitro fertilization- embryo transfer using sibling oocytes

○Jung K Choe, Jerome H Check, Theresa Jamison

UMDNJ, Robert Wood Johnson Med. School at Camden, Cooper Hosp./Univ. Med. Cntr., Dept. OB/GYN, Div. Repro. Endo. & Infertility, Camden, NJ, U.S.

P-007 Testicular sperm retrieval using a new multiple-holes puncture needle

○Hong-Hua Wang, Li-Yi Cai, Hong-Ying Yu, Jing-Ying Xiang, Lin-Qing Hu, Xiao-Jin Zhou

Department of Reproductive Medicine, the Affiliated Wuxi Hospital for Maternal & Childers Health Care of Nanjing Medical University, China

P-008 The impact of body mass index on sperm recovery and serum reproductive hormone levels in an infertility setting -An analysis of 445 azoospermic cases

○Hatsuki Hibi[1], Tadashi Ohori[1], Yoshiaki Yamada[2], Yoshimasa Asada[3]

[1]Kyoritsu General Hospital, Nagoya, Japan, [2]Department of Urology, Aichi Medical University School of Medicine, [3]Asada Lady's Clinic, Aichi, Japan

P-009 The frequency of males with sperm with low hypoosmotic swelling test scores (which prevents morphologically normal embryos from implanting) in couples having in vitro fertilization-embryo transfer

○Gabrielle Citrino, Jerome H Check, Jung K Choe, Ann DiAntonio

UMDNJ, Robert Wood Johnson Med. School at Camden, Cooper Hosp./Univ. Med. Cntr., Dept. OB/GYN, Div. Repro. Endo. & Infertility, Camden, NJ, U.S.

P-010 Effects of several culture conditions on the primordial germ cell proliferation

○Zohreh Makoolati[1], Mansoureh Movahedin[2], Mehdi Forouzandeh-Moghadam[3]

[1]Department of Anatomical sciences, Medical Sciences Faculty, Fasa University, Fasa, , [2]Department of Anatomical sciences, Medical Sciences Faculty, Tarbiat Modares University, Tehran, Iran , [3]Department of Biotechnology, Medical Sciences Faculty, Tarbiat Modares University, Tehran, Iran

P-011 Early development of *in vitro* aged eggs after intracytoplasmic sperm injection

○Gaku Shimoi[1], Masato Hayashi[1], Yuichi Kameyama[1], Ryoichi Hashizume[1], Ken-ichi Kudoh[2], Masao Ito[1]

[1]Faculty of Bioindustry, Tokyo University of Agriculture, Hokkaido, Japan, [2]School of Veterinary Medicine, Kitasato University, Aomori, Japan

P-012 One step closer to the development of a rapid bioassay to determine if adequate luteal phase progesterone supplementation is provided during in vitro fertilization-embryo transfer cycles

○Ann DiAntonio[1], Jerome H Check[1], Maya D Srivastava[2], Rachael Cohen[1], Ebony Dix[1]

[1]1UMDNJ, Robert Wood Johnson Med. School at Camden, Cooper Hosp./Univ. Med. Cntr., Dept. OB/GYN, Div. Repro. Endo. & Infertility, Camden, NJ, [2]Department of Medicine, Division of Allergy and Immunology, SUNY at Buffalo, Buffalo, NY, U.S.

P-013 Effect of ovarian induction on the ultrastructure of corpus luteum during luteal phase at implantation period

○Mandana Beigi Boroujeni[1], Nasim Beigi Boroujeni[2], Mojdeh Salehnia[3], Elahe Marandi[4], Sadegh Rezapour[4], Masoud Beigi Boroujeni[5]

[1]Department of Anatomy, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran, [2]Department of clinical science, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran., [3]Department of Anatomy, School of Medicine, Tarbiat Modares University, Tehran, Iran, [4]School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran, [5]Department of Biology, School of Basic Science, Shahrekord University, Shahrekord, Iran

P-014 Autophagy in the ovarian granulosa cells

○Eri Ishida, Hirohiko Tani, Miwa Shimizu, Akiyoshi Urano, Takakazu Saito, Hidekazu Saito

National Center for Child Health and Development, Department of Women's Health, Division of Reproductive Medicine and Infertility, Tokyo, Japan

P-015 The promoter -1031(T/C) polymorphism in tumor necrosis factor-α associated with polycystic ovary syndrome

○Kwang-Hyun Baek[1], Ji-Hyun Yun[1], Jin-Woo Choi[2], Hyo Young Jeoung[3]

[1]Department of Biomedical Science, CHA Stem Cell Institute, CHA University, CHA General Hospital, Seoul, Korea, [2]St. Paul's School, Concord, NH, USA, [3]Department of Obstetrics and Gynecology, CL Hospital, Gwangju, Korea

P-016 Association between INS-VNTR polymorphism and polycystic ovary syndrome in a Korean population

○Bum-Chae Choi[1], Sang Jin Song[1], Ji-Hyun Yun[2], Bon-Hee Gu[2], Kwang-Hyun Baek[2]

[1]Department of Obstetrics and Gynecology, CL Hospital, Gwangju, Korea, [2]Department of Biomedical Science, CHA Stem Cell Institute, CHA University, CHA General Hospital, Seoul, Korea

P-017 Application of non-elective blastocyst culture

Mengying Gao, ○Yanping Ma, Yonggang Li, Ze Wu , Lian Deng, Bo Deng

The Department of Reproduction and Genetics, Yunnan first people hospital, Kunming, P.R. China

P-018 Oocyte activation with Ca ionophore A23187 and roscovitine for ovulated mouse oocytes to produce haploid parthenogenones

○Yuya Yano, Yuri Yamamoto, Yu Tanaka, Kenji Hinokio, Akira Kuwahara, Minoru Irahara

Department of Obstetrics and Gynecology, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, Japan

P-019 The Availability of Recombinant FSH (recFSH) in Minimum Ovarian Stimulation by the Deference of the Anti-Müllerian Hormone (AMH) Level

○Yuki Nagase[1], Miki Ikegami[1], Maiko Yoshioka[1], Tomijirou Nishihara[1], Mitsuru Usui[2], Toshiki Matsuura[1]

[1]Kaba Clinic Reproduction Center, Hamamatsu, Japan, [2]Kyoritsu Juzen Hospital, Hamamatsu, Japan

P-020 Results of 1027 office-based diagnostic hysteroscopies before IVF and evaluation the pregnancy and take home baby percentages between patients with normal and abnormal uterine findings.

Selcen Bahadir, ○Mujdegul. Z Karaca, Sertac Batioglu

Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey.

P-021 Serum levels of macrophage inhibitory cytokine 1 (MIC 1) as a predictor of miscarriage in early pregnancy of women treated for repeated reproductive failures

○Daniela. N Baltadzhieva, Kalinka. L Penkova, Pepa. A Angelova, Meglena. M Metodieva

Center for Reproductive Health "Nadejda", Sofia, Bulgaria

P-022 A drop in serum estradiol the day after human chorionic gonadotropin (hCG) shot does not adversely affect pregnancy rates per embryo transfer in women with very decreased oocyte reserve

○Ann DiAntonio, Jung K Choe, Jerome H Check

UMDNJ, Robert Wood Johnson Med. School at Camden, Cooper Hosp./Univ. Med. Cntr., Dept. OB/GYN, Div. Repro. Endo. & Infertility, Camden, New Jersey, U.S.

P-023 Analysis of the limitations for the numbers of attempts of infertility treatment using ART.

○Hiroaki Shibahara, Tatsuya Suzuki, Kenro Chikazawa, Tomoe Ikeda, Yuki Hirano, Mitsuaki Suzuki

Department of Obstetrics and Gynecology, School of Medicine, Jichi Medical University, Tochigi, Japan

P-024 The predictive value of AMH for ovary reserve and response during IVF-ET

○Xiaomei Zhang

The reproductive medicine center of Subei People's hospital, China

P-025 Whether Day 3 FSH/LH ratios is a good predictor of IVF prognosis in GnRH antagonist protocol?

○K.H. Lee[1], J.D. Cho[2], H.G. Sun[1], S.K. Kim[1], J.H. Lee[1], Ilhae Park[1]

[1]Mamapapa&baby OB&GY, [2]Ellemedi Infertility Clinic, Changwon, Gyoung Nam, republic of Korea

P-026 Luteal blood flow in patients undergoing GnRH agonist long protocol
○Kumiko Mizumoto, Akihisa Takasaki, Maki Okada, Yuuko Sakaguchi, Katsunori Shimamura, Hitoshi Morioka
Saiseikai Shimonoseki Hospital, Yamaguchi, Japan

P-027 Comparison of the effect of GnRHa long and short protocol in older women during IVF/ICSI
○Xun Zeng, Shangwei Li, Lang Qing, Xiaohong Li, Han Hu
Reproductive Medical Center of West China 2nd Hospital,Sichuan,China

P-028 Small amount of testosterone administration improves ovarian response to ovulation induction in poor responders
○Kohzo Aisaka[1], Haruko Hiraike[1], Hiroe Hyodo[1], Seiichiro Obata[1], Osamu Hiraike[2], Hironobu Hyodo[2]
[1]Department of OB/GYN, Hamada Hospital, Tokyo, [2]Department of OB/GYN, University of Tokyo, Japan

P-029 rFSH combined with uhMG had significantly increased pregnancy compared with rFSH alone undergoing ovarian stimulation following a long protocol in IVF
○K.H. Lee[1], J.D. Cho[2], H.G. Sun[1], I.H. Park[1], J.H. Lee[1], S.K. Kim[1]
[1]Mamapapa&baby OB&GY, [2]Ellemedi Infertility Clinic, Changwon, Gyoung Nam, Repubilic of Korea

P-030 A comparison of pregnancy outcome in women with normal oocyte reserve according to the use of high or low dose follicle stimulating hormone (FSH) stimulation
○Gabrielle Citrino, Jerome H Check, Jung K Choe
UMDNJ, Robert Wood Johnson Med. School at Camden, Cooper Hosp./Univ. Med. Cntr., Dept. OB/GYN, Div. Repro. Endo. & Infertility, Camden, NJ, US

P-031 The effects of laser assisted hatching on pregnancy rates
○Forouzan Absalan[1,2]
[1]Anatomical department, Jundishapoor university of medical sciences, Ahvaz, , [2]Iranshiraz infertility and limited surgical center, Shiraz, Iran

P-032 Correlation between semen intracellular parameters and fertilization rates following ICSI
○Mina Sharbatoghli, Bita Ebrahimi, Mojtaba Rezazade Valojerdi
Embryology Department, Cell Sciences Research Center, Royan Institute, ACECR, Tehran, Iran

P-033 Whole ooplasmic transfer by direct injection method using Piezo driven system for prevention of mutated mitochondrial DNA transmission
○Akiko Yabuuchi[1], Noriko Kagawa[1], Kenji Ezoe[1], Chiemi Mori[1], Yuko Takayama[1], Masashige Kuwayama[2], Keiichi Kato[1], Fumihito Aono[1], Yuji Takehara[1], Osamu Kato[1]
[1]Advanced medical research institute of fertility, Kato Ladies Clinic, Shinjuku, Tokyo, Japan, [2]Repro-support medical research center, Shinjuku, Tokyo, apan

P-034 Elevation of the Risk of Monozygotic Twinning by Expanded Blastocyst Transfer
○Kazusuke Nagoshi
Nagoshi Ladies Clinic, Okayama, Japan

P-035 Evaluation of sperm chromatin integrity following IMSI or HBA for pre-ICSI sperm selection
○Satoshi Ueno, Kazuo Uchiyama, Keiichi Kato, Yuji Takehara, Osamu Kato
Kato Ladies Clinic, Tokyo, Japan

P-036 Differential effects of urinary and recombinant chorionic gonadotropin on oxidative stress responses in decidualizing human endometrial stromal cell.
○Takeshi Kajihara, Japarath Prechanich, Hideno Tochigi, Osamu Ishihara
Saitama Medical University, Saitama, Japan

P-037 Heparin prevents programmed cell death induced by oxidative stress in human decidualized endometrial stromal cells
Takeshi Kajihara, ○Japarath Prechanich, Hideno Tochigi, Osamu Ishihara
Saitama Medical University, Saitama, Japan

P-038 The role of 5'AMP-activated protein kinase (AMPK) in human endometrial stromal cells
○Yasushi Kawano, Sinya Karakida, Yufuko Utsunomiya, Hisashi Narahara
Oita University, Oita, Japan

P-039 Pinopode Formation upon Progesterone, hCG, and Clomiphene Citrate treatment
M Kabir-Salmani, ○M Shahali
National Institute of Genetic Engineering and Biotechnology, Dept. Molecular Genetics, Tehran. Iran

P-040 IS THERE ANY CORRELATION OF UTERUS POSITION AND DEEPTH EMBRYO TRANSFER ON PREGNANCY RATE IN IVF
○Agus Supriyadi
Harapankita hospital, Republic of Indonesia

P-041 Influence of antisperm antibodies (ASA) on the outcome of infertility treatment
○Yasufumi Shimizu, Takeshi Yorimitsu, Hiroshi Motoyama, Motohiro Ohara, Toshihiro Kawamura
Denentoshi Ladies Clinic, Kanagawa, Japan

P-042 The effect of ovarian stimulation on expression level of E-cadherin, Leukemia inhibitory factor, Progesterone receptor and αvβ3 Integrin genes in mouse blastocysts
○Bahar Movaghar, Saeedeh Askarian
Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

P-043 Usefulness of elective single blastocyst transfer in the first treatment cycle for infertile women aged less than 35.
○Hiroaki Shibahara, Shoko Hashimoto, Shiho Nagayama, Kazuhiko Shimada, Tatsuya Suzuki, Mitsuaki Suzuki
Department of Obstetrics and Gynecology, School of Medicine, Jichi Medical University, Tochigi, Japan

P-044 A Summary of The clinical outcome of minimal stimulation IVF/ ICSI
Yan Ping Ma, Ze Wu, Bo Deng, ○Yonggang Li
Department of Reproduction and Genetics, Reproductive Medicine Centre, The First Peoples Hospital of Yunnan Province, KunMing,China

P-045 Ovarian Response and Reserve Evaluation in consecutive IVF/ICSI-ET cycles
○Mujdegul Karaca, A. Sertac Batioglu, Murat Ozel, Selcen Bahadir, Simla Karaca, Beril Gurlek
Dr ZTB Women's Health Hospital, Ankara, Turkey

P-046 Our 11-year experience with sperm cryopreservation for patients with malignant disease

○Syuichi Iida, Masakuni Suzuki, Kazuhiro Hirayama, Ikuo Tachibana, Masahiro Katou, Kouhei Tanaka

M. Suzuki's Memorial Hospital, Miyagi, Japan

P-047 A Successful pregnancy and live birth after intracytoplasmic sperm injection with cryopreserved limited numbers of spermatozoa stored in the practical container

○Yuji Endo, Yoshitaka Fujii, Hiroaki Motoyama

Kurashiki Medical Clinic, Okayama, Japan

P-048 An evaluation of a modified slow cool cryopreservation technique and the efficacy of a graduated estrogen regimen vs. natural cycles on pregnancy rates following frozen embryo transfer

○Jung. K Choe, Jerome H Check

UMDNJ, Robert Wood Johnson Med. School at Camden, Cooper Hosp./Univ. Med. Cntr., Dept OB/GYN, Div. Repro. Endo. & Infertility, Camden, New Jersey, U.S.

P-049 The effect of vitrification on Histone Modification of regulatory regions of H19, Igf2 and Mest imprinted genes in mouse embryo

○Bahar Movaghar[1], Saeideh Sahraei[1], Maryam Shahhoseini[2], Ali Farrokhi[3]

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P-050 Withdrawn

P-051 Which is more effective for pregnancy freezing embryos: in day-2, day-3 stage or blastocyst?

○Tomio Sawada, Kaori Yoshikai, Sayumi Hori

The sawada women's clinic Nagoya reproduction center, Aichi, Japan

P-052 Highest liquid nitrogen quality for vitrification process : Micro bacteriological filtration of LN2

Ana Cobo[1], ○Damia Castello[2], B Weiss [3], C Vivier[4], Angel De La Macorra [5], F Kramp[6]

[1]IVI clinica de Fertitidad, Valencia, Spain, [2]IVI clinica de Fertitidad, [3]Air Liquide Head Office Industrial Merchant, [4]Air Liquide Sante International, [5]Air Liquide Medicinal, [6]Soprodi S.A.S., Quai du canal, Spain

P-053 Comparing developmental potential and ultra structure of human blastocysts after re-vitrification

○Mansoureh Movahedin[1], Zahra Alamolhoda[2], Nasim Ghorbanmehr[1,2], Mojgan Moradkhani[3]

[1]Tarbiat Modares University, Tehran, Iran, [2]Shayamehr IVF Center, Tehran, Iran, [3]Khatamolanbia Hospital, Tehran, Iran

P-054 Examination of blastocyst transfer after pronucleate embryos were slowly freeze-thawed

○Hiroei Ohhashi, Yukio Ohmomo, Osamu Arakawa

Arakawa Ohmomo Angel Mother Clinic, Niigata, Japan

P-055 5′-(N-Ethylcarboxamido) adenosine (NECA) improves angiogenesis in transplanted human ovarian tissue: potential for fertility preservation

○Maryam Hormozi[1], Saeed Talebi[2], Amir Hassan Zarnani[3,4], Mahmood Jeddi-Tehrani[5], Ladan Hosseinigohari[6], Mohammad Mehdi Akhondi[2]

[1]Biochemistry Department, lorestan university of medical sciences, Khorramabad,Iran, [2]Reproductive Biotechnology Research Center, Avicenna Research Institute, (ACECR), Tehran, Iran, [3]Nanobiotechnology Research Center, Avicenna Research Institute, (ACECR), Tehran, Iran, [4]Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran, [5]Monoclonal Antibody Research Center, Avicenna Research Institute, (ACECR), Tehran, Iran, [6]Biochemistry Department, Tehran university of Medical Sciences, Tehran, Iran

P-056 Assessing risks of birth defects in ART babies

○Coralia V. Stefanescu, Ion M Rusa, George Costea, Lenuta Malcea

Euromaterna Hospital, Constanta, Romania

P-057 Two cases of ovarian abscess after oocyte retrieval

○Aki Oride, Haruhiko Kanasaki, Kohji Miyazaki

Shimane University School of Medicine, Shimane, Japan

P-058 An experience of an infertility treatment for a woman with benign metastasizing leiomyoma

○Tsuyoshi Hashiba, Toshio Hamatani, Naoaki Kuji, Kou Sueoka, Yasunori Yoshimura

Keio University School of Medicine, Tokyo, Japan

P-059 A Transgenic Mouse Model of Breast Cancer Using tissue specific MMTV Promoter

○Maryam Shahali[1,2], Ehsan Ranaei[1], Shams-Ara Mahdi[1], Javad Mowla[2], Maryam Kabir-Salmani[1], Karim Nayernia[3]

[1]National Institute of genetic engineering and Biotechnology, Tehran, Iran & Tarbiat Modares University, Tehran, Iran, [2]Tarbiat Modares University, Tehran, Iran, [3]GENOCELL CO.

P-060 Effect of genistein administration on intratesticular testosterone level and spermatogenesis in rats treated with busulfan

○Heejun Chi, Kangwoo Cheon, Jonghyun kim, Giyoung Kim, Jaeseok Lee, Sungil Roh

i-Dream Center, Mizmedi Hospital, Seoul, Korea

P-061 The role of Doppler in predicting endometrial thickness, pattern and blood flow in infertile women undergoing IVF cycle in a tertiary care institute

○Anupama Bahadur, Neeta Singh, Neena Malhotra, Ashok Bhatt, Suneeta Mittal

All India Institute of Medical Sciences, New Delhi, India

P-062 Reduced blastcyst formation rate in women in advanced reproductive age with high Anti-Mullerian hormone level

○Takahiro Noda, Shinako Hashimoto, Mitsuru Iwamoto, Syuichi Iida, Kohei Tanaka, Masakuni Suzuki

Suzuki Memorial Hospital, Miyagi, Japan

P-063 Effects of salpingectomy on ovarian response in controlled ovarian hyperstimulation for in vitro fertilization: a reappraisal

○Israel Wagman[1], Benny Almog [1,2], Ishai Levin[1], Gali Barkn[1], Dina Kovalsky[1], Togas Tulandi[2]

[1]Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel., [2]Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada

P-064 Semen analysis and evaluation of ICSI outcome in patients infected with hepatitis B virus

○Mounir Ajina[1], Neila Hannachi[2], Khaled Youssef[2], Souhir Mehri[1], Jalel Boukadida[2], Ali Saad[3]

[1]Unit of reproductive Medicine, University Hospitol Fahat Hached, Sousse, Tunisia, [2]Laboratory of Bacteriology, University Hospital Farhat.Hached, Sousse, Tunisia, [3]Laboratories of Cytogenetic, Molecular Biology and Human Biology of Reproduction, Farhat Hached Hospital, Sousse, Tunisia.

P-065 Effect of Chemotherapy on Spermatogenesis in Testicular Cancer Patients

○Miki Fuse[1], Takashi Imamoto[1], Takanobu Utsumi[1], Takumi Endo[2], Naoki Nihei[1], Tomohiko Ichikawa[1]

[1]Department of Urology, Graduate School of Medicine, Chiba University, Chiba, Japan, [2]Department of Urology, Toho University Sakura Medical Center, Chiba, Japan

P-066 Reproductive outcomes following laparoscopic myomectomy in patients with infertility

○Kohei Tanaka, Masakuni Suzuki

M. Suzuki Memorial Hospital, Miyagi, Japan

P-067 The role of CINC/gro in the rat ovulatory process

○Yu Tanaka, Akira Kuwahara, Yuya Yano, Yuri Yamamoto, Kenji Hinokio, Minoru Irahara

Deparatment of Obstetrics and Gynecology, The University of Tokushima, Institute for Health Biosciences, Tokushima, Japan

P-068 A Commercially Available Dual-Buffered IVF Handling Medium Containing HEPES and MOPS Maintains Stable pH and Supports Human Sperm Survival, Normal Fertilization Following ICSI and Embryo Development

○Jason E Swain[1], Marlane Angel[2], Nadir Cira[3], Juergen Liebermann[4], Thomas Pool[5]

[1]University of Michigan, Ann Arbor, MI, [2]Laurel Fertility Care, San Francisco, CA, USA, [3]Bacchi IVF Center, Istanbul, Turkey, [4]Fertility Center of Illinois, Chicago, IL, USA, [5]Fertility Center of San Antonio, San Antonio, TX, USA

P-069 Development of Mouse and Human Embryos in a Low Humidity Incubator

○Chiemi Mori, Tadashi Okimura, Fumihito Aono, Yuji Takehara, Osamu Kato

Kato Ladies Clinic, Tokyo, Japan

P-070 Contribution of oocyte and embryo cleavage quality in obtaining blastocysts

○Mounir Ajina[1], Sonia Jallad[1], Souhir Mehri[1], Sawsen Meddeb[2], Hedi Khairi[2], Ali Saad[3]

[1]Unit of reproductive Medicine. University Hospital Farhat.Hached, Sousse, Tunisia, [2]Department of Obstetrics and Gynaecology, University hospital Farhat.Hached, Sousse, Tunisia., [3]Laboratories of Cytogenetic, Molecular Biology and Human Biology of Reproduction Farhat Hached Hospital, Sousse-Tunisia

P-071 Involvement of first cytokinesis on gene expression at blastocyst stage

○Satoshi Sugimura, Tadayuki Yamanouchi, Yutaka Hashiyada, Eiji Kobayashi, Kei Imai

National Livestock Breeding Center, Fukushima, Japan

P-072 Zona-free oocyte formed a one pronucleus zygote following piezo intracytoplasmic sperm injection can subsequently develop to the blastocyst in micro-well culture system. (Case Report)

○Xinzhi Yang, Akina Takamura, Emi Fukunaga, Tomoyo Kusuda, Shinichiro Okano, Masayuki Kinutani

Kinutani Women's Clinic, Hiroshima, Japan

P-073 A CUMULATIVE EMBRYO SCORING SYSTEM USED TO PREDICT PREGNANCY OUTCOME

○Yoon Jeong Choi, Jung Ho Kim, Chan Park, Kwang Rae Kim, Sung il Roh, Hee Jun chi

i-Dream Center, Mizmedi Hospital, Seoul, Korea

P-074 Cytoplasmic halo as an additional indicator to predict the quality of the results of IVF and ICSI embryos

○Devi Natalia, Harris Harlianto, Sintya Jatnikasari, Tono Djuwantono, Wiryawan Permadi
Aster Clinic, RS.Hasan Sadikin, Bandung, Indonesia

P-075 Withdrawn

P-076 Effects of L/D stimuli and the circadian clock on reproductive physiology in female mice

○Tomoko Amano[1], Juergen Ripperger[2], Urs Albrecht[2]
[1]Kinki University, Japan, [2]Dep. of Medicine, Div. of Biochemistry, University of Fribourg

P-077 Numbers of CGG repeats on the FMR1 gene in Japanese patients with premature ovarian insufficiency

○Naoki Okamoto, Naomi Hamada, Yodo Sugishita, Nobuhito Yoshioka, Noriyuki Takahashi, Bunpei Ishizuka
Department of Obstetrics and Gynecology, St.Marianna University School of Medicine

P-078 Genetic analysis of spermatozoa in four infertile patients with macrocephalic sperm head syndrome

○Ghaya Merdassi[1,2], Pierre Ray[3], Meriem Chaabouni[4], Nedia Memmi[1], Fethi Zhioua[1], Amel Zhioua[1]
[1]IVF Center, Aziza Othmana Hopsital, Tunisia, [2]Faculty of pharmacy.Monastir.Tunisia, [3]Department of genetic and procreaction. Tronche Hospital .grenoble. France , [4]Department of human genetic. Charles Nicolle Hospital .Tunisia

P-079 Gene Expression Profiling of Karyotypically Normal, Trisomy 12 and XXY Human Embryonic Stem Cells

Hye Won Seol[1], ○Sun Kyung Oh[1,2], Baik Seol Cho[2], Kyung Eui Park[2], Young Min Choi[1,2], Shin Yong Moon[1,2]
[1]Institute of Reproductive Medicine Population, Medical Research Center, Seoul National University College of Medicine, [2]Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul 110-799, Korea

P-080 Effect of dibutyryl cAMP during in vitro maturation on the developmental competence of bovine oocytes after ICSI

○Chikako Kani[1,2], Tomohisa Wada[2], Akiko Kuwahata[2], Masanori Ochi[2], Toshitaka Horiuchi[1]
[1]Graduate School of Comprehensive Scientific Research, Prefectural University of Hiroshima, Hiroshima 727-0023, Japan, [2]Ochi Yume Clinic Nagoya, Aichi, Japan

P-081 Androstenedione induces abnormalities in morphology and function of developing oocytes, which impairs oocyte meiotic competence

○Wataru Tarumi[1], Sanae Tsukamoto[1], Yuki Okutsu[1], Noriyuki Takahashi[1], Masanori Itoh[2], Bunpei Ishizuka[1]
[1]Department of Obstetrics and Gynecology, St. Marianna University School of Medicine, Kanagawa, Japan., [2]Department of Biology, College of Liberal Arts and Sciences, Tokyo Medical and Dental University, Ichikawa, Chiba 272-0827, Japan.

P-082 Preimplantation genetic diagnosis for spinal muscular atrophy using mini-sequencing and genetic linkage analyses at the blastocyst stage

○Chia-Cheng Hung[1,2], Shee-Uan Chen[3], Shin-Yu Lin[3,4], Yi-Ning Su[1,2,4]
[1]Graduate Institute of Clinical Genomics, National Taiwan University College of Medicine, Taipei, Taiwan[2], Department of Medical Genetics, National Taiwan University Hospital, Taipei, Taiwan, [3]Department of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei, Taiwan, [4]Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan

P-083 Preimplantation Genetic Diagnosis (PGD) for translocation carrier with mild stimulation and single vitrified blastocyst transfer : Case Report
○Naoki Aoyama,Yuji Takehara, Satoshi Kawachiya,Tomoko Kuroda, Nami Kawasaki, Rie Yamadera,Keiichi Kato, Osamu Kato
Kato Ladies Clinic, Tokyo, Japan

P-084 Noninvasive morphological examination for germinal vesicle oocytes predict their maturity process of completion
○Tsuyoshi Okubo[1], Ryoko Matsumoto[1], Naoko Shimada[1], Teruaki Hayashi[1], Tomoya Segawa[1], Shoukichi Teramoto[1], Masashige Kuwayama[2]
[1]Shimbashi Yume Clinic, Tokyo, Japan, [2]Repro-Support Medical Research Center, Tokyo, Japan

P-085 hCG is critical for the uterine decidual response in mice
○Hiroomi Kawano[1], Kenji Ezoe[1], Noriko Kagawa[1], Akiko Yabuuchi[1], Keiko Ochiai[2],, Hiroshi Nagashima[2],, Hisao Osada[1], Fumihito Aono[1],Yuji Takehara[1], Osamu Kato[1]
[1]Kato Ladies Clinic, Tokyo, Japan [2]Meiji University, Kanagawa, Japan

P-086 Cytogenetic study in early spontaneous abortion after IVF and ICSI
○Satoshi Kawachiya, Yuji Takehara, Keiichi Kato, Hisao Osada, Naoki Aoyama, Osamu Kato
Kato Ladies Clinic, Tokyo, Japan

P-087 Day 7 β-hCG level after frozen-thawed blastocyst transfer can be a predictor for pregnancy outcomes?
○Keiichi Kato[1], Tomoya Segawa[2], Hisao Osada[1], Tamotsu Kobayashi[1], Yuji Takehara[1], Osamu Kato[1]
[1]Kato Ladies Clinic, [2]Shimbashi Yume Clinic, Tokyo, Japan

11. Luncheon Seminars:

Luncheon Seminar 1

Sunday, September 11 12:00～13:00 (Concord Ballroom A)

Chairperson: Takafumi Utsunomiya (Japan)

12:00～13:00 GnRH antagonists in ovarian stimulation for IVF and ICSI from practical perspective
-Atsushi Tanaka (Japan)

Luncheon Seminar 2

Sunday, September 11 12:00～13:00 (Concord Ballroom B)

Chairperson: Osamu Ishihara (Japan)

12:00～13:00 Clinical aspects of GnRH analogue use in Assisted reproduction
-Naoaki Kuji (Japan)

Luncheon Seminar 3: Potential of Piezo-assisted micromanipulation brought by PMM from the fundamental research to the clinical application

Sunday, September 11 12:00～13:00 (Concord Ballroom C)

Chairperson: Fumihito Aono (Japan)

12:00～12:20 Impact of piezo impact drive unit; it reversed the common sense of life
-Teruhiko Wakayama (Japan)

12:20～12:40 Would you try Piezo -ICSI without mercury?
-Hiroshi Morita (Japan)

12:40～13:00 Advantage of the use of Piezo driven system (PMM) in ARTs; Whole ooplasmic replacement in Germinal Vesicle oocytes
-Akiko Yabuuchi (Japan)

Luncheon Seminar 4

Monday, September 12 12:00～13:00 (Concord Ballroom A)

Chairperson: Yoshiharu Morimoto (Japan)

12:00～13:00 The role of AMH in female reproduction
-Budi Wiweko (Indonesia)

Luncheon Seminar 5

Monday, September 12 12:00～13:00 (Concord Ballroom C)

Chairperson: Yuji Takehara (Japan)

12:00～13:00 Fertility tourism in the U.S.
-John Zhang (USA)

Luncheon Seminar 6

Tuesday, September 13 12:00～13:00 (Concord Ballroom A)

Chairperson: Osamu Ishihara (Japan)

12:00～13:00 Global inequity: Access to Assisted Reproductive Technologies
-Sheryl Vanderpoel (Switzerland)

SPECIAL GUEST LECTURE

●Special Guest Lecture

Induction of Pluripotency by Defined Factors

Shinya Yamanaka

Director, Center for iPS Cell Research and Application (CiRA),
Professor, Institute for Integrated Cell-Material Sciences (iCeMS),
Kyoto University

Induced pluripotent stem (iPS) cells were originally generated from mouse and human fibroblasts by retroviral introduction of *Oct3/4*, *Sox2*, *c-Myc*, and *Klf4*. iPS cells are similar to embryonic stem (ES) cells in morphology, proliferation, gene expression, and most importantly, pluripotency. Compared to ES cells, iPS cells have less ethical controversy and can be generated from various genetically identified individuals including disease patients or those having specific human leukocyte antigen (HLA) types. Patient-specific iPS cells provide unprecedented opportunities in disease research, drug screening, and toxicology. A bank of iPS cells constructed from HLA-homozygous donors would provide significant resources for stem cell therapy. However, recent reports of tumor formation following transplantation, and the large diversity between iPS cell clones highlight potential problems. Furthermore, the mechanism of reprogramming remains unclear.

In addition to fibroblasts, iPS cells can be generated from various somatic cells, such as hepatic cells, gastric epithelial cells, neural cells, dental pulp cells, peripheral blood cells, and cord blood cells. As alternatives to retroviral transduction, iPS cells can be generated by lentiviruses, adenoviruses, plasmids, transposons, recombinant proteins, or synthesized mRNA. Recently, we reported an integration-free induction method using episomal vectors. This method can induce human iPS cells efficiently and reproducibly. Regarding iPS cell induction factors, we discovered that L-Myc and the transcription factor Glis1, which is strongly expressed in the unfertilized egg, can establish iPS cells with a high efficiency and quality, replacing the oncogene c-Myc. Other reports suggest that chemicals can further enhance induction efficiency.

Each induction experiment can result in up to 100 or more independent iPS cell clones. These iPS cell clones may vary qualitatively, considering responses to in vitro directed differentiation protocols and their propensity to produce tumors. In fact, we have previously shown that the origins of mouse iPS cells have profound effects on tumorigenicity. It is therefore essential to determine the best origins, the best induction protocols, and the best methods to evaluate iPS cell clones and subclones for future clinical applications. From this point of view, the need for genetic and epigenetic analyses, such as DNA methylation, histone modification, and genomic imprinting becomes more significant. It is also important to note that iPS cell within a clone can be heterogeneous, despite their common derivation from a single progenitor cell. This is likely because the process requires multiple cell division and cannot be completed by the four exogenous factors alone. Additional endogenous factors are required to achieve full reprogramming. Better understanding of the reprogramming mechanism will facilitate more uniform and complete reprogramming during iPS cell generation.

◆Biosketch

Titles: *Director*, Center for iPS Cell Research and Application (CiRA), Kyoto University
Professor, Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University

Educational History:

1981–1987	Kobe University, Kobe, Japan School of Medicine (M.D. awarded in March, 1987)
1989–1993	Osaka City University Graduate School, Osaka, Japan Division of Medicine (Ph.D. awarded in March, 1993)

Professional History:

1987–1989	*Resident*, National Osaka Hospital, Osaka, Japan
1993–1995	*Postdoctoral Fellow*, Gladstone Institute of Cardiovascular Disease The J. David Gladstone Institutes, San Francisco, CA, USA
1995–1996	*Staff Research Investigator*, Gladstone Institute of Cardiovascular Disease The J. David Gladstone Institutes, San Francisco, CA, USA
1996–1999	*Assistant Professor*, Osaka City University, Medical School, Osaka, Japan
1999–2003	*Associate Professor*, Nara Institute of Science and Technology, Nara, Japan
2003–2005	*Professor*, Nara Institute of Science and Technology, Nara, Japan
2004–	*Professor*, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan
2007–	*Professor of Anatomy*, University of California, San Francisco, CA, USA
2007–	*Senior Investigator*, Gladstone Institute of Cardiovascular Disease The J. David Gladstone Institutes, San Francisco, CA USA
2007–	*Professor*, Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto, Japan
2008–	*Director*, Center for iPS Cell Research and Application (CiRA), iCeMS, Kyoto University, Kyoto, Japan
2010–	*Director*, Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan

Honors and Awards:

2007 JSPS Prize, Japan Society for the Promotion of Science
2008 The Special Prize for Science and Technology, the Minister of Education, Culture, Sports, Science and Technology, Japan
2008 The Shaw Prize in Life Science and Medicine, Hong Kong
2008 Robert-Koch Preis 2008, Germany
2009 Albert Lasker Basic Medical Research Award, USA
2009 Canada Gairdner International Award, The Gairdner Foundation, Canada
2010 100th Imperial Prize and Japan Academy Prize, The Japan Academy, Japan
2010 Selected as Person of Cultural Merit (“Bunka Korosha”), Japan
2010 26th annual Kyoto Prize in Advanced Technology, Japan
2010 Balzan Prize for Stem Cells: Biology and Potential Applications, Italy
2011 King Faisal International Prize, Saudi Arabia
2011 Elected as National Academy of Sciences Members and Foreign Associates, USA
2011 Wolf Prize in Medicine, Israel etc.

PRE-CONGRESS WORKSHOPS

Vitrification

Pre-implantation Genetic Diagnosis

Basic science of vitrification

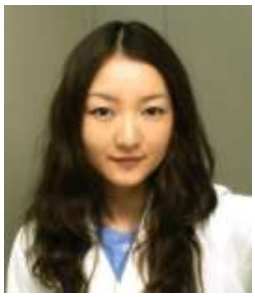
Stanley P. Leibo
Department of Biological Sciences, University of New Orleans,
New Orleans, LA, U.S.A.

AIMS: The purpose of this lecture is to briefly review basic principles of cryopreservation as applied to vitrification.
METHODS: Over the past decade, ~800 articles have described cryopreservation of oocytes and embryos by "vitrification", the reversible transition of liquid into an amorphous non-crystalline glass. Vitrification presumably requires high concentrations of cryoprotective additives (CPAs) and very high cooling rates to produce the glassy state. Vitrification of reproductive cells and tissues has been achieved by suspending them in solutions containing 15 to 30% permeating CPAs plus 18% saccharide, and cooling very small samples at rates ≥10,000°C/min. One criterion considered to be essential if the best results are to be achieved is that the extracellular solution itself must form a glass when cooled at high rates.
RESULTS: Many experimental and clinical studies have been published in which survival rates of vitrified samples ranging from 75% to close to 100% have been reported. Comparison of results attained by vitrification with results from standard, equilibrium cooling methods have almost always demonstrated that vitrification is as good and usually better than standard freezing. One limitation of many vitrification methods is that human specimens, to be cooled at high rates, are immersed directly into liquid nitrogen. This may expose them to potential contamination by viruses or microbes.
CONCLUSIONS: Recent results and re-examination of older results reveal that it may not be necessary to vitrify the extracellular solution to cryopreserve oocytes and embryos. The key to survival is whether the intracellular contents vitrify or not. Ultimate survival of cryopreserved cells and tissues may be more dependent on the rate at which the specimen is warmed, rather than the rate at which it is cooled.

◆Biosketch

Stanley P. Leibo received his Bachelor of Arts degree from Brown University, Master of Science degree from the University of Vermont, and Master of Arts and Doctor of Philosophy degree in Biology from Princeton University. He began his career in cryobiology with Peter Mazur at Oak Ridge National Laboratory in Tennessee. He then became Vice-President of Research and Development at Rio Vista International, a cattle company in San Antonio, Texas. In 1988, he was appointed Associate Professor of Obstetrics and Gynecology and of Urology at Baylor College of Medicine in Houston. From 1991 to 1998, Dr. Leibo was Professor of Biomedical Sciences at the Ontario Veterinary College of the University of Guelph in Ontario, Canada. In 1998, he was named to his present positions as Professor of Biological Sciences at the University of New Orleans, and Senior Scientist at the Audubon Center for Research of Endangered Species in New Orleans.
In addition to his permanent positions, Dr. Leibo has served as a Fellow of the Japan Society for the Promotion of Science at Kyoto University, and for four years was Visiting Professor on the medical faculty at the Dutch-speaking Free University in Brussels, Belgium. He was elected president both of the Society for Cryobiology and of the International Embryo Transfer Society, and was named an Honorary Lifetime Member of the American Embryo Transfer Association. In 2009, Leibo received the Pioneer Award of the International Embryo Transfer Society to recognize his role in the derivation of the original methods to cryopreserve gametes and embryos. He has published more than 135 scientific articles and book chapters, and has delivered more than 180 lectures in the United States and in twenty-nine other countries.

Ovarian reserving



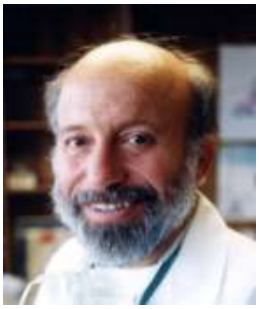
Noriko Kagawa
Kato Ladies Clinic, Tokyo, Japan

Recently, the adaptation of the cryopreservation has been expanding and dramatically changing in human assisted reproduction field because of the development of the ultra rapid vitrification method. This technique enables to establish oocyte bank which preserve fertility of cancer women after the therapy by combination with IVF cycle. To preserve fertility in female cancer patients, oocyte vitrification is one solution. However, it does not work for the children and the urgent patients who do not have enough time for one or more IVF cycles. Ovarian tissue cryopreservation has the potential to solve these problems, and to preserve their natural fertility after chemo and radiotherapy. Similarly to oocyte freezing, ovarian tissue cryopreservation has utilized a conventional slow freezing method, resulting in limited success. However, encouraging good results with ovarian tissue freezing are recently being obtained by using vitrification. More recently, we also established the efficient ovarian tissue cryopreservation by ultra rapid vitrification, the Cryotissue method, and already applied for the young unmarried cancer patient for the first time in the world. So we had have also the first success of vitrified ovarian tissue transplantation between sisters who HLA matched. She had the first period in April 2008 after ovarian tissue transplantation since she finished cancer therapy in 2004. And Second success of vitrified ovarian tissue transplantation between identical twins in February 2010. She had the first increasing of estradiol and decreasing of FSH and LH at 42days after ovarian tissue transplantation. This ovarian tissue vitrification can preserve sex of female patients after cancer therapy, and help to improve their quality of life to be happy as women. In this lecture, I introduce our detailed protocols, experimental/clinical results of ultra rapid vitrification for human ovarian tissue.

◆Biosketch

Dr. Noriko Kagawa obtained her Ph.D. in 2005 from the Kyoto University, Japan. She is currently the senior scientist at the Advanced Medical Research Institute of Fertility in Kato Ladies' Clinic, Tokyo, Japan, the world's largest human IVF unit. In 2000, she started animal embryology and studied Assisted Reproductive Technologies, for example, IVM, IVF, vitrification, embryo culture in pig, and established the porcine follicular growth system using SCID mice. She moved to the human IVF field in 2004, and has developed vitrification methods for mammalian ovaries and ovarian tissues in mice, cat, dog, lion, giraffe, bovine, human. Her major research interests have focused on in vitro growth and in vitro maturation for preantral follicles of adult mammals as well as on vitrification of Germinal Vesicle stage oocytes and various organs for unlucky patients.

Ovary Transplant: Ovary allotransplantation between non-homozygous sisters: Success and failure, an immunologic puzzle



Sherman J. Silber
Infertility Center of St. Louis

Objective: To see whether prior bone marrow transplantation from an allogeneic ovary donor can allow an ovary cortical graft from the same donor to be successful without immunosuppression.

Design: Two cases of ovary donation from a previous allogeneic bone marrow donor to her sister who became sterile as a result.

Materials & Methods: In two pairs of non-identical, non-twin, sisters, leukemia was treated in the younger sister (one at 4 years of age and one at 28 years of age) with total body irradiation, ablative chemotherapy, and bone marrow transplant. Both sisters were deemed cured by their oncologists, and both underwent ovarian cortical transplant from their sister who had previously donated bone marrow.

Results: In both cases, prior to transplantation, DNA fingerprinting of tissue and peripheral lymphocytes indicated complete engraftment of donor bone marrow. Yet in one case, whose cancer treatment and bone marrow transplant was 3 years earlier, rejection of the graft occurred, and rejection of a later frozen cortical graft also occurred. In the other case, menstruation and ovulation resumed at 63 days, and there was no rejection, and the graft continues to function perfectly with no immunosuppression. In this case, HLA typing had shown a perfect match prior to the transplant. In the other case, which surprisingly rejected, the HLA match was not perfect before the bone marrow transplant, and the recipient had an increase in eosinophil count, indicating a mild "graft versus host" condition (GVH).

Conclusions: Successful bone marrow transplant recipients thought to have complete engraftment of donor bone marrow might still have undetectable, clones of their original bone marrow that could mount a rejection against an ovary cortical graft.

◆Biosketch

Dr. Sherman Silber is one of the world's leading authorities on IVF, microsurgery, vasectomy and tubal reversal, egg and embryo freezing, ovary and testis transplantation, and the reproductive biological clock. He is director of the Infertility Center of St. Louis at St. Luke's Hospital in St. Louis, and author of the best-selling *How To Get Pregnant*.

The Cryotop Methods (Live Demonstration)

Masashige Kuwayama
Repro-Support Medical Research Center, Tokyo, Japan

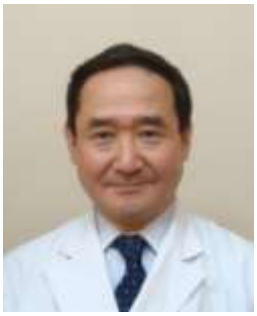
Recent drastic advances in cryobiology have made it possible to preserve various types of reproductive cells with little viability loss. Vitrification, the alternative cryopreservation method is a powerful tool to any biological specimens, which cannot be preserved by the conventional slow freezing. The Cryotop method, effective and safety ultra-rapid vitrification realized the successful clinical use of vitrification not only for human PN zygotes, cleavage stage embryos and blastocysts but also for oocytes. The Cryotop method has been clinically applied to more than 500,000 clinical cases for human oocytes and embryos for these 10 years in 40 countries with excellent results without any serious problems like virus contamination during storage. The reasons of the advantage of Cryotop method is 1) Reliable 2) Universal and 3) Simple. In this session, I show these advantages of the Cryotop method by a live demonstration of the standard Cryotop method, and other 2 variations of the Cryotop methods adapted to the regulations in different countries.

1. Cryotop method for human oocytes (Standard version)
2. Cryotop method with Cold Air Sealing (Open cooling/Closed storage)
3. Cryotop method with Closed Cooling (Closed Top method)

◆Biosketch

Name: Masashige Kuwayama Repro-Support Medical Research Center
Ph.D.: Hokkaido University
Occupation:
Present - CEO, Repro-Support MRC
2010- General Academic Supervisor, Kato Ladies group
2007- Board Internal Society of MAAR
2005- Editor, J. Reproductive Bio Medicine Online
2005- Board, JSClE
2005- Lecturer, IUMW, Meiji, Azabu Univ.
Publications: 81 Papers on Scientific Journal: JRF, Reproduction, Cryobiology, CryoLetters, BOR etc..
290 Papers on Scientific Meeting: IETS, ICAR, ASRM, ESHRE etc.
Topics: Scientific Director, largest IVF center for 10 years.
R & D Director: vitrification, GVT, spermatogenesis, ES-cells.
Obtained first calf after vitrification of blastocyst (1991), MII oocyte (1992). First vitrification porcine pre-hatch blastocyst, oocyte (1997, 1998). Developed Cryotop method for human oocyte (1999), embryo (2000), first oocytes bank (2001), in USA (2003). Vitrified 500,000 oocytes and embryos in 40 countries by Cryotop method.

Overview



Kou Sueoka
Keio University School of Medicine, Tokyo, Japan

Preimplantation genetic diagnosis (PGD) is going into the new era according to the dramatically developed technologies of genetic analysis. It is a medical approach to support transmission of genes to be hopefully healthy, which is the aim and the principle of reproductive medicine. On the other hand, there has been a lot of ethical arguments to relate to criticism that it is medical treatment to lead to selection of life including on the aspects of the indication and its range. The situation of PGD is still different in the countries. There are the countries which are not accepted at all, while there are positive countries approving it legally. Japan is a country that asks strict correspondence to require double ethical deliberation and approval on the restrictive acclimated indications and also the diagnostic method. ESHRE PGD Consortium has been collected the accumulated data on PGD outcome from the cooperated institutions in the world and reported for a fixed interval. As a technical aspect of PGD the large changes have occured both in the alteration of biopsy and genetic diagnosis. However, biopsy method of next generation PGD is shifting to that from polar body and blastocyst. Polar body biopsy is useful to distinguish mosaicism of numerous anomalies. In addition, the method of blastocyst biopsy is also another alteration to improve diagnostic accuracy by obtaining many cells, and to cope with wide variety of genetic diagnosis. As for the technologies for genetic diagnosis, while FISH has been conventionally used for diagnosis of chromosomal information, nested PCR has been used for diagnosis of DNA information. However, the technical limitations and problems have been experienced in both diagnostic methods so far. However, particularly diagnostic significance of aneuploidy screening using FISH for limited number of chromosomes has been asked from the standpoint of the evidence, and desired to shift to the new analytical technologies. Moreover, FISH is given as a basic problem in diagnostic accuracy from single cell based on the extremely restrictive information only with a number of detected signals. On the other hand, a lot of problems about PCR used for DNA diagnosis has been noticed; for example, that tailor-made diagnostic method needs to be suffered in most cases corresponding to a wide variety of genetic type; problems relating to gene amplification such as DNA contamination and allele drop out; that there is limitation of DNA amount to diagnose for single copy gene of single cell; and the subject for the cost and spending time. Genetic diagnosis is going into the new era of genome wide analysis. Array technology has been developed as a new trend of genetic diagnosis, and the method of whole genome amplification (WGA), which is required prior to the array analysis for single cell, has been introduced into PGD. These technologies would be reaching the possibilities not only of largely expanded diagnostic width, but also of improvement of diagnostic accuracy. However, from the viewpoint of genetic information management, we should pay more attention to perform enough genetic counseling for a client.

◆Biosketch

Birth Date: July 7, 1954.
Present Status: Keio University School of Medicine, Department of Obstetrics and Gynecology. Associate Professor, Director of Reproductive. Obstetrics and Gynecology.
Subspecialty: Reproductive medicine, Clinical human genetics, Gynecologic endoscope surgery.
Title: National license of medical doctor of Japan, Doctor of Medical Science (Ph.D.),
Specialty Board: Obstetricians and Gynecologist, Endoscopy, Clinical Genetics, Reproductive Medicine.
Career of Medical Background:

1980.8 Graduation of Keio University School of Medicine,

1983.6 Keio University fellow,
1986.7 Johns Hopkins University post doctoral fellow (U.S.A., Maryland),
2001.11 Director of Genetic Counseling Section Keio University Hospital,
2000.4 Keio University Associate Professor.

Major Appointments: Vice president of the Alumni Society of Keio University School of Medicine.

Whole genome amplification is a new basic technology for next-generation preimplantation genetic diagnosis on mendelian inheritance disease

Suguru Sato, Kou Sueoka, Akira Nakabayashi, Yasunori Yoshimura
Department of Obstetrics and Gynecology, Keio University School of Medicine, Tokyo, Japan

Preimplantation genetic diagnosis (PGD) is an established procedure of embryo genetic analysis, allows couples carrying disorder to have an unaffected child, without invasive prenatal diagnosis and termination of pregnancy. The scarcity of DNA is a limiting factor in many reseach fields. For PGD on Mendelian inheritance diseases, because of difficulty in analyzing target single gene from a single cell, two rounds of PCR, termed nested PCR is once a essential basic technique. Genetic diagnosis has been made by testing PCR products for a variety genetic disorder. It requires appropriate primers based on genetic information derived from the client's family and optimization of multiplex PCR condition and primer setting are labor-intensive. There are limits to multiplexing that multilocus simultaneous analysis from a single cell is restrictive. In cases with multiple exon deletions or more than two copies of mutation with autosomal gene, the contamination risk increases. Development of new gene amplification method instead of PCR is desirable. Whole genome amplification (WGA) is expected to be a solution for it. Multiple displacement amplification (MDA) is a non-PCR based WGA which includes the bacteriophage ϕ 29 DNA polymerase, random hexamers expected to allow multilocus parallel analysis by any combination of conventional genetic testing. For genetic diagnosis of multiple loci, when one round WGA can amplify the template DNA instead of each 1st PCR on the relevant mutation site, risk of contamination reduces, leads time and cost reduction. One of the potential problem associated with WGA is so called allele drop out (ADO), defined as the random non-amplification of one of the alleles present in a heterozygous sample. To avoid that phenomenon, optimization of cell-lysis is necessary. Method for cell-lysis using alkaline lysis, proteinase K, and other enzymes or detergents have been expected for more efficient DNA collection. The combination of MDA and alkaline lysis extraction on single cells by KOH has been highest efficiency for the amplification success rate from the target part from single cell, which was stably more than 70%. Repetitive assay for mutation sites is favorable in PGD to avoid misdiagnosis by ambiguous result especially for autosomal dominant diseases and compound heterozygote. One of these diseases is Fukuyama congenital muscular dystrophy (FCMD), which is an autosomal recessive disorder indigenous to Japan. The incidence of FCMD is 0.7-1.2 per 10,000 births, next to Duchenne muscular dystrophy. Fukutin gene, responsible for FCMD is located on 9q31 and it is well known that almost all patients have the 3-kb retrotransposal insertion in Fukutin gene and the disease often occurs as a result from combined homozygous for the mutation or compound heterozygous with other copy of point mutation. MDA amplify scarce DNA enough to be served for multilocus simultaneous assay and abundant WGA products can be analyzed repeatedly. Unbiased and high fedility WGA is an essential technique for the construction of accurate and high through-put diagnostic system, will enable comprehensive mutation analysis by combination of array-based technology. Further validation will be needed for PGD in next-generation.

◆Biosketch

2002 Graduated from Iwate Medical University School of Medicine
2002 Residents of Obstetrics and Gynecology, Keio University School of Medicine
2004 Obstetrics and Gynecology, Toyoshina Red Cross Hospital
2005 Obstetrics and Gynecology, Otawara Red Cross Hospital
2006 Research Associate, Keio University School of Medicine, Obstetrics and Gynecology
2010 Research Associate, Tokyo Dental College Ichikawa General Hospital, Obstetrics and Gynecology
2011 Research Associate, Keio University School of Medicine, Obstetrics and Gynecology

To PGD or not to PGD? How many oocytes are needed to start PGD with?

Ilan Tur-Kaspa
Institute for Human Reproduction (IHR), the Department of Ob/Gyn,
The University of Chicago, Chicago, IL, USA

Introduction: The optimal number of retrieved oocytes for successful IVF outcome depends primarily on women's age and the ovarian stimulation protocol. PGD results significantly and independently of embryo development reduce the number of embryos available for transfer, by 25 - 88 percent, depending on the type and number of PGD tests performed. We investigated if there is a magic number of oocytes to start PGD with. This information is valuable for professionals who consul women on ovarian stimulation and possible outcome of IVF with or for PGD.

Material and Methods: In addition to literature review, outcome data from personal clinical experience with 562 consecutive IVF-PGD cycles in 4 years (168 cycles for 51 single gene disorders, 99 cycles for HLA matching, 44 cycles for translocations, and 251 PGD cycles for aneuploidy) were analyzed by women's age, number of oocytes retrieved, and type of PGD performed. Patient's ovarian reserve was estimated before treatment by age, antral follicle count, and day-3 FSH levels, and accordingly changes in the stimulation protocol or in medication dosage to try to safely reach oocyte yield of 10-15, were implemented.

Results: An increase in the number of oocytes retrieved improved the patients chance to have an ET with normal embryos, as well as to have surplus blastocysts cryopreserved. Responding poorly to stimulation may result from an inadequate stimulation protocol or because of low ovarian reserve, which may or may not be related to the genetic disorder itself. Most young patients may have an ET with good outcome even with only 1-7 oocytes. However, for patients at age 35-42 years old, more oocytes significantly improved outcome.

Conclusions: Individually adjusted ovarian stimulation based on estimated ovarian reserve for patients undergoing IVF-PGD improves outcome. When the patient is a poor responder, there is practically no risk of OHSS and therefore, a mild stimulation protocol should not be offered. Once patients were adequately stimulated and wish to conceive with their own eggs, oocyte retrieval and PGD may be continued even with very low number (1-7) of oocytes or embryos. Routine canceling of PGD cycles because of poor response should be reconsidered, especially for young women.

◆Biosketch

Professor Ilan Tur-Kaspa is the President and Medical Director of the Institute for Human Reproduction (IHR), Director of the Clinical IVF-PGD Program, Reproductive Genetics Institute, and Professor at the Dept of OB-GYN at the University of Chicago. Prof. Tur-Kaspa is one of the world's most experienced Reproductive Specialists involved in advanced IVF-ICSI treatment with PGD. He specializes in advanced ART, reproductive imaging, and PGD. He is the author and co-author of over 90 scientific publications and book chapters. He serves as a Reviewer for the leading Journals in Reproductive Medicine, such as Fertility and Sterility, Human Reproduction Update and Reproductive BioMedicine Online. He is a co-Editor of the book on Biotechnology of Human Reproduction. He was awarded the 2010 Star Award of the American Society for Reproductive Medicine (ASRM) for over 10 years of continuous scientific contributions in the field of Reproductive Medicine.

Introduction to performing efficient FISH-based PGD

Naoki Aoyama
Kato Ladies Clinic, Tokyo, Japan

Since Alan Handyside first established preimplantation genetic diagnosis (PGD) more than 20 years ago, it has become a widely accepted clinical technique available in many centers around the world. Currently PGD's indication is well-established for couples at high-risk for having children affected by hereditary diseases, habitual abortion, etc. Recently, microarray-based techniques have also been developed for the screening applied to the general population. Nonetheless the "classical" FISH technique is still an extremely useful, cost-effective and powerful tool for PGD, especially for chromosomal translocation carriers.
The present workshop will provide you with an introduction to the fundamental techniques necessary for practicing FISH-based PGD. Hopefully it will be a valuable aid to everybody wanting to start a PGD program at his/her center.

・Embryo biopsy
PGD implies the removal of polar bodies, blastomeres or trophectoderm cells from oocytes, cleavage-stage embryos or blastocysts, respectively. Embryo biopsy performed at cleavage-stage by the mechanical opening of the zona pellucida and suction of a single blastomere is considered the most fundamental technique and will be demonstrated at the present workshop.

・Blastomere fixation
Blastomere fixation is one of the most critical steps in FISH-based PGD. Three methods for blastomere fixation or spreading have already been described and currently all of them are considered acceptable.
Carnoy method : Methanol/Acetic acid
Cell spreading : Tween/HCl
Combined : Tween/HCl and Methanol/Acetic acid
Although the Combined method is the simplest and the easiest to perform, it was reported that the Carnoy method might generate better nuclear quality by obtaining a wider nuclear diameter after fixation on a glass-slide. Our own experience also supports the higher efficiency of the Carnoy method. An introduction will be given to the three above-mentioned techniques highlighting the differences between each of them and how to use them correctly to obtain accurate and reliable PGD results.

◆Biosketch

Major: Cytogenetics & Animal Physiology

Naoki Aoyama began his studies in biology in 1988 at Faculty of Animal Science University of Meij (Japan).

In 1994 he obtained his MSc degree in Agricultural Faculty from the same University.

After graduation he began working at Mitsubishi Biomedical Laboratories Co., Ltd and moved to Kato Ladies Clinic in 2000.

He started to study Cytogenetics at Advanced Institute of Fertility in 2001 and developed his technique for Preimplantation Genetic Diagnosis.

PLENARY LECTURES

Natural/Clomiphene Cycle IVF: The Japanese Experience

Osamu Kato, M.D.
General Director, Kato Ladies Clinic, Shinjuku, Tokyo, Japan

During the some thirty years since Steptoe and Edwards delivered Louise Brown in 1978, in vitro fertilization and embryo transfer (IVF-ET) have come to be practiced all over the world. At the same time, Assisted Reproductive Technology has continued to make progress by leaps and bounds with brilliant results, and various revolutionary techniques have been developed, solving even those problems which had been thought impossible to remedy. These techniques include Microsurgical Epididymal Sperm Aspiration (MESA) (R. H. Asch and S. J. Silber, 1991), Intracytoplasmic Sperm Injection (ICSI) (Palermo et al., 1992), Percutaneous Epidydimal Sperm Aspiration (PESA) (O. Kato et al., 1993), and Testicle Sperm Extraction (TESE) (R. Schoysman et al., 1993) for male infertility; the Towako Method (transvaginal-transmyometrial embryo transfer for difficult transcervical embryo transfer cases) (O. Kato and R. H. Asch, 1993), Pre-implantation Genetic Diagnosis (PGD) for screening embryos with abnormal genes, and vitrification of unfertilized embryos for unmarried young cancer patients (M. Kuwayama, 2001) for female infertility. However, as regards the ovulation stimulation method, protocols for Controlled Ovarian Hyperstimulation (COH) have not improved greatly, save for the use of different drugs. As a result, there have emerged various questions, specifically:

(1) Why do we retrieve so many oocytes despite the fact that only 2-3 oocytes are of good quality even when more than ten oocytes are retrieved?

(2) Why do we need to transfer more than one embryo, increasing the multiple birth rate, when patients do not wish to have more than one child? (Is it because it is difficult to select good quality oocytes or because we want to improve the pregnancy rate?)

(3) Why do we use COH which requires as the maturation trigger the use of HCG which has the potential to induce severe Ovarian Hyperstimulation Syndrome (OHSS)?

(4) Why are we increasing the financial burden on patients with the use of drugs?

In our clinic, in order to address these questions, we have developed and practiced unique protocols for oocyte retrieval and embryo transfer. Today, I would like to talk about the history of our protocols, from our first Minimal Stimulation protocol up to our present protocol, the single follicle-single embryo transfer (SF-SET), which requires no ovulation inducing drugs at all.

◆Biosketch

Osamu Kato, M.D., General Director, Kato Ladies Clinic
Born in1946 and graduated from Kanazawa University, School of Medicine in March 1972.
Medical Doctor in December 1972.
President, The International Association of Private Assisted Reproductive Technology
Vice-President, The World Association of Reproductive Medicine (WARM)
Executive Board Member, Japan Society of Fertilization and Implantation(JSFI)
Trustee, The International Society for Mild Approaches in Assisted Reproduction (ISMAAR)
Congress President, 3rd World Congress on MAAR, 2010
Congress President, 16th World Congress on IVF, 2011
Congress President, 29th Annual meeting of JSFI, 2011

Non-invasive assessment of embryo quality

Zsolt Peter Nagy
Reproductive Biology Associates

Introduction. The traditional procedure for embryo assessment is performed by morphological evaluation which provides useful information, however, there are numerous factors contributing to oocyte/embryo quality that are not necessarily reflected by morphology. Review of alternative techniques on embryo viability assessment. In recent years, there have been numerous reports on different technologies, which may aid embryo assessment by providing additional information on the viability of the embryos. One of them is birefringence imaging which utilizes polarized microscopy (polscope) to study the status of the meiotic spindle and the zona pelludica and can provide information on embryo development ability. Time-lapse video monitoring of embryos is another, more complex method of visual assessment which is based on monitoring by real-time, time-lapse imaging of individual embryos. One of the advantages of these systems is that they minimize handling and exposure to sub-optimal environmental effects, while enabling the continuous monitoring of embryo development and through analyses of developmental (cleavage) data with sophisticated software it also can provide prediction on embryo viability. Measurement of uptake and secretion of single or specific molecules in the culture medium has also been suggested by several studies to aid embryo assessment. Pyruvate and glucose can give information on a switch from carboxylic acid to glucose metabolism that occurs in the early embryo between pre- and post-compaction thus their concentrations can relate to the ability of the embryo to develop further. Amino-acid measurements by ultramicrofluorometric assays or by reverse-phase high performance liquid chromatography (HPLC) can be used to predict blastocyst formation, clinical pregnancy rate and live birth. Soluble Human Leukocyte Antigen G (HLA G), which is believed to play a role in immuno tolerance during pregnancy was also suggested as indicator for pregnancy outcome, as well Leptin and Platelet Activating Factor (PAF) have also been suggested as potential markers. Oxygen consumption yet another single marker measured by Nano and Embryo Respirometer, that can provide information relating to embryo implantation potential. Proteomics, which refers to the study of proteins, is another technology that can be used, specially the method called surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI TOF MS) is particularly suited for analysis of relatively low concentrations of secreted proteins from culture media with higher sensitivity of than other platforms, and has been described to relate embryo development to the blastocyst stage. Metabolomics is another newly introduced approach; which refers to the study of factors present in the culture medium that are secreted by the embryo into its environment as a result of metabolomic processes that occur within the cells. The secreted metabolites (also called secretome) are readily available and can be collected in a simple, non-invasive way using Raman on near infrared spectroscopy. Several studies have demonstrated strong correlation between metabolomic profile of embryo and its ability to implant.

Conclusions. There are several novel techniques that are suggested to be able to predict embryo viability using non-invasive approaches. Further studies will clarify, which technique(s) are best suited to aid current embryo assessment procedures.

◆Biosketch

Dr. Nagy obtained his MD (1986) and his Ob&Gyn degrees (1996) at the Semmelweis Medical University in Budapest. His PhD was granted at the Free University of Brussels in 1997, where he worked in the team that developed the ICSI procedure. More recently, he has contributed to the development of a highly efficient oocyte cryopreservation procedure that gave bases to establishing the first donor cryo-bank in USA. He is author of more than 150 publications and book chapters. Dr. Nagy serves on the board of several different societies; as well he is Associate Editor for Human Reproduction and RBMonline. Currently, Dr. Nagy is the Scientific and Laboratory Director at Reproductive Biology Associates, Atlanta, USA.

The Prospect of Embryonic Stem Cell Research

Shin Yong Moon M.D., Ph.D.
Department of Obstetrics and Gynecology,
Director of the Institute of Reproductive Medicine and Population
Medical Research Center, College of Medicine, Seoul National University

There has been no argue on the importance of stem cell research in developing of future medicine and therapeutic bioengineering. Lots of peoples and the patients who supper from incurable diseases are enthusiastic about clinical feasibility of stem cells since the first establishment of human embryonic stem cells (hESCs). As the strong candidate of clinical application, tissue-specific stem cells and induced pluripotent stem cells (iPSCs), in addition to hESC, have strongly been suggested. Based on recent development of novel cytogenetic and cytological tools, however, all of these candidate cells showed the limitations, which should be cleared before clinical application. In the case of hESC, ESCs derived from surplus embryos is very much likely to induce immune reaction to a recipient patient. Cellular heterogenicity in self-renewal, differentiation and localization characteristics should be controlled. Extremely low efficiency in establishing patient-specific stem cells by somatic cell nuclear transfer is critical, which concomitantly evokes serious ethical issue on its clinical application. In iPSCs, the processes of reprogramming and adaptation to in vitro-environment cause lots of genetic and epigenetic abnormalities including copy number variation and multiple point mutations as well as aneuploidy. As a matter of fact, human iPSCs show more genetic abnormalities and cellular heterogenticity than precursor fibroblasts or even hESCs and recent reports strongly concerned the acquisition of immunogenicity to iPSC donor after reprogramming. More basically, genetic manipulation of somatic cells for deriving iPSCs makes its clinical application impossible. Oncogene transfection to precursor somatic cells for the cell derivation remarkably increases concerns on clinical application of iPSCs. Regardless of its significance, inefficient isolation of tissue-specific stem cells from various adult tissues has been criticized for long time and the establishment of homogenous stem cell population is a prerequisite factor for clinical application of the stem cells. Cell niche to control stem cell fate and to mobilize tissue-specific stem cells has not been defined yet.

In conclusion, more detailed study on genetic and cellular characteristics of stem cells will guarantee the development of stem cell engineering for clinical application, and the state-of-the-art cellular and genomic technology can be utilized for the novel studies.

◆Biosketch

Name: Shin-Yong Moon M.D.
Born April 1, 1948
Address: Department of Obstetrics and Gynecology
Seoul National University Hospital
Yonkeun-Dong, Chongno-Gu, Seoul 110-744, Korea

Shin-Yong Moon is currently Professor of Obstetrics and Gynecology, College of Medicine, Seoul National University Hospital, and Director of the Institute of Reproductive Medicine and Population, Medical Research Center, at Seoul National University. Completing all the necessary courses and requirements for Obstetrics and Gynecology specialists in Seoul National University Hospital, he started to study “In Vitro Fertilization Program” at Jones Institute for Reproductive Medicine, Norfolk, Virginia in United States in 1983.

After coming back to Seoul, he could have first successful IVF baby in Korea in 1985. During the last 30 years he studied Cytogenetics, Reproductive Endocrinology and Infertility and Embryonic Stem Cell. In 2001

he succeeded in establishment of “Human Embryonic Stem Cell” from frozen 2 pronuclear stage cryopreserved embryos in his laboratory.

Professor Moon has been a major contributor to the development of reproduction medicine in Korea and is actively involved in numerous local medical societies; he was President of the Korean Society of Human Reproduction and the Korean Tissue Engineering and Regenerative Medicine Society, President of the Korean Society for Assisted Reproduction and Emeritus President of the Korean Society of Medical Genetics.

He is also author or co-author of more than 30 papers published in leading international journals, including Molecular Therapy, Stem Cells, Human Reproduction and Fertility and Sterility.”

Preimplantation genetic diagnosis: an update

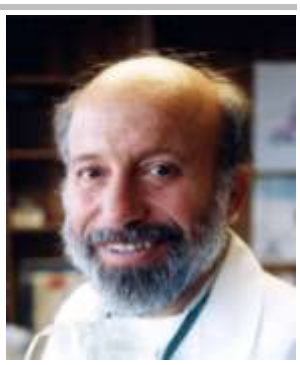
Alan H. Handyside
London Bridge Fertility, Gynaecology and Genetics Centre

Twenty one years on from the first pregnancies and births in July, 1990 preimplantation genetic diagnosis (PGD) is now clinically well established as an alternative to prenatal diagnosis. As genome wide analysis becomes quicker, cheaper and more widely available over the next decade, genetic testing of both patients and their embryos will be revolutionised. Ultimately it may even be possible to sequence the entire genome of an embryo though it is more likely that targeted methods will be more cost effective. The benefits to patients are that this will enable accurate testing of embryos not only for virtually any chromosomal or single gene defect identified in the parents but also for genetic defects formed spontaneously in the gametes and early embryo, many of which are likely to compromise viability and development. As the unveiling of the first personal genomes has demonstrated, however, genome wide analysis raises challenging clinical, ethical and social issues. Increasingly it may be possible, for example, to provide information about physical characteristics and predisposition to common diseases and late onset disorders. On the other hand, thousands of genetic variants are being discovered for which the consequences are unknown. There is a danger that this will increase demands for PGD from prospective parents who want to know (or control) how their child will turn out and whether they will be healthy. Future regulation of PGD needs therefore to focus on not only what inherited conditions should be allowed for embryo selection but also what genetic information should be provided to patients about those embryos that are selected for transfer.

◆Biosketch

Background in mouse and human embryology and genetics with over 200 publications. Created one of the first mouse transgenic mice using embryonic stem cells. With Prof Lord Robert Winston, achieved the first pregnancies following IVF and PGD worldwide in 1990. First Chairman ESHRE Special Interest Group in Reproductive Genetics and founder and first Chairman of the ESHRE PGD Consortium.

Preservation of Fertility



Sherman J. Silber[1], Noriko Kagawa[2], Masashige Kuwayama[2]
[1]Infertility Center of St. Louis, [2]Kato Ladies Clinic

Objective: Cryopreservation of ovarian tissue is widely performed to preserve fertility in cancer patients, but its efficacy has been difficult to assess because most patients do not request transplantation of the thawed tissue for over a decade. Our objective was to evaluate the long-term results of transplantation of fresh ovarian versus frozen ovarian tissue in patients with ovarian failure (OF), to evaluate its usefulness in preservation of fertility for cancer patients.

Design: Seven year follow-up of a consecutive series at one center of women with OF who underwent either fresh (11) or frozen (4) ovary transplantation, and evaluation of the tissue of 62 patients who underwent ovarian cryopreservation, but not yet transplantation.

Materials and Methods: 12 women with ovarian failure were recipients of 15 ovarian tissue transplants, 11 fresh from a sister, and 3 frozen autotransplants. In 9 of the fresh cases, the sisters were identical twins, and in 2 cases, they were non-identical siblings. In 4 women, the cause of OF was cancer chemotherapy and radiation. In the other 8 women, the cause was idiopathic premature OF. A small portion of tissue was tested for post-thaw viability in all cases of ovarian tissue freeze.

Results: There have been 17 pregnancies, and 14 healthy babies (11 from fresh transplant and 3 from frozen tissue), with 3 miscarriages. 5 babies were delivered to women who had received pelvic irradiation. With fresh transplants, menstruation resumed from 63 to 130 days post-transplant, whereas with slow frozen transplants, menstruation resumed after 147 days. Fresh transplants continued to function from 2 years to over 7 years (4 of the 8 cases) depending on the donor's pre-operative antral follicle count. The average duration of function was over 5 years. Frozen (slow freeze) grafts, however, functioned for 1-1⁄2 to 2 years. Examination of vitrified grafts revealed no difference between pre-freeze and post-freeze viability.

Conclusions: Ovarian cortical grafting, fresh or frozen, particularly with vitrification gives robust results with healthy babies, and good, long-term function.

◆Biosketch

Dr. Sherman Silber is one of the world's leading authorities on IVF, microsurgery, vasectomy and tubal reversal, egg and embryo freezing, ovary and testis transplantation, and the reproductive biological clock. He is director of the Infertility Center of St. Louis at St. Luke's Hospital in St. Louis, and author of the best-selling *How To Get Pregnant.*

The role of reproductive surgery in the era of IVF & ART

Victor Gomel
Department of Obstetrics and Gynecology,
Faculty of Medicine, University of British Columbia,
Vancouver BC. Canada

The indications for reconstructive surgery in the era of IVF and ART are two-fold: 1. Primary treatment of tubo-ovarian disease, adhesions and other pelvic pathology to give the patient the opportunity to achieve spontaneous conception; and 2. Treatment of uterine and other conditions that would adversely affect the outcome of assisted reproduction

Tubo-ovarian disease, adhesions and other pelvic pathology are the principal cause of infertility in approximately one third of cases. While, reconstructive surgery was at one time the only treatment option, this is no longer the case. Improvement in the outcomes, simplification of the techniques and much wider availability of the services of in vitro fertilization (IVF) and assisted reproduction (ART), provide such couples with a realistic therapeutic alternative.

Further to significant improvement in IVF results in the nineties, the pregnancy rate per cycle initiated appears to have stabilized in the USA at around 28% during the last several years (2002-2008). Success with transfer of cryopreserved embryos has also improved significantly. The introduction of intra-cytoplasmic sperm injection (ICSI) proved to be a panacea in the treatment of male infertility. Indeed the outcomes of IVF with ICSI produce similar outcomes in couples with and without male factor.

This enormous progress in the live birth rates with ART has been accompanied with the commercialization of the technology and its services, all over the world. In parallel fashion there has been a significant decline in the practice and teaching of reproductive surgery. IVF now is offered as primary treatment option, in most cases of tubal factor infertility. These changes have occurred despite the major progress that gynecologic surgery has experienced; introduction of microsurgical techniques and the predominant use of laparoscopic surgical access that readily permits the application of these techniques in reproductive surgery.

ART is associated with a tremendous increase in preterm births and its important sequelae of perinatal mortality and morbidity, including cerebral palsy. The rates of preterm births for singletons, singletons from multiple fetuses, twins, triplets and greater order of multiples are 12.5%, 18.3%, 63.3% and 95.3% respectively. The perinatal mortality was 1.9% in the in-vitro fertilization group almost double that of the controls. In the USA, in 2007, of the 36,079 pregnancies resulting from ART 28.8% were twins and 3.7% were triplets and greater order of multiples; and of the resulting 29,556 births 29.4% were twins; greater order of multiples decreased to 1.8% either by fetal reduction and/or spontaneously. The large proportion of multiple births, with the associated increased obstetrical complications, neonatal complications and deaths, causes great societal costs and significant financial burden and emotional costs for parents.

Based on the current US outcomes of a birth rate of 28% per initiated cycle, the cumulative probability of live birth after 3 cycles of treatment would be around 52%. However, several studies have shown conclusively that a large percentage of couples do not wish to complete 3 cycles of IVF, even when these are paid by the state or covered by insurance. Reports from France, where IVF is covered by the state, corroborate with these findings. Others refuse to have IVF for religious or ethical reasons, and many would find the cost of IVF prohibitive, since the procedure is not covered in many states and countries, while other treatment modalities are, as is the case in British Columbia in Canada.

For the infertile woman with tubo-ovarian and pelvic pathology there are only two realistic options to achieve a pregnancy: reconstructive surgery or IVF. The presence of a credible alternative, in IVF, permits the reproductive surgeon to operate on cases with a better prognosis, which was not the case before the end of the

eighties. We have known for a long time that one of the important factors influencing surgical outcome was the degree of tubal damage and extent of pelvic disease and adhesions. Operating on patients with better prognosis translates in superior outcomes, which has been well demonstrated.

With appropriate preparation, many reconstructive procedures can be performed during the initial diagnostic laparoscopy using microsurgical techniques: salpingo-ovariolysis, salpingostomy and even tubo-tubal anastomosis for reversal of sterilization. This is also applicable to other pelvic pathology, such as endometriosis, uterine myoma etc. Intrauterine conditions may be treated performing a concomitant hysteroscopy. In the absence of any other infertility factors, laparoscopic salpingo-ovariolysis yields intrauterine pregnancy rates of 50% to 60%; and salpingostomy yiels rates ranging from less than 20% to 50%, depending largely on the degree of tubal damage and the extent and severity of adhesions.

Microsurgery is ideal for tubo-tubal anastomosis and produces excellent results that are principally dependent on the length and status of the reconstructed tube. In cases of reversal of sterilization, live birth rates of 60% to 80% can be achieved. In cases of true pathologic proximal tubal occlusion microsurgical tubo-cornual anastomosis yields an intrauterine pregnancy rate of about 50%. Microsurgery also permits reconstruction in complex situations, for example a tubo-ovarian transposition, with preservation of their vascular pedicles. In such cases, the prognosis that the surgical reconstruction offers is not necessarily proportional to the technical difficulty of the procedure.

Assisted reproductive techniques are being used increasingly as primary treatment for infertility. This is largely the result of the commercialization of the ART technology and its services. The industry is well capitalized and is lucrative. Yet it is important to stress that even if couples were to undergo three successive cycles of IVF, nearly 50% would fail to obtain a baby.

Many patients need to have reproductive surgery before IVF for various conditions such as myomas, adnexal tumors, endometriosis, etc. Yet, there has been a significant decline in the practice and teaching of reconstructive surgery and microsurgery in gynecology. Such teaching and practice made the gynecologist a more refined surgeon, attributes that would be regrettable to loose.

As evident from the preceding, reconstructive surgery when well performed, in properly selected cases offers satisfactory results. Furthermore, it offers the couple the opportunity to attempt a pregnancy over a long period of time and to conceive more than once out of the same procedure. This and the available data suggest that there should be a real place for reconstructive surgery. The preservation of this place will require a concerted effort on the part of the teaching institutions.
The development of operative laparoscopy, tubal microsurgery, and IVF in the last 30 years has significantly improved the outlook of couples suffering from tubal infertility. These are complementary approaches that can be used singly or in combination. When both alternatives are equally available to the patient and used a much greater overall cumulative birth rate can be obtained.

In the preface of my book, Microsurgery in Female Infertility, published by Little Brown, in early 1983, I wrote: “This manuscript has been completed during a time of rapid change and expansion with the understanding that it represents not an endpoint but merely an accounting at a given point in time. Further developments are also occurring in the area of IVF and embryo transfer (IVF & ET), which will undoubtedly produce improved results. Nonetheless, I do not consider the techniques of microsurgery on the one hand and IVF & ET on the other as competitive; on the contrary, I see them as complementary, enabling us to achieve a greater success rate among those patients presenting with complex fertility problems.” This statement is still valid today.

◆Biosketch

(For Prof. Gomel’s Biosketch please refer to his abstract for Concurrent Symposium C-4.4.)

Recent advance of controlled ovarian stimulation

Klaus Diedrich
Department of Obstetrics and Gynecology of the University of Lübeck, Germany

Ovarian stimulation contributes to the overall effectiveness of in vitro fertilization treatment. However, ovarian stimulation is also associated with health risks, adverse events, treatment burden for the patient and high financial costs. Ovarian stimulation therefore needs to be continuously improved. In this literature review, three important new developments in the field of ovarian stimulation have been selected for discussion. Human chorionic gonadotropin as the triggering agent for ovarian hyperstimulation syndrome (OHSS) can now safely be replaced with a bolus dose of a gonadotropin-releasing hormone agonist. This has been shown to reliably prevent OHSS, the most serious complication of ovarian stimulation. To reduce the injection frequency of gonadotropins, a long-acting follicle-stimulating hormone molecule (C-terminal peptide, FSH-CTP) has been developed and tested in a large set of clinical trials. It was shown that long-acting FSH-CTP is able to stimulate the ovaries for 7 days at doses of 150 and 100 µg, respectively, and that the outcome in terms of pregnancy likelihood is similar to conventional gonadotropin stimulation by daily injection. Orally active non-peptide mimetics of luteinizing hormone and FSH are currently being developed. However, no data on the administration to humans have been published to date, and only scarce data on in vitro and animal experiments are available.

◆Biosketch

Prof. Dr. med. Dr. h.c. mult. Klaus Hermann Rolf Diedrich

Birth: 28.04.1946

Birth-place: Nordenham

Parents: Dr. med. Hermann Diedrich, M. D.
Rita Diedrich (maiden-name: Ehrlich), lady of the house

Education:

1966:	High school graduate at the Matthias-Claudius-Gymnasium in Hamburg
1966:	Start of studies in human medicine at the University of Hamburg
1967:	Naturwissenschaftliche Vorprüfung in Hamburg
1968:	Ärztliche Vorprüfung
1972-73:	Junior house officer in surgery and internal diseases
1973-74:	Military service as M. D.
1974-78:	Senior house officer in OB/GYN at the university clinic of Hamburg
1979:	Consultant at the department of OB/GYN at the university clinic of Lübeck
1981:	Ph. D. in Obstetrics and Gynecology
1982:	Marriage with Christa Diedrich, M. D.
1983:	Birth of a son
1984:	First consultant at the department of OB/GYN of the university clinic of Bonn; University Professor
1984:	Founding member of the European Society of Human Reproduction and Embryology (ESHRE)
1985-91:	Secretary of the European Society of Human Reproduction and Embryology (ESHRE)

1991-93:	Chairman-elect of the European Society of Human Reproduction and Embryology (ESHRE)
1993:	Head of department of Obstetrics and Gynecology of the University Clinic of Lübeck
1993:	President of the European Society of Human Reproduction and Embryology (ESHRE)
1994-2000:	Secretary of the German Society of Obstetrics and Gynecology (DGGG)
2001:	Member of the German Academy of Science Leopoldina
2000:	Vice President (chairman-elect) of the German Society of Obstetrics and Gynecology (DGGG)
2002:	President of the German Society of Obstetrics and Gynecology (DGGG)
2004:	2. Vice President of the German Society of Obstetrics and Gynecology (DGGG)
2006:	Vice Dean of the University of Lübeck
2006:	Fellow of the Royal College of Obstetrics and Gynecology
2006:	Foundation of the German-Greek Society of Obstetrics and Gynecology by Prof. Agorastos (President) and Prof. Diedrich (Vizepresident)
2009:	Medical Director of the University Hospital of Lübeck
2009:	Doctor honoris causa of the Aristoteles University of Thessaloniki
2009:	Doctor honoris causa of the University of Alexandropoulis
2010:	Medical director of the University Hospital Schleswig- Holstein, Campus Lübeck

Member of 18 national and international Editorial boards.
Publications: 480 in national and international journals.

Tending to Male Factor Couples

Gianpiero D. Palermo, M.D., Ph.D.
The Ronald O. Perelman & Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY, USA

In the USA infertility affects about 13.3%, approximately 7.3 million, of all couples of reproductive age, but only about 1.5 million of those benefit from assisted reproductive technologies (ART). Unexplained and combined male and female factor infertility accounts for 34%, the remainder being evenly attributed to female (33%) or male (33%) problems.

Since the inception of in vitro fertilization (IVF) in 1978, it has become evident that half of the couples failed to achieve fertilization because of sperm-related problems. This stimulated the development of several micromanipulation procedures that resulted in the establishment of intracytoplasmic sperm injection (ICSI), a reliable procedure through which to achieve fertilization. ICSI has now been employed successfully even in cases where spermatozoa can only be aspirated from the epididymis and/or the testis of azoospermic men.

The causes of male infertility include the presence of a varicocele in some 30% of such men, followed by unexplained reasons in 25%, and accessory gland abnormalities and cryptorchisdism in 13% and 9%, respectively. The obvious problem of azoospermia due to vas obstruction or testicular failure is seen in about 9% and 14% of male factor couples, respectively.

As a further element, chromosome anomalies are a significant cause of infertility, pregnancy loss and the birth of children with mental and physical disorders. Understanding the genesis of such anomalies requires the direct study of human spermatozoa as such. Since the great majority of errors occur during meiosis, analysis at other spermatogenic stages will minimize the ascertainment bias due to the loss of chromosomally abnormal conceptuses during development. Even males with normal peripheral karyotype may have chromosomal abnormalities in their germ cells, these often arising through instances of nondisjunction during spermatogenesis. Therefore, it is important to screen for sperm aneuploidy.

The root of poor sperm production is often genetic. Male subfertility has been associated with a higher incidence of genomic defects, ranging from aneuploidy to Yq microdeletions, and concerns have been raised as to the risk of transmitting these to the offspring. For this reason, screening for such defects can be an important adjunct to appropriate counseling prior to ICSI treatment. So far, only a few reports of father/son cohorts have evaluated the heritability of mutations associated with male factor infertility, particularly the well-being of the children. Although early studies on neonatal outcomes failed to reveal any differences between ICSI and IVF babies, some recent reports have claimed that there is a greater incidence of abnormalities in ART children, and also an increased risk of low birth weight (and very low birth weight) when compared to those conceived normally. Anxieties have also been voiced in regard to rare imprinting disorders, as well as cancer following ART.

Since 1993, ICSI has been employed in over 30,000 ART cycles at our center and has resulted in positive pregnancies in 40.1% of cases, based on the presence of at least one fetal heartbeat, comparable to those obtained with conventional IVF.

A total of 902 cycles were performed with epididymal spermatozoa and 1,143 cycles with testicular spermatozoa. Both fertilization (71.5%) and clinical pregnancy rates (52.5 %) were higher ($P < 0.001$) with epididymal versus testicular samples (TESE fertilization 56.9% and clinical pregnancy rate 40.8%). When we assessed whether or not cryopreservation affected clinical outcome and observed that fresh MESA (61.9%) had a higher clinical pregnancy than TESE (41.3%; $P = 0.04$) and a similar result was observed when specimens were cryopreserved (frozen MESA 47.6 versus frozen TESE 36.1%; $P = 0.003$). When we looked within a particular surgical retrieval method, the clinical pregnancies with TESE was comparable regardless of

the sample being fresh or cryopreserved. However, fresh MESA showed a higher pregnancy rate in comparison to its frozen counterpart (61.9 vs 47.6%, P = 0.0001).
In 8,293 patients a viable fetal heart was observed by ultrasound, where 898 patients miscarried, aborted, or had an ectopic. The ongoing pregnancy rate was 36.1% per retrieval (7,444/19,226) and 38.7% per replacement procedure (7,444/10,676). So far, we have 8,695 neonates born from 6,537 deliveries.
In reproductive medicine, ART has played a major role in the treatment of couples with all forms of infertility, and in this arena ICSI has distinguished itself in shedding light on the intricate mechanisms of gamete interaction, fusion, and early embryo development. In addition, ICSI has been a research tool through which to study the male gamete not only in regard to its role as the carrier of the male genome but also in its ability to trigger ooplasmic calcium release and its provision of the precursor of the mitotic spindle — of paramount importance for the generation of a healthy embryo.
The recent trends in ART have also raised concerns regarding genetic and epigenetic risks to the offspring. This experience has enabled us to better counsel couples contemplating reproductive treatment, to meticulously monitor the safety of all procedures applied, and, not least, to carefully assess the physical and psychological wellbeing of the children involved.

◆Biosketch

Gianpiero D. Palermo, M.D., Ph.D.

Gianpiero D. Palermo is the developer of intracytoplasmic sperm injection (ICSI), the revolutionary procedure that has been able to virtually cure male infertility. He established the ICSI programs with Prof. André Van Steirteghem at the Brussels Free University in Belgium and later with Prof. Zev Rosenwaks at Cornell University in New York. There are over 2 million babies born from ICSI worldwide.
Dr. Palermo completed his clinical training in Obstetrics and Gynecology at the University of Bari in Italy, attended the Masters and Ph.D. programmes in Brussels Free University and is currently completing additional post-doctoral training in New York and Melbourne. Dr. Palermo has won many prestigious prizes and awards for his pioneer work in Reproductive Biology, including two presentation awards from Serono Laboratories, the Barbara Eck Menning from RESOLVE, the Shackman Memorial Lecture at Johns Hopkins, the Buckeye Lecture of the ASA, Jacob Heskel Gabbay Award in Biotechnology and Medicine, and The Crystal Tube Award.
Dr. Palermo has delivered almost 300 lectures before international audiences, presented over 250 abstracts, several book chapters, and authored/co-authored over 100 peer-reviewed articles. Since 1993, he has been the Director of the ICSI Program at The Ronald O. Perelman & Claudia Cohen Center for Reproductive Medicine and Professor at the Weill Cornell Medical College. He leads a team of talented researchers actively involved in molecular and genetic aspects of fertilization, follow-up of ICSI babies, genetic aspects of male infertility as well as devising new procedures to treat age-related female infertility, harvesting and differentiation of embryonic stem cells, and in vitro maturation of male germ cells.

In-Vitro Maturation (IVM) for fertility treatment and preservation of fertility

Seang Lin Tan
McGill University

IVM is a relatively new technology with limited clinical experience compared to conventional IVF. IVM should be regarded as a complementary assisted reproductive technology of IVF that provides unique opportunities for patients at high risk of OHSS, those with unexpectedly hyper- or poor responses during controlled ovarian hyperstimulation, those with recurrent unexplained IVF failures, and those who are facing imminent gonadotoxic chemotherapy. Essentially, all ART laboratory procedures can be performed with in-vitro matured oocytes if the need arises. The first successful IVM cycles combined with preimplantation genetic screening and percutaneous testicular sperm aspiration have already been reported by our team. We have found aneuploidy rates to be similar in IVM and IVF embryos. IVM has enabled successful treatment of patients with empty follicle syndrome in previous stimulated IVF cycles. Patients can undergo several IVM cycles and we previously reported a series of patients who achieved repeated live births with IVM treatment. Currently, IVM represents the least invasive and the simplest option, and with further improvement in success rates, could become the ultimate patient-friendly protocol. Current clinical pregnancy rates per cycle started reach 50% in young women. Improved treatment methods have resulted in a steady increase in cancer survival rates over the last decades, and a growing number of women are facing the risk of infertility resulting from gonadotoxic oncologic treatment. Patients exposed to gonadotoxic agents for the treatment of non-oncologic diseases such as systemic lupus erythematosus, who are undergoing surgery for endometriosis and who suffer from genetic disorders such as Turner syndrome and fragile-X pre-mutation face similar risks. With an increased awareness of the options available, more women are being offered and are utilizing fertility preservation technologies. The methods can differ for women with medical conditions and for those who wish to defer child bearing for social reasons in the absence of a medical condition. IVF followed by embryo cryopreservation is regarded as the only established method for fertility preservation in female cancer patients; however, concerns about IVF/embryo freezing include: (1) possible delay in treatment of the primary disease due to ovarian stimulation in relation to onset of the next menstrual cycle (2) exposure to supraphysiologic estrogen levels induced by ovarian stimulation (3) the need for a male partner or donor sperm for embryo production (4) legal, ethical, religious issues related to embryo cryopreservation in general.IVM avoids treatment delay or exposure to increased estradiol levels associated with IVF and, combined with embryo or oocyte vitrification, provides previously unavailable options, such as immature oocyte collection in the luteal phase for some patients, and improves the services provided by a fertility preservation program. Primary-care physicians and oncologists should be made aware of the available fertility preservation options in order to allow referral of their patients, if desired, to an ART center that offers the full range of fertility preservation options. We have preserved fertility for over 200 women with various medical conditions. In a clinical trial of IVM and oocyte vitrification, we achieved a live-birth rate of 20% and the birth of the first four healthy babies.

◆Biosketch

Dr. Tan is Professor and James Edmund Dodds Chair in ObGyn at McGill University, internationally renowned infertility expert and a pioneer of IVM and oocyte vitrification. He is founding director of McGill Reproductive Center, founding President of GCARM and ISIVM, founding Treasurer of ISIVF and Medical Director of the Montreal Reproductive Centre. He led the team that produced the first air transport IVF/ICSI pregnancies in the world, first IVM twin birth, first IVM/PGD birth, first 4 IVM/oocyte vitrification (OV)

births and first serial OV and PGD birth. His many awards include the Howard Eddey, MRCOG, Benjamin Henry Sheares and RCOG Singapore Lecture Gold Medals, Resolve Award, John Collins Lectureship, CFAS/ESHRE Exchange Speaker Award, Montreal Arts and Trade Medal and the College of ObGyn Gold Medallion lectureship. He has published 12 books, 248 original articles in many major journals, including Nature, The Lancet and NEJM, and 92 book chapters and reviews.

CONCURRENT SYMPOSIA

Androgenic spectrum concept of PCO morphogenesis

Takahide Mori
Academia for Repro-regenerative Medicine,
Daigo Watanabe Clinic, Kyoto, Japan

Polycystic ovarian (PCO) morphology is commonly observed both in women with polycystic ovary syndrome and with regular menstruation. Hormonal regulation for morphogenesis has not yet been scrutinized. In 1991, we reported that PCO syndrome (PCOS) could be divided into three discrete types based on pituitary-ovarian androgenicity; classical Stein-Leventhal syndrome, hyper-LH with hyper-androgemia and simple hyper-LH. The world-widely accepted classification of Rotterdam consensus on diagnostic criteria seems ignored androgen-producing potency by theca cells under LH control. Therefore, we examined In recognition of complementary interaction of androgen-induced expression of FSH receptor on granulose cells with FSH-augmented expression of the androgen receptor on granulosa cells in early antral follicles until selection of the dominant follicle, a possible correlation of antral follicle count (AFC) and pituitary-ovarian androgenic function was investigated in 180 infertile women during 3-5 days of the menstrual cycle under the understanding that PCO morphology is composed of a mixture of healthy and atretic antral follicles. AFC, taken as an indicator of PCO morphogenesis, was compared with clinical signs of androgen excess and laboratory measures of baseline LH, LH/FSH ratio, and total testosterone concentrations as parameters for pituitary-ovarian androgenic function. Six discrete types with decreasing pituitary-ovarian androgenic activity were identified in parallel to the declining number of AFC: Type I (classical Stein-Leventhal syndrome), Type II (hyperandrogenism), Type III (singular hyper-LH), Type IV (cryptic hyperandrogenism), Type V (relative LH dominancy) and Type VI (relative FSH dominancy). Since AFC reflects the basal number of antral follicles generated, these six different types with each characteristic androgenic pattern of the pituitary ovarian androgenic activity is thought to represent dynamic wave of PCO morphogenesis under the regulation of enhancing androgenic activity of the pituitary-ovarian axis. Considering that antral follicles over 3 to 5 days of the menstrual cycle consist of newly emerged selectable healthy follicles plus atretic antral follicles that are left unovulated from previous cycles, we proposed the hypothesis that the six identified types of PCO with increasing androgenic function represent the folliculogenetic spectra along which PCO morphogenesis may proceeds in normally cycling as well as PCOS patients, though additional factor(s) may be involved in the latter.

◆Biosketch

Education: MD degree from Kyoto Univ in '60, PhD degree in '68.
Research carrier: Follicular steroidogenesis in Miami, and reproductive immunology at Population Council.
Academic carrier: Chairman of OB/GYN Dept Univ of Tokushima in '81. He organized WHO Workshop on Reference Bank Sera during the term.
Academic interest: Since the first discovery of progesterone suppression on lymphocytes in '77, endocrine-immune interaction in reproduction became his major academic interest with remarkable success.
Academic achievements: A total of 1310 articles including 516 English papers.
IVF: Establishing the first IRB at Univ of Tokushima on IVF into Japan which was highly evaluated as a monumental attempt in the country. He organized 8th World Congress on IVF as President in Kyoto in '93.
Post-retirement activities: After retirement from Kyoto Univ. he continues research and practice at Daigo-Watanabe Clinic, concurrently serving as Director of Academia for Repro-regenerative Medicine.

Do phenotypic differences in PCOS affect fertility treatment and outcomes?

Roger A. Lobo M.D.
Professor Obstetrics and Gynecology
Columbia University, New York, NY, USA
President American Society for Reproductive Medicine

The diagnosis of PCOS has broadened considerably in recent years, with many different phenotypes now being possible, including that of "ovulatory" women. Phenotypic differences vary among regions of the world, with the more severe (or classic) phenotype more commonly seen in the US, and also being the focus of most studies on PCOS. If hyperandrogenism is considered to be a key feature for the diagnosis, then there is evidence that this feature may have a negative effect on fertility even in ovulatory women. This may be manifest in subtle abnormalities in ovarian function as well as in endometrial maturation. Phenotypic variation also may influence the miscarriage rates in PCOS, which have been considered to be increased, yet may only be increased in a subset of the disorder, namely obese women with a more severe phenotype. The Thessaloniki ESHRE/ASRM guidelines for treatment of infertility in PCOS stated that clomiphene is still first line therapy, followed by either ovarian "drilling" or gonadotropin therapy, and then followed by IVF-ET as third line therapy. These guidelines discouraged the use of metformin except for glucose intolerance and the use of aromatase inhibitors. However, depending on the phenotype involved, there is ample accumulating evidence that metformin and letrozole both have a role in treating women with PCOS. There remains controversy as to whether oocytes from women with PCOS are normal or abnormal, although it has been established that IVF pregnancy rates are comparable to that of women with tubal infertility. Here, it appears that phenotypic variability does not affect IVF outcomes except for the influence of increased BMI/obesity being negative. Uses of metformin and antagonist therapy appear to have a role in reducing the risk of OHSS in PCOS. Poorer obstetrical outcomes and a higher neonatal death rate have been reported in women with PCOS, but this is probably related to the more severe phenotypes of PCOS, and is less evident in ovulatory or non hyperandrogenic women.

◆Biosketch

Rogerio A. Lobo, M.D. received his medical degree from Georgetown University Medical School in Washington, D.C., and completed his residency in Obstetrics and Gynecology at the Chicago Lying-in Hospital at the University of Chicago. He went on to complete a clinical research fellowship in the Division of Reproductive Endocrinology and Infertility at the University of Southern California Medical Center in Los Angeles.

He has since held numerous teaching positions, including Assistant and Associate Professor in the Department of Obstetrics and Gynecology at the University of Southern California in Los Angeles, where he was later promoted to full Professor. In 1995 he came to the Columbia University College of Physicians and Surgeons in New York City where he was named the Willard C. Rappleye Professor of Obstetrics and Gynecology, and Chairman of the Department. He was also appointed Director of the Center for Reproductive Sciences at the College of Physicians and Surgeons and Director of the Sloane Hospital for Women, Columbia University Medical Center, one of the most prestigious women's health centers in the country.

In addition to a successful career in academics, Dr. Lobo has excelled in other aspects of professional medicine, serving as the Director of the Reproductive Endocrinology and Infertility Training Program at the

University of Southern California for 11 years. He has provided outside consulting services for many large pharmaceutical laboratories. He has functioned as Editor, Editorial Board member and/or Consultant for over 30 peer-reviewed medical journals in his field, and has authored over 400 articles and 20 books. Dr. Lobo helped found the Journal of the Society for Gynecologic Investigation and served as Editor-in-Chief from its inception until July, 2006. He was President of the Society for the years 1997-1998. He has contributed Chapters to and edited several important medical textbooks. His book **Treatment of the Post-Menopausal Women** is in its Third Edition and published this year. (2007) Another text, **Menopause**, was published in early 2000.

Dr. Lobo has done extensive research in various areas of reproductive endocrinology and infertility. His primary research interests are in reproductive endocrinology, specifically in hyperandrogenic disorders and polycystic ovary syndrome. He also carried out extensive research in gamete biology, induction of ovulation, IVF, and estrogen metabolism and the treatment of postmenopausal women. He has excelled in his areas of clinical interest and practice which include reproductive endocrinology, infertility, and menopause. In 1910 he became President of the American Society For Reproductive Medicine.

Laser assisted ICSI for oocytes matured in vitro from PCO patients

Muchsin Jaffar, Yuslam. E Fidianto, Hadi Sjarbaini,
Soegiharto Soebiyanto, Dianing Amalia, Malvin Emeraldi
Family Fertility Center, Family Children and Maternity Hospital, Jakarta, Indonesia

INTRODUCTION: ICSI is a microinvasive procedure that is often used in In Vitro Maturation (IVM) treatment to handle zona changes of in vitro matured oocytes that have resulted from long term culture. In ICSI procedure, penetration of the injection pipette into the oocytes can sometime very difficult when there is resistance of the zona pellucida and/or the fragility of the oolemma. Application of high pressure in this situation can damage the oocytes. The high degeneration rate may result in losing the cycle, especially when very few oocytes are available because there are no embryos available for transfer. In order to avoid such deformations and to minimize degeneration of the oocytes, in this study spermatozoa was injected into the oocyte through a microhole on the zona pellucida drilled by laser beam just before ICSI to facilitate penetration of the injection needle into ooplasm.

MATERIAL & METHODS: As many as 389 immature oocytes were collected from 32 PCO patients (36 cycles) from November 2008 to December 2010. All female patients received pretreatment with FSH and hCG priming. The oocytes were cultured in maturation medium for 24 to 48 hours in the 5% CO2 incubator. After the oocytes reached metaphase II, they were grouped according to their morphology at the insemination time into the study group [oocytes with less favourable morphology where laser-assisted (L-ICSI) was used as method of insemination] and the control group [oocytes with more favourable morphology where conventional ICSI (C-ICSI) was used].

RESULTS: A total of 315 MII oocytes were obtained after in vitro maturation for 48 hours. Of this, 299 oocytes were subjected to ICSI [L-ICSI (n=224); C-ICSI (n=75)]. Oocytes treated by L-ICSI survived better [95.1% (213/224) versus 85.3% (64/75), P=0.005] and tended to give higher fertilization rate [61.2% (137/224) versus 50.7% (38/75), P=0.071] but lower number of good embryos [2-4 cells on the second day of development, fragment <10%; 62.8% (86/137) versus 68.4% (26/38), P=0.179] compared to oocytes treated by conventional ICSI. Four healthy babies were born from 9 pregnancies achieved after transferring embryos from L-ICSI, C-ICSI or mixed.

CONCLUSIONS: Laser asissted ICSI by drilling microhole on the zona pellucida just before ICSI is a safe, simple and easy to perform to facilitate penetration of the injection needle into ooplasm. Our preliminary results show that laser assisted ICSI could give higher oocyte survival rate in patients who have higher risk for oocyte degeneration such as in IVM .

◆Biosketch

Dr Muchsin Jaffar is the Chief Embryologist and Director of Family Fertility Center, Jakarta Indonesia. He has been trained at some prominent IVF centers in Europe such as: Academisch Ziekenhuis Dijkzigt, Rotterdam, The Netherlands in 1987; Academisch Ziekenhuis-Vrije Universiteit Brussel, Belgium in 1992 and at National University Hospital, Singapore in 1994. He is one of the pioneer of IVF in Indonesia with over 20 years experience in the area of Infertility and IVF practice. He is involved in the developments of many IVF centers in Indonesia. His work has been significant for the advancement and growth of the field of Assisted`Reproductive Technology in Indonesia. Dr Muchsin Jaffar is a founder member and has served on a number of senior comittees of the Indonesian Society of In Vitro Fertilization. His current interests are in IVF, IVM, ICSI, PGD as well as in Male Factor Infertility.

The use of GnRH antagonist in the controlled ovarian stimulation for IVF in PCOS patients

Khaled Mahmoud
Centre of Reproductive Medicine and Prenatal Diagnosis, Tunis-Tunisia

Polycystic Ovarian Syndrome (PCOS) is a common endocrinopathy that affects 5 to 10% of Women of reproductive age. Regarding diagnosis, a consensus was reached on the criteria that identify and define the syndrome (The Rotterdam PCOS consensus workshop sponsored ESHRE/ASRM- group-2004), but as regards the treatment of infertility caused by this syndrome, the debate remains wide open despite the 2008 publication of the work of Thessaloniki ESHRE / ASRM-sponsored PCOS Consensus Workshop Group.

Ovarian stimulation of PCOS patients entering in IVF program is difficult to control because we should take into account not only of the quantitative aspect with a production of an excessive number of oocytes and the major risk of OHSS but also of the qualitative aspect to having a good quality of oocytes that would give good embryos.

It was reported that, compared with GnRH agonists, GnRH antagonists that have been introduced recently in the controlled ovarian stimulation, offer several advantages that could secure these women with PCOS specially as in term of live birth rate the 2 products are identical (kolibiannakis, 2006) The main advantages are: (i) Requirements for exogenous gonadotropins are reduced, rendering ovarian stimulation less costly. (ii) Duration of ovarian stimulation protocols is shortened, improving patient discomfort. (iii) The incidence of OHSS is lower.

In our center we conducted a randomized controlled trial from January 2004 to June 2009 included 420 PCOS patients who came for treatment with IVF / ICSI. All patients received oral contraceptive pill (Diane35) starting on day 2 or 3 of the cycle prior to ovarian stimulated cycle for IVF/ICSI either by a long GnRH agonist down regulation protocol (n=210, agonist group) or by a flexible GnRH antagonist protocol (n=210, antagonist group).

The starting dose of rFSH (GonalF) was 150IU per day for all patients in both group, this dose was adjusted after 4 or 5 days of stimulation depending on the ovarian response.

The triggering of final maturation of oocytes was made according to usual criteria by rHCG (Ovitrelle 6500 IU) or by 0.2 mg of Decapeptyl in case of hyper-response with E2 greater than 3500 pg / ml predicting women at risk for ovarian hyper stimulation syndrome.

Both groups of patients had similar baseline characteristics regarding their age, hormonal profile and duration of infertility.

The analysis of our results found that the COS with rFSH and antagonist in PCOS patients is as efficacy as rFSH and agonist and also more safe: less duration of stimulation, less consumption of gonadotrophins and less of risk of OHSS especially if the triggering of ovulation is made by GnRH agonist rather than by HCG.

◆Biosketch

Name: Khaled MAHMOUD
Date of birth: December, 10, 1954
Place of birth: Tunisia
Married and Father of 2 girls.
Scientific Degrees:
1985: Diploma in Obstetrics and Gynaecology University of Tunis
1985-1987: Diploma in the ART University of Marseille, France

Training and ART practice at the Institute of Medicine of the Reproduction in Marseille France

Current position:

Founder and Head of the first Centre of IVF in Tunisia since April 1988

General Secretary of the Mediterranean Society of the Reproductive Medicine

Vice president of the Tunisian Society of Gynaecology and Obstetrics

Awards and distinction:

Medal of Merit as a pioneer of ART in Africa offered by the Federation of North African societies of infertility. Casablanca, Morocco decembre1997

Award of the best oral communication in the 4th MSRM congress

Member of the National Commission - within the Ministry for the Public health for the development and the study of the Assisted Reproductive Techniques (ART) in Tunisia.

Active participant in the preparation and writing of the law governing the ART at the Health Ministry in Tunisia

Former Congress President of the 7th annual Meeting of Mediterranean Society for Reproductive Medicine May 2008 Hammamet, Tunisia

Area of Special Interest and Accomplishments

My special areas of interest in Gynaecology and Obstetrics include infertility, both classical and endoscopic surgery and high-risk pregnancy.

In the field of infertility I had the first babies born in Tunisia after GIFT (1989), ZIFT (1989), Embryo reduction of Multiple IVF pregnancy (1992) and after ICSI (1996).

My works published or presented during the different meetings focused on ovarian stimulation, quality of gametes and embryos, hysteroscopy and laparoscopy, male infertility, embryo implantation, genomics and metabolic

Polycystic ovary syndrome is associated with metabolic abnormalities in the purine mononucleotide pathway and tricarboxylic acid cycle

Makio Shozu[1], Guiwne Wang[1], Tomoya Segawa[2], Shokichi Teramoto[2], Hiroshi Ishikawa[1]
[1]Department of Reproductive Medicine, Graduate School of Medicine, Chiba University, [2]Shimbashi Yume Clinic

Introduction: Obesity associated with insulin resistance is a characteristic metabolic abnormality of polycystic ovary syndrome (PCOS), but is not the only feature of the metabolic status of such patients. To investigate alternative therapeutic targets for PCOS, we analyzed the metabolomic profiles of PCOS patients.
Materials and methods: Plasma was obtained from women with PCOS (n = 5) presenting with obesity and insulin resistance and women (n = 5) with regular menstruation presenting with simple obesity (BMI < 27). Metabolites in plasma samples were analyzed using capillary electrophoresis followed by time-of-flight mass spectrometry. Each metabolite was identified based on the measured variables and migration time using the Kyoto Encyclopedia of Genes and Genomes database. Differences were considered significant when $p < 0.05$.
Results: The most striking difference was found in the levels of purine mononucleotides. There were increased levels of ATP, ADP, AMP, GDP, and IMP (the key intermediates of purine synthesis) in the PCOS samples. In cells, levels of purine nucleotides are determined by equilibrium along the de novo synthesis pathway, recycling pathway (salvage pathway), and catabolic pathway. In PCOS, the level of hypoxanthine, a key intermediate of both the salvage and catabolic pathways, was not different from that of the control subjects, suggesting that enhancement of de novo synthesis is responsible for the increase. In contrast to the purine nucleotide levels, the level of pyrimidine mononucleotides was unchanged. The next striking difference was the levels of intermediates of the tricarboxylic acid (TCA) cycle. Succinic acid, pyruvic acid, oxoglutaric acid, malic acid, and isocitric acid, but not citric acid, were increased in PCOS compared to the control subjects.
Conclusion: PCOS is obviously associated with systemic changes in purine and TCA cycle metabolites, which would not be a consequence of co-existing obesity. These metabolic abnormalities might be a new therapeutic target of PCOS.

◆Biosketch

1981: Graduated Kanazawa University, School of Medicine
1982-1986: Graduated Ph.D. program of Postgraduate School, School of Medicine, Kanazawa University
1986-1989: Department of Obstetrics and Gynecology, Fukui Prefecture Hospital
1990-1999: Lecture, allied Health Science School, Kanazawa University
1995-1998: Senior fellow, Cecil and Ida Green Center for Reproductive Medicine, University of Texas at Dallas (Professor Evan Simpson).
1999-2005: Associated Professor, Center for Fetal and Maternal Medicine, Kanazawa University
Dec. 2005-present: Professor and Chairman, Reproductive Medicine, Chiba University.

How to get pregnancy in women with PCOS

Chii-Ruey Tzeng
Taipei Medical University

Polycystic ovary syndrome (PCOS) is an extremely common disorder in women of reproductive age. Diagnosis of PCOS is principally based on clinical and physical findings. Diagnostic criteria and PCOS definitions used by clinicians and researchers are almost as heterogeneous as the syndrome. Of those diagnosed with PCOS using the 2003 Rotterdam criteria, 61% fulfilled 1990 NIH criteria for unexplained hyperandrogenic chronic anovulation. The patient populations with the new phenotypes had less severe ovulatory dysfunction and less androgen excess than patients diagnosed using the 1990 NIH criteria. These findings might be common across all female populations with PCOS, whether in Oriental or Occidental countries. Data for clinical hyperandrogenism indicated that the prevalence of hirsutism in Taiwanese PCOS women is lower than that for Caucasians/Western women.The extent of metabolic abnormalities in women with PCOS may vary with phenotype, age and ethnicity. Obesity represents a major risk factor for metabolic syndrome and insulin resistance. Approximately 40-50% of all women with PCOS are overweight or obese. Obese subjects with PCOS had a higher risk of developing oligomenorrhea, amenorrhea and biochemical hyperandrogenemia than non-obese women with PCOS. Moreover, obese women with PCOS had significantly more severe insulin resistance, lower serum LH levels, and lower LH-to-FSH ratios than non-obese women with PCOS. PCOS women in Taiwan presented with higher LH-to-FSH ratio and lower insulin resistance than PCOS women in Western Countries. However, the average body mass index (BMI) was significantly lower in Taiwanese PCOS women than Western women, which might partially explain the difference between these two populations in terms of clinical and biochemical presentations.The treatment of infertile women with PCOS is surrounded by many controversies. Recently consensus meeting of ESHRE/ASRM are recommended as following:1.First-line treatment for ovulation induction remains the anti-estrogen clomiphene citrate (CC).2.Second-line intervention is either exogenous gonadotropins or laparoscopic ovarian surgery (LOS).3.Third-line treatment is in vitro fertilization (IVF).(The Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome, Fertil Steril 2008)Retrospectively reviewed our clinical data, women with PCOS had excellent pregnancy outcome in Reproductive Medicine center of Taipei Medical University Hospital.

◆Biosketch

Professor Tzeng is currently Dean of the College of Medicine at Taipei Medical University since 2004, and Chairman of Ob/Gyn at TMU since 1992. He graduated from the School of Medicine at TMU in 1976 and obtained a MPH degree from Harvard School of Public Health from 1980-1981. He completed his fellowship training in Reproductive Endocrinology and Fertility from 1981-1983 in the Department of Ob/Gyn at Brigham & Women's Hospital, Harvard Medical School. He has undertaken two projects; the first test-tube baby in 1985 and the first mitochondria transfer in 2002. He and his group have received the Prize Poster Award at the annual meeting of ESHRE in 2001, 2003 and 2007 respectively. He received 2009 Gold Medal for the invention of diagnosis method of endometriosis by detecting biochemical markers and the STAR Award from ASRM in 2010. He served as President of the TSRM from 1996-1998, and PRSFS since 2008. He was on the board member of the International Ovarian Conference and ISFP.

New criteria of diagnosis and treatment in polycystic ovary syndrome (PCOS) in Japan

Toshiro Kubota
Comprehensive Reproductive Medicine, Graduate School,
Tokyo Medical and Dental University

Polycystic ovary syndrome (PCOS) which is the most frequent endocrine disorder in women of reproductive age could present a variety of clinical pictures. In Japan, compared to other countries, the usual clinical presentation of PCOS is slightly different - with less frequently encountered cases of hyperandrogenism - therefore established European or US guidelines are clinically less useful. In 2006 the Japanese Society of Obstetrics and Gynecology (JSOG) has proposed new, revised diagnostic criteria that in the future could also be valued internationally. Diagnostic criteria are based on three main features: 1) cycle irregularities; 2) polycystic changes in the ovary by ultrasonography; 3) endocrine anomalies (LH or androgen hypersecretion). Based on the above-mentioned diagnostic criteria in 2008, the JSOG has also proposed revised treatment guidelines summarized as follows:

1. PCOS patients wishing to have children: if obese (BM≥25 kg/m2), as a first option, weight loss and exercise is recommended. Non-obese patients or those obese women who do not ovulate after lifestyle changes are submitted to ovulation induction therapy with clomiphene citrate. Obese clomiphene-resistant patients who have impaired glucose tolerance or insulin resistance are treated with a combination of metformin and clomiphene citrate. If the previously mentioned treatments options are unsuccessful, ovulation induction with exogenous gonadotropin therapy or laparoscopic ovarian drilling (LOD) is recommended. During ovulation induction a chronic, low-dose, step-down regimen is recommended with careful monitoring in order to reduce the risk of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies. Alternatively with LOD high success pregnancy rates of around 60% are expected with a low risk of multiple pregnancies. If ovulation induction is unsuccessful, *in vitro* fertilization (IVF) treatment is indicated. During ovarian stimulation for IVF, FSH-only preparations are used. In high OHSS-risk patients, systematic embryo freezing and subsequent frozen embryo transfer cycles are recommended.
2. PCOS patients not desiring for children: in non-obese, anovulatory patients, pharmacological treatments such as Holmström, Kaufmann regimens or low-dose oral anticonceptives are used to induce regular withdrawal bleedings which are especially important for preventing endometrial hyperplasia and endometrial cancer.
These new diagnostic and treatment guidelines hopefully will contribute to an improved care of PCOS patients in Japan.

◆Biosketch

1975	Graduated from Tokyo Medical and Dental University, Faculty of Medicine
1982-1991	Tokyo Medical & Dental University, Research Assistant
1988-1989	University of Virginia(U.S.A.), Research Fellow
1991-1996	Tokyo Medical & Dental University, Lecturer
1996-2006	Tokyo Medical & Dental University, Associate Professor
2006-	Tokyo Medical & Dental University, Professor

PCO and transvaginal hydrolaparoscopic ovarian drilling

Hiroaki Shibahara, M.D., Ph.D.
Department of Obstetrics and Gynecology, School of Medicine,
Jichi Medical University, Tochigi, Japan

Polycystic ovary syndrome (PCOS) is a syndrome of ovarian dysfunction showing cardinal features of hyperandrogenism and polycystic ovarian morphology. It is one of the most common reproductive endocrine disorders in young adult women, showing clinical signs of menstrual disorder, anovulation, hirsutism, acne, and obesity. Frequently, this group of patients present with infertility due to chronic oligoovulation or anovulation. In cases that do not respond to medical induction of ovulation, ovarian surgery such as laparoscopic ovarian drilling (LOD) by the trans-abdominal approach has been widely used to induce ovulation in PCOS women after failure of treatment with clomiphene citrate. So far, many authors have reported high rates of ovulation (~ 80%) and pregnancy (~ 60%) following LOD.

In 1998, transvaginal hydrolaparoscopy (THL) was introduced as the first line procedure in the exploration of the adnexal structures in infertile women[1)]. Because of the advantages of THL, including accurate inspection of adnexal structures without manipulation, it became clear that THL is a less traumatic and more suitable outpatient procedure than diagnostic laparoscopy. The risks of a general anesthetic are avoided, and there is less chance of trauma to major vessels[2)]. Therefore, we have been performing THL for a diagnostic laparoscopy on infertile women based on any of the following four indications[3-6)]: i) tubal obstruction and/or peritubal adhesion is suggested by hysterosalpingography, ii) serum antibody against C. trachomatis is positive, iii) diagnosis of early-stage endometriosis, and iv) unexplained infertility. Our group also reported the application and usefulness of THL for transvaginal salpingoscopy[7)].

Although THL was initially developed as a method for diagnostic laparoscopy, THL has more recently been performed for operative laparoscopy, especially for ovarian drilling in women with PCOS8). This novel technique, transvaginal hydrolaparoscopic ovarian drilling (THLOD) using a laser or bipolar electrosurgery, appears to be an effective minimally invasive procedure to induce ovulation in women with PCOS9-13). In a report, the cumulative pregnancy rate was 60 % for spontaneous and stimulated cycles, with 40% imputed to drilling alone. It was also reported that there were no complications.

The usefulness of diagnostic and therapeutic THL for the purpose of infertility investigation and treatment will be presented.

◆Biosketch

Hiroaki Shibahara, M.D., Ph.D.
Professor, Department of Obstetrics and Gynecology, School of Medicine, Jichi Medical University, and Center for Reproductive Medicine, Jichi Medical University Hospital, 3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan

Education :

Graduate:	1984	Kochi Medical School (Nangoku, Kochi, Japan)
Medical Doctor:	1984	
Ph.D.:	1994	Department of Obstetrics and Gynecology, Hyogo College of Medicine (Nishinomiya, Hyogo, Japan)

Awards:

1995: Hyogo Prefectural Society of Obstetrics and Gynecology
1998: Japan Society of Fertilization and Implantation
2003: Japan Society of Fertility and Sterility

Roles in the Medical Societies:

International

1) Councilor,
American Society of Reproductive Immunology

Domestic

1) Chair of the annual meeting
2007: the 5th annual meeting of the Japan Association of Psychological Counseling for Reproductive Medicine
2011: the 6th annual meeting of the Japan Society for Reproductive Regeneration

2) Exective Board Members
Japan Society of Fertilization and Implantation
Japan Society of Mammalian Ova Research
Japan Association of Psychological Counseling for Reproductive Medicine
Japan Society for Reproductive Regeneration

3) Board Members
Japan Society of Gynecologic and Obstetric Endoscopy
Japan Society for Reproductive Immunology
Japan Society of Andrology

4) Ethics Committee
Japan Society for Reproductive Medicine

5) Editorial Board:
Reproductive Medicine and Biology (Editor-in-associate chief, Reviewing editor)
Current Women's Health Reviews
Open Women Health Journal

Available tools to personalize ovarian stimulation-Dose Prediction/Protocol selection

Bruno Lunenfeld
Faculty of Life Sciences, Bar-Ilan University, ISRAEL

The response to ovarian stimulation is a key element and a major determinant in the outcome of ART procedures. The response will depend on two variables: the dose of stimulant administered and the protocol used and, the patient's response, depending on previous response, biological age (ovarian reserve), as well as genetic variants, like the FSH and LH receptor and mutations of FSH and LH by themselves. Age, antral follicle count and anti-Müllerian hormone seem to be the best indicators to predict initial dose and protocol. Inhibin on day 3 of treatment will permit protocol assessment and dose correction. Oestrogen and follicle count and size on day 6 will permit protocol adjustment adding LH, increasing or decreasing the amount of FSH. Follicle count and oestradiol levels on the last day of treatment will help to avoid hyperstimulation by permitting the choice of agent to be used for ovulation induction, hCG, LH or GnRH agonist, or freezing of the embryos

◆Biosketch

Bruno Lunenfeld, MD, PhD, FRCOG, FACOG (hons), POGS (hons)
Born in Vienna 1927, married +2

Bruno Lunenfeld is Professor Emeritus at the Faculty of Life Sciences, Bar-Ilan University, and Ramat Gan, Israel. He is also President of the International Society for the Study of the Aging Male, General Secretary of The Asian-Pacific Initiative on Reproductive Endocrinology, Treasurer of the International Society of Gynaecological Endocrinology and Editor of *The Aging Male*.

He graduated from the medical School in Geneva Switzerland. Following his post graduate work in Geneva he joined the Weitzman Institute as senior Scientist, and then headed the WHO International Reference Center for Fertility Promoting Drugs and acted as a Consultant and member of Expert Committees at the World Health Organization.

Professor Lunenfeld is best known for his pioneering work in human reproduction. After describing the clinical use of hMG in men and women in 1960, his group was the first to achieve a pregnancy with hMG in 1961, demonstrated binding of hCG to LH receptors in theca cells in 1967 and were the first to induce ovulation followed by pregnancy with GnRH in an hMG-stimulated cycle in 1975. His research interests pertain to the physiology and pathology of male and female reproduction and include stimulators, modulators and regulators of sex steroids, spermatogenesis, spermiogenesis, folliculogenesis and ovulation, and the mechanism of action of gonadotrophins, GnRH and growth factors on gene expression and steroidogenesis.

He was **instrumental** in the study of the aging male, was the founder of the International Society for the study of the aging male (ISSAM. He **organized** in collaboration with the World Health Organization the first, second and third world Congress of the aging male in Geneva.

He published more than 400 papers 19 books including a textbook on Male and Female Infertility and one the "first textbooks on Men's Health. He gave more than 700 invited lectures and chaired or co-chaired more

than 100 sessions at national/international Meetings. He trained more than 200 local and foreign physicians and supervised more than 50 MD, MSC, and Ph.D. Students

Professor Lunenfeld has received several distinguished international awards for his scientific achievements, including the special recognition award of the United States Public Health Service for his outstanding contribution in the promotion of health from the Surgeon General (1983), the Verdienstkreuz 1 Klasse – one of Germany's highest honours – signed by the President of Germany, Roman Herzog (1995), the Bertarelli Foundation Award for his lifetime achievements in women's health (2002) and the World Fertility Awareness Month Lifetime Achievement Award (2005).

Professor Lunenfeld is honorary member of European Society of Human Reproduction and Embryology (ESHRE), the International Federation of Fertility Societies (IFFS, German, Polish and Italian Society of Gynecology and Obstetrics, German Endocrine Society of Reproduction, Austrian Society of Fertility and Sterility, and the Asian Society of Andrology

Advances in ultrasound monitoring of IVF cycles by automated volume measurement

Baris Ata[1,3], Ayse Seyhan[1], Hai-Ying Chen[2], Alper Mumcu[2], Seang Lin Tan[1,2]
[1]Dept. Of Obstetrics and Gynecology, McGill University, Montreal, Canada
[2]Montreal Reproductive Centre, Montreal Canada
[3]Dept. of Obstetrics and Gynecology, Uludag University, Bursa, Turkey

Accurate assessment of follicular size is crucial for good IVF treatment outcome. A major problem hitherto has been large inter- and intra-observer variation in follicular measurements with conventional 2 dimensional (2D) ultrasound (US). Sonography-based Automated Volume Count (SonoAVC) is a new three-dimensional (3D) US technology, which automatically identifies and quantitatively analyses hypoechoic structures within a volume of interest. It generates a set of measurements including the mean diameter (MFD) and a volume-based diameter (d (V)) for each hypoechoic structure in the scanned volume. Although SonoAVC was designed for follicle measurement in the IVF setting, it has also been used to conduct volumetric measurements of embryonic or fetal structures.

Several studies have evaluated the accuracy of SonoAVC measurements and compared with actual follicular volume or other validated methods of US volume measurements under a variety of conditions. In the IVF setting, SonoAVC follicular measurement results are in very good agreement with conventional real time 2D follicle measurements, actual follicular volume, and follicular measurements by VOCAL. SonoAVC results maintain accuracy across different follicular sizes above 10mm. We have reported that the mean difference between the size of the leading follicle on hCG day as measured by SonoAVC or conventional 2D US is less than 1mm. Using conventional hCG criteria for oocyte collection, exclusive use of SonoAVC for follicle tracking does not improve or worsen clinical outcome as shown in a randomized controlled trial. The technique seems to provide reliable and highly reproducible results. Automated measurements take less time than manual measurements. Time savings can increase up to 5 minutes per patient. Most importantly, SonoAVC measurements are highly reproducible. We have reported intracluster correlation coefficients exceeding 0.8 for 4 different operators analyzing same volumes with SonoAVC. These results suggest very low inter-observer variability. This is especially important for busy IVF programs where a patient can be scanned by different sonographers during the course of one IVF cycle. SonoAVC can facilitate remote monitoring of women undergoing ovarian stimulation.

During early pregnancy scans, such as viability scans, SonoAVC allows accurate volume measurements of irregular structures such as the embryo. It has been used to measure embryonic volume which can be a better measure of embryonic growth than traditional crown rump length measurements. It has also been used to assess fetal heart and cardiac blood flow, volumes of cerebral ventricles. Again, SonoAVC yields accurate results in shorter time compared other validated methods such as VOCAL, and makes it possible to introduce these measurements into clinical practice. This new technology is expected to play a more important role in the future of our practice.

◆Biosketch

Dr.Baris ATA,
Age 37
M.D. Istanbul University **1998**
Residency Ob&Gyn Istanbul **2003**

Assisted Reproductive Technologies certification with Prof. Bulent Urman at the American Hospital of Istanbul **2006**
Attending Physician at the American Hospital of Istanbul IVF Unit **2006 – 2008**
M.Sc. in Clinical Trials from the London School of Hygiene and Tropical Medicine, **2007 - 2010**
REI Clinical Fellowship at McGill University, Montreal **2008 – 2010**
REI Research Fellowship at McGill University, Montreal **2010 – 2011**
Director of the Assisted Reproduction Unit of Uludag University, Bursa, Turkey **Jan 2011 – ongoing**
References: Prof. Seang Lin Tan and Prof. Victor Gomel
Authored – co-authored 35 papers indexed by SCI and 18 book chapters.

Volumetric measurements of the follicular development

Maximilian Murtinger
IVF Center Prof. Zech – Bregenz

Follicle tracking is commonly employed to assess the response to ovarian stimulation. In the majority of cases two dimensional transvaginal ultrasound (2D) is performed to monitor follicle growth and to determine the optimal time for administering human chorionic gonatotrophin (hCG) to trigger oocyte maturation. The presence of numerous follicles of different sizes and shapes makes such an assessment much harder as the observer has to identify each and every follicle individually and measure it only once. The reliability and validity of such measurements are likely to reduce as the number of follicles increase. 2D only provides an approximation of the real volume of follicles and therefore cannot be used to guarantee standards for follicle tracking.
This presentation will give an overview for the use of three dimensional ultrasound (3D) techniques combined with automated measurement of follicular size which might provide an objective, fast, valid and reliable standard for such measurements in reproductive medicine.

◆Biosketch

Dr. Maximilian Murtinger received his MD at the University of Vienna in 1986. After specialization as a trauma surgeon in 2003, he changed to the field of reproductive medicine and endocrinology and finished his specialty in OB/GYN. since 2010.His current interest is in ultrasound techniques in reproductive medicine, especially related to follicular measurements in stimulated cycles and in establishing clinical standards related to the quality criteria set by the European Union directive on tissues and cells (2004/23/EC). In addition, Dr. Murtinger is developing requirements for software used in obstetrics and gynecology, especially for reproductive medicine and endocrinology. He is Deputy Medical Director at the IVF Center Prof. Zech – Bregenz.

Ovarian stimulation and endometrial receptivity

Pak Chung Ho
Department of Obstetrics and Gynaecology, The University of Hong Kong

Introduction: Ovarian stimulation is now used routinely in most in-vitro fertilization (IVF) programs. There was controversy as to whether excessive stimulation and high oestradiol (E2) level would be associated with a reduction in the endometrial receptivity and implantation rate of the embryos in IVF treatment. In a retrospective analysis, we have shown that the embryo implantation rates and clinical pregnancy rates were significantly reduced in IVF treatment cycles only when the serum E2 level was more than 20,000 pmol/l. Previous differences in results may be due to the use of different levels of serum E2 to define excessive responders. Therefore, we conducted a series of studies on the endometrium of women undergoing ovarian stimulation.

Materials and methods: We conducted a series of studies on the morphometry, blood flow and gene expression in the endometrium of women undergoing ovarian stimulation for assisted reproduction as well as normal women in natural cycles. We also studied the effects of oestrogens on the attachment of trophoblastic spheroids (from choriocarcinoma cell lines) to the endometrial cancer cell lines.

Results: In a morphometric study of the glands and stroma in endometrial biopsies of women in whom embryo transfer could not be performed for a variety of reasons, we found that the glands and stroma in moderate responders were in phase as in women in unstimulated ovulatory cycles. In excessive responders, there was evidence of gland-stroma dys-synchrony. Doppler studies of the endometrial blood flow also showed that there was a significantly higher percentage of excessive responders with no endometrial color signals in the endometrium. Our results on the endometrial gene expression patterns in natural cycles, moderate responders and excessive responders showed that there was a significant difference (more than 2 fold) in the expression patterns of 411 genes among the 3 groups. A subsequent study showed that the gene expression pattern of the excessive responders on day LH+7 was more similar to that of day LH+10 in natural cycles, indicating that there was advancement in the endometrial development in excessive responders. Using an in-vitro spheroid-endometrium attachment model, we also demonstrated that the attachment of spheroids to endometrial cancer cell lines was adversely affected by high levels of oestrogen.

Conclusion: In conclusion our series of studies showed that excessive response to ovarian stimulation will affect the morphological development, the blood flow, gene expression patterns and receptivity of the endometrium. There is a need to avoid excessive ovarian stimulation in IVF programs.

◆Biosketch

Professor P.C. Ho is the Chair Professor of Obstetrics and Gynaecology, Director of Centre of Reproduction, Development and Growth, and Associate Dean in Clinical Affairs in Faculty of Medicine, University of Hong Kong. He is in charge of the assisted reproduction program at the University of Hong Kong/Queen Mary Hospital Centre of Assisted Reproduction and Embryology. His research interests include medical abortion, emergency contraception, infertility and assisted reproduction. He has published more than 350 papers in peer-reviewed journals. He is serving on many national and international professional bodies. He is currently the President of Asia-Oceania Federation of Obstetrics and Gynaecology, and President of Family Planning Association of Hong Kong.

Minimal ovarian stimulation (mini-IVF) for IVF utilizing vitrification and cryopreserved embryo transfer

John Zhang[1], Lyndon Chang[1], Yoshie Sone-Endo[1], Sherman Silber[1,2]
[1]New Hope Fertility Center, New York, U.S.A.,
[2]Infertility Center of St. Louis at Luke's Hospital, Missouri, U.S.A.

Gentle ovarian stimulation protocols, such as mini-IVF, have several potential advantages over conventional IVF protocols, including less medication and fewer injections, producing fewer eggs, but eggs of higher quality. The particular mild stimulation protocol called mini-IVF is described. This protocol requires a reliable and cheap method for embryo cryopreservation such as vitrification, because of the negative impact of clomiphene citrate on the endometrium and since cryopreserved embryo transfers with this protocol have yielded much higher pregnancy rates than fresh transfers. In this series, patients were not denied treatment based on their day-3 FSH value or ovarian reserve. Yet very acceptable pregnancy rates were achieved (20% for fresh embryo transfers and 41% for cryopreserved embryo transfers). These results strengthen the argument for a mini-IVF protocol and vitrification as an alternative to standard conventional IVF stimulation protocols. Now a randomized control trial with cryopreserved single-embryo transfer is required.

◆Biosketch

Dr John Zhang graduated in 1984 from Zhejiang University School of Medicine in China. He was awarded his PhD from Cambridge University for his IVF studies in 1991. In addition, he also has a Masters degree in male infertility from Birmingham University, UK. He is a reproductive endocrinology and infertility specialist whose academic training includes residency training in obstetrics and gynecology and a fellowship in Reproductive Endocrinology and Infertility in 2004 from New York University School of Medicine. Dr Zhang is the director and founder of New Hope Fertility Center, an independent IVF facility in New York.

Poor responder patients undergoing IVF treatment

Svend Lindenberg Professor Dr.Med.
Copenhagen Fertility Center, Copenhagen

The definition of poor responder is still being debated although ESHRE 2011 has added much better clarification to this issue.
The presentation will use evidence based methodology to elucidate several aspects of the possible medical target points for poor responder patients. However very little new information has been generated during the last 10 years and very little has added to the treatment modalities although several new drugs have been introduced on the marked.
Specifically we present data on Growth Hormone and luteal phase modulation providing hope for better and more efficient treatment for these patients.
However the general conclusion is that we still need a better understanding of the pathophysiology in poor responder patients to improve treatments.

◆Biosketch

Professor dr. med Svend Lindenberg is a pioneer in IVF and has partcipated in the group of scientist producing the first IVF baby in Denmark in 1982. Since then published more than 150 papers and given numerous presentations in internatinal and national societies and holds several international patents in ART.
Today director for Copenhagen Fertility Center and Research Institution with focus on low stimualtion and IVM.
Copenhagen Fertility Center: Copenhagen, Lygten 2c, DK-240 NV
In 2012 Congres President for the 2012 internationa Meeting in ISMAAR: www.ismaar2012.dk

Prediction and prevention of the ovarian hyperstimulation syndrome (OHSS) - an evidence-based approach

Hassan N. Sallam, MD, FRCOG, PhD (London)
Professor of Obstetrics and Gynaecology, the University of Alexandria, Clinical and Scientific Director of the Alexandria Fertility Center, and Director of the Alexandria Regional Center for Women's Health and Development, Alexandria, Egypt

Ovarian hyperstimulation syndrome (OHSS) is an uncommon but serious iatrogenic complication of ovarian stimulation occurring during the luteal phase or during early pregnancy. It is potentially fatal and is difficult to predict.

Prospective controlled studies have shown that good predictors include young age, the presence of polycystic ovarian syndrome or polycystic ovaries on ultrasound, the measurement of the antral follicular count (AFD), estradiol levels, insulin resistance, ovarian volume or antimullerian hormone (AMH) before starting ovulation induction. Bad predictors include the measurement of body mass index (BMI), genetic predisposition, serum vascular endothelial growth factor (VEGF), von Willebrand factor and perifollicular blood flow.

Similarly, randomized controlled trials (RCTs) have shown that primary prevention (before starting HMG/FSH) can be achieved by giving FSH rather than HMG (without GnRHa), using the step-up rather than the conventional protocol, giving GnRH antagonists rather than agonists (but with a lower LBR), performing IVM rather than IVF and using the sequential rather than the step down protocol. They have also shown that the alternate day protocol is comparable to the conventional protocol and that the sequential protocol is comparable to the step-up protocol, while the superiority of either the step-up compared to step down protocol needs further evaluation.

RCTs have also shown that secondary prevention can be achieved by triggering ovulation with GnRH agonists, metformin administration, intravenous albumin, hydroxyethyl starch, cabergoline (for early OHSS) and laparoscopic ovarian electrocautery. They have also shown that the following approaches are equivocal in preventing OHSS: coasting versus unilateral oocyte aspiration and GnRH antagonists versus coasting, while the following approaches await further evaluation: cancellation of the cycle, coasting, diminishing the dose of HCG, embryo freezing and triggering with GnRHa + embryo freezing.

◆Biosketch

Hassan Nooman Sallam, MB, ChB, DGO, DrChO&G (Alex), FRCOG (England), PhD (London)

Hassan Nooman Sallam is professor and former head of the department of obstetrics and gynaecology in Alexandria University and clinical director of the Alexandria Fertility and Assisted Reproduction Center. He is also the director of the Alexandria Regional Center for Women's Health and Development, a training and research center active in all areas of women's health based in Alexandria, Egypt. He is the former vice-dean and director of research in Alexandria University Faculty of Medicine. In 1998, he served as the overseas examiner to the Royal College of Obstetricians and Gynaecologists in London.

Besides the fellowship of the Royal College of Obstetricians and Gynaecologists (FRCOG), he holds a doctorate degree in Obstetrics and Gynaecology from Alexandria University and a PhD in reproductive biochemistry from the University of London. He has published more than 100 papers in national and

international refereed journals in English and French as well as 3 books. He is the co-author of “Infertility and Assisted Reproduction”, the reference textbook published by Cambridge University Press.

Professor Sallam serves/served on the editorial boards of many journals including "Human Reproduction", "Reproductive Biomedicine Online", "Middle East Fertility Society Journal" and “Facts, Views and Visions in OB/GYN” (Official Journal of the Flemish Society of Obstetrics and Gynecology) and is an *ad hoc* reviewer for 11 other journals. He is a founding board member of the Middle East Fertility Society, based in Beirut, Lebanon and the founding chairman and current president of the Mediterranean Society for Reproductive Medicine, based in Florence, Italy.

What should we prefer for IVF-The Agonist or Antagonist?

Prof Zion Ben-Rafael
Tel Aviv, Israel

The answer seems clear. While the combination of GnRH-Antagonist for IVF is controversial, the use of GnRH-Agonist is widely accepted. Therefore, after two decades of experience and 200 publications one can't escape from the conclusion that for most clinician the Antagonist is a second choice reserved for special cases. Yet this clear cut preference by clinicians is not fully supported by the evidence.

Combining GnRH –analogs (agonists or antagonist) became the standard of care in all IVF treatments, with the Agonist accounting for more than 2/3 of the treatments. GnRH antagonist with it direct –competitive blocking action on the receptors to GnRH in the pituitary was suppose to be better than the old time agonist, which is plugged by several side effects.

The advantages to the use of the antagonist which includes; Short treatment duration, Lower gonadotropins requirement, Lower OHSS rate, Lack of severe hypoestrogenism and its consequences, Fitness to be used in spontaneous cycles. The disadvantages include: A novel approach which is still under evaluation and needs more optimization. If not given early enough, might fail to prevent high endogenous LH and most importantly pregnancy rate per cycle is consistently lower than with the agonist. Most protocols allow minimal programming; luteal phase support is also needed.

Conclusions: The GnRH agonist long luteal phase protocol should be the protocol of choice for all young IVF patients. All other protocols of agonist or antagonist protocols should be reserved for special cases and situations; apparently the best Antagonist protocol is the fixed daily dose protocol which should be preferred over the flexible or one dose protocol. Other Antagonist protocols should be developed to match the analog results. Patients at higher risk for OHSS might enjoy a milder stimulation and lower overall risk with the GnRH-Antagonist.

◆Biosketch

Prof. Zion Ben-Rafael,
Married + 3
Graduated from Tel Aviv University, Board certified in Israel and USA.
84-86, NIH Fellow in Reproductive Biology and Endocrinology, in Hospital of University of Pennsylvania, Philadelphia.
Full Professor (1993), Associate Professor (1990), Tel Aviv University and Incumbent of the Tarnesby- Chair of Fertility regulation Tel Aviv University

Previously:
President, Israeli Menopause Association.
1997-2007 Chairmen, Department of OBGYN, Rabin Med Center.
1991-2007 Chairmen, Department of OBGYN, Golda-Hasharon.
1989-1991 Director, Katowitz Women's Health Center, Tel Aviv
Currently in charge of the IVF unit, Laniado hospital

Founder and Chairman, COGI - World Congress on Controversies in Obstetrics, Gynecology and Infertility. 1999 - Prague, 2001 - Paris, 2002 - Washington, 2003 - Berlin, 2004 - Las Vegas, 2004 - Bangkok, 2005 - Athens. 2007, Barcelona 2007 - Shangahi, 2008 - Paris, 2009 - Beijin, 2010 - Berlin, 2011 - Paris, 2011 - Hainan china.
Founder and Chairman of the Mediterranean Congress 1993-96
Founder and Co-Chairman, Gender based medicine, World Congress Berlin 2006
Co-Founder World congress on controversies in Obesity, Diabetes and Hypertension 2006
Scientific Publications:
Total over 350 Scientific Publications; 280 original articles, 2 books, editor 12 congress proceedings

Evidence-based management of endometriosis-associated infertility

Hassan N. Sallam, MD, FRCOG, PhD (London)
Professor of Obstetrics and Gynaecology, the University of Alexandria, Clinical and Scientific Director of the Alexandria Fertility Center, and Director of the Alexandria Regional Center for Women's Health and Development, Alexandria, Egypt

Various therapies have been used in the management of endometriosis-associated infertility. These include surgical therapy, medical therapy, combined medical and surgical therapy, controlled ovarian hyperstimulation as well as assisted reproductive techniques. Observational studies have shown that expectant management for 18 months is associated with about 50% cumulative pregnancy rate in patients with stage I and II endometriosis, while patients with severe degrees of endometriosis rarely become pregnant (Olive et al, 1985). Surgical management of endometriosis includes ablation and/or resection of laparoscopic lesions as well as the drainage of endometriomas. In a meta-analysis, Hughes et al found that surgical intervention in general was associated with a higher pregnancy rate compared to either medical therapy or no therapy, but the studies were heterogeneous making this conclusion doubtful (Hughes et al, 1993). In a study by Jones and Sutton, drainage of endometriomas with ablation or resection of their walls was associated with a high pregnancy rate but there are no randomized trials to confirm these findings (Jones and Sutton, 2002). In women with minimal-to-mild endometriosis, pregnancy rates following laparoscopic ablation and/or resection were found to be significantly higher than in women who had diagnostic laparoscopy in a multi-center randomized study conducted in North America (Marcoux et al, 1998), but these results could not be confirmed in another multi-center study conducted in Italy (Parazzini, 1999). On the other hand, meta-analyses of randomised studies have shown that danazol, gestrinone, medroxyprogesterone acetate and GnRH agonists do not enhance pregnancy rates over placebo or no treatment (Hughes et al, 1993). Similarly, the combination of laparoscopic surgery and medical therapies did not improve pregnancy rates over danazol-only therapy (Yap et al, 2004). Controlled ovarian hyperstimulation combined with intrauterine insemination (COH+IUI) improves the pregnancy rates significantly compared to expectant management (Tummon et al, 1997). Finally, in-vitro fertilization (IVF) is associated with a high pregnancy rate in women with endometriosis, although this is lower than in those with tubal factor infertility (Barnhart et al, 2002). In a recent meta-analysis, we have shown that long-term (3 to 6 months) administration of GnRH agonists prior to IVF in these patients improves the pregnancy rate significantly (Sallam et al, 2005).

◆Biosketch

(For Professor Sallam's Biosketch, please refer to his abstract for Concurrent Symposium C-3.7.)

Surgery of Endometriosis in Infertility: State of the Art!

Issam Lebbi
Professor in gynaecology-obstetrics, Dream Center, 1002, Tunis, Tunisia

In the field of endometriosis associated infertility, we know since perhaps 15 years now that:

1-The surgical treatment of the mild stages (stages 1 and 2) improve significantly the spontaneous pregnancies rates (17.7 % to 31.7 %, ENDOCAN study, NEJM; 1997).

2-The surgical treatment of the moderate and severe stages (stages 3 and 4) can or should improve the spontaneous pregnancies rates (evidence level 3 by expert opinions).

The gold standard treatment of endometriosis-associated infertility is done by laparoscopic surgery. Therefore, the place and the interest of this procedure in the management of the disease is highly discussed in the era of ART and the main question is: how many laparoscopies should we perform in this group of patients to obtain one pregnancy with regard to the physical, psychological, social and financial costs of laparoscopy?

Indeed, the place of laparoscopy in an integrated approach with ART techniques in the fields of endometriosis associated infertility and unexplained infertility is not resolved yet.

Since five years now, surgical treatment for endometriosis associated infertility is “gaining popularity supported mostly by uncontrolled studies”!

These studies reported a good pregnancies rate (55 to 65 %) with surgery after IVF failure, surgery as a first line treatment and/or before IVF-ET and surgery before IVF-ET in severe stages and deep infiltrative endometriosis.

In this lecture we will try to propose an integrated approach of the management of endometriosis associated infertility but as more controlled studies are needed in this field, we can’t, in my opinion, talk about a state of the art in the management of these situations.

◆Biosketch

Professor Issam Lebbi
Professor in gynaecology-obstetrics
Dream Center, 1002, Tunis; Tunisia
Polyclinique Alyssa, Les Berges du Lac, Tunis
Medical Director of IMBR (Institute of Medicine and Reproductive Biology)
Past-President of STGO (Tunisian Society of Gyn-Obs)
Past-President of FGOM (Mediterranean Federation of the National Societies of Gyn-Obs)
Member of the Board of Directors of ISIVF
Honorary Member if the CNOGF (French College of Gyn-Obs)

Surgical management of recurrent endometrioma: prior to IVF or not

Hiroshi Nabeshima, M.D., Ph.D.
Department of Obstetrics and Gynecology, Tohoku University Hospital

Since ovarian endometrioma is frequently diagnosed in women of reproductive age, laparoscopic excision of the endometrioma is performed for most cases. However, endometrioma frequently recurs even after repeated surgical procedures.
When, we perform the IVF for the patient with endometrioma, we wonder whether the endometrioma should be removed or not before the egg collection, especially the endometrioma is recurrent one.
In this session, we show our data of recurrent endometrioma of IVF/ET patients and literature review at first. After that, we show the laparoscopic minimal invasive technique for the removal of endometrioma by VIDEO, and our surgical management to prevent postoperative ovarian dysfunction in IVF/ET patient.

◆Biosketch

Hiroshi Nabeshima, M.D., Ph.D.

EDUCATION

1997 M.D., Akita University School of Medicine
2004 Ph.D. (Dr. of Medical Science), Tohoku University Graduate School of Medicine.
2010 Attending to the Frontier In Reproduction course of Marine Biological Laboratory (Woods Hole, MA)

PROFESSIONAL TRAINING and EMPLOYMENT

2009- Present Medical Staff and Researcher of Reproduction Medicine Unit in Obstetrics and Gynecology, Tohoku University Hospital.
2005- 2009 Medical Staff in Obstetrics and Gynecology, Iwate Prefectural Iwai Hospital, Ichinoseki, Iwate, Japan.
(2007-2008 Kurashiki Medical Center for laparoscopic fellow training)
2004- 2005 Medical Staff in Obstetrics and Gynecology, Iwate Prefectural Miyako Hospital, Miyako, Iwate, Japan
2000- 2004 Resident and fellow in Department of Obstetrics and Gynecology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan
1999-2000 Resident in Obstetrics and Gynecology, Sanuma Hospital, Toyoma, Miyagi, Japan
1997-1999 Resident in Obstetrics and Gynecology, Yuri Kumiai General Hospital, Yurihonjo, Akita, Japan
1997 Passed the Examination of National Board

CERTIFICATION and LICENSURE

1997 Japanese Medical License Registration
2002 Diplomate, Japanese Society of Obstetrics and Gynecology, Certificate
2005 Technical Fellow of the Japan Society of Gynecological and Obstetric Endoscopy, Certification
2006 Technical Fellow of the Japan Society for Endoscopic Surgery, Certification
2009 Japan Society for Reproductive Medicine Board Certified Reproductive medicine specialist by Japan Society for Reproductive Medicine, Certification

MANAGEMENT OF HYDROSALPINGES PRIOR TO IVF AND ET

Victor Gomel
Professor, Department of Obstetrics and Gynecology,
Faculty of Medicine, University of British Columbia,
Vancouver BC. Canada

We have been aware of the deleterious effects of hydrosalpinx on the outcome of IVF treatment. This deleterious effect is more evident with large hydrosalpinges, visible at sonography. It has also become evident that the major detrimental effect is due to a "wash-out effect" owing to the passage of the collected tubal fluid to the uterine cavity at the time, or soon after the transfer of the embryos to the uterine cavity. This "wash-out" may also occur sometime after transfer; as evidence suggests many embryos ascend to the tube after transfer and eventually return to the uterus when the tube assumes a pro-uterine transport.
The following therapeutic options have been used to overcome this adverse effect:

1. Aspiration of the hydrosalpinx at the time of oocyte retrieval or embryo transfer.
2. Proximal occlusion of the tube.
3. Salpingectomy.
4. Salpingostomy.

Several studies reported on aspiration of the liquid contained in the hydrosalpinges. While some report significantly higher pregnancy rates with aspiration others do not. This approach remains controversial.
Laparoscopic tubal occlusion has been shown to improve the odds of clinical pregnancy after meta-analysis. However results for ongoing pregnancy, reported by only one trial, were not significant.
It has been demonstrated that salpingectomy before IVF, clearly improves the outcome of IVF treatment; this is more evident with large hydrosalpinges, visible at sonography.
Salpingostomy as opposed to salpingectomy may be used in appropriately selected cases. The beneficial effect of this approach in IVF has been demonstrated in a small number of cases. Obviously salpingostomy, in addition, offers the woman the potential of achieving a pregnancy naturally.
The place for each one of these treatment modalities and the selection of the appropriate option will be discussed in detail.

◆Biosketch

Dr. Gomel holds the rank of Professor in the Department of Obstetrics and Gynecology, Faculty of Medicine of the University of British Columbia. He served as Chairperson of his Department for fifteen years, during which the Department was greatly expanded and attained international recognition.

Dr. Gomel's special research interests lie in the fields of gynecologic surgery and reproductive medicine. In the field of human reproduction, his work included infertility associated with tubal and peritoneal causes, fertility control, reproductive surgery, operative laparoscopy and in vitro fertilization. His other research interests include reproductive physiology and formation and prevention of peritoneal adhesions.

He is internationally known for his pioneering work in both microsurgery and operative laparoscopy. He was responsible for the introduction of laparoscopy in Canada; he was the first to demonstrate the efficacy of laparoscopic fertility-promoting procedures and one of the first to use laparoscopic surgical access for other gynecologic procedures.
For nearly four decades, he has been involved in work to better understand the tubal and peritoneal factors that cause infertility, and to improve the surgical techniques to correct them. His experimental work was designed

to elucidate the function of the fallopian tube. Microsurgical techniques were employed to alter the structure of the fallopian tube and thus gain understanding of the function of its various parts. Another aspect of his animal work was designed to better understand the formation of peritoneal adhesions. Various techniques and numerous substances were used in the attempt to diminish or prevent the formation of peritoneal adhesions. This work was carried out with the collaboration of members and various trainees (fellows) in his Department. These efforts led to the development and introduction of microsurgical techniques in gynecology, which produce significantly better results. These techniques have gained international acceptance and recognition.

He established an in vitro fertilization (IVF) program in Vancouver in 1981. Under his leadership this program was the first to achieve success, resulting in the birth (on December 25, 1983) of the first IVF baby in Canada.

He was responsible for the establishment of a masters and PhD. Program "Reproductive and Developmental Sciences", within his Department, which has been operative since 1984; at present this program has more than 40 graduate students. He was also instrumental in the creation of the "Women's Health Centre," which was the first such tertiary facility in Canada, and has been operational since early 1992.

In addition to medical students and residents he has trained, in Vancouver, post doctoral fellows from all over the World, many of whom currently hold important positions, in Canada and in their own countries.

Dr. Gomel authored a long list of scientific articles published in prestigious international journals. He wrote numerous book chapters, and authored and edited several books. His authored books, among others, include "Gynecology: A Practical Approach", co-authored with Malcolm Munro and Timothy Rowe (Williams and Wilkins 1990), a problem oriented text designed for medical students, interns, junior residents and general practitioners. Published in 1983 (Little Brown & Co.), his book "Microsurgery in Female Infertility" is considered a classic. His book "Diagnostic and Operative Gynecologic Laparoscopy" (Mosby 1995) was highly acclaimed. In addition he has been the editor or co-editor of several other books including "Female Genital Prolapse and Urinary Incontinence" (Informa 2008). His most recent book is "Reconstructive and Reproductive Surgery in Gynecology" (Informa 2010).

He is on the editorial boards of numerous important international scientific journals. He has been on the Board of Trustees and Executive Boards of many national and international scientific societies, including among others, the American Society of Reproductive Medicine (previously AFS), the American Association of Gynecologic Endoscopists (AAGL) and the International Society of Gynecologic Endoscopy. He served as president several societies, including the Society of Reproductive Surgeons, the AAGL, and the Canadian Fertility and Andrology Society. He is currently the Vice president of the Peritoneum and Surgery Society and the Vice President of the International Society of In Vitro Fertilization (ISIVF).

He has received honorary memberships and awards of excellence from numerous international scientific societies and universities, including his own Faculty of Medicine, in recognition of his pioneering work in reproductive medicine and gynecologic surgery. Professor Gomel was also the recipient, of the prestigious distinction of Chevalier of the order of Légion d'Honneur, awarded by Jacques Chirac, President of France in 2003. In 2008, he was elected "Fellow" to the World Academy of Art and Science, and in 2009 he received the degree of Doctor of Science, Honoris causa, from the Simon Fraser University.

Surgery of intramural leiomyomas in infertility patients

Jean-Bernard Dubuisson, Victor Gomel
Department of Obstetrics and Gynecology (HUG, Genève)

Uterine myomas are the type of pelvic tumour found most frequently, and this raises the problem during everyday practice of how to manage them. This is particularly clear in infertility patients. When uterine myomas are diagnosed this does not necessarily mean surgery is required. The development of endoscopic procedures (hysteroscopy and operative laparoscopy), of embolization and IVF procedures have modified the over the past few years the operating indications for uterine leiomyomas. Only complicated or large intra mural leiomyomas distorting the uterine cavity need surgical treatment together with those giving rise to symptoms which do not respond to properly conducted medical treatment. Technical advances mean that surgical treatment today for uterine myomas can for certain indications be carried out by operative laparoscopy.

The two most crucial elements for infertility patients in laparoscopic myomectomy are the quality of the uterine suture and the risk of adhesions.

The problem of the quality of the uterine suture is above all important for women desiring pregnancy. The risk is low and depends mostly of the quality of the surgery.

The second problem with laparoscopic myomectomy is the risk of adhesions. Once again the risk is not specific to laparoscopic surgery, for adhesions have been observed in 80% of the cases after myomectomy via laparotomy. In our experience the rate of adhesions we observed after laparoscopic myomectomy is less.

All these elements enable us to stress that just as for any surgical technique, once it has been shown to be feasible the most important element is the indication for surgery. We believe that laparoscopic surgery is indicated for intramural leiomyomas, only in selected indications that will be discussed.

◆Biosketch

Professeur Jean-Bernard DUBUISSON
Date of Birth: 10th May, 1946 in Paris (France), Gynaecological Surgeon
Head department of obstetrics and gynecology (HUG, Genève) since 2004
Head unit of gynecology (HUG, Genève) since 2003
Ordinary Professor of the University of Geneva since 2003
Docteur Honoris Causa, Faculté d'Athènes, septembre 2005
Professor of the University of Paris (Université Paris V, Centre Hospitalier Universitaire Cochin Port-Royal, Paris) 1980 - 2003
Head Department of Gynecology (Hôpital Baudelocque, Centre Hospitalier Cochin - Paris) 1993 – 2003

DIPLOMAS :
Interne des Hôpitaux Paris (1970), Medical Doctor (thesis of Medicine Paris 1975), General Surgeon (1977), - Obstetrician and Gynaecologist (1978)

SCIENTIFIC SOCIETIES :
France :
Member of the French Commission of the Universities 1998 - 2003
Director of University Certificate "Etudes Relatives à la Stérilité et aux Troubles de la Reproduction" (Paris) 1988 - 2003

Director of University Certificate "Applications de l'Endoscopie en Gynécologie" (Paris) 1992 - 2003
President of FIV-NAT Association 1987-1989
Vice-President of the French Society of Gynaecological Endoscopy since 1989 (SFEG)
Member of the French Academy of Surgery 1995-2006
Europe :
Member of the Executive Board of the European Society of Gynaecological Endoscopy (1993-1999)
Member of the Editorial Board of HUMAN REPRODUCTION, Coordinator of the Special Interest Group of Reproductive Surgery of ESHRE (European Society of Human Reproduction and Embryology)
USA :
Member of the Board of Trustees of the American Association of Gynecologic Laparoscopists (1998-1999), member of the Editorial Board of AAGL (1999-2005) and International Advisor of AAGL (since 2006)
Switzerland :
Member of the Swiss Society of gynecology & obstetrics (since 2003)
Member of the Management Committee of Swissendos (since 2003)

PUBLICATIONS: 200 publications in international journals, 150 publications in French journals, 100 publications for teaching, 100 chapters in books, 4 books :

. *Coelioscopie et cancérologie en gynécologie*
J.B. Dubuisson, C. Chapron, J. Bouquet de la Jolinière
Editions Arnette 1993
. *Les fibromes utérins*
J.B. Dubuisson, C. Chapron, J. Bouquet de la Jolinière
Editions Arnette 1994
. *L'endométriose*
J.B. Dubuisson, C. Chapron, J. Bouquet de la Jolinière
Editions Arnette 1995
(traduction anglaise : endometriosis)
. *La douleur en gynécologie*
C. Chapron, D. Benhamou, J. Belaisch Allart, J.B. Dubuisson
Editions Arnette 1996 sous presse

These publications concern:
Microsurgery in infertility (1978 – 1988)
IVF (natural cycles, CIVETE) (1985 – 1990)
Operative laparoscopy (1990 – 2006):

- tubal infertility (laparoscopic salpingostomy, laparoscopic tubal anastomosis),
- myomas (laparoscopic myomectomy, laparoscopic hysterectomy),
- endometriosis (laparoscopic treatment of endometriosis of the bladder, deep endometriosis),
- genital prolapse (new procedures of laparoscopic lateral colpo-uterine suspension with or without meshes),
- tubal pregnancy (laparoscopic salpingectomy, management)

Myomectomy during pregnancy

Yuji Hiramatsu
Department of Obstetrics & Gynecology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

There are two kinds of treatment for pregnant women complicated with uterine myomas. One is conservative therapy while the other is myomectomy during pregnancy.

Today, I would like to talk about a myomectomy during pregnancy. This operation is still prohibited according to most textbooks. About 20 years ago, a pregnant women experienced deep vein thrombosis and pulmonary embolism with a 15 cm myoma. She had a D/C and was treated in the ICU for 2 weeks. We also experienced two mid-trimester fetal deaths in other patients. Thereafter, we began to perform myomectomies during pregnancy.

There are some merits and demerits in both therapies. In conservative therapy, there is no risk of abortion caused by operation, but many other complications may occur during pregnancy which may result in long hospitalization, birth troubles and the incidence of cesarean section may increase. On the other hand, with a myomectomy, there is a risk of a spontaneous abortion after the operation; however, these symptoms disappear in 87% of all such patients.

We have so far performed 92 myomectomies during pregnancy. The size of myomas was 4-25cm and the number of enuclated myomas was 1 - 25. The indications of myomectomy include, 1) symptoms of threatened abortion do not disappear in spite of conservative therapy, 2) a rapidly enlarging myoma, 3) a past history of a spontaneous abortion or IUFD which might have been caused by the myoma, 4) myomas which may cause complications during pregnancy, 5) myomas which cause recurrent degeneration and intense pain.

The contra-indications are 1) the margin of the myoma is not clear (adenomyosis), 2) a posterior cervical myoma.

The most common symptoms are abdominal pain, genital bleeding and rapidly enlarging myomas. Some patients present with more than 2 symptoms.

We followed up 83 patients, excluding 9 that experienced spontaneous abortions. The symptoms disappeared in 87% of the patients and they could enjoy as same lifestyle as other normal pregnant women. Thirteen percent of the patients required some therapy for a threatened spontaneous abortion or preterm labor. Seventy-nine cases (87.3%) had term delivery and 34 cases (43%) of those had a vaginal delivery. Four cases (2.5%) were preterm delivery and 9 cases (10.1%) had a spontaneous abortion. However, one IUFD occurred after 5 weeks and one placental abruption occurred 9 weeks after the operation. In results, 7 cases (7.6%) experienced abortions caused by a myomectomy.

A myomectomy during pregnancy is considered to a safe and beneficial treatment when performed by doctors who are Knowledgeable and experienced in using the proper techniques for a myomectomy.

◆Biosketch

Education

1971-1977 Okayama University Medical School

1981-1985 Graduate School of Okayama University Medical School

Academic Appointments:

1989-1995 Instructor of Okayama University Hospital

1995-1999 Assistant Professor of Okayama University Hospital

1999-2003 Associate Professor of Okayama University

2000 Visiting Associate Professor of Harvard Medical School, Joslin Diabetes Center

2003- Professor & Chairman of Okayama University Graduate School, Department of Obstetrics & Gynecology

Major Committee Assignment:

Japan Society of Obstetrics and Gynecology

The Japan Society of Diabetes and pregnancy

The Japan Society of Gynecologic Oncology

Japan Society of Gynecological and Obstetrical Surgery

Memberships:

American Diabetes Association (ADA)

International Association of Diabetes and Pregnancy Study Group (IADPSG)

Major interests in research

1) Gestational diabetes mellitus

2) Preeclampsia

3) Metabolism and nutrition of pregnant women and fetus

4) Gynecological and Obstetrical Surgery

Uterine fibroids and IVF – What is the controversy?

Prof Zion Ben-Rafael
Tel Aviv, Israel

It is generally accepted that fibroids may cause infertility or pregnancy failure depending on the number, size and location. Since our original study in 1995, many studies have confirmed that in IVF settings, fibroids that distort the uterine cavity (submucous) may affect implantation. Some of the studies also suggested that large intramural fibroids without submucous part can negatively affect the results of IVF and hence should be surgically removed prior to IVF treatment. The current dilemma mainly resides in the possible deleterious effect that intramural fibroids <7 cm, and even <5 cm may have on implantation. The current results on the issue are conflicting which is probably related more to the inability to properly evaluate the exact location of the fibroid and its imprint on the cavity.
The role of uterine fibroids as a cause of infertility remains controversial. The lack of randomized control trials does not provide the means of establishing definitive causal relationships. The sharp decline in pregnancy rates in cases of submucous myomas appears to be quite convincing; however, the less significant effect of intramural fibroids on the outcome is probably one of the reasons for the diverse results reported in different studies. New randomized studies that evaluate the size and location by 3-D ultrasound or MRI, should be able to define more precisely which fibroids should be excised and when we can proceed with IVF, despite the fibroids.

◆Biosketch
Prof. Zion Ben-Rafael,
Married + 3
Graduated from Tel Aviv University, Board certified in Israel and USA.
84-86, NIH Fellow in Reproductive Biology and Endocrinology, in Hospital of University of Pennsylvania, Philadelphia.
Full Professor (1993), Associate Professor (1990), Tel Aviv University and Incumbent of the Tarnesby- Chair of Fertility regulation Tel Aviv University
Previously:
President, Israeli Menopause Association.
1997-2007 Chairmen, Department of OBGYN, Rabin Med Center.
1991-2007 Chairmen, Department of OBGYN, Golda-Hasharon.
1989-1991 Director, Katowitz Women's Health Center, Tel Aviv
Currently in charge of the IVF unit, Laniado hospital
Founder and Chairman, COGI - World Congress on Controversies in Obstetrics, Gynecology and Infertility. 1999 - Prague, 2001 - Paris, 2002 - Washington, 2003 - Berlin, 2004 - Las Vegas, 2004 - Bangkok, 2005 - Athens. 2007, Barcelona 2007 - Shangahi, 2008 - Paris, 2009 - Beijin, 2010 - Berlin, 2011 - Paris, 2011 - Hainan china.
Founder and Chairman of the Mediterranean Congress 1993-96
Founder and Co-Chairman, Gender based medicine, World Congress Berlin 2006
Co-Founder World congress on controversies in Obesity, Diabetes and Hypertension 2006
Scientific Publications:
Total over 350 Scientific Publications; 280 original articles, 2 books, editor 12 congress proceedings

A novel operative treatment of severe adenomyosis prior to embryo transfer

Hisao Osada
General Director, Shinjuku ART Clinic
Former Professor, Nihon University School of Medicine

Introduction: Severe case of adenomyosis is not only a cause of infertility, but may include severe dysmenorrhea, thus may interfere with a woman's well-being. Routine conservative surgery for adenomyosis involves a wedge resection of the involved uterine tissue, followed by approximating the remaining myometrium and serosa. However, this method may retain unexcised affected tissue, and thus result in an unsatisfactory post surgical prognosis such as being incapable of sustaining a normal pregnancy. Our proposed treatment for severe cases of adenomyosis involves wide complete excision of affected tissues to reduce post surgical dysmenorrhea, followed by a triple-flap reconstruction of the uterine wall to prevent ruptures in subsequent pregnancies.

Methods:

1**. Resection and removal of all adenomyosis-affected myometrium:** The affected tissue is vertically incised, to split the area to be excised in two, the incision is extended to the uterine cavity. The tissue to be excised is grasped and placed under tension with Martin forceps. The tissue is adequately dissected free with scissors, with care taken to retain a serosal flap with a layer of myometrium, as well as a medial flap containing both endometrium and myometrium. The tissue flaps, both medial and distal must be more than 5 mm in thickness to assure adequate material for the reconstruction of the uterine wall. It is essential to introduce an index finger into the uterine cavity to assure maintenance of an adequate medial flap thickness. Special care must be taken to prevent damage to the Fallopian tubes.

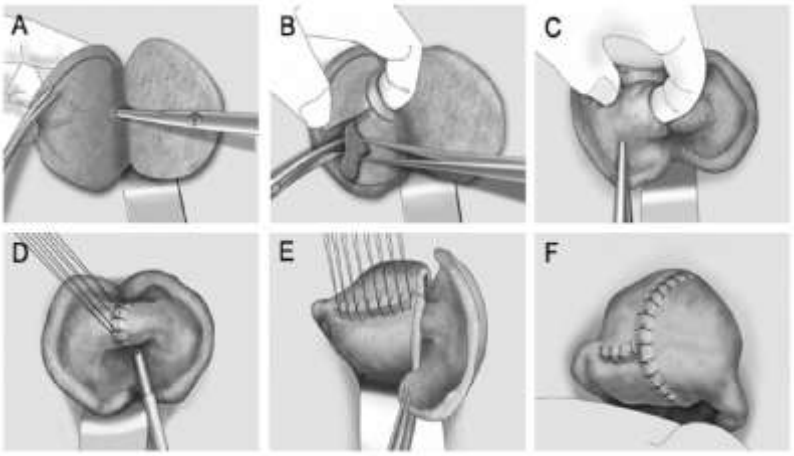
2. **Reconstruction of the uterine cavity:** Care must be taken to retain sufficient endometrium to allow reconstruction of an adequate uterine cavity. In cases of an over abundance of endometrial tissue, excess amounts must be removed to secure a more physiological uterine cavity.

3**. Reconstruction of the uterine wall:** Reconstruction of the middle portion of the uterine wall involves approximation of the myometrial musculature to ablate the space created by the excision of diseased tissue. The serosa including adequate myometrium is dissected free with a scalpel to form the third flap. The serosal or distal and third flap is then approximated to finish the reconstruction.

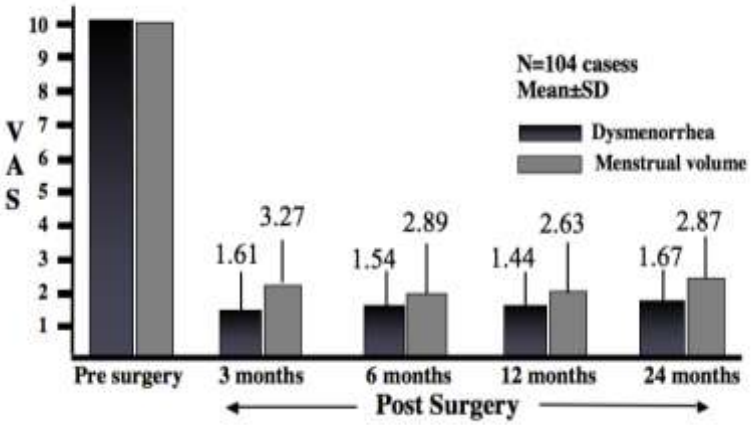
4. **Hemostasis and application of hemostatic barriers for prevention of adhesion:** The last step of this method is to apply TachoComb®, a Fibrin adhesive in sheet form, to the uterine surface for the control of oozing. The applied TachoComb® is firmly anchored and works as physical barriers, thus contributing to the reduction of post surgical adhesions.

Results: Clinical post surgical evaluation was performed using the Visual Analog Scale (VAS) to assess dysmenorrheal and hypermenorrhea at 3, 6, 12, 24 months after surgery. We performed the procedures on 104 patients during the period between June 1998 and August 2008. Of the 26 women desired to conceive, 16 (61.5%) subsequently conceived. Of these, 4 women conceived spontaneously and 12 women conceived by in vitro fertilization and embryo transfer (IVF-ET). Two women who had IVF-ET experienced a spontaneous abortion; 14 went to term and all were delivered by elective Caesarean section. There were no cases of uterine complications to the pregnancies. The triple-flap reconstruction of the uterine wall following wide adequate excision of adenomyosis tissue in women with hypermenorrhea and/or dysmenorrhea resulted in a dramatic

reduction in both menstrual cramping and menstrual flow volume post surgically and gave women chances to become pregnant.



Severe Cases of Adenomyosis Surgically Treated by the Triple-flap Method



The mean (SD) VAS findings for menorrhagia were 3.27 (2.17) at 3 months, 2.89 (1.77) at 6 months, 2.63 (1.3) at 1 year, and 2.87 (1.77) at 2 year post-surgery

◆Biosketch

Dr. Hisao Osada was born in 1946 and trained in the Department of Obstetrics and Gynaecology at Nihon University School of Medicine in Tokyo, where he later served as Professor until 2009. He was at Mainz University, Germany, from 1979 to 1981. His specialization includes laparoscopic surgery and reproductive medicine. Dr. Osada currently teaches and practices at his university and at Shinjuku ART Clinic (General Director) in Tokyo. He is the Vice-President of International Association of Private Assisted Reproductive Technology Clinics and Laboratories, as well as the Vice Congress President of the 16th World Congress on In Vitro Fertilization to be held in Tokyo in 2011.

The surgical management of uterine fibroids and adenomyosis

Michael DeRosa
Infertility Center of St. Louis

Uterine fibroids are present in approximately 70% of all women and 5 - 10% of infertile females. It has been found to be the sole abnormal finding in 1 - 2.4% of infertile women. Since childbearing is being delayed, couples are attempting pregnancy when female fertility is in decline while the incidence of fibroids and adenomyosis are increasing. Numerous surgical procedures such as hysteroscopy, laparotomy, laparoscopy, and robotics have evolved over the years giving us a myriad of choice. We will discuss the indications and methods of each of these procedures and the rational for when they should be utilized for the treatment of uterine leiomyomas. We will also briefly discuss the surgical approach to adenomyosis.

◆Biosketch

Dr. Michael DeRosa, a graduate of St. Louis University School of Medicine and Residency program, has been practicing Obstetrics and Gynecology for nearly 25 yrs. His interest has always been directed towards the treatment of the infertile patient and became affiliated with Dr. Sherman Silber and The Infertility Center of St. Louis 17 yrs ago. Since that time he has been involved in IVF, ovarian tissue freezing and transplantation, and the surgical management of the infertile patient.

The oncofertility: consequences of a previous oncological treatment and possibilities

Rufino García-Otero Reina
Director, medico

The use of chemotherapy and radiation methods in patients of both sexes may have irreversible consequences on fertility. Team work between the oncologist and the infertility specialist is considered essential, especially in young patients who have not become mothers yet or those who already have become mothers but still have a desire to increase their family.

The current use of methods such as the freezing of testicular or ovarian tissue, or vitrification of gametes has improved the prognosis of such patients.

Oncofertility was born to provide a specialist, who masters both fields, and can devise pre or post cancer treatment strategies to preserve fertility and who can integrate the work of oncologist and infertility specialist together.

◆Biosketch

Specialist in Gynecology and Obstetrics by Landesaerztekammer Bayern Munich (Germany).

Third generation of gynecologists in the García-Otero family after his grandfather Dr.Rufino García-Otero and his father, Dr. Eduardo García-Otero González. He studied in Germany, as well as his predecessors, because the best and leading schools in Gynecology and Breast Pathology in the world are there.

5 years of work at the Klinikum Bayreuth. (Academic Hospital of the University of Erlangen-Nürnberg). Prof. Dr. med. A. H. Tulusan (grade 1 reference centre in Germany for the treatment of gynecological and breast cancer with a chemotherapy unit independent from the Frauenklinik, in addition to high risk perinatal medicine).

During his stay at the Klinikum Bayreuth, participate in the following studies for patients with breast and gynecological carcinomas: ABCSG21, Compact, FACE, GAIN, Hera, BIS, NECTA, Pact, Panther, Preface, Success.

Also, in the Klinikum Bayreuth , he manage the women's chemotherapy unit clinic for 6 months.

Since 2 years medical director of "Embryocenter CIVTE" (Clinic for IVF with 26 years experience) and Senocur-Institute for Breast cancer". Both in Sevilla (Spain).

www.civte.com

www.senocur.com

Fertility Sparing in Stage I a2 to I b1-Cervix Carcinoma

Werner Lichtenegger
Charité Berlin

The incidence of pelvic lymph node metastases according to stage I a1 is very low. It was shown by different publications from Bellin, Benedetti-Panici, Burghardt, under 3 mm invasion it lays by less than 1%, between 3-5 mm up to 3%. For the cervix important are the parametrial nodes. Parametrial lymph node metastases without pelvic lymph node positivity are very rare. So, parametrial involvement in patients with tumors less than 2 cm are under 1%. Rational for radical trachelectomy was the quality of life, the trend to more conservative surgery and the fact that in some cases the removal of the uterine fundus is not necessary. The question is: radical trachelectomy or only conization? When you look at the literature radical vaginal trachelectomy shows positive nodes in about 4% and a lymph vascular space invasion of about 29%. The recurrence rate in tumors less than 2 cm is very low, about 3%, over 2 cm it increased up to 21%. In cases of radical vaginal trachelectomy pregnancy loss or delivery before 32 weeks was in 44% of the patients. In cases of radical abdominal trachelectomy pregnancy loss or delivery before 32 weeks were 38% of the patients. Therefore we looked at the possibility of neoadjuvant chemotherapy followed by conization in patients with I b1 and we found in these cases a neoadjuvant chemotherapy pregnancy loss or delivery before 32 weeks only in 23% of patients. So, in conclusion fertility sparing in early cervical cancer patients should be very well selected. Trachelectomies are an option in patients with I a2 but in patients with negative nodes they are an overtreatment. In some series of trachelectomy up to 40% of the patients did not have residual tumor after conization and trachelectomy results in about 40% pregnancy loss. Neoadjuvant chemotherapy and conization had a pregnancy loss only for 23% and Paclitaxel/Carboplatin weekly seems to be a very good and effective regimen.

◆Biosketch
Born on December 31st, 1942 in Graz/Austria

Since 1989 Chairman of the Gynecological Clinic of University Clinical Centre Rudolf Virchow in Berlin/Germany

Since 1998 Director of the Clinic of Gynecology and Obstetrics of University Clinical Centre Charité in Berlin/Germany, Campus Virchow Clinic

Research activities: surgical treatment and adjuvant therapy of cervical cancer, ovarian cancer and endometrial cancer; conducting of numerous monocentric and multicentric phase I, II and III studies; identification of new molecular biologic prognosis factors in breast cancer, ovarian cancer and cervical cancer; more than 300 publications and articles

Member of multiple international societies

Abdominal radical trachelectomy in our experience of 126 cases

Takuma Fujii, Hiroshi Nishio, Kazuhiro Minegishi, Naoaki Kuji, Yasunori Yoshimura, Daisuke Aoki
Keio University, School of Medicine

In our institution, 140 abdominal radical trachelectomy (RT) were planned and 126 were performed from September 2002 through March 2011. The median age of the patients was 33 (23-44) years. The majority of the lesions were stage Ia (21.4%) or Ib1 (78.6%). In histology, 87% (110/126) were squamous and 13% (16/126) were adenocarcinomas. The median follow-up was 26 (2-104) months. Based on the accumulated our experience in the past ten years, patients were offered radical trachelectomy if they met the following criteria: 1.FIGO stage Ia with lymph-vascular space involvement or confluent invasion, stage Ia2, stage Ib1 disease; 2. squamous carcinoma or well differentiated adenocarcinoma; 3.tumors less than 2cm in size or exophytic tumor less than 3cm in size but with little stromal invasion; 4. desire to preserve fertility; 5. No evidence of disease outside the cervix. This criteria was reported by several authors, and we also reported our experiences (Gynecol Oncol 115,51-55,2009). If the criteria above were satisfied in the candidate patients, no recurrence was observed in two years. However, eligible criteria has not been established yet. The risk of early invasive adenocarinoma was controversial because skip lesion of adenocarcionma are present higher in the endocervical canal. Even if the surgical margin of the removed cervix was negative, safety was not guaranteed. In our experience, 10% of patients planned RT were abandoned and converted to hysterectomy due to positive lymph nodes or positive margins of the removed cervix by frozen section in the original operation. Furthermore, additional 10% of patients who underwent RT received adjuvant chemotherapy and/or radiation for those reasons. As a whole, 20% of candidate patients did not meet the eligible criteria, consequently. There were some post operative complications; cervical stenosis, amenorrhea and lymphocele. In order to prevent lymphocele, ligation of lymph vessels was critical and non-suture technique for the retroperitoneum followed by Seprafilm (genzyme) treatment after lymphadenectomy was also important. In the process of anastomosis of the neo-cervix to the vagina, intrauterine device or cervicalplasty will improve to prevent cervical stenosis. In the obstetrical outcome, 11 babies were delivered by Caesarean section. Eight of 11 patients conceived with artificial reproductive technology. Nine babies were delivered after 32 weeks. Since no standard protocol for perinatal management was established yet, perinatologists corresponded to individual pregnant women. RT seems to be an oncologically safe procedure in well-selected patients with early-stage diseases. Obstetrical outcomes post RT was tolerable. However, the patients need to be fully informed about perioperative and late complications especially for the risk of premature delivery. Collaboration with gynecologic oncologists, perinatologists, ART specialists and professional nurses were also critical issues for establishing this procedure.

◆Biosketch

1987 Graduated from Keio University, School of Medicine
1991 Research resident in Genetics Division of National Cancer Center Research Institute in Japan
1996 Research associate in Yale University, School of Medicine, Department of Internal Medicine, Medical Oncology
1999 Gynecology, National Tochigi Hospital, Japan
2000 Assistant, Keio University, School of Medicine
2004 Assistant Professor, Keio University, School of Medicine

Laparoscopic radical trachelectomy

Masaaki Ando
Kurashiki Medical Center

Introduction: Vaginal trachelectomy with type II radical parametrectomy has been well reported and although rare, recurrences were reported at the lateral parametrium. Our concerns with the inadequate resection of the parametrium and operability in narrow vaginal access cases led us to develop a Type III total laparoscopic counterpart focusing on the isolation and removal of the total length of the parametria. Although there are some concerns that a Type III radicality may have a negative impact on fertility, to date we are happy to report a total of 9 births.

Methods: From 2001 to 2010, 50 patients with stage 1a2 to stage 2a1 cervical cancer underwent our total laparoscopic radical trachelectomy. The inclusion criteria was exophytic type with a size of less than 2.5cms with at least 5mm residual cervix with a surgical clearance of at least 1cm. Squamous cell carcinoma and adenocarcinoma cases were included. Cases with lymph node metastasis discovered after MRI or CT were excluded.

To begin the procedure, first we create a vaginal cuff in order to define the exact length of the vaginal and prevent spillage of the tumor into the abdominal cavity. We begin the pelvic lymphadenectomy using our umbilical ligament suspension technique. After isolation and preservation of the uterine artery, and complete isolation of the ureter the pararectal space is developed isolating the cardinal ligament. The cardinal ligament is then transected at the pelvic sidewall in a Type III radical fashion using a vessel sealing system or linear stapler. All other paracervical ligaments as well as the upper 1.5cms of the vagina are transected. Then the cervix was cut from the uterine body at the level of 5mm below the internal os. The sump of the vaginal was anastomosed to the residual cervix laparoscopically.

Results: Average duration of the procedure was 393±109min and blood loss measured 447±330mL. No complications occurred except for temporary lymphedema. Although one case with lymphatic space invasion died from recurrences at multiple sites, the other 24 cases are alive with no evidence of disease after 6 to 72 months.

At the time of the operation 33 patients were single with no plans for pregnancy. Only 12 cases have reportedly attempted pregnancy, of these 7 cases have reportedly become pregnant one time, 3 cases have become pregnant a second time. All but 2 cases have become pregnant as a result of ART or other fertility treatment. After pregnancy, three cases experienced blighted ovum. One case, terminated at 21 weeks in her first pregnancy terminated again at 18 weeks in her second pregnancy both times due to PROM and intrauterine infection. Other pregnancies have resulted in 9 live births all delivered by cesarian section. One patient delivered twins (MCT) at 23 weeks due to PROM. Although one twin died before delivery the other survived and is healthy at time of writing. Two cases delivered at 26 weeks, one case at 28 weeks and one case at 31weeks, one case at 34weeks one case at 36weeks. Two case was delivered at term.

One baby died due to congenial heart disease. Two cases underwent Sailing technique to prevent intrauterine infection. One procedure deemed effective as the case achieved term delivery. Another procedure failed and terminated at 23 weeks (twin pregnancy case).

At time of writing there are 2 other on going pregnancies. One is 23 weeks while another is 29 weeks. It has been impossible to follow up on all cases as some were referred from distant places and have not reported their condition.

Discussion: Type III radical transection of the cardinal ligament is possible totally laparoscopically preserving the uterine artery so even cases suffering from malignant disease can benefit from new procedures that improve the quality of life. In spite of the radical nature of the procedure compared with the radical vaginal

trachelectomy, pregnancy is possible. A recent paper related to vaginal radical trachelectomy reported a 4% recurrence rate with half of those recurrences happening in the pelvic sidewall. To date we have experienced no recurrent cases meaning that the oncologic requirements have been fulfilled while preserving the patients' fertility potential.

◆Biosketch

1973-1980 Jichi Medical School
1980.04 Okayama Red Cross Hospital: Obstetrician
1984.04 Kurashiki Medical Center: Head of Gynecology Department.
2001.04 Osaka University: Visiting Lecturer of Obstetrics and Gynecology.
2003.04 Kyoto University: Visiting Lecturer of Obstetrics and Gynecology.
2004.08 Beijing Capital University: Visiting Professor of Obstetrics and Gynecology.
2007.04 International Advisory Board: Society for Laparoscopic Surgeons (USA)
2009.03 Thai-German Multidisciplinary Endoscopic Training Center Visiting Professor of Obstetrics and Gynecology
2006.03 Keio University School of Medicine: Visiting Associate Professor of Obstetrics and Gynecology
2009.03 Kurashiki Medical Center: Head of Center for Endoscopic Surgery
2009.03 Kurashiki Medical Center: Vice Director
2009.03 Kurashiki Medical Center: Trustee
2009.04 Keio University School of Medicine: Visiting Professor of Obstetrics and Gynecology
2010.04 Clinical Professor Kyoto University School of Medicine: Clinical Professor Obstetrics and Gynecology
2010.04 Clinical Professor Osaka University School of Medicine: Clinical Professor Obstetrics and Gynecology
2011.04 Honorary member of Austlarian Gynecologic Endoscopic Society

Cryopreservation of human oocytes of cancer patients in Japan

Shokichi Teramoto
Shinbashi Yume Clinic

Given the established egg-freezing technology, institutions in Japan affiliated with the International Association of Private Assisted Reproductive Technology Clinics and Laboratories are currently engaged in clinical research projects to collect oocytes from women who may become infertile due to treatment of hematological malignancies, such as leukemia, before or during treatment. The oocytes will be cryopreserved so that the affected women can still become pregnant in the future. This activity is based on the belief that the preservation of fertility in women who wish to give birth will contribute to a better quality of life once they have overcome the underlying disease.

The most important thing to consider when collecting oocytes from patients with hematological malignancy is not to interfere with the treatment protocol for the underlying disease. To ensure this, an ovarian stimulation technique that is compatible with the cycle of the treatment for the underlying disease and has flexible start and end dates for ovarian stimulation is required. In addition, since antitumor therapy is usually administered repeatedly after each hematological recovery, the egg collection technique also needs to be repeatable. For these reasons, the maximum stress that can be imposed by egg collection on the ovary and the patient's body are strictly limited, making the conventional long protocol the least appropriate strategy for ongoing hematological malignancies with poor hematological status, except for very early-stage malignant lymphoma. Both the short protocol;SP and antagonist protocol;AP are less stressful than the LP, but are not repeatable and less flexible in terms of start and end timing. On the other hand, the clomiphene protocol;CP is repeatable and flexible. The letrozole protocol;LP and the natural cycle protocol;NP are highly comparable in terms of repeatability and flexibility and also the least stressful, but are associated with a relatively high probability of cancellation. Thus, the SP and AP can be used for low-grade malignant lymphoma for which the initiation of remission induction can be delayed, whereas the CP should be chosen for hematological malignancies for which remission induction has been successfully completed and treatment is proceeding to consolidation therapy. The LP and NP are applicable to all stages of disease as they are associated with relatively low stress on the ovary and the patient's body. They also carry a low risk for complications, such as ovarian hemorrhage, although efforts are required to reduce cancellation and increase the number of oocytes that can be collected.

When collecting oocytes, safety considerations are required for anesthesia and prevention of infection and bleeding. Anesthesia is not required when collecting one or two oocytes, but is required when the procedure is expected to take a long time and when multiple punctures are required. General anesthesia may cause a delay in implementing urgent measures, such as blood transfusion, as it may mask signs of ovarian hemorrhage e.g., abdominal pain. Therefore, pain management should be done with local anesthesia, which does not reduce consciousness and enables information indicating abnormality, such as abdominal pain, in to be obtained in a timely manner.

Since 2007, we have succeeded in effectively removing pain by injecting a small amount of lidocaine hydrochloride into a gap between the vaginal muscularis and peritoneum under ultrasound guidance. Infection can be completely prevented by vaginal disinfection using povidone iodine and eliminating the process of follicle washing. This requires a technical basis to avoid the entrance of povidone iodine into the collection solution and ensure reliable egg collection with only one aspiration procedure. To prevent ovarian hemorrhage, it is essential to introduce an egg collection technique that can reduce the number of punctures into the ovarian parenchyma and a fine egg collection needle. We have developed unique fine egg collection needles since 1998, and while constantly improving these tools, we have used them for collecting oocytes from leukemia

patients since 2001. We have also used 22- and 23-gauge tapered needles in practice since 2006 and have succeeded in collecting oocytes with a very high level of safety.

The best practice is required for egg collection from patients with hematological malignancy in all fields of assisted reproduction technology, including freezing, ovarian stimulation and egg collection techniques. The following presentation describes our efforts and achievements, which have been made over a period of more than 10 years.

◆Biosketch

Born in 1957 in Japan, Shokichi Teramoto graduated in 1990 from Kanazawa University School of Medical Science, Ishikawa, Japan. Subsequently he trained in the Department of Obstetrics and Gynecology at the Graduate School of the same university. From 1994 he trained as an infertility specialist under the guidance of Dr Osamu Kato, in 1996 becoming Vice Director of Kato Ladies Clinic, an IVF center in Tokyo. Since then, he has devoted himself to establishing an ovulation stimulation method using clomiphene citrate. Other interests include obtaining oocytes from leukemia patients and building an oocyte retrieval-cryopreservation system for leukemia patients.

Cryopreservation of human ovarian tissue, clinical data and scientific considerations

Claus Yding Andersen, MSc, DMSc, Professor,
Laboratory of Reproductive Biology, Section 5712,
University Hospital of Copenhagen, 2100 Copenhagen, Denmark

Girls and women suffering from a cancer or another disease that require treatment with gonadotoxic drugs may as a side effect reduce the ovarian pool of follicles. The gonadotoxic effect is dependant on the specific treatment and is influenced by the dose of the therapeutical agent and the possible use of radiotherapy. Especially, alkylating substances and radiation to the abdomen in case of cancer diseases often cause irreversible damage to the ovaries. When the ovaries are depleted of follicles many women experience profound effects on the physical and psychological status. Menstrual cycles ceases and it is not possible to become pregnant. To young girls it may further mean that normal puberty fails.

Cryopreservation of ovarian tissue is a new method, which has been developed in an attempt to circumvent the long-term ablative effect on reproductive performance by gonadotoxic treatment. Removing one whole ovary or part of an ovary from women in their reproductive years prior to treatment and cryopreserving the tissue can keep a viable pool of follicles. When the women have been cured and is considered fit, the thawed ovarian tissue may be transplanted to those who entered menopause.

In Denmark cryopreservation of ovarian tissue has been organized with one central laboratory that freezes all the tissue in close collaboration with three fertility clinics round the country. Totally more than 500 girls and women have had ovarian tissue cryopreserved in Denmark. The youngest girl was 0,5 years old and the oldest 38 years. We have currently cryopreserved ovarian tissue from around 100 girls younger than 18 years of age. The ovarian tissue is excised at the local hospital and transported on ice to the freezing facility, where cryopreservation and storage is performed. In case of transplantation the frozen tissue will transported to the local hospital for the operation. This transport model has been validated and has now been used for more than 250 cases.

In Denmark a total of 18 women have experienced transplantation of frozen/thawed ovarian tissue a total of 25 times (7 women having tissue transplanted twice). All women regained ovarian function and none have experienced relapse as a consequence of the transplantation. Over a period of 20-25 weeks levels of FSH gradually return to pre-menopausal levels and menstrual cycles are regained. The longevity of the tissue depends on the age of the woman at tissue retrieval and the amount of tissue transplanted. Most women experience return of ovarian function for some years with just a fraction of tissue from one ovary being replaced. Recently, one child has had ovarian tissue transplanted for natural induction of puberty; this case will be presented in detail.

Six women have been pregnant; in most cases following natural conception. Two women have delivered three healthy babies as a result of transplanted frozen/thawed ovarian tissue. In the latter two cases the tissue was transported 4-5 hours prior cryopreservation. The presentation will review our experiences and results with transplantation of cryopreserved ovarian tissue.

◆Biosketch

Claus Yding Andersen is professor in human reproductive physiology at the Faculty of Health Sciences, University of Copenhagen, Denmark and has since 2009 been heading Laboratory of Reproductive Biology at the University Hospital of Copenhagen, Denmark. Claus Yding Andersen qualified first as MSc from the Danish Technical University (1979) and obtained his Doctor of Medical Science degree from University of Copenhagen in 1997. His main research interests include cryopreservation of gonadal tissue, ovarian endocrinology and human embryonic stem cells. He has published more 200 scientific papers and has given a large number of international presentations.

Conservative Treatment of Borderline Ovarian Cancer

A.H.Tulusan[1]; V.Russu[1]; B.Lex1; M.Popovic[1]; I.Sopov[1]; H.Volkholz[2]
Dept. Gynecology and Obstetrics[1] and Dept. Pathology[2]; Klinikum Bayreuth, Germany

Borderline ovarian Carcinoma (BOC) are epithelial neoplasm with pathological characteristic in between benign and frank malignant ovarian cancer. So the WHO define the BOC as a Ovarian Tumor with low malignant potential (LMP). Despite peculiar histopathological features they generally are associated with an excellent prognosis. Most series reported a 5-years survival rates of 100% for LMP stage I or IIA. Even for tumors involving the pelvis or abdomen still the 5-years survival rates are about 80%. Still much effort has been and to be done to identify the features associated with poor prognosis of some of the LMP.

Beside architectural complexity with papillary and detached atypical cell clusters also nuclear atypia, mitotic activity and lack of invasion are used for the diagnosis of LMP. Even then there are still often difficulties to distinguish between LMP and frank invasive ovarian cancer especially in cases with dissemination and implantation of LMP tumor cells seeds at the peritoneal cavity.

Histological different types of LMP was distinguished: serous, mucinous, endometroid, clear celland they could have different prognosis.

Patients with LMP are generally younger (45Y) than patients with invasive ovarian cancer (56Y).

Only 30% of the LMP (stage I) are growing bilaterally, higher stage cases are bilateral in 60%.

Only 25% of stage I LMP have elevated CA125.

Even treated with complete hysterectomy and bilateral adnexectomy some LMP has a late recurrence (16 years) some with a fatal outcome. Some investigator showed a poor prognosis for cases with high MIB-1 or Ki-67 on cases with late recurrence.

Surgery is still the corner stone of Ovarian Cancer management. This is also true for LMP. Even without adjuvant chemotherapy the survival rates of LMP ranged from 90%-100%. To have the better possibility of distinguishing prognosis of LMP the same detailed staging like for invasive ovarian cancer has to be done. More than 80% of the LMP present with stage I disease.

Since many LMP patients are in the childbearing years the possibility of conservative treatment must be seriously considered.

No evidence of adverse effect of conservative fertility preserving surgery for stage I LMP in terms of survival has been seen. But conservative treated LMP patients can have a higher recurrence rate. The need of adjuvant systemic therapy for higher stages of LMP is still a subject of debate. Randomised studies on LMP stage I showed no necessity for adjuvant treatment (Creasman et al.1982; Young et al. 1990).

For more advance stages of LMP (II – IV) the surgical therapy of LMP is similar to patients with invasive ovarian cancer. Patients with aneuploid tumors have a worse prognosis compared with diploid tumors (Trope et al, 1990). In these cases Platin containing chemotherapy are indicated.

Experience with the conservative treatment of 5 cases of LMP in our institution will be presented.

Conclusions: For the individualised risk adapted treatment of LMP ovarian tumors and considering the preservation of fertility following must be done:

1. Interpreting the stage and histological and biological risk factors.
2. Determine surgical management and the possibility and need for fertility preservation.
3. Determine the appropriate medical chemotherapy management for advanced disease.

◆Biosketch

Agustinus Harjanto TULUSAN, M.D., Ph.D., German, born on 21 July 1945

Present Position:

Director, Dep. of Gynecology and Obsterics Bayreuth, Academic Teaching Hospital of the Erlangen University, Klinikum Bayreuth, Preuschwitzer Str. 101, 95445 Bayreuth, Germany

Professional Qualification:

Medical Education: University Erlangen 1966-1972
Habilitation/Ph.D 1982 University Erlangen: "Carcinoma lobular in situ and breast cancer"
Dept. of Pathology: 1972-1976 University Erlangen
Dept. Gynecology and Obstetrics: 1976-1994 University Erlangen
Dept. Gynecology and Obstetrics: since 1994 Klinikum Bayreuth

Previous appointments:

Professor of Gynecologic Oncology and Head of the Dept. of Gynecologic Onkology
Womens Hospital University Erlangen: 1985-1994
Vice Director of the Dept. of Gynecology and Obstetrics, Univ.Erlangen: 1986-1994

Membership of medical scientific societies:

1. German Gynecologic and Obstetrics Society (DGGG)
2. German Senology Society
3. Bavarian Gynecology and Obstetrics Society
4. German Cancer Society (Deutsche Krebsgesellschaft)
5. Gynecology Oncology Study Group (Arbeitsgemeinschaft Gyn. Onkologie)
6. Reconstructive Surgery Study Group (Arbeitsg. Wiederherstellende Operation)
7. Gynecologic Pathology Study Group (Arbeitsg. Gyn. Pathologie)
8. Society Pelvic Surgeons since 2003
9. Member of the American Society of Clinical Oncology since 2007

Infertility, ovulation induction treatments and the incidence of breast, ovarian and endometrial cancers – Thirty years of follow up

Lunenfeld[1] B and Gertner L[2]
Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel[1]
Women and Children's Health Research Unit, Gertner Institute for Epidemiology and Health Policy Research, Tel Hashomer, Israel[2]

Objective:
To evaluate the possible risk for cancer development in infertile women treated with ovulation inducing agents.

Design: The study is a historical cohort analysis of 2431 women who were treated for infertility at the Sheba Medical Center during the period 1964-1974 and contributed more than 84,000 women years of follow-up.

Material & Methods: Cancer development was assessed through linkage with the National Cancer Registry updated to 31.12.2005. Standardized incidence ratios (SIR) were calculated between the observed cancer cases and the expected cancer rates in the general population with respect to age and continent of birth. Birthrate was assessed through linkage with the National Birth registry.

Results: The cohort includes 33.8 years of follow-up with a mean age at the end of follow-up of 62.7 years. Over all 350 cases of all-site cancer were observed as compared to 338.4 expected (SIR=1.03; 95%CI 0.93-1.15). Eighteen cases of ovarian cancer were observed as compared to 18.1 expected (SIR=1.0; 95%CI 0.59-1.57). For breast cancer, 153 cases were observed as compared to 131.9 expected (SIR=1.16; 95%CI 0.98-1.36) and for endometrial cancer 30 cases were observed as compared to 17.8 expected cases (SIR=1.69; 95%CI 1.14-2.41).

Conclusion: Hormonal causes of Infertility were found to be associated with increased risk of endometrial cancer. Borderline increase in the risk for breast cancer was observed in infertile patients. Ovarian cancer risk was not found to be elevated. No significant changes in the risk were found following treatment with gonadotropins

◆Biosketch
(For Professor Lunenfeld's Biosketch, please refer to his abstract for Concurrent Symposium C-3.1.)

Highly efficient and safety vitrification using hydroxypropyl cellulose as a macromolecular supplement for cryopreservation of oocytes and blastocysts

Luis Ruvalcaba, Martha Isolina Garcia Amador, Rocio Martinez Armas
Instituto Mexicano de Infertilidad

Introduction: Vitrification as an alternative method of cryopreservation on human assisted reproductive technologies (ART), resulting significantly improved survival rates of oocytes and embryos. Reduce risk of viral contamination resulting from use of media containing biological macromolecules, government regulators in some countries have stipulated that non-serum substitutes must be used in human ART, including cryopreservation. Study was to test efficacy of a synthetic macromolecule, hydroxypropyl cellulose (HPC), in vitrification.

Methods: Four experiments were conducted using bovine oocytes, blastocysts, and human oocytes, 4-cell embryos. Bovine oocytes retrieved from ovaries harvested at slaughter at an abattoir were subjected to in vitro maturation (IVM), in vitro fertilization (IVF), in vitro culture (IVC). Used either as Metaphase II oocytes, blastocysts. In each of two experiments, three groups of bovine oocytes (Experiment 1) and bovine blastocysts (Experiment 2) were cryopreserved with Cryotop method. Group 1, the vitrification medium with 0.6 mg/ml HPC, and Group 2, with 1% commercial Synthetic Serum Substitute; Group 3 contained no added macromolecule. Experiments were repeated twice with total of 90 oocytes, 90 bovine blastocysts were used. (Experiment 3), human oocyte were vitrified and medium containing 0.6% mg/ml of HPC. This experiment was repeated several times for total of 30 oocytes had been vitrified. After vitrification, survival was based on development into blastocysts of oocytes subjected to IVF and IVC. Blastocysts were classified as survivors if they reformed a blastocoelic cavity after 3 hours in culture. (Experiment 4) 35 human oocyte from 9 patient and 7 blastocysts from 4 patient were cryopreserved with ES, VS, TS, DS, WS containing 0.6 mg/ml HPC. We excluded patient with PCO, Low response, patient over 35 years old or implantation failure.

Results: (Experiment 1), The respective morphological survival rates of oocytes Groups 1 (100%), 2 (100%) and 3 (87%). After fertilization and culture, the corresponding rates of two-cell formation in these three groups were 63%, 67% and 37%. The respective rates of blastocyst formation were 20%, 17%, and 7%. (Experiment 2), survival rates of bovine blastocysts of the three groups were 100%, 100% and 93%, respectively. (Experiment 3), all 30 human oocyte vitrified in HPC exhibited normal osmotic responses during removal of the cryoprotectants. (Experiment 4) survival rates were 100% on oocytes and on blastocysts all 42.

Conclusion: High morphological and functional survival of bovine oocytes vitrified in medium supplemented with HPC, as well as morphological survival of bovine blastocysts and human oocyte suggest that (HPC) may be a safe and effective supplement for vitrification in human ART.

◆Biosketch

Luis Arturo Ruvalcaba Castellón M.D. Graduated from University of Autonoma de Guadalajara, Mexico. Director of the Mexican Institute of Infertility in Guadalajara, MexicoChief of the Department of Gynecology and Obstetrics at the Medical Center Specialty Hospital of Puerta de Hierro in Guadalajara, Mexico. Laparoscopic Surgery Fellow at the University of Kiel, Germany, 1994 Fellow Biology of Reproduction, Assisted Reproduction at the Central Hospital Baptist, Lexington, Kentucky, USA and North Carolina Chapel Hill, 1997. Fellow Assisted Reproduction and Vitrification in Kato Ladies Clinic, Tokyo, Japan 2004. Active member of International Society for Preservation Fertilty, ESHR, AMMR, APART. Robotic Surgery Da Vinci, Florida 2010. Pioneer in Vitrification in Latino America.

Role and Possibility of Vitrification in Human Oocyte

Masashige Kuwayama
Repro-Support Medical Research Center, Tokyo, Japan

Recent drastic advances in cryobiology have made it possible to preserve various types of reproductive cells with little viability loss. Ultra rapid vitrification, the alternative cryopreservation method seems to be a powerful tool to any biological specimens, which cannot be preserved by the conventional slow freezing and vitrification. Ultra rapid vitrification realized the successful clinical use of vitrification not only for human PN zygotes, cleavage stage embryos and blastocysts but also for oocytes. Cryopreservation of oocyte means to give a time between the day of aspiration of oocytes and IVF for ET. This is called Oocytes Bank. Oocyte bank keeps the possibility to have a baby in the future family life for all the women who have any risks of future infertility. The women of blood cancer, reproductive organ cancer and breast cancer have risks of losing ovarian function by a side effect of the treatments. Turners syndrome girls will lose ovarian function in their early 20s. Ovarian function can be also lost by a case of accidental exposure to radiation by a nuclear power station. Oocytes bank gives patients not only a time but also a distance for IVF treatment. Oocytes bank eliminates the difficulties to synchronize the cycles between the donor and recipient in the egg donation IVF programs over national borders. Sex reassignment surgery has been recently legally approved in some countries including Japan. Some FTM women want to have babies with her own gene in their future family life after the surgery and marriage with women. In addition, all women actually lose their ovarian function in their advanced age by senescence of the body. Self oocyte banking has been already used for career women for the happy family life with own children for the delayed delivery over 40 years old, as an option of the personal fertility plan in many countries. Ultra rapid vitrification, the Cryotop method has been clinically applied to more than 500,000 cases (30,000 cases for oocytes) for these 10 years in 40 countries with excellent clinical results without any problems like virus contamination during storage. Safety and effective technology of oocytes cryopreservation has been already established, and women have now choices of the solutions for the loss of ovarian function toward having their own babies to improve quality of life as women.

◆Biosketch

Name: Masashige Kuwayama Repro-Support Medical Research Center
Ph.D.: Hokkaido University
Occupation:
Present- CEO, Repro-Support MRC; 2010- General Academic Supervisor, Kato Ladies group; 2007- Board Internal Society of MAAR; 2005- ditor, J. Reproductive Bio Medicine Online; 2005- Board, JSClE; 2005- Lecturer, IUMW, Meiji, Azabu Univ.

Publications: 81 Papers on Scientific Journal: JRF, Reproduction, Cryobiology, CryoLetters, BOR etc.
290 Papers on Scientific Meeting: IETS, ICAR, ASRM, ESHRE etc.

Topics: Scientific Director, largest IVF center for 10 years.
R & D Director: vitrification, GVT, spermatogenesis, ES-cells.
Obtained first calf after vitrification of blastocyst (1991), MII oocyte (1992). First vitrification porcine pre-hatch blastocyst, oocyte (1997, 1998). Developed Cryotop method for human oocyte (1999), embryo (2000), first oocytes bank (2001), in USA (2003). Vitrified 500,000 oocytes and embryos in 40 countries by Cryotop method.

Blastulation and pregnancy rates after vitrified human zygote culture for 4 days: Preliminary results

Safaa Al-Hasani, Beate Schöpper, Ehab Abu Marar, Tim Cordes, Askan Schultze-Mosgau, Klaus Diedrich and Georg Griesinger
Women Hospital, University of Schleswig-Holstein, Campus Luebeck, Ratzeburger Allee 160, 23538 Luebeck-Germany

Introduction: Blastocyst culture has been introduced with the aim of increasing the efficacy of embryo selection. In this preliminary study, blastocyst culture was offered to patients, who had shown excessive ovarian response and thus had all 2 PN stage oocyte frozen by vitrification in order to prevent ovarian hyperstimulation syndrome (OHSS).

Material and Methods: 29 patients were included in this study till now. These patients were stimulated with either corifollitropin alfa or recombinant FSH in a GnRH-antagonist protocol. Final oocyte maturation was triggered with GnRH agonist to avoid OHSS. The fresh embryo transfer was cancelled and all 2PN stage oocytes were vitrified by the Cryotop method (Al-Hasani et al. (2007). Four to six zygotes were warmed per attempt to transfer and cultured in "Sage sequential media" for further 4 days under oil. A maxiumum of two blasotcysts were transferred in a programmed cycle and if more blastocysts were available they were re-vitrified.

Results: A total of 160 zygotes were warmed from 29 patients till now and 42 embryos reached the early and expanding blastocyst stage, while 9 reached the morula stage (32%).
The scoring system used was according to Gardner et al. (1999). The implantation rate achieved in this study was 31.4 % and the pregnancy rate was 34.5%.

Conclusion: These results show that blastulation rate after vitrification is high, and thus can be offered to patients with a sufficiently high number of 2 PN stage oocytes. In combination with agonist triggering, OHSS can be avoided while efficacy is high.

References:

Al-Hasani S, Batuhan Ozmen, Nikoleta Koutlaki, Beate Schoepper, Klaus Diedrich, Askan Schultze-Mosgau (2007): Three years of routine vitrification of human zygotes: is it still fair to advocate slow-rate freezing? RBM online *14, 288-293*

Gardner DK, Schoolcraft WB. In vitro culture of human blastocyst. In: Jansen R, Motimer D, eds. Towards reproductive certainty: infertility and genetics beyond 1999. Carnforth: Parthenon Press, 1999: 378-88.

◆Biosketch:
Dr Safaa Al-Hasani graduated from the University of Baghdad in 1977. In 1980 he undertook his doctorate degree, as well his specialty, in the field of reproductive veterinary medicine from Tieraeztliche Hochule in Hannover. He has been the director of assisted reproduction laboratories in Erlangen, Bonn and Lübeck since that time. In 1982, he was in the scientific group that achieved the first German IVF baby in Erlangen. Thereafter, he became a staff member of the University of Lübeck where he became an extra-ordinary Professor in 2002 and where he is still working. He is the author or co-author of more than 250 scientific publications.

Cryopreservation in human Assisted Reproductive Technology

Stanley P. Leibo
Department of Biological Sciences, University of New Orleans,
New Orleans, LA, U.S.A.

AIMS: The purpose of this lecture is to briefly review applications of cryopreservation to the practice of human Assisted Reproductive Technology (ART).

DESCRIPTION OF RESULTS: In 1953, the first human pregnancies resulting from artificial insemination with frozen-thawed spermatozoa were reported. Exactly thirty years later, the first human pregnancy resulting from transfer of a frozen-thawed embryo was reported, and the first birth from a cryopreserved embryo was reported one year later. Since then, cryopreservation of reproductive cells and tissues has become an essential technique in the ART clinical laboratory. Babies have been born from women impregnated with semen that has been stored at -196°C for more than 25 years. As for embryos, in 2005 in Canada alone 464 babies were born from frozen-thawed embryos; in that same year in Europe more than 13,000 pregnancies were produced by transfer of frozen-thawed embryos. From 2005 to 2009 in the United States more than 37,000 pregnancies resulted from cryopreserved embryos. Although difficult to document precise figures, it can be roughly estimated that many more than 1,000 babies have now been born after fertilization of oocytes cryopreserved by any one of several techniques. More recently, pregnancies and births have resulted from various methods to utilize reproductive tissues, such as ovaries and testes, that have been cryopreserved.

CONCLUSIONS: Thirty-three years after the remarkable report by Steptoe and Edwards of the birth of the first human child by assisted reproduction, millions of other children have been born by application of similar methods. Cryopreservation of reproductive cells and tissues has contributed to the efficiency and success of human ART.

◆Biosketch

Stanley P. Leibo received his Bachelor of Arts degree from Brown University, Master of Science degree from the University of Vermont, and Master of Arts and Doctor of Philosophy degree in Biology from Princeton University. He began his career in cryobiology with Peter Mazur at Oak Ridge National Laboratory in Tennessee. He then became Vice-President of Research and Development at Rio Vista International, a cattle company in San Antonio, Texas. In 1988, he was appointed Associate Professor of Obstetrics and Gynecology and of Urology at Baylor College of Medicine in Houston. From 1991 to 1998, Dr. Leibo was Professor of Biomedical Sciences at the Ontario Veterinary College of the University of Guelph in Ontario, Canada. In 1998, he was named to his present positions as Professor of Biological Sciences at the University of New Orleans, and Senior Scientist at the Audubon Center for Research of Endangered Species in New Orleans.

In addition to his permanent positions, Dr. Leibo has served as a Fellow of the Japan Society for the Promotion of Science at Kyoto University, and for four years was Visiting Professor on the medical faculty at the Dutch-speaking Free University in Brussels, Belgium. He was elected president both of the Society for Cryobiology and of the International Embryo Transfer Society, and was named an Honorary Lifetime Member of the American Embryo Transfer Association. In 2009, Leibo received the Pioneer Award of the International Embryo Transfer Society to recognize his role in the derivation of the original methods to cryopreserve gametes and embryos. He has published more than 135 scientific articles and book chapters, and has delivered more than 180 lectures in the United States and in twenty-nine other countries.

Evaluate the IVF Success Rates of Accumulated Pregnancy Rates per Oocyte Retrieval

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The Center for Clinical Reproductive Medicine, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China, 210029

Objective: To assess the efficiency of oocyte utilization after controlled ovarian stimulation.
Methods: We estimated cumulative pregnancy rate per oocyte retrieval among patients undergoing IVF/ICSI between 2009 and 2010 at the Center for Reproductive Medicine, Nanjing Medical University. Couples were followed-up until either discontinuation for no embryo to transfer or pregnancy with visualization of a fetal heartbeat on ultrasound. Analyses were stratified according to maternal age.
Results: A total of 1,593 oocyte retrievals yielded 16,118 oocytes. Mean age (±SD) was 30.3 (±4.3) years, mean number of transfer per oocyte pickup was 1.2, mean number of embryo transferred was 1.7. The cumulative pregnancy rate per oocyte retrieval was 70.2%, the multiple pregnancy rate was 27.9% and the miscarriage rate was 8.6%. While the same number of embryos transferred, no statistically significant difference was detected between fresh and the FET cycles with respect to pregnancy rate, implantation rate, multiple pregnancy rate and miscarriage rate. Among patients who were older than 35 years of age, the cumulative pregnancy rate was significant decreased ($P<0.05$), and the miscarriage rate was significant increased ($P<0.05$).
Conclusion: During ART cycles, maternal age remain a factor that influence the cumulative pregnancy rate per oocyte retrieval, reducing the number of embryos transferred does not influence the final success rate, while the multiple pregnancy rate was significant lower, this strategy yields a lower pregnancy in a fresh IVF cycle, but the difference is almost completely overcome by an additional frozen embryo transfer cycle.

◆Biosketch

JIAYIN LIU, M.D., Ph. D.

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Vice- director of Group on Gynecologic Endocrinology in Chinese Society of Obstetrics and Gynecology,
Vice- chair of Jiangsu Society of Obstetrics and Gynecology,
Vice- chair of Jiangsu Society of Genetic Medicine.
Vice-chair of Jiangsu Society of Women's Health Care
President of Jiangsu Association of Integrative Medicine on Reproductive Medicine

Genetics and Health of Children Born from Cryopreserved Oocytes

Ilan Tur-Kaspa, MD.
Institute for Human Reproduction (IHR) and the Department of Obstetrics and Gynecology, The University of Chicago, Chicago, IL, USA.

Introduction: The safety of new technologies or treatments in ART is of critical importance to patients and practitioners. Oocyte cryopreservation has major benefits in modern ART: Fertility preservation; circumvention of the ethical, legal and religious implications associated with embryo storage; optimization of egg donation programs; and for embryonic stem cell research. We investigated the safety of the clinical use of oocyte cryo-banking in order to allow proper patient counseling in the decision making process in modern ART.

Materials and Methods: A search of published manuscripts and abstracts on human oocyte cryopreservation was conducted in MEDLINE and other bibliographic databases. The genetics of oocytes, embryos, fetuses, and newborns derived from cryo-thawed human mature (MII) oocytes tested by chromosomal studies were reviewed. In addition, perinatal data and follow-ups outcome of children conceived from such treatment were analyzed.

Results: The cryopreservation process may alter the oocyte spindle. However, in most *survived* oocytes the spindle apparatus has the ability to reform properly upon thawing and to recuperate its functionality. Oocytes were investigated by preimplantation genetic diagnosis (PGD) of the 2nd polar bodies and by karyotyping after fertilization and 90% were normal. The origin of the sperm used to fertilize the thawed oocytes was reported not to affect the clinical outcome. The rate of embryonic aneuploidy, tested by PGD, is within the expected range encountered in embryos derived from fresh oocytes. None of the aneuploidy found showed any repetitive pattern. The number of live births derived from cryo-thawed oocytes has rapidly increased in the last decade. Better post-thawing survival rates and encouraging pregnancy rates have been demonstrated with vitrification as compared with slow freezing, and it became the method of choice worldwide. The rate of congenital anomalies is comparable to naturally and IVF/ICSI conceived children. Post-natal follow-ups reported normal development.

Conclusions: No increase rates of aneuploidy or malformations were reported, and normal development was found in post-natal follow-up in children born from cryo-thawed oocytes. While this data reassure the safety of the clinical use of oocyte cryo-banking and is valuable in the decision making process in modern ART, further prospective follow-up of the children is highly recommended.

◆Biosketch

Professor Ilan Tur-Kaspa is the President and Medical Director of the Institute for Human Reproduction (IHR), Director of the Clinical IVF-PGD Program, Reproductive Genetics Institute, and Professor at the Dept of OB-GYN at the University of Chicago. Prof. Tur-Kaspa is one of the world's most experienced Reproductive Specialists involved in advanced IVF-ICSI treatment with PGD. He specializes in advanced ART, reproductive imaging, and PGD. He is the author and co-author of over 90 scientific publications and book chapters. He serves as a Reviewer for the leading Journals in Reproductive Medicine, such as Fertility and Sterility, Human Reproduction Update and Reproductive BioMedicine Online. He is a co-Editor of the book on Biotechnology of Human Reproduction. He was awarded the 2010 Star Award of the American Society for Reproductive Medicine (ASRM) for over 10 years of continuous scientific contributions in the field of Reproductive Medicine.

What has happened on epigenetics in ART children?

Yukiko Katagiri
Toho University, Tokyo, Japan

In these years, an increase in imprinting disorders; such as Beckwith-Wiedemann syndrome (BWS), Angelman syndrome (AS), and Silver Russell syndrome (SRS) in children born after ART. Work on animal models suggests that in vitro culture may be a source of these imprinting errors. Various clinical and experimental studies also suggested alterations of epigenetics of the male gamete, the female gamete, and embryogenesis in vitro culture to compare natural conception. Culture conditions can also make a chance of imprinting alterations. ART has a possibility to change epigenetics with the handling of gametes and early embryos as well as with in vitro culture because imprinted genes are likely to be reprogramming at that times. It is also known that superovulation may effect on DNA methylation. Oogenesis is one of an importance on epigenetics.

It needs to think here whether only ART gives the opportunity when epigenetics changes. There are the backgrounds that increase risks of epigenetic alterations in couples requiring ART. Methylation of spermatozoa in male infertile patients was different from in fertile men. For infertile couples with male factor, in vitro fertilization or sperm injection can be used to assist conception; however, this could result in abnormally methylated sperm fertilizing an oocyte. In late years, with the change of the social background, the women needing infertility treatment has been ageing. We need to know that the expression of some genes was altered in ageing oocytes.

Though BWS and SRS are growth disorder diseases, some imprinted genes are associated with fetal growth. In ART cases, imprinted gene expression and DNA methylation with human placental tissue were different from neonates conceived naturally. It also has been known that epigenetic disorders are associated with some adult diseases. The early life events in the development are very important for long-term health in their future. ART was spread out very much in worldwide. However, there is not yet often reverse and others about the long-term health of next generation. It is important epigenetic study and follow-up study in ART children.

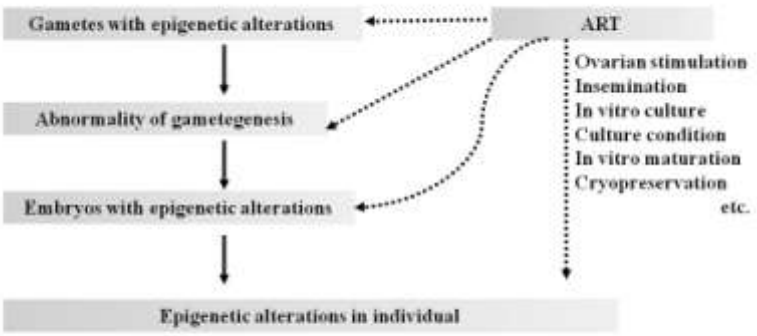

Figure. The possibility that ART has an influence on epigenetics

◆Biosketch

Dr. Yukiko Katagiri obtained her specialty in Gynecology and Obstetrics and in Human Reproduction and Genetics from Toho University. From 2001 to 2004, she had been in a research program in Cornell Institute for Reproductive Medicine, Weil Medical College of Cornell University leaded by Dr. Gianpiero D Palermo. She is Associate Professor in Department of Obstetrics and Gynecology, Toho University and Chief of the Reproduction Center for women at Toho medical center, Omori Hospital in Tokyo, Japan. She is also Director in Department of Clinical inheritance, Omori Hospital.

Pregnancy and Child Outcome after Assisted Reproductive Technologies

Dov Feldberg
Helen Schneider Hospital for Women, Rabin Medical Center,
Jabotinsky St., Petah Tiqva, Israel

INTRODUCTION: One of the greatest achievements in the medical profession in the previous century was the delivery of Louise Brown in 1978 and the first publication of Edwards and Steptoe in Lancet that year. The second most important breakthrough in the field of Assisted Reproduction was the introduction by Palermo of Intracytoplasmic Sperm Injection (ICSI) procedure in order to solve the problem of severe Male Factor Infertility. Until now about 4 millions of children were born from these novel technologies and the major question that troubles the scientific world is Are ART Children healthy as their "brothers and sisters" that were born from natural conception?

MATERIALS AND METHODS: The prevalence of major congenital malformations including imprinted gene defects was investigated in ART children compared with natural conception neonates that were treated and born in our Hospital for Women between the years 1986-2006. We investigated as well all the reports on major congenital malformations in ART children, compared to natural conception born children in the whole state of Israel between the years 1997-2004. Our department is a part of Israel Birth Defects Monitoring System and a part of the International Center for Birth Defects in Rome. We investigated the impact of the use of IMSI (Intracytoplasmic Morphologically Selected Sperm Injection) sperm selection technolopgy in severe male factor infertility cases on the early abortion rate and prevalence of major congenital malformations in ART neonates. All the global relevant literature concerning this crucial issue of congenital malformations and ART was surveyed and extrapolated.

RESULTS: According to the results from our Hospital for Women, the Israeli Registry and the global literature, the incidence of congenital malformations in ART children is nearly double as compared to natural conception group. We could not present any specific malformations among the ART neonates and there was no discrepancy in congenital malformation rate between standard IVF and ICSI micromanipulation children. We found a possible linkage between ART children and increasing risk of Angelman and Beckwith-Wiedeman Syndromes that are associated with imprinted gene clusters. We present for the first time a significant improvement in neonatal outcome in terms of early abortion and congenital malformations rates in IMSI children as compared to regular ICSI neonates.

CONCLUSIONS: There is no doubt that neonates of ART are at increased risk for major congenital malformations including imprinted gene defects, as compared to natural conception neonates. No specific congenital malformation was documented in ART children. No discrepancy was presented between standard IVF and ICSI children. IMSI technology seems to reduce significantly the early abortion and congenital malformation rates. This information should be presented to couples seeking ART treatment.

◆Biosketch

Born in Vilnius, Lithuania, 1947. Married with 2 children. Graduated Medical School of Hadassah and Jerusalem University in 1971. Fellowship in Reproductive Medicine in 1984-1985 at the Yale New Haven Hospital with Prof. Alan Decherney and Prof. Fred Naftolin. In 1985 establishes the fourth IVF Unit in Israel, at the Rabin Medical Center. Sabbatical in Reproductive Medicine in 1990-1991 at the Center for Reproductive Medicine, Cornell University Hospital, New York, with Prof. Zev Rosenwaks. In 1993 receives Professor degree at the division of OB/GYN Medical School at Tel-Aviv University. In 1994 appointed Vice Chairman of OB/GYN Division, Rabin Medical Center. In 2001 appointed Acting Chairman OB/GYN Division, Rabin Medical Center. From 2008 Executive Vice President of the World Association of

Reproductive Medicine (WARM). About 120 publications in various journals mainly in the field of Reproductive Medicine. Several chapters in books and organization of National and International meetings.

Just Twis? Perinatal data from multiple pregnancies

Peter Brockerhoff
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Johannes Gutenberg-University Mainz, Germany

With the expansion of reproductive medicine worldwide the rates of multiple pregnancies have considerably increased during the last decades. The rates of multiple pregnancies after artificial reproduction differ enormously between the European countries. By data from literature as well as by an own analysis of the nations perinatal database it can clearly be demonstrated that twin pregnancies compared to singleton pregnancies carry increased risks like pre-eclampsia, diabetes mellitus or perinatal haemorrhage and increased fetal risks like prematurity, intrauterine growths retardation and higher perinatal morbidity and mortality. In higher multiple pregnancies these risks occur with dramatically higher incidence rates. Further collection of perinatal data from pregnancies after IVF or ICSI is on demand. Reproductive methods therefore, which lead to higher rates of multiple pregnancies should be banned. Selective Single-Embryo-Transfer (eSET) should be legalized also in those countries which still forbid this method by their embryonic protection laws.

◆Biosketch

Peter G. Brockerhoff , M.D.

29th of October 1948: born at Oldenburg, Germany

1955 – 1968	School education at Bremen
1968 – 1973	University of Heidelberg Faculty of Medicine and Faculty of Chemistry Scholarship by the Students' Foundation of the German People
1971	Exchange student at Osaka/Nishinomiya , Japan (sponsored by LIONS Club)
1972	Exchange student at Tyler , Texas, USA
1973	Doctor's degree by the University of Heidelberg
1973	Marriage to Claudia Trommsdorff , High School Teacher
1974	Junior Doctor at Deptm. of Surgery University of Mannheim, Germany
1975 – 1984	Assistant Doctor of the Deptm. of. Obstetrics and Gynecology, Univ.-Mainz
1985 – 1993	Associate Professor and Consultant
Since 1994	Full Professor and Vice-Chairman Deptm. of Obstetrics and Genecology, Univ.of Mainz, Germany

Board Member of the Medical Faculty of the University of Mainz
Board Member of the Senat Council for Education of the University of Mainz
Board Member of the Senat Board for Internationalisation University of Mainz
Member of the State Council for Medical Quality Control
Member of the Medical Association's Ethic Board

Perinatal Outcomes of ART Pregnancies

Fethi Zhioua
IVF Center, Aziza Othmana hospital,
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In-vitro fertilisation has been done for nearly 30 years; in developed countries at least 1-2% of births are from assisted reproductive therapy (ART). Perinatal outcomes, such as preterm delivery, low birth weight and some obstetric complications seems to be increased after in vitro fertilisation (IVF) compared with spontaneously conceived pregnancies (J.Hallyday.2004, Fujii.2010). But, it is difficult to obtain accurate outcome information because of unmeasured confounders parameters. Besides, some of the morbidity associated with ART does not result from the techniques but from the underlying health risks of being subfertile (Et.Reefhuis, 2009). So it is unknown whether IVF technologies or patient infertility is the major contributor to adverse outcomes. Further, much of the amplified risk associated with ART is related to high birth order. Multiple births continue to be the major risk of couples needing fertility treatment. Percent countries range from 25% to nearly 50%, with the rate of high order pregnancies as much as 40%.

The risk of birth defect after ART has been also discussed extensively in published studies. The subject remains of concern, especially since the introduction of ICSI, with microinjection of single spermatozoa. The overall prevalence of birth defects in the general population is approximately 4 %, with children born following IVF/ICSI having a 25 – 40% higher risk of birth defects than children conceived spontaneously. Further large population-based study are needed to explain specific risks.

There are many important deliberations for couples when considering the success of achieving a live birth and the adverse outcomes potentially associated with ART. The most accurate and up-to-date information possible should be given to them, along with counselling to help with understanding of the potential adverse outcomes. Only then can couples make the choice about IVF that may be best for them.

◆Biosketch

PRESENT ADDRESS:
IVF Center. Hopsital of Aziza Othmana, Place de la kasbah Tunis, Tunisia

EDUCATION AND TRAINING:
Presidency of the department of gynecology and obstetrics (2004 - 2005)
Professorate degree in gynecology obstetrics: University of medicine Tunis 1992
Medical doctorate in gynecology obstetrics: University of medicine Tunis 1986

PROFESSONAL ACTIVITIES:
Several national positions including:
Presidency of the National society of gynecology and obstetrics (2008-2011)
Member of the National Committee of reproduction (2005 - 2011)
Counselor of the Minister in gynecology and obstetrics (2004-2011)
Member of ESHRE since 1992
Member of ASRM since 1998

AREAS OF INTEREST:
Gynecology and Obstetrics include infertility, high-risk pregnancy and gynecologist surgery
Biomedical ethics
Aneuploidy screening and PGD

Outcome of IVM babies

Ri-Cheng Chian
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Introduction: Initial oocyte in vitro maturation (IVM) treatment was performed for women infertility with polycystic ovary syndrome (PCOS), and then IVM technology was expanded to treat the over responders and the poor responders for gonadotropin stimulation. It has been estimated that more than 2,000 babies born with IVM treatment in the worldwide, and the clinical pregnancy and implantation rates were approximately 30-35% and 10-15% per embryo transfer, respectively (Chian et al., 2004). Although it has been believed that IVM treatment is not associated with any additional risk in terms of obstetric outcomes and congenital abnormalities (Buckett et al., 2007), it was based on a small number of live births at one fertility center. In this study, we performed a large scale survey for IVM babies born in the worldwide.

Methods: Data were collected from the worldwide with where IVM babies born. Congenital abnormality, gestational age, birth weight, Apgar scores, cord pH, growth restriction, pregnancy complications, mode of delivery, and multiple pregnancies were analyzed.

Results: A total of 1,421 IVM babies born world widely were analyzed. The data showed that congenital abnormality, Apgar scores, cord pH, growth restriction, and pregnancy complications were comparable with the control database.

Conclusions: Based on the data collected, it seems that IVM treatment is not associated with any additional risk in terms of congenital abnormalities for the IVM babies.

◆Biosketch

Currently, Dr. Chian is Professor (rank as Associate Professor with Tenure) at Division of Reproductive Biology, Department of Obstetrics and Gynecology, McGill University, Montreal, Canada.

Dr. Chian is the key person developed in vitro maturation (IVM) of human oocytes for clinical application and responsible for cryopreservation of human oocytes produced pregnancies and live births at McGill University Health Center (MUHC) in Canada. Dr. Chian published numerous research papers and presentations in refereed journals and international/regional conference. Dr. Chian edited two books, and contributed many book chapters. As a guest or invited speaker, Dr. Chian delivered numerous lectures in different societies and countries.

Dr. Chian was former Associate Editor for the journal, Human Reproduction and is an Editorial Board Member for Journal of Assisted Reproduction and Genetics and other two journals. Dr. Chian is acting as a reviewer for many other scientific journals, including Fertility and Sterility, Reproduction, Biology of Reproduction, Reproductive BioMedicine Online, Endocrinology, The Journal of Clinical Endocrinology & Metabolism, and The Lancet, etc.

Dr. Chian's research interests are focused on 1) Ovarian function; 2) The mechanism of oocyte maturation and activation; 3) Fertility cryopreservation.

Dr. Chian speaks English, Chinese, Korean and Japanese as well as some knowledge of French.

Perinatal outcome of vitrified human blastocysts in 11 years experience (5434 attempted cycles) including the rate of monozygotic twinning (MZT)

Tetsunori Mukaida[1], Chikahiro Oka[2], Tetsuya Goto[2], Katsuhiko Takahashi[1]
[1]Hiroshima HART Clinic, [2]Tokyo HART Clinic

Introduction: Vitrification has been recognized as a useful method for cryopreservation of human blastocysts (BLs). However, a possible drawback of vitrification could be the use of a relatively high concentration of cryoprotectants, which may affect the embryo and subsequent development. Also, increased incidences of MZT related to BL transfer including vitrified BL compared with natural conceptions have been reported. Therefore, we analyzed reproductive outcomes and perinatal incidences after 13 weeks of gestation in infants born after vitrified BL transfer.

Materials and Methods: Clinical and perinatal outcome of vitrified BL transfer was summarized between Jan. 2000 and Dec. 2010. Mean age was 36.4 years. Procedures of vitrification were carried out at 37^0C containing 7.5% DMSO and 7.5% ethylene glycol (EG) and 15% DMSO, 15% EG, 1% Ficoll 70 and 0.65 M sucrose. For cooling, the BLs were loaded on a small nylon loop and plunged directly into LN_2. Warming was performed by placing the tip of the cryoloop into 0.5 M sucrose for 2 min and then 0.25 M sucrose for 3 min. For expanded BLs, the blastocoelic cavity was artificially collapsed prior to vitrification, and zona hatching was performed after warming with laser pulses Survival was assessed based on the morphological integrity and re-expansion of the blastocoele. Embryo transfer was performed 2-3 hours after warming. The uterine endometrium was prepared with exogenous E_2 and P_4. Pregnancy was defined as the presence of gestational sac(s) (GS), and MZT was defined as two fetus with fetal heart beat in the same GS.

Results: A total of 8960 vitrified BLs from 5434 cycles were warmed and 8432 survived (94.1%). Transfer was cancelled in 87 cycles (1.8%), since warmed BLs did not survive or were not suitable for transfer. In 5437 transfers, 2568 cycles resulted in pregnancy (48.0%), and 686 cycles (26.7%) ended with miscarriage. Seven thousand, five hundred and thirty two vitrified BLs were transferred and 2882 were implanted (38.3%). Mean number of BLs transferred per cycle was 1.41. Two hundred two (13.6%) were multiple pregnancies. Seventeen hundred and seventy one children were born in 1553 deliveries. Forty one clinical pregnancies were confirmed as MZT (2.6%). Cesarean section was performed in 804 deliveries, and mean gestational age was 38.4 weeks. No bias in the sex ratio was observed with 909 boys and 862 girls. Mean birth weight was 2819g. Thirty two babies in 32 deliveries had either congenital birth defects or perinatal complication (1.9%), including six chromosomal abnormalities (one 18 trisomy, three 21 trisomy), three multiple anomaly, one death due to hydropus, four stillbirth of unknown cause (25, 29, 30, 37 weeks of gestation), one anencephaly, one spina bifida, nine congenital heart malformation and three minor anomaly in hands and/or foot, one congenital esophageal, and one biliary duct obstruction, one Cornelia De Lange syndrome (CdLS), and one Treacher Collins syndrome.

Conclusion: Clinical results showing above section as well as the incidence rate of MZT (2.6%) and rate of congenital defect and neonatal complication (1.9%) were both similar to fresh BL transfer as we reported previously (2.3% & 2.0%) confirm that vitrification of BLs using the cryoloop technique are effective, practical, and confirming the safety of our procedure. Also, the high implantation rate encourages us to establish single BL transfer.

◆Biosketch

He completed his board certified OB&GYN training in OB&GYN dept. at Kochi Medical School and Hospital, Japan in 1990. During that training, he studied abroad to the Univ. of Miami, School of Medicine, in the Biochemistry and Molecular Biology Dept. After the training in Japan, he became a lab director in a private fertility clinic, the Diamond Institute for Infertility in New Jersey, USA until 1995. For the last 15 years, he became co-director of the Hiroshima HART Clinic. His main interest is vitrification for gametes and embryos: he is one of the pioneers for vitrification including reports of successful birth of day 2-3 vitrified embryos with straw (1998), and the vitrified blastocysts using a cryoloop (2000). His original video for vitrification was received the prize in Video: ART Category in 61st ASRM. His abstract of perinatal outcome in vitrified BL program in 11 years experiences was pre-selected for the Clinical Science Award for oral presentation at the 2010 ESHRE in Rome.

Novel embryo culturing and monitoring systems

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In vitro culture of mammalian embryos may be remarkably successful when the embryos are kept in relatively large groups during the whole culture period. Although preimplantation stage embryos are autonomous organisms, their developmental competence is considerably increased by factors secreted by other embryos cultured in close vicinity. The selection of the best embryo(s) for transfer is of utmost importance, to achieve high pregnancy rates and to avoid multiple pregnancies. Most selection methods require individual culture of embryos. Very recently, systems have been developed to meet both, seemingly conflicting requirements. The group or communal effect can be mimicked by culturing embryos in small amount of media. In these systems paracrine effects may be replaced by autocrine ones, ligands produced by a single embryo may stimulate the embryo proper. Another possible benefit of decreasing the amount of culture media is that the embryo may establish and maintain a microenvironment, a mixture of physical, chemical and biological factors that support appropriately metabolism and development. A similar situation may occur in the virtual space of the oviduct; however, the high medium / embryo rate of most in vitro culture systems may require disproportional effort from embryos to accommodate continuously to the suboptimal conditions that are artificially provided. Microwells produced on the bottom of the wells of the culture dishes (well of the well, WOW system) may provide an appropriate solution. The quantity of medium in the well is minimal, allowing accumulation of autcrine ligands and establishment of the appropriate microenvironment. On the other hand, the free contact with a large amount of medium outside the microwell allows exchange of components by slow diffusion, hampers accumulation of potentially dangerous end products of metabolism, and ensures supply of nutrients. Application of such micro wells has considerably improved in vitro development of both zona-intact and zona-free bovine and porcine embryos, human zona intact, in vitro produced embryos, and increased pregnancy rates after transfer. Versions of the WOW system can also be applied for monitoring embryo metabolism as well as morphological features of individual embryos during the whole period of development. With proper arrangement of wells, a single objective can monitor 9 or more embryos in a single field of view. Recently, a compact time-lapse microscope / camera system (Primo Vision; Cryo-Innovation, Budapest, Hungary) has been developed to exploit this possibility, while avoiding the usual complicated structure and potential harmful effects of time-lapse instruments. Experiments performed in mouse have proved the value of the system, the time of the first and second cleavage strongly indicates the probability of blastocyst development, while occurrence of fragmentation - even if completely reabsorbed in hours - markedly decrease the developmental competence. This system has resulted in the first baby in the world born after time-lapse investigation, followed with hundreds worldwide. The combination of WOW and compact time-lapse system offers new perspectives for improvement and proper monitoring of cultured embryos.

◆Biosketch

Artur Bernard obtained his MD degree (1969) specializing in Obstetrics and Gynecology (1974) and earned a PhD degree in human reproduction (1991) in Hungary. In 1978-79 he spent more than one year as a Lalor Fellow at Washington University, St. Louis, Mo. USA. Between 1987 and 1990 he worked in Germany, one year in the IVF Team of the University Erlangen, and later in Dusseldorf. 1993 he has been appointed as Visiting Associate Professor in the Dep. of Ob/Gyn. at the Albert Einstein College of Medicine, Bronx, New York. Since 1992 he has served as the scientific director of the Kaali Institute IVF Center in Hungary.

Effects of cumulus cells removal after 6 h co-incubation of gametes on the outcomes of human IVF

Guoning Huang
Chongqing Reproductive and Genetics Institute

OBJECTIVE: To investigate the effects of cumulus cells removal after 6 h co-incubation of gametes on the fertilization, polyspermy, multinucleation and clinical pregnancy rates in human IVF.

MATERIAL AND METHODS: A total of 1200 IVF-ET cycles undergoing 6 h co-incubation of gametes in 2009 were included in this study. Inclusion criteria were: female age younger than 38 years, first IVF treatment, with bi-ovary and normal ovarian response, e.g., 4~20 oocytes can be obtained. A 6 h period of co-incubation was applied in all IVF cycles. According to the history of infertility, cumulus cells were mechanically removed either 6 h post-insemination or 20 h post-insemination. For couples with primary infertility, or unexplained infertility, or mild oligospermia or asthenospermia, the cumulus cells were removed at 6 h of insemination for the polar body observation (6 h group, n=565). Of which, 80 cycles received early rescue ICSI due to fertilization failure or low fertilization rate at 6 h of insemination. For couples with secondary infertility and normal semen analysis, the cumulus cells were removed at 20 h of insemination as routine (20 h group, n=635). Of which, 3 cycles received late rescue ICSI due to fertilization failure at 20 h of insemination. Fertilization, polyspermy (more than 3PN), multinucleation and clinical pregnancy rates were compared between the two groups (rescue ICSI cycles were not included in the comparison in both groups).

RESULTS: Significant difference (Pless than 0.05) was observed between the two groups regarding polyspermy rates (7.48% in 6 h group and 9.22% in 20 h group). No difference was observed between the two groups regarding normal fertilization rates (2PN rate) (64.89% in 6 h group and 65.74% in 20 h group). No difference was observed between the two groups regarding multinucleation and clinical pregnancy rates (11.01% and 65.15% in 6 h group, 10.75% and 66.93% in 20 h group, respectively). The clinical pregnancy rate was 51.43% in cycles receiving early rescue ICSI, while no clinical pregnancy was obtained in cycles receiving late rescue ICSI.

CONCLUSION: The present results indicate that cumulus cells removal at 6 h of insemination is a relatively safe operation, which yielded comparable normal fertilization rate, multinucleation and clinical pregnancy rates compared with 20 h group. This protocol may be beneficial for early obsevation of fertilization failure and make early rescue ICSI possible.

◆Biosketch

Guoning Huang is the professor and director of Chongqing Reproductive and Genetic Institute in Chongqing Obstetrics and Gynecology Hospital. He is the Secretary-General of Chinese Society of Reproductive Medicine.

An integral view on therapeutic options and lab-techniques for individualized therapy in IVF

Nicolas Zech, M.D., PhD
IVF Center Prof. Zech - Bregenz

In the early days of IVF the consultation of patients was a rather easy task with only limited options available. Nowadays, there are various – sometimes very complex - treatment modalities available to improve the individualized IVF outcome of which all should be discussed with the couple.
This presentation will give an overview on new treatment modalities and lab-techniques which continuously evolve with sometimes radical changes in the approach on how patients are being consulted and treated as well as the challenges and complexities we face as a team to guarantee the highest individualized chance for a safe conception.

◆Biosketch

Univ. Doz. Dr. med. Nicolas Zech studied medicine at the Medical University Innsbruck, Austria. His research interests have focused on stem cells since completing his thesis on expansion of mobilized peripheral blood progenitor cells. At the IVF Centers Prof. Zech he focused on in-vitro culture, embryology and micromanipulation techniques. He has been trained as Obstetrician and Gynecologist at the University Hospital Zurich, Switzerland, and has finished his specialty in OB/GYN in 2005. Nicolas holds a Diploma for Genetics from the Austria Society for Medical Doctors. He was for 2 years on a research fellowship at the Reproductive Genetics Institute in Chicago (Yury Verlinsky – Pioneer of Preimplantation Genetic Diagnosis), USA. Habilitation (Venia docendi) in Obstetrics, Gynaecology, Reproductive Medicine and Endocrinology at the Medical University Graz 01/2010 - Topic: Stem Cells and Assisted Reproductive Technologies - Title: Characterization, isolation/ establishment, culture and storage of clinical grade stem cells with input from assisted reproductive technologies. Nicolas joined the Chief Executive Program at Harvard Business School (HBS) in August 2008. He is a Harvard Business School Alumnus (Owner-President-Management Program - OPM41) since March 2011. He was winner of HBS-OPM41 Idol in 2009. He is Medical Director at the IVF Center Prof. Zech - Bregenz.

How to manage the modern IVF laboratory

Zsolt Peter Nagy
Reproductive Biology Associates

Introduction. Managing appropriately a contemporary IVF laboratory is one of the most complex and challenging work, that is critically important to achieve success in assisted reproduction treatment. The objective of the present submission is to provide a review of the most important contributing factors to manage an IVF Laboratory successfully. Laboratory design is the cornerstone of a well functioning laboratory, usually it can be considered when setting up a new laboratory or when moving to a new space. It is critical to assign a sufficiently large enough space (relative to the expected work volume) that provides conditions to a seamless flow of procedures. High quality of materials with easy to clean surfaces are required to build out the space. HVAC system providing high pressure of clean air of all time is a principal condition. Automated gas systems (providing N2, CO2 and gas mix/triple gas) with highest purity gases are essential, with alarms and sufficient back up. There are plenty of choices of different instruments, most importantly, microscopes, micro-manipulators and incubators, important that those chosen are high quality and reliable. Incubators should be able to provide conditions to low O2 culture conditions. Disposables that are used in the IVF lab should be specifically manufactured to fulfill related requirements and be approved for routine use. Another most critical component of the laboratory work is the culture system, the choice of culture medium, protein source and oil. The embryo culture system should be established in a way to provide conditions for successful extended culture as well. Those items used in the IVF lab should be obtained from trusted sources but independently, they should be tested before regular use to verify appropriateness. Quality control and quality assurance systems must be in place and should be used rigorously to verify for appropriateness all components of the laboratory, including daily checks on all critical components of the lab. Laboratory personal has to be appropriately trained having both adequate theoretical knowledge and procedural skills. Regular evaluation on individual performance is critical, as well as continuing update on knowledge and skills. Team work within the laboratory and with clinicians as well with other supportive staff is essential. Participation in proficiency testing and laboratory inspection by independent third party organizations is highly recommended.

Conclusions. There are several hundreds of variables that are constantly influencing laboratory performance, thus to manage it successfully it requires a well established system and well trained, knowledgeable professionals.

◆Biosketch

Dr. Nagy obtained his MD (1986) and his Ob&Gyn degrees (1996) at the Semmelweis Medical University in Budapest. His PhD was granted at the Free University of Brussels in 1997, where he worked in the team that developed the ICSI procedure. More recently, he has contributed to the development of a highly efficient oocyte cryopreservation procedure that gave bases to establishing the first donor cryo-bank in USA. He is author of more than 150 publications and book chapters. Dr. Nagy serves on the board of several different societies; as well he is Associate Editor for Human Reproduction and RBMonline. Currently, Dr. Nagy is the Scientific and Laboratory Director at Reproductive Biology Associates, Atlanta, USA.

Chemical substances exposure on IVF-ET environment

Tsunehisa Makino
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Introduction: Very little has been discussed on chemical substances exposure on ART environment, although recent estimates have indicated that more than 50,000,000 chemicals exist in the global environment.

Materials and Methods: Several candidate chemicals were selected from lists of chemicals previously reported to have the potential or capability of inducing possible health risks and/or congenital disorders. Sensitive and specific quantitation methods have been developed for the measurements of these chemicals, which included phthalates (DEHP,MEHP) and perfluorinated compounds (PFCs), polybrominated diphenyl ethers (PBDEs), nonylphenol (NP), bisphenol A (BPA), nicotine and its metabolites, organophosphate and pyrethroidal insecticides, and heavy metals. Peripheral blood and urine samples from mothers, cord blood, amniotic fluid, and breast milk samples were collected without manual contamination and the levels of the selected chemicals in these various samples were measured. After establishing reference standards for chemical contamination on human specimens, especially during the perinatal period, we investigated how these endogenous amounts of chemical substances might induce health hazards in the next generation by analyzing epigenetic alterations in mouse embryonic stem cells (ESCs) and in part in human induced pluripotent stem cells (iPS). We also analyzed the levels of DEHP,MEHP,PBDEs, Np, BPA,PFCs in culture media used for assisted reproductive technology (ART).

Results and Discussion: Though the genetic bases of physiological variations as well as those of many pathologic abnormalities are rapidly being elucidated, hereditary diseases and congenital malformations are still major concern in mordern obstetrics. In the present study, the concentrations of chemical substances in the fetomaternal eivironment, as detected using newly developed and very sensitive and specific chromatographic and spectrometric asay systems, were relatively low (approxomately 1 - 10 ppb) and were far from the pharmacologic doses utilized in teratogenic or toxic studies in animals. However we demonstrated that 0.1ppb of DEP (diethyl phosphate, a metabolite of organophosphate insecticides), cotinine (a metabolite of nicotine), mercury (Hg), selenium (Se) or S-421 (octachlorodipropyl ether, a synergist of insecticide) and 1 ppb level of MEHP and deca BDE (brominated diphenyl ether) are capable of causing significant epigenetic changes. The selection of an appropriate medium for each step in gamete preparation and zygote cleavage is widely known to have a crucial impact on the success of implantation during in vitro fertilization. We investigated varous kinds of IVF media, including sperm and oocyte buffers and media, culture oil, fertilization and cleavage media and blastocyst media, and detected 10 - 100 times higher levels of MEHP and PBDE in some groups, indicating that some IVF media are highly contaminated by these substances.

Conclusion: Several environmental chemical substances at doses detectable in fetomaternal specimens and IVF media can induce significant alterations in the epigenetic profile. The present study also showed that our method using mouse ESCs and human iPS cells to assess epimutagens is simple and sufficiently sensitive to evaluate the toxic and/or teratogic influences on environmental chemical substances. (This study was supported by a Health Science Reserch Grant from the Ministry of Health, Labour and Welfare, Japan)

◆Biosketch

Dr. Tsunehisa Makino was graduated from School of Medicine, Keio University, Japan, 1964. Dr. Makino has been a postdoctoral fellow at The Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School between 1970-1973 and was Assistant Professor of the Department of Obstetrics and Gynecology, Harvard Medical School in 1973. Dr Makino promoted his position to Professor and Chairman, Department of Obstetrics and Gynecology, Tokai University Hospital, Japan, in 1995. Dr. Makino was chairman of the Executive Board Meeting of the Japan Society for Immunology of Reproduction (JSIR), also the president of IVth Conference of the Pacific Rim Society of Fertility and Sterility, held in Okinawa, and IXth International Congress of Reproductive Immunology held at Hakone, Japan, in 2004. Dr. Makino has been the director of Tohbu Hospital, At Gotemba City, Shizuoka, Japan, since 2005.

Recent insights into the oocyte maturation in ART

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College of Medicine, CHA University, Seoul, Korea

Gonadotropin stimulation for human in vitro fertilization-embryo transfer (IVF-ET) program has been used to retrieve multiple numbers of mature oocytes, subsequently leading to increased number of embryos to be transferred and the pregnancy rate. However, this regime has potential disadvantages such as ovarian hyperstimulation syndrome, especially in women suffering from polycystic ovarian syndrome (PCOS). In vitro maturation (IVM) followed by IVF (IVM-IVF program) could be an alternative to bypass disadvantages of conventional IVF and give better chances to achieve pregnancy to patients suffering from infertility. Recent works have clearly showed that unfavorable conditions that could occur during growth and maturation of immature oocytes greatly affect embryo development and further clinical outcome. Thus, age, etiology, menstrual cyclicity of patients could influence maturity and growth as well as the number of retrieved oocytes in ART program. Furthermore, follicle size, oocyte diameter, and hormonal environments have an impact on cytoplasmic maturation of oocytes. With respect to advance of IVM-IVF programs, it is necessary to investigate underlying mechanisms and regulatory factors which contribute to achieving higher efficacy of IVM as well as in vitro culture system. Composition of culture media and other laboratory factors such as hormones, growth factors, energy substrates, and scavenger system have been continuously fine-tuned to improve nuclear and cytoplasmic maturation of immature oocytes in vitro. Increased number of oocytes with good quality for IVF-ET by employing an optimized IVM system would provide practical benefits to patients with PCOS, POF and repeated IVF-failure. Moreover, it could reduce an ethical burden about the disposal of retrieved oocytes with low developmental competence. Current knowledge and achievements in IVM of human oocytes for clinical application should be further expanded to enhance clinical outcome in IVM-IVF-ET programs.

◆Biosketch

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EDUCATION:

1969 - 1971 Premedical College, Yonsei University, Seoul, Korea
1971 - 1975 Medical School, Yonsei University, Seoul, Korea
1983 - 1986 Department of Medical Science, The Graduate School, Yonsei University, Seoul, Korea

POSTGRADUATE TRAINING:

1978 - 1979 Intern, Paik Hospital, Inje University, Seoul, Korea
1979 - 1983 Resident, Department of Obstetrics and Gynecology, Severance Hospital, Yonsei University, Seoul, Korea
1988 - 1989 Postdoctoral Fellow, Reproductive Endocrinology and Infertility Department of Obstetrics and Gynecology, Yale University, New Haven C.T., USA

PROFESSIONAL POSITIONS HELD:

2009 - 2011	President, Korean Society of Assisted Reproductive Technologies (KOSAR)
2009 -	Member of Korea National Bioethics Deliberation Committee
2004 - 2008	Chair of Committee Member, Technical Expert of Artificial Fertilization, Korean Bioethics Review Board
2004 - 2008	Chairman of ART Subcommittee, Korean Society of Obstetrics & Gynecology
2004 - 2008	Medical Treatment Consultant, Korean Broadcasting System
1997 - 2008	Professor and Chair, Department of Obstetrics and Gynecology School of Medicine, CHA University
2011 -	Professor and Dean, School of Medicine, CHA University
1990 -	Director, Fertility Center, CHA Gangnam Medical Center
1983 - 1987	Clinical Staff, Department of Obstetrics and Gynecology

AWARDS, HONORS & DISTINCTIONS: 11 times (International and Domestic)
PUBLISHED BOOKS AND CHAPTERS: 10 times (International and Domestic)
MEDICAL JOURNALS: 52 publications (International, SCI journals), 120 publications (Domestic journals)
INVITED FACULTY AT INTERNATIONAL CONFERENCES: More than 25 times

Use of high concentrations of cryoprotectants: is it a justified argument to prefer slow freezing instead of vitrification?

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Introduction

The use of high levels of cryoprotectants (CPs) in solutions applied to vitrify oocytes or embryos is an argument to still prefer slow freezing procedure. Is it a justified argument?

Material and Methods

Cinematographic analysis of the change in volume in relation with different concentration of cryoprotectants

Conclusions

Out of three studies using mice zygotes we may assume:

- The intracellular concentration of CPs is far lower than the one in the vitrification solutions
- The intracellular concentration of CPs in the vitrified zygote is in contrary to the common beliefs even lower than the one observed after a slow freezing procedure
- Survival after slow freezing reflects the presence of an intracellular vitrified state in these cells

◆Biosketch

Pierre Vanderzwalmen, embryologist, is currently working as scientific coordinator at the IVF center of Prof Zech in Austria and in the CHIREC IVF institute of Prof Lejeune in Brussel.

His scientific investigations focused: on the relationship between selection of spermatozoa and embryo selection criteria on the outcome of embryo development, and on vitrification of oocytes and embryos.

Embryo culture: Can we perform better than Nature?

Gábor Vajta[1], Aniko Reichart[2], Laura Rienzi[3]
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Culture of preimplantation stage embryos has always been a key element of laboratory embryology, and contributed substantially to the success of many assisted reproductive procedures. During the past decade, its importance has increased even more, as extended in vitro embryo culture and single blastocyst transfer have become indispensable parts of the approach to decreasing the chances of multiple pregnancies while preserving the overall efficiency of the treatment. Based on the impressive achievements during the past decade there is an increasing, although rarely declared doubt if the present efficiency of in vitro embryo culture can be considerably expanded in the future. The basis of this opinion is that there are natural limits of advancement, and essentially we cannot cross the laws of Nature. However, in spite of the scientific and commercial challenge stimulating research worldwide to optimize embryo culture conditions, a consensus is missing even in the basic principles including composition and exchange of media, the required physical and biological environment, or even the temperature of incubation. Basic elements of embryo culture are derived from somatic cell cultures and have been applied mechanically in embryology, disregarding the special needs of preimplantation embryos. Unfortunately most producers of commercially available media fail to provide the exact composition of their products, hampering further development, as any comparison can only be made between different brand names instead of constituents. In this presentation we summarize the controversies, demonstrate the fragility of some widely accepted dogmas, and try to generate an open-minded debate towards rapid and efficient optimization. New approaches expanding the traditional frames of mammalian embryo culture are also discussed including application of new culture devices (microwells, or microchannel-microfluidic system), the need of selection of the right animal model for testing devices and media, and the optimal balance between undisturbed quiet environment and well-defined stress treatment to improve developmental competence. Authors are confident that substantial improvement may be achieved that may expand considerably the possibilities of future assisted reproduction in humans.

◆Biosketch

Gábor Vajta has obtained M.D. degree (1976) specialty degree (1979) and Ph.D. (1988) in human pathology in Budapest, Hungary. In 1990 he has started to work in mammalian embryology. In 1999, he obtained a DSc degree in Copenhagen, and is now Affiliated Professor of the University of Copenhagen, Honorary Professor of Beijing Institute of Genomics, and Adjunct Professor of James Cook University, Australia. He was author of more than 160 scientific publications and innovator in 5 patents His incubation, embryo culture, cryopreservation and nuclear transfer methods are known and used worldwide. He has led the research group that produced the first cloned animal of Africa as well as that of Scandinavia, and in collaboration with his Chinese and Danish colleagues he contributed in establishment of the first porcine model in the world for the Alzheimer disease. His current work includes propagating/teaching innovative new technologies in the field of domestic animal and human embryology.

Superovulation/IUI - An Alternative to IVF

Bill Yee, M.D., F.A.C.O.G.
Long Beach, CA

Infertility affects 12-15% of couples the United States, but only a very small percentage will seek IVF because of its cost. The cost of an IVF cycle, including medications, range from $12,000 - 15,000 USD. Of the couples undergoing IVF, over 50% of the women have patent tubes and over 75% of the men have motile sperm totaling more than 10 million. Superovulation/IUI, where gonadotropin is used to increase the number of eggs, is not only a viable option but also an attractive financial alternative instead of IVF for these couples. The cost of an IVF cycle is five to six times more than a SO/IUI cycle, which is typically only $2,000 - 3,000 USD. Compared to IVF, the success rate with SO/IUI is only about one-third less.

A typical SO/IUI cycle consists of using 75 to 150 IU of gonadotropin for 8 to 10 days. When two follicles reach a mean diameter of 18-20 mm, hCG is usually given and a single insemination is performed 36 hours afterward. In a series of over 5,500 SO/IUI cycles performed this way, in women less than 35 years old and with more than 10 million motile sperm, the clinical pregnancy rate was 21%.

The success rate was higher in a subset of patients who had two inseminations instead of one and when hCG was given at a much larger follicular size. The pregnancy rate in this group was 29%, compared to 53% in the same age group of women undergoing IVF.

The methodology and the rational for this approach, along with a probable reason that would account for such high success rate with technique will be discussed.

◆Biosketch

Bill Yee, M.D., the Medical Director of Reproductive Partners Medical Group, Inc., started the In Vitro Fertilization (IVF) program at Long Beach Memorial Medical Center in 1986. Dr. Yee is internationally recognized for his expertise in the field of Reproductive Medicine.

His work resulted in the first successful frozen embryo transfer resulting in a healthy baby in the United States. Dr. Yee is well known for his expertise in microsurgical, laparoscopic, and hysteroscopic surgical techniques. He is the lead author of *Transvaginal Sonography in Infertility* and co-authored *Atlas of Gynecological Surgery*. His pioneering IVF work also produced the first frozen embryos in the lowland gorilla.

Recently, he completed a three year term on the Board of Directors of The American Society for Reproductive Medicine (ASRM), the national and international leader in multidisciplinary information, education, advocacy and standards in the field of reproductive medicine.

Dr. Yee also served on the RESOLVE National Board of Directors. RESOLVE is the National Infertility Association, established in 1974, dedicated to providing education, advocacy and support to men and women facing infertility issues.

Dr. Yee's commitment to quality IVF care has led to the positions of President of the Society for Reproductive Technology (SART), Deputy Commissioner of the College of American Pathologists (CAP) for Reproductive Laboratory Accreditation, and Director of the American Society for Reproductive Medicine. Numerous publications have recommended him as one of this country's top doctors, including Ladies' Home Journal, Good Housekeeping, and Top and Best Doctors in America.

He is the longest sitting member on the Executive Council of SART, since 1994. Dr. Yee also confounded the Pacific Rim Society for Fertility and Sterility.

Nurturing the ovaries: Treatment of patients with high FSH with delayed stimulation using low dose gonadotrophins

MKH Leong, AKN Doo
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Hong Kong and the IVF Centre, Hong Kong Sanatorium and Hospital,
Hong Kong

One of the major problems in IVF has been that of patients who are extreme poor responders. These patients are identified by their previous history, by raised FSH, low antral follicle count, low AMH, or a combination of some or all of these. Probably the commonest criteria of diagnosis are low AFC and raised FSH.

FSH levels over 12 or 15 U/L is generally the cut off level as raised. Over these levels, these patients are excluded in any 'normal' IVF studies. They are considered 'abnormal' and this group of patients also commonly face canceling their cycle, and denied an IVF treatment. Most of these patients are older also, and so not to treat them in consideration of IVF outcome amounts to a humiliating decision. In present times, especially in the Western world, these unfortunate couples are usually offered egg donation (sometimes forced) and dissuade from routine IVF. Egg donation has its problems, genetic, social, and psychological, and may not be acceptable to a lot of couples. A better solution has to be found.

We looked at this particular problem, and asked "why not?" - these are cycling patients, most of them quite regularly. Our reasoning is this: if they are cycling, they must produce estrogen sometime during their cycle, and this would be from the granulosa cells lining the follicles. In other words, follicles must develop to make a regular cycle. They are not responsive to especially high doses of FSH in the beginning because their follicles are not ready to respond, the receptors are not ready; and they are producing high doses of FSH by their own Pituitary gland already. But, given the right timing, we should be able to take over from when their own secretion of FSH drops off by adding exogenous FSH to continue follicular maturation. This event starts when a follicle reaches diameter of 10mm and more. FSH and sometimes gonadotrophin antagonists is required because, for these patients, even if respond to natural events, their functioning follicles may, stop growing, or prematurely luteinised if left to themselves.

With this assumption, we started a protocol of delayed stimulation. These patients usually presented with raised FSH (>15U/L) on day 2-3, and typically AFC would be 0 or less than 2. No FSH is given as in routine IVF then, but we would just do US every 3-5 days, depending on findings, until at least one follicle reaches a diameter of over 10mm, and then a low dose FSH of 75-150U sc per day is added. Antagonist is added when follicle diameter is 14mm or over, and egg collection when at least one follicle reaches 17mm. We presented our preliminary data at the ISIVF in Montreal. We termed our treatment nurturing of these follicles to maturity instead of ovarian stimulation.

This presentation deals with our collective data of over 200 cycles by two doctors working in the same clinic and doing IVF in the same IVF embryology laboratory. We will show that in over 95% of cases at least 1 egg was collected which was fertilized. Most of these patients will then participate in our embryo banking service where they will bank these single embryos until at least three, and then replaced as thawed-frozen transfer. Overall, we have a clinical pregnancy rate of over 10% in this very difficult group of patients.

In conclusion we described a novel way of preparing the ovary for a group of patients who otherwise may not be accepted into an IVF program. If they start IVF a lot ends up in cancelled cycles. We have shown that with patience and care, a gentle "nurturing" of the ovary gives better result than super doses.

◆Biosketch

Dr. Milton Leong has been involved in IVF since 1985. He received his medical education at McGill University and after post graduate training also taught at McGill. In 1979 he returned to Hong Kong, and out of demand he started a practice in infertility. In 1985 he set up the IVF Centre, together with Dr. Clement Leung, at the Hong Kong Sanatorium & Hospital and produced Hong Kong's first IVF baby in 1986.

In 2000 he was a leader to set up the Hong Kong Society for Reproductive Medicine, an interest he still maintains. Dr. Leong is still in active practice and contributes to the specialty in publication and lectures. He is a frequent speaker in regional and international meetings with current interest in identifying the three portals of IVF: eggs, embryos and endometrium; and in poor responders and older patients.

Currently he is an adjunct professor of O & G of McGill University and a founder of the ivf-worldwide.com – the website for practicing IVF professionals.

Use of hCG in ovarian stimulation

Johan Smitz, MD PhD.
Research Director, Follicle Biology Laboratory (FOBI)
Faculty of Medicine and Pharmacy, Free University Brussels

Untimed LH increases are associated with reduced chances of ongoing pregnancy. The etiologies of a high endogenous LH might be diverse: they may be part of WHO type 2 anovulation or might be iatrogenically induced by increasing E2 levels (due to stimulation therapy). Observing the relationship between a high basal or a premature rise of LH and reduced success-rates in ART patients has led to the policy that LH should always be banned from the therapeutic arsenal of the reproductive endocrinologist, with as only exception: the WHO-type I anovulation.

In 30 % of the patients having ovarian superovulation treatment using rFSH combined with GnRH agonist therapy leads to an iatrogenic depletion of LH.

It was observed that making use of molecules with a longer halflife (hCG) in Highly Purified Human Menopausal Gonadotrophins (HP-hMG) adds a significant qualitative element to treatment outcome (Diedrich et al., 2001; Andersen et al., 2006; Platteau et al., 2006, 2008). HCG concentrations in patient's serum (after using HP-HMG) on stimulation day 6 or day of the ovulatory stimulus was significantly positively correlated with the occurrence of a higher quantity of top quality embryos, higher pregnancy- and life birth rates. Statistical modeling demonstrated that circulating hCG levels – independent from LH baseline concentrations – positively impact on the pregnancy outcome (Arce and Smitz, 2011). The precise molecular background behind these observations is now under further research.

Besides the differences in clearance there might be differences at the level of molecular interaction between LH and hCG and the LH-receptor (LHR). The way that both molecules (LH or hCG) bind to the receptor might induce a different cascade of molecular events downstream the receptor and/or lead to differences in receptor metabolisation. It is known that a mutation in exon 10 of the LH receptor precludes effects of natural LH, but in contrast hCG remains effective in stimulating the LH receptor. Mechanisms driving the superior results by hCG over LH need to be investigated in relevant physiological models such as follicle cells and endometrial tissue.

◆Biosketch

Professor Johan Smitz (MD, PhD) is currently leading a research laboratory on ovarian and oocyte biology at the Free University Brussels (VUB) and is Head of the Hormone Laboratory of the University Hospital VUB, named UZBrussel. His clinical activity involves endocrine follow-up of infertility patients at the Center For Reproductive Medicine at the UZBrussel.

His interests are in Oocyte Development in Vitro, Reproductive Physiology, culture systems and Clinical Biochemistry.

Follicle stimulation by transdermal application of FSH

Herbert Zech, M.D., Professor
IVF Center Prof. Zech - Bregenz

This report establishes for the first time the effectiveness of transdermal delivery of molecules as large as follicle stimulating hormone (FSH, 32 KDa protein).
We chose IVF as a model to test the transdermal device because a clinical response can be seen within days (by analysis of follicle growth) and the pregnancies testify to the quality of the retrieved oocytes. In the presentation, broader implications for the use of such a transdermal device (e.g.: the transport of other peptides, proteins and vaccines of large molecular size or of hydrophilic nature across the skin) will also be discussed.

◆Biosketch

President of the Austrian Society for Reproductive Medicine and Endocrinology 2003-2006. Since 2007 President of Honor of the Austrian Society for Reproductive Medicine and Endocrinology.
After medical school at the University of Innsbruck, Herbert moved to the University of Graz and Innsbruck for a professional training as a gynaecologist finishing this specialty in 1982. Herbert was always research oriented and moved to the United States for a post doc at the University of Louisville Kentucky working on basic cancer research with steroid receptors and vitamin A-receptors in rodents and in man. From there he went back to the University of Innsbruck and became Head of the Department of Endocrinology. In 1984 he moved to Bregenz and started the first IVF-Clinic realizing the big need for infertility treatment. The first IVF children in southern Germany, Switzerland and western Austria were the results of the initial months of therapy in Bregenz. In order to further develop this technique and the potential for the future he founded the first private clinic for in vitro fertilization in Austria and was also the first to be ISO certified worldwide in the field of reproductive technologies. Realizing the deep desire for children, Herbert founded further IVF-centers in Italy, in Switzerland, in Liechtenstein, in the Czech Republic and in Salzburg, Austria. The total workforce in all centers is 120 and is increasing. In recognition of his ongoing research efforts, Herbert was promoted professor at the University of Innsbruck in 1999, where he teaches reproductive technologies and endocrinology. He is Harvard Business School Alumnus (Owner-President-Management Program – OPM39) since 2010. Herbert brings into the company excellent knowledge of a broad spectrum in endocrinology, genetics, embryology and stem cell research, a global network of medical collaborators, as well as proven entrepreneurial commercial business development experience.

IVF Center Prof. Zech - Bregenz, Römerstr. 2, 6900 Bregenz, Autria

Quality evaluation of human & mouse IVM embryo and epigenetic evaluation of human born IVM babies

Hiroaki Yoshida
Yoshida Ladies Clinic, Center for Reproductive Medicine, Miyagi, Japan

Introduction: IVM oocyte exhibit lower viability and developmental potential after fertilization. We compared the oxygen consumption rate of human IVM and COH embryo with SECM non-invasively. Mitochondria regulate metabolism and influence the developmental potential of oocytes and embryos. We evaluated basic research to clarify for IVM culture problem due to mitochondrial function using mouse IVM oocytes, and also evaluated chromosomal numbers and premature chromosomal separation (PCS) with control and different medium (HTF & Waymouth). There was the report of imprinting disorder with ART patients recently. So we checked the epigenetic disorders for born IVM and natural pregnant babies.

Material and method: The oxygen consumption rate was calculated with SECM system for human oocytes, IVM and COH embryos. We evaluated the number of oocytes, maturation rate and fertilization rate in human IVM cases. And we compared the imprinting genes from umbilical cord of IVM and natural pregnant babies. Basic research was performed using mice GV oocytes. We used different culture medium for oocyte maturation and MII oocytes have done IVM/IVF, after that we evaluated embryo developments and the several mitochondrial function, and also evaluated, chromosomal number count, and PCS.

Results: The oxygen consumption rate for a single oocyte was as follows: GV 0.49, MI 0.47, MII 0.41 F × 1014/mols-1. There were no significant difference in the mean oxygen consumption rate at each embryo in both IVM & COH-IVF embryos (0.26-0.56 × 1014/mols-1). There were mean number of oocyte (10.4±5.3), maturation rate (54.7±18.7) and fertilization rate (85.8±15.4) in delivered cases. There was no significant difference of epigenetic finding in all human IVM and natural pregnant babies. In the basic research, HTF group was observed mitochondrial aggregation from like a spot in mitochondrial staining in mice. There was lower MT membrane potentials in the HTF group than in vivo and Waymouth group. Although there was no significant difference in total chromosomal count, PCS has much increased in Waymouth with small layer group.

Conclusion: There was no significant difference both IVM & COH embryo oxygen consumption rate after fertilization. This means IVM embryo has same mitochondrial activity with COH-IVF embryo. The mitochondrial function and chromosomal count & PCS were influenced from different IVM culture media. Further study of cytoplasmic maturation and mitochondrial function from IVM is required.

◆Biosketch

1988 Assistant Professor : Division of Reproduction Dept. of OB/GYN Tohoku Univ.
1991 After acquired Ph.D. MIAMI School of Medicine Division of Reproductive Medicine Dept. OB/GYN Director of IVF Lab.
1998 Opened Yoshida Ladies Clinic
2005 Division Chief of Center for Reproductive Medicine
2008 Clinical Associate Professor of Tohoku University Dept. of OB/GYN

Lessons from the mouse follicle culture and in vitro maturation models

Johan Smitz, MD PhD.
Research Director, Follicle Biology Laboratory (FOBI)
Faculty of Medicine and Pharmacy, Free University Brussels

Primordial follicles from juvenile mice ovaries can be grown in a petri dish during 3 weeks and produce oocytes capable being fertilised by sperm. Good health of the offspring has now been confirmed on a large series of animals by John Eppig's Laboratory. Making the In-vitro culture technology work for a broad range of follicle classes requires a whole range of different culture techniques.

An unresolved question is how primordial follicles could be induced to grow in an ordered manner, instead of activating massively once they feel in vitro. Following the initial work from A. Hsueh's laboratory who could induce invitro activation of primordial follicles in mouse and human ovarian tissue -which was xeno-transplanted- and developed normally, our research addresses entire in-vitro conditions which could induce controlled growth of prinordial follicles in ovarian tissue.

Work from several teams (Newton et al. (1995) in Sheep, Xu et al. (2009a) in Rhesus monkey , Xu et al. (2009b) in human) have been able to grow secondary ovarian follicles in-vitro up to meiotic competence. By making use of an extracellular matrix (matrigel, alginate) in a basal medium which maintains granulosa cell differentiation (i.e. estrogenic) it is possible to grow these follicles and obtain oocytes with a capacity that is sufficient to be fertilised. However at any time of the cycle there are only a limited number of such secondary follicles present.

The fear that a prolonged culture period could change the imprinting pattern in oocytes during theit growth phase has proven to be unjustified (Anckaert et al., 2009).

As our knowledge advances, the chances of having one day an artificial ovary that could support full follicular growth becomes more realistic.

◆Biosketch

Professor Johan Smitz (MD, PhD) is currently leading a research laboratory on ovarian and oocyte biology at the Free University Brussels (VUB) and is Head of the Hormone Laboratory of the University Hospital VUB, named UZBrussel. His clinical activity involves endocrine follow-up of infertility patients at the Center For Reproductive Medicine at the UZBrussel.

His interests are in Oocyte Development in Vitro, Reproductive Physiology, culture systems and Clinical Biochemistry.

Laboratory aspect of natural cycle IVF/M treatment

Ri-Chen Chian
McGill University, Montreal, Canada

Currently most infertility treatments employ ovulation stimulation with gonadotropins to increase the number of oocytes available for in vitro fertilization (IVF), because it has been believed that the success rate of IVF treatment relates directly to the number of embryos available for transfer. Although the side effects of ovarian stimulation with gonadotropins, including ovarian hyperstimulation syndrome (OHSS), have been well documented, the long-term side effects of repeated ovarian stimulation with protocols involving a gonadotropin-releasing hormone (GnRH) agonist or antagonist in combination with gonadotropins are largely unknown. Immature oocyte retrieval followed by in vitro maturation (IVM) of these oocytes is an attractive infertility treatment for women with infertility. In comparison with ovary stimulated IVF treatment, the major advantages of IVM treatment include avoidance of the risk of OHSS, reduced cost, and simplified treatment. Immature oocyte retrieval followed by IVM of these oocytes was initially shown to be a successful treatment for infertile women with polycystic ovary syndrome (PCOS), because there are numerous antral follicles within the ovaries in this group of patients. Immature oocyte retrieval followed by IVM might be useful in up to approximately 30% of women undergoing IVF treatment who have large numbers of antral follicles including patients with PCOS. In general, clinical pregnancy and implantation rates of IVM treatment have reached approximately 35% and 15%, respectively. Attempts have been made using IVM technology to treat patients with normal menstrual cycle and intact ovaries. As protocols, the patients were given 10,000 IU human chorionic gonadotropin (HCG) 36 h prior oocyte collection in natural cycle when the leading follicle reached to 12-14 mm in diameter. The results indicate that mature oocytes can be retrieved when the leading follicles reached 12-14 mm in diameter after HCG administration. It is important to identify the mature oocytes at egg collection. This new technology has been defined as natural cycle IVF combined with IVM, namely Natural cycle IVF/M, in order to distinguish with traditional concept of IVM treatment. In this lecture, we will discuss laboratory aspects of natural cycle IVF/M treatment in term of 1) Preparation of IVM media; 2) Identification of immature and mature oocytes; 3) In vitro maturation of immature oocytes; 4) Insemination of in vitro matured oocytes; 5) Embryo transfer.

◆Biosketch

Currently, Dr. Chian is Professor (rank as Associate Professor with Tenure) at Division of Reproductive Biology, Department of Obstetrics and Gynecology, McGill University, Montreal, Canada. Dr. Chian is the key person developed in vitro maturation (IVM) of human oocytes for clinical application and responsible for cryopreservation of human oocytes produced pregnancies and live births at McGill University Health Center (MUHC) in Canada. Dr. Chian published numerous research papers and presentations in refereed journals and international/regional conference. Dr. Chian edited two books, and contributed many book chapters. As a guest or invited speaker, Dr. Chian delivered numerous lectures in different societies and countries. Dr. Chian was former Associate Editor for the journal, Human Reproduction and is an Editorial Board Member for Journal of Assisted Reproduction and Genetics and other two journals. Dr. Chian is acting as a reviewer for many other scientific journals, including Fertility and Sterility, Reproduction, Biology of Reproduction, Reproductive BioMedicine Online, Endocrinology, The Journal of Clinical Endocrinology & Metabolism, and The Lancet, etc. Dr. Chian's research interests are focused on 1) Ovarian function; 2) The mechanism of oocyte maturation and activation; 3) Fertility cryopreservation. Dr. Chian speaks English, Chinese, Korean and Japanese as well as some knowledge of French.

Cytoplasmic maturation and mitochondrial activity in human IVM

Yoshiharu Morimoto
IVF Namba Clinic, Osaka, Japan

The treatment for patient of polycystic ovary syndrome (PCO) may be the most important by an increase of patients. At the time male factor infertility was almost solved, the PCO is one of remained diseases that is difficult to manage. In order to achieve ovulation in those patients, it needs ovarian stimulation with more risk for ovarian hyperstimulation syndrome (OHSS). In vitro maturation (IVM) of oocyte procedure would be a best option for PCO treatment in terms of complete prevention of OHSS. Since the technology has been clinically applied, the number of centers undergoing it has increased. Moreover it became to be applied for the normal menstrual cycle cases, poor quality embryo cases and PGD source. However it cannot be said to be alternative for conventional IVF procedure, because the clinical outcome has not been reached to the acceptable extent.

In IVM procedure, the immature oocytes in germinal vesicle or metaphase I stage are cultured with medium, gonadotropins and serum for 24 to 48 hours. About 50 to 60 % of immature oocytes commonly maturate to metaphase II stage. In order to acquire better outcome, it is essential to elucidate maturation process of human oocytes. Oocytes maturate in their nucleus and cytoplasm. The investigation has been concentrated to the nuclear maturation process, but not for cytoplasmic maturation. In the cytoplasmic maturation, mitochondria may have a key role for oocyte maturation and further development for embryogenesis in the organella.

They produce ATP as an energy source for resumption of meiosis, fertilization and cleavage. By our ultrastructural observation of oocytes in maturation process during IVM procedure, mitochondria have showed their unique appearances. In the germinal vesicle stage of unstimulated oocytes, mitochondria were dense and scattered in cytoplasm. In the second metaphase stage, mitochondria increased in number and aggregated in the center of cytoplasm.

We have investigated to express active mitochondria using porcine and human immature oocytes. Active mitochondria were stained by DAB (3,3'-diaminobenzidine tetrahydrochloride) on the ultrathin sections.

The appearance of mitochondrial distribution changes dynamically at each stage of maturation. We have observed it in JC-1 stained oocytes by confocal laser microscope.

In order to estimate viability of maturing oocyte, the cellular respiration during oocyte maturation with a scanning electrochemical microscopy was evaluated.

◆Biosketch

Prof. Yoshiharu Morimoto is currently Chief Executive Officer and Chairman of IVF JAPAN group which includes two big infertility centers of IVF Namba Clinic and IVF Osaka Clinic running 6000 periods of IVF cycles in total. He is also a visiting professor of Pochon CHA University, Saint Marianna University and Kinki University Faculty of Biology-Oriented Science and Technology, and associate professor of Kyoto University and Kansai Medical University. He was graduated from Kansai Medical University in 1977, national board certified and obtained a degree of PhD in 1987. He is active in research area and his main research field is ultrastructure in reproduction and concerns on oocyte maturation mechanism. He is mainly focused on mitochondrial activity. Prof. Morimoto is the president of Japan Society of Assisted Reproduction, former president of Japan Society of Fertilization and Implantation, and the president of the Asia Pacific Initiative on Reproduction (ASPIRE).

Correct embryo selection improves embryo implantation rates in IVM cycles

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Montreal Reproductive Centre, Montreal, Canada[1] and Department of Obstetrics and Gynecology, McGill University, Montreal, Canada[2]

In vitro maturation (IVM) was introduced into clinical practice in the late 1980s. Since then, it has become an increasingly important method in human ART. A successful IVM program provides several advantages including avoidance of ovarian stimulation, reduction of treatment costs, and elimination of the side effects of medication, including the risk of ovarian hyper-stimulation syndrome. Treatment outcomes have improved substantially since we started our IVM program in 1998. In women younger than 35 years of age, our IVM clinical pregnancy rates have been comparable with average IVF clinical pregnancy rates in North America. However, embryo implantation rates have been lower in IVM cycles and similar clinical pregnancy rates have been achieved by transferring a higher number of embryos.

Between 1998 to June of 2003, we performed 279 IVM cycles reaching embryo transfer (ET). Mean female age was 32.7 years, the mean number of embryos transferred was 3.4, resulting in a clinical pregnancy rate of 19.4%, with implantation rate of only 6.9%. Between 2003 July to 2010 December, we performed 502 IVM cycles reaching ET. The mean female age was 32.8 years, and the mean number of embryos transferred was 3.5, resulting in a clinical pregnancy rate of 35.6%, with an implantation rate per embryo of 13.2%. Despite the relative increase, figures were still low compared to IVF results. Since January 2011, contrary to the former period when we essentially used to do cleavage stage embryo transfers, we adopted a policy of preferential blastocyst transfer for IVM patients. Based on 11 IVM cycles, with a mean female age of 31.4 years, and mean number of embryos transferred of 1.8, significantly less embryos transferred than in former periods, we achieved a clinical pregnancy rate/cycle started of 36.4% with a mean embryo implantation rate of 31.8%.

Conclusion: Preferentially extending the culture period to five days and choosing the blastocyst with best morphological characteristics seems to significantly increase embryo implantation rate. This also enables elective single embryo transfer (SET) in IVM cycles with comparable clinical pregnancy rate with IVF as achieved by most IVF centers. Multiple pregnancies can also be avoided with elective SET policy for IVM patients.

◆Biosketch

Dr. Hai Ying Chen obtained his medical degree from Beijing University Medical School followed by a residency in Beijing University First Hospital. He completed Master of Science degree at the Department of Experimental Medicine in McGill University, Montreal, Canada. He continued as a research associate in the Department of Obstetrics and Gynecology, McGill University. He worked as a clinical embryologist at McGill Reproductive Center between 2008 and 2010. Dr. Chen is currently working as the director of in vitro maturation and oocyte vitrification programs of the Montreal Reproductive Center. He has conducted several research projects on IVM and oocyte vitrification. He has authored or co-authored several publications and has been invited to lecture in various international conferences.

IVM Application in PCOS Patients

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Over the next decade, immature oocyte retrieval followed by IVM becomes a widespread treatment for infertile women with PCOS because there are numerous antral follicles within the ovaries in this group of patients. Compared with ovary-stimulated in vitro fertilization (IVF) procedure, the major advantages of IVM include avoidance of the risk of ovarian hyperstimulation syndrome, reduced cost, and simplified treatment. Although good results have been reported by some clinics, in-vitro maturation has not yet become a mainstream fertility treatment. The most important reason for this is the lower chance of a live birth per treatment compared with conventional in-vitro fertilization.

Gonadotropin plays an important role in the regulation of oocyte growth and maturation. In order to mimic the preovulatory luteinizing hormone (LH) surge in spontaneous menstrual cycle, human chorionic gonadotropin (hCG) as a surrogate for LH is administered at a dose of 5000-10000IU at the end of follicular stimulation to trigger the meiosis resumption and nuclear maturation of oocytes in IVF technology. The traditional applications of hCG have proven to be highly successful and valuable tools in the treatment of infertility for over four decades. However, the effect of hCG priming on oocyte maturation and developmental competence in IVM cycle has remained a debatable issue for several years. Chian and colleagues demonstrated that hCG priming could hasten the maturation time of the oocytes in PCOS women. Subsequently, a multicentre study by the same investigators have further supported this finding by reporting pregnancy rates of 30-35% in hCG-treated IVM cycles in patients with PCO and PCOS. However, another studies did not demonstrate any beneficial effect of HCG priming. Although there are many reports of pregnancy rates after IVM with gonadotropin priming, there is little data of clinical controlled randomized study. Therefore, the present controlled randomized study was designed to determine whether hCG priming prior to oocyte aspiration can improve embryonic developmental competence and clinical result generated from IVM oocytes of unstimulated women with PCOS.

Objective: To investigate whether hCG priming prior to oocyte aspiration can improve embryonic developmental competence and clinical result generated from IVM oocytes of unstimulated women with PCOS.

Patients: 82 women with polycystic ovary syndrome (PCOS) undergoing 83 IVM treatment cycles.

Interventions: Each patient was randomly assigned either to be primed with 10000IU hCG or not primed 36-38 hours before oocyte retrieval. After the oocytes had matured in vitro, fertilization and embryo transfer were performed.

Result: The average number of cumulus-oocyte complexs (COCs) recovered were 13.8 and 14.7 in the hCG-primed and non-primed groups respectively ($p>0.05$). The maturation rate of COCs was significantly improved in the hCG-primed group (55.43% versus 42.56%; $p<0.05$). The fertilization and cleavage rates were comparable between the two groups. There was no significant difference in the clinical pregnancy (37.50% vs 48.84%), live birth (22.50% vs 30.23%) and implantation rates (32.86% vs 31.46%) between

hCG-primed and non-primed group. The number of pregnancy loss were 6 (hCG-primed group) and 8(non-primed group) cases respectively,

Conclusion: While a significant improvement of maturation rate of immature oocytes were observed in hCG-primed IVM cycle with PCOS patients, the use of hCG prior to oocytes retrieval does not improve the subsequent embryo developmental competence. Higher rate of pregnancy loss was observed in IVM cycle.

◆Biosketch

Qiao Jie is Chief Physician and head at the Department of Obstetrics and Gynecology of Peking University Third Hospital and is now the president-elect for reproductive medical branch of Chinese medical association. She went to Queen Mary Hosp, the University of Hong Kong as a visiting scholar from 1996 to 1997 and Stanford University Medical Center as Postdoctoral Research Scholar from 2002 to 2003. She specializes in clinical treatment, education and medical researches on female fertility preservation and improvement, as well as the diagnoses and treatment for infertility and reproductive endocrine diseases, the focus of PCOS. She published over 200 articles in medical journals in the field of OB/GYN. Her researches have been funded through variety of grants, includes National Outstanding Youth Fund and Key Technologies R&D Program. She is Treasurer of Asia-Pacific Society for Reproductive Medicine, Board member of AEPCOS Society and Asian Journal of Andrology, Associate Chief Editor of Chinese Version for Fertility and Sterility, Editorial Member for Reproductive Biology and Endocrinology and Seminar Reproductive Medicine. She has received many awards including National Science and Technology Progress Award Ministry of Scientific Progress Award first prize. Qiao becomes to be the head scientist of Major State Basic Research Development Program of China (973) in 2011.

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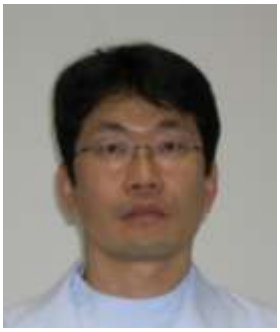

Oocyte maturation from tiny follicles in human ovarian tissues

Shu Hashimoto[1], Yodo Sugishita[2], Nao Suzuki[2], Bunpei Ishizuka[2], Aisaku Fukuda[3], Yoshiharu Morimoto[1]
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Ovarian tissue cryopreservation has been shown to be effective measure for fertility preservation. However, this method is inadequate for freezing of antral follicles. To preserve Immature oocytes in antral follicles, cryopreservation of IVM oocytes is a valid procedure after the culture of immature oocytes retrieved following follicle aspiration or follicle isolation from ovarian tissue excised by laparoscopy. Thus, the combination of in-vitro maturation (IVM) of immature oocytes and oocyte freezing is one of useful measure to preserve the fertility of patients who undergo chemotherapy and/or radiotherapy, and are short of time for controlled ovarian hyperstimulation (COH, Hashimoto et al., in press). After the approval of IRB of St. Marianna University School of Medicine, we started the cryopreservation of mature oocytes and ovaries for patients who were at risk of gonadal dysfunction from cytotoxic chemotherapy or radiotherapy. Thus far, 63 immature oocytes were obtained from 1- to 5-mm follicles of five patients, of which 32 were matured (51%). Thirteen mature oocytes were cryopreserved. The maturation rate was similar to that of oocytes retrieved from approx. 10-mm follicles in routine IVM protocol in which immature oocytes are retrieved from larger follicles because of a technical challenge of low recovery rate from smaller follicles. IVM of immature oocytes following their retrieval has been proposed as a potential alternative to conventional IVF treatment following COH. However, low developmental competence of IVM oocytes compared with that of in vivo matured oocytes prohibits the progress of IVM protocol. To overcome this challenge, several studies have examined the effects of holding oocytes at the germinal vesicle (GV) stage before IVM in cattle and mice, because oocytes might require time to acquire developmental competence during meiotic arrest. This idea is based on the fact that mammalian oocytes are arrested at the diplotene stage until the GnRH surge occurs and immature oocytes resume meiotic maturation spontaneously following the release from follicles. Several groups achieved the improvement of the developmental competence of IVM oocytes in cattle and mice (Hashimoto et al., 2002; Albuz et al., 2010). An adaptation of these new culture systems for clinical application might have significant implications for infertility management. Hashimoto et al., Reprod Med Biol in press. Hashimoto et al., Biol Reprod 2002; 66:1696-1701.Albuz et al., Hum Reprod 2010; 2: 2999-3011.

◆Biosketch

Dr Hashimoto obtained his PhD in Reproductive Physiology at Kyoto University in 2001. He started his research career in 1989 by production of transgenic mice, rat, rabbits and cattle. He also developed assisted reproduction technology (ART) in cattle (super ovulation, transvaginal oocyte retrieval, IVM, IVF, ICSI, embryo culture and vitrification) in Snow Brand Milk Products Co., Ltd. He moved to IVF Namba Clinic in 2004 and started human ART. Currently, he is the research director of IVF Namba Clinic. He was recently secretary-general of the Japan Society of Fertilization and Implantation. He received the JSAR (Japanese Society of Animal Reproduction) innovative technology award in 2008 and JSMOR (Japanese Society of Mammalian Ova Research) outstanding presentation award in 2009.

Natural Cycle IVF combined with IVM

Jin-Ho LIM, MD
Maria Fertility Hospital

IVM has been developed to prevent the side effects of ovarian hyperstimulation, and the efficiency and safety of IVM were already proved.
The clinical pregnancy rate of IVM reached 35-40%/ET, and more than 2000 healthy IVM babies have been born in the world.
But the main indication of IVM was PCOS patients with irregular anovulatory cycles.
To expand the indication of IVM for patients with regular ovulatory cycles, we designed the new procedure what we like to call ' Natural cycle IVF combined with IVM (Natural IVF/M)'.

We check baseline ultrasound on MCD #2-3.
If the number of AFC is more than 7, we recommend Natural IVF/M for patients with regular cycles.
When the leading follicle reaches 12-14mm in diameter, we give 10,000IU of HCG, and collect the oocytes 36-38 hours later.
The matured oocytes collected at the time of OPU are inseminated by ICSI on the same day, and the immature oocytes are cultured in vitro for 24-48 hours.
In vitro matured oocytes are then inseminated by ICSI subsequently.
On day 3 or 4 after OPU, the embryos are pooled together and the best 1-3 embryos are selected for transfer.
We could confirm that the Natural IVF/M together with IVM are an efficient treatment for more than 50% of infertile women with acceptable clinical pregnancy rate and we could do embryo transfer in almost every cases.

◆Biosketch

Dr. Jin-Ho Lim is the founder of Maria Medical Group.
He is an internationally recognized infertility expert and a pioneer in simplifying IVF treatment. He established the Maria Fertility Hospital in 1989 and produced the first natural cycle IVF baby in Asia.
In the end of 2006, more than 30,000 IVF babies had been born in his 9 IVF Centers in Korea, and they are performing more than 10,000 OPU cycles per year for infertile couples.
He also has an IVF center in Beijing, China.
Maria IVF Centers have been offering IVM or Natural cycle IVF/IVM treatment for patients who has more than 7-8 antral follicles at the beginning of menstruation.
In addition, Dr. Lim is the President of Maria Biotech. Institute in Seoul, Korea, where they first generated human embryonic stem cell (HESC) lines in Korea, and the first 3 of them were registered by NIH, the United States.
Dr. Lim has published many original scientific papers and review articles, and delivered many lectures and presentations in national and international conferences.
Dr. Lim's recent research interests include: 1) Simplification of IVF treatment; 2) Human placental stem cells.

The effective approach for IVM-IVF using Metformin

Aisaku Fukuda
The Centre for Reproductive Medicine and Infertility

Introduction: Although IVM-IVF (In vitro maturation, in vitro fertilization and embryo transfer) is a relatively new option for ART with significant benefits for patients, its success rate is still less than conventional ART. First successful IVM-IVF in the world was achieved in 1994, 16 years after first IVF. The present study was conducted to determine if Metformin administration benefited on outcomes of IVM-IVF in PCO patients.

Materials and Methods: We have performed 914 cycles of IVM-IVF (571 fresh and 343 frozen cycles) and have achieved 122 successful pregnancies (23%) from Oct. 1999 through Mar. 2011, since our first success in Japan. When we divide the last 12 years into two terms (term 1: 2000-2005 and term 2: 2006-2011) and compare them, the pregnancy rates of term 2; total, fresh and frozen (33.8%, 35.6% and 30.6%) was significantly higher ($P<0.01$) compared to term 1 (16.2%, 19.1% and 15.7%, respectively). Multiple strategies such as double needle system, frozen cycles, low dose FSH, various culture media, HCG pretreatment and Metformin, alone or in combination have been attempted in the meantime. We focused on Metformin in the present study, because Metformin was routinely used last 6 years. Fresh cycle IVM-IVF was performed on 172 PCO patients in term 2, either with Metformin of 1500mg/day for at least four weeks (Group A; n=74) or without Metformin (Group B; n=98). Follicular monitoring began from day 7, and if needed, 150 units/day of FSH were administered until follicles reached 10 mm in diameter. HCG was administered 36 hours before retrieval. Immature oocytes were cultured in IVM medium (MediCult) supplemented with 10% SSS (Irvine) for 26 hours, and ICSI was performed on in vitro-matured oocytes. Fertilization was confirmed 18 hours after ICSI. Day 3 embryos were transferred after assisted hatching.

Results: The pregnancy rate, number of oocytes retrieved and good quality embryos in group A (51.1%, 12.1 and 2.1) were significantly higher ($P<0.01$) than in group B (29.6%, 8.4 and 1.0, respectively). However, there were no significant differences in the rates of maturation (53.7% vs. 51.9%), fertilization (81.9% vs. 83.4%), and good quality embryos (36.2% vs. 29.3%).

Conclusions: The present study suggests that Metformin pretreatment of PCO patients improves clinical outcome of IVM-IVF by increasing the number of oocytes retrieved and good quality embryos by altering intrafollicular environment. IVM-IVF might be applied as a first choice of ART for PCO patients with Metformin pretreatment.

◆Biosketch

Aisaku Fukuda, M.D., Ph.D., HCLD (ABB) Chairman; IVF Osaka Clinic, Clinical Prof.; Kansai Medical University, Osaka Japan, Clinical Prof.; East Tennessee State University, USA
1978: M.D. Kansai Medical University, Osaka Japan
1978-1980: Residency Kyoto University, Kyoto Japan
1980-1984: Director, Maizuru City Hospital, Dept. OB/GYN. Kyoto Japan
1984-1988: Graduate school, Kyoto University Faculty of Medicine, Japan
1989: Ph.D. Kyoto University
1990-1992: Research associate, East Tennessee State University (ETSU), USA
1996: High Complexity Laboratory Director (HCLD) by American Board of Bioanalyst (ABB)
1993-1998: Assistant professor, (ETSU), USA
1998-present: IVF Osaka Clinic, Osaka Japan
Awards: AFS (ASRM) Poster Prize Award 2nd place (1992),Kansai Medical University Alumni Association Award (1993), Sigma scientific photography prize (1995), Japan Society of Fertilization and Implantation Award (2000), Good speech Award from International Laser Medicine Congress (2009)

Clinical implications of IVM

Svend Lindenberg Professor dr. med.,
Mette Munk, Chief Embryologist
Copenhagen Fertility Center, Copenhagen

With the recent update of IVM children born (Ri-Cheng Chian), it is obvious, that IVM has a place in routine ART. Indeed also in combination with the newly reported excellent results following vitrification of oocytes.
Three different aspect of IVM are today applicable to routine IVF:
1) IVM of immature oocytes from single women wanting to preserve fertility by freezing oocytes
2) IVM as backup to IVF in high responder to reduce the OHSS risk
3) IVM/IVF in routine ART as the efficient treatment option for all IVF. Specifically to allocate women in real IVM, real IVF and IVM/IIVF cycles.

By applying this approach in the IVF treatment several goals are archived:
1) Reducing the medication
2) Reducing the risk for OHSS syndrome

◆Biosketch

Professor dr. med Svend Lindenberg is a pioneer in IVF and has partcipated in the group of scientist producing the first IVF baby in Denmark in 1982. Since then published more than 150 papers and given numerous presentations in internatinal and national societies and holds several international patents in ART.
Today director for Copenhagen Fertility Center and Research Institution with focus on low stimualtion and IVM.
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Early embryo development: What's critical

Moncef Benkhalifa[1,2], Paul Cohen Bacrie[2], Alain dalleac[2], Yves Menezo[3]
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In functional biology, the fertilisation success is depending on oocyte maturation at genomic and epigenetic level, in parallel to sperm gemone integrity. Sperm-oocyte fertilization and activation is a complicated fascinated phenomenon in human reproduction and its molecular elucidation is still totally unknown because of the limitations of the material and difficulties to analyse the complicate proteins-proteins interactions. Parallelly the oocyte surface is covered with microvilli with the exception of the region overlying the meiotic spindle (Kaji et al 2004). Recent studies showed that genes targeting is a powerful tool to identify the function of candidate molecules involved in sperm oocyte fusion and activation (Cuasnico et al 2001). Ubiquitous tetraspanin family including CD9 are involved in sperm-fusion and for multi-molecular complexes with these associated molecules on the plasma membrane, and thus have been implicated in cell adhesion, motility, proliferation and differentiation (Boucheix et al 2001).

Participation of oocyte-specific GPI-anchored proteins in the sperm-oocyte interaction was suggested and oocyte specific phosphatidylinositol glycan class A (PIGA) deficient showed severely reduced fusibility of oocyte with sperm (Alfeiri et al 2003). Also intention had been given to integrins on oocytes and their ligands as members of ADAM family. After the interaction between gametes to activate the oocyte, the G protein receptor is activated by the sperm binding proteins which activate tyrosine kinase which then activate PLC. The inositol system is implicated as the pathway in egg activation via the phospholipase C (PLC).

It's possible also that soluble sperm factors diffuses from the sperm into the egg cytosol upon sperm oocyte fusion by activating a signal transduction pathway that uses second messengers. A new PLC isoform, PLC zeta may be equivalent of the mammalian sperm factors. Oocyte activation after fertilization go via a complex pathway of genomic et epigenics maturation for maternal RNA reservoir to follow early zygote formation and embryo development until 4 cells, the stages of embryo genome expression

◆Biosketch

Dr. Moncef Benkhalifa obtained his Ph.D. in 1992 from Clermont Ferrand University. He is qualified in Reproductive Biology and Medical Cytogenetics from the school of Medicine and mainly involved in ART and Genetics practice. Since 2006 he is consultant in reproductive biology & genetics at Eylau laboratories / Unilabs Paris and technical director of Unilabs, France.

Dr Benkhalifa is collaborating with different teams involved in ART and Genetics for clinical practice and research. He is author and co-author of more than 80 national and international publications with a main interest for R&D in Embryology and Genetics.

Implantation window

Atsushi Azumaguchi
KKR Sapporo Medical Center Tonan Hospital

1. What is an implantation window? By resecting the ovaries of a female rat after mating and continuously administering progesterone to the rat, a neutral state can be created in which the blastocyst can survive in the uterus, but cannot implant. When estrogen is administered during this neutral state, a receptive state for the blastocyst appears within 24 h and lasts only 12 h. This 12-h receptive state is referred to as the implantation window, and the blastocyst cannot implant after the end of the implantation window (state of non-receptivity). Establishment of an implantation window in humans has not yet been achieved. However, in histological studies of uteri following total hysterectomy, blastocysts were observed floating in the uterine cavity, but no implantation was confirmed up to day 19 of the estimated menstrual cycle and blastocyst implantation occurred only on day 21 or later, suggesting that an implantation window similar to that in rats exists in humans.
2. Are pinopodes an index of implantation window? Since 1971, pinopodes have been considered to represent an index of the implantation window. However, pinopodes have recently been claimed to be observed before ovulation as well as during the luteal phase and pregnancy. If such observations are correct, pinopodes do not represent a suitable index. We speculate that the above claim has been made because no detailed definition of pinopodes has been established. Regarding pinopodes during pregnancies, we have also confirmed the existence of pinopodes in the endometrium of tubal pregnancy. However, one possibility could be that pinopodes disappear when pregnancy is not established and continue to exist when pregnancy is maintained. Therefore, in our view, pinopodes can be used as an index of the implantation window.
3. Pinopodes in the natural cycle, human menopausal gonadotropin (HMG) cycle and hormone replacement cycle: Pinopodes have been reported to be triggered by progesterone. In the HMG cycle, the amount of premature secretion of progesterone is larger and pinopodes have been suggested to appear earlier compared with natural cycle. In the hormone replacement cycle, pinopodes have been suggested to appear later than in the natural cycle, due to a lack of progesterone secretion. We have obtained similar findings. In Japan, frozen embryo transfer has resulted in a higher pregnancy rate than fresh embryo transfer, suggesting the possibility that pinopodes developing early in the HMG cycle contribute to low pregnancy rates.
4. Pinopodes in abnormally thin endometrium: We found that pinopodes appear significantly earlier in abnormally thin endometrium less than or eqaul to 6 mm in thickness during the implantation phase than in normal endometrium more than or equal to 7 mm. We also found that the surface of the endometrial epithelium was deteriorated with abnormally thin endothelium. We therefore speculated that early development of pinopodes and deterioration of endometrial epithelium are factors for the low implantation rate with abnormally thin endometrium.

◆Biosketch

Name: Atsushi Azumaguchi
Nationality: Japanese
Education: Medical school, Sapporo Medical University Post Graduate School, Sapporo Medical University
Major Research: Reproductive Endocrinology, hysteroscopic surgeryPresent
Position: KKR Sapporo Medical Center Tonan Hospital, Dept. of Reproductive Endocrinology

Luteal phase support

Svend Lindenberg Professor dr. med.
Copenhagen Fertility Center, Copenhagen, Lygten 2c, DK-240 NV,

It is obvious that any stimulation regime for IVF involving a high Estradiol and progesterone serum level in the patient at the peri ovulatoric phase involves corpus luteum malfunction. To rescue this several treatments protocols has been devised such as:

1) Low dose hCG
2) Vaginal progesterone
3) Low dose antagonist

Today the intramuscular and the vaginal supplementation are mostly used due to the risk of OHSS by using hCG. The most interesting findings during the last year is that no patients need more than 16 days of progesterone supplementation following routine IVF treatment according to new published data. Further data on low doses hCG as supplementation might again be a convenient part of the IVF treatment in the luteal phase.

◆Biosketch

Professor dr. med Svend Lindenberg is a pioneer in IVF and has partcipated in the group of scientist producing the first IVF baby in Denmark in 1982. Since then published more than 150 papers and given numerous presentations in internatinal and national societies and holds several international patents in ART.
Today director for Copenhagen Fertility Center and Research Institution with focus on low stimualtion and IVM.
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In 2012 Congres President for the 2012 internationa Meeting in ISMAAR: www.ismaar2012.dk

Predictors of IVF outcome with a particular interest in the amount of gonadotropin administered

Peter Kovacs, Steven G Kaali
Kaali Institute IVF Center, Budapest, Hungary

Background: Recently interest was renewed in the benefits of using less gonadotropin (G) for IVF. Mild stimulation is patient friendly and has economic advantages but the literature is split on its efficacy. Our aim was to assess predictors of IVF outcome with a particular interest in G dose.

Methods: All fresh, non-donor cycles that progressed to embryo transfer in 2009 were considered for the analysis (N=1385). Cycles using GnRH agonist/ antagonist protocols were included. Patient (age, CD3 FSH, order of treatment cycle, indication) and stimulation (protocol, G dose, # of follicles/eggs, embryo quality, # of embryos transferred, cryopreservation, day of transfer) were compared between cycles with and without clinical pregnancy (CP). The impact of medication dose was compared in cycles where below and above median dose was used and across tertiles and quartiles of medication dose. Univariate & multivariable logistic regression analyses were used.

Results: The overall pregnancy rate (PR) was 32.9%. In those cycles that resulted in a pregnancy the CD3 FSH was lower, the # of eggs and the proportion of good quality embryos was higher. Blastocyst stage transfer was associated with a 58% improved chance for CP. The availability of surplus embryos for cryopreservation was associated with better outcome. The use of the GnRH agonist long protocol was more likely to result in a CP when compared to the flare or antagonist protocol and the diagnosis of tubal infertility was associated with lower pregnancy rates. According to our analysis the number of embryos transferred and endometrial thickness were not associated with CP. In those cycles that resulted in a pregnancy the mean daily medication dose administered was less (175 IU +-59sd vs 195 IU +-73sd; p"<"0.00001). When comparisons were made across dose tertiles pregnancy rates (36.8% vs 34.6% vs 26.8%; p=0.004) and implantation rates (20.5% vs 19.2% vs 13.8%, p=0.003) decreased as higher amount of G was used. Logistic regression analysis showed that every additional ampoule of G daily decreased the chance of achieving a pregnancy by 17% (OR: 0.83, 95% CI: 0.70-0.98, p=0.03).

Conclusions: Our results confirm findings of previous reports of predictors of CP. In addition we found that the use of less G is associated with higher pregnancy rates. These results lend support to the use of mild stimulation but the ideal candidate and clinical setting for this still needs to be clarified.

◆Biosketch

Short bio for Peter Kovacs

I graduated from the Albert Szent-Gyorgyi School of Medicine in Szeged, Hungary in 1994. Upon graduation I decided to follow my grandfather's and father's footsteps and chose to become an obstetrician gynecologist. I did my ObGyn and Reproductive Endocrinology and Infertility training at the Albert Einstein School of Medicine in New York. Upon completion of residency and fellowship training I moved back to Hungary to my country of origin where I currently serve as the medical director of the largest IVF Center, the Kaali Clinic in Budapest. I earned a PhD degree for studies assessing the effect of diabetes/insulin on GnRH neuron activity using a rat model. My current research interest focuses on stimulation options for IVF, predictors of IVF outcome and on various tools that could be used to improve laboratory and clinical outcome. Besides work my 4 sons keep me busy and have a long standing love for tennis that I try to play when time allows.

Whole ooplasmic replacement in Germinal Vesicle oocytes

Akiko Yabuuchi
Advanced medical research institute of fertility, Kato Ladies Clinic

Oocytes from aged individuals have been known to have higher rates of aneuploidy and higher proportions of mutated mtDNA compared to those from younger individuals. Aneuploidy has been reported to be one of the major causes of infertility in older women. It has been proposed that cytoplasmic factors in oocytes are responsible for the construction of meiotic spindle and indeed, dysfunctional cytoplasmic factors which have been shown in some aged oocytes increase the frequencies of abnormalities in spindle formation and chromosome segregation. Therefore, these abnormalities may be avoided by replacement of compromised cytoplasm with healthy cytoplasm through GV transfer (GVT) before the start of chromosome segregation. This strategy can also be adopted for mitochondrial disease patients by transferring patients' GV into enucleated GV stage oocytes containing normal mtDNA. However, currently available methods for GVT is cell fusion method which karyoplast is fused with enucleated oocyte by inactivated Sendai virus (HVJ) or electric pluses. Karyoplast contains nucleus and cytoplasm (mitochondria), therefore, the resulting reconstructed oocytes have mixture of two different mtDNA in the cytoplasm which so called mtDNA heteroplasmy and the heteroplasmy persists if mtDNA from karyoplast actively replicate throughout embryo development and possibly will transmit in germ cells, as determined by mitochondrial bottleneck. Therefore, the best way to reduce the risk of heteroplasmy is to remove patient mtDNA completely when GVT are performed. As an alternative to cell fusion methods which results in various degrees of mtDNA heteroplasmy, we have established a new method in which we inject GV without any visible residual ooplasm into enucleated GV stage oocyte using a Piezo-driven system (Germinal Vesicle injection: GVI). We used a bovine model to explore the feasibility of GVI, as the size of GV stage bovine oocyte is similar to that of human. Germinal vesicles were aspirated from the oocytes, cytoplasm surrounding GVs were removed by pipetting and directly injected into enucleated GV stage oocytes. In our study, the frequencies of in vitro maturation and blastocysyt development after fertilization of GVI oocytes were 81% and 22%, respectively. These developmental endpoints were comparable to those of non-manipulated control GV stage oocytes (maturation: 81%, and blastocyts development: 25%). After staining GVs with MitoTracker CMXRos, we confirmed that barely detectable amounts of mitochondria were attached to GV membrane. Preliminary results of our study suggest that GVI is very effective method in order to reconstruct GV stage oocytes without compromising their ability to complete meiosis. Although we believe that the residual cytoplasm transferred in to the reconstructed oocyte through GVI is extremely small, actual fate of these mitochondria in developing embryos and offspring needs to be further evaluated in order to bring the promise of clinical application.

◆Biosketch

2003.3 PhD at Laboratory of Animal Reproduction, Graduate School of Kinki University, Nara, Japan
2003.3-2004.12 Postodoctoral Research Fellow at Cellular Reprogramming Laboratory, Michigan State University, East Lansing, MI, USA
2005.1-2009.10 Staff Scientist at Department of Hematology and oncology, Children's Hospital Boston, Harvard Medical School, Boston, MA, USA
2009.11-2011.5 Scientist at Advanced Medical Research Institute of Fertility, Kato Ladies Clinic, Tokyo, Japan
2011.6-Present Senior Scientist at Advanced Medical Research Institute of Fertility, Kato Ladies Clinic, Tokyo, Japan

Dynamic genome dysfunction and chromosomes abnormalities

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[3]Genetics & IVF, Gurgan Clinic, Ankara, Turkey

The contribution of chromosomes abnormalities, genes disorders and functional biology process miss fit in reproductive failure are well documented. The pre zygotic gametes maturation and competency are mandatory for early embryo cleavage, and development. More than the culture conditions, genetic and epigenetic status investigation of the early embryo are important to select and predict the chance of clinical pregnancy in ART programme. To select and predict the best gametes and embryo, Cytological, Cytogenetics, Molecular techniques (karyotype, FISH, PCR and sequencing) or imaging methods are routinely applied sine many years at pre and post zygotic stages to assess gametes and embryos quality. Recently, Genomic or expression microarray technology becomes a common tool for genome and gene investigation in research and development and clinical diagnostic. For genome profiling and high resolution molecular karyotyping, array comparative genome hybridisation (array CGH) methods appear to be far better and have much higher sensitivity and specificity for subtle genomic changes. In Reproductive Medicine and IVF, nanotechnology (genomic, expression or proteins arrays) is becoming an important clinical assay for genomics, transcriptomics and proteomics testing. For non invasive testing cumulus cells, endometrial cells transcriptome profiling, the metabolic monitoring of culture media can give valuable information on embryo implantation competency and IVF success rate improvement.

◆Biosketch

Dr Moncef Benkhalifa obtained his Ph.D. in 1992 from Clermont Ferrand University. He is qualified in Reproductive Biology and Medical Cytogenetics from the school of Medicine and mainly involved in ART and Genetics practice. Since 2006 he is consultant in reproductive biology & genetics at Eylau laboratories / Unilabs Paris and technical director of Unilabs, France.

Dr Benkhalifa is collaborating with different teams involved in ART and Genetics for clinical practice and research. He is author and co-author of more than 80 national and international publications with a main interest for R&D in Embryology and Genetics.

A novel trial of nuclear transfer for repairing an aged oocyte

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The main causes of repeated failure in assisted reproduction such as IVF-embryo transfer are believed to be ooplasmic deficiencies, abnormalities and ageing rather than nuclear deficiencies. It is a common phenomenon that pregnancy rates decrease, but miscarriages increase, as women grow older. Also, the percentage of fetal chromosomal abnormalities in miscarriages increases according to female age, reaching >90% when women are over 40 years old; surprisingly, about 90% of them are cases of autosomal trisomy. Such aneuploidy is mainly induced by the chromosomal pre-division, in which homologous chromo-somes fail to pair during meiosis I and segregate before it is complete, resulting in disomic gametes. Nuclear transfer into the metaphase-II (M-II) oocytes shows promise as a means of repairing female infertility due to ooplasmic deficiency and abnormalities. We therefore conducted nuclear transfer of in vitro matured metaphase-II oocytes (recipient oocytes) into enucleated freshly ovulated metaphase-II oocytes (donor oocyte).

In both in-vitro matured oocytes and freshly ovulated oocytes, the M-II chromosomes were easily recognized as a round transparent substance in which the chromosome body was centrally located, and they were usually beneath or adjacent to the 1PB with the aid of an inverted microscope equipped with a Normarski differential interference contrast system. The aspirated M-II karyoplast of recipient oocytes was transferred into the perivitelline space of an enucleated donor oocyte. The grafted oocyte was transferred in Zimmerman cell fusion medium. Membrane fusion was facilitated by electrical stimulation (10V for 1 second AC + 10V for 45 microsecond DC) with an electro cell fusion generater (LF 201). After fusion, the constructed oocytes were cultured in HTF medium for 2 hours and ICSI was performed.

The percentage of identification of M-II chromosome was 91.1 % (41 out of 45) in freshly ovulated oocytes and 96.0% (48 out of 50) in vitro-matured oocytes.

The M-II karyoplast was removed successfully in 35 of 41(85.4%) of the donor oocytes and 40 of 48(83.3%) of the recipient oocytes. All of 35 karyoplasts of recipient oocytes were replaced in the periviteleine space of enucleated donor oocytes and 28 of these 80.0% were fused to form a reconstituted oocyte. The fertilization rate, cleavage rate and blastocyst formation rate following ICSI for constructed oocytes and recipients oocytes were [77.1%(27/35), 65.7%(23/35), 25.7%(9/35)], [59.0%(58/98), 26.1%(25/98), 3.4%(3/98)] respectively. Chromosomal analysis of 4 embryos following nuclear transfer indicated they were all diploid sets of 46 chromosome.

In conclusion, it has been demonstrated that oocytes constructed following the karyoplast transfer of in-vitro matured M-II oocytes into enucleated freshly ovulated M-II oocytes clearly had more efficient and chromosomally normal embryonic development than did in-vitro matured oocytes after ICSI. These results demonstrate that this technique can be applied to the treatment of female infertility due to ooplasmic deficiency and abnormalities in aged oocytes.

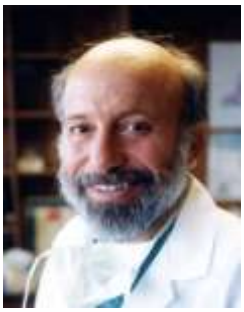
◆Biosketch

Name: Atsushi Tanaka, M.D. Ph.D.

Date of Birth: December 22, 1949 - Niigata, Japan

Education:
1970-1976 Juntendo University School of medicine, Tokyo Degree: M.D.
1976 Juntendo University Hospital, Department of Obstetric and Gynecology
1978-1982 Juntendo University Graduate School of Medicine
1982 Juntendo University Hospital, Department of Pathology
1983 Koshigaya city hospital, Chief Doctor of Obstetric and Gynecology
1990 Saint Mother Hospital, Director

TESE and Male Infertility



Sherman J. Silber
Infertility Center of St. Louis

Objective: To determine the relative impact, if any, of the degree of spermatogenic defect, and sperm origin (testis, epididymis, or ejaculate), on pregnancy and delivery rates with ICSI.

Design: A prospective study of unselected consecutive IVF cycles was conducted in a single center where all cycles were performed with ICSI regardless of the degree of male factor if any.

Materials & Methods: 719 consecutive cycles of ICSI with sperm retrieval were performed for azoospermic men, whether obstructive (OA) or non-obstructive (NOA). During the same time interval, 1,849 consecutive cycles of ICSI were performed using ejaculated sperm for couples with normal spermatogenesis as well as with varying degrees of male factor defects. Conventional rather than minimal stimulation was used for all these cycles.

Results: The age of the wife and the number of eggs retrieved had the greatest effect on pregnancy and delivery rate for every sperm category evaluated, and sperm category had no effect on results. For ejaculated sperm, whether severe oligospermia (less than $5x10^6$), normospermia, or even donor sperm, pregnancy rate varied only from 42% to 47%. However, pregnancy rates varied significantly from 54% to 18% related to the increasing age of the wife, and to her decreasing ovarian reserve.

Testicular sperm (TESE) gave poorer results than epididymal (MESA) or ejaculated sperm, even when there was normal spermatogenesis. The pregnancy rate for epididymal sperm (MESA) with obstructive azoospermia (OA) was 55%, but for TESE for OA was 38% ($p<.001$), and with TESE for NOA was 37%.

Conclusions: The degree of spermatogenic failure has no impact on pregnancy or delivery rate with ICSI. However, proximal motile epididymal sperm and motile ejaculated sperm using ICSI gave consistently better pregnancy and delivery rates than testicular sperm. There may be some aspect of sperm maturation which occurs when sperm leave the testis that is beneficial for ICSI.

◆Biosketch

Dr. Sherman Silber is one of the world's leading authorities on IVF, microsurgery, vasectomy and tubal reversal, egg and embryo freezing, ovary and testis transplantation, and the reproductive biological clock. He is director of the Infertility Center of St. Louis at St. Luke's Hospital in St. Louis, and author of the best-selling *How To Get Pregnant*.

Microdissection Testicular Sperm Extraction (MD-TESE)

Atsumi Yoshida, M.D., Ph.D, MBA
Kiba Park Clinic

Introduction

About 20% of men who visit an outpatient clinic or department of male infertility have azoospermia. Of such patients, about 20% have obstructive azoospermia (OA), while about 80% have non-obstructive azoospermia (NOA). NOA was considered to be absolute infertility in the past; however, a case of pregnancy achieved by ICSI using testicular sperm from a man with NOA was reported in 1995. Thus, even for men with NOA, pregnancy has become achievable if a trace amount of sperm is present in the testes. In the past, a greater volume of testicular tissue than required was extracted to retrieve sperm by the conventional method (conventional testicular sperm extraction [C-TESE]). Recently, however, microdissection testicular sperm extraction (MD-TESE) has been developed to retrieve sperm efficiently (high sperm retrieval rate) and safely (low rate of adverse reactions), without extracting testicular tissue at random. At this symposium, I would like to present the results of MD-TESE-ICSI that have been performed at our clinic.

Results of MD-TESE

Azoospermia was diagnosed if no sperm is detected in the sediment in centrifuge tubes after centrifugation on repeated semen tests. MD-TESE was performed in men with NOA, and the rates of retrieval of "genuine" sperm (excluding pseudo-sperm and late-stage spermatids) according to the cause of NOA were as follows: 28.7% (109/380) of 46,XY men with NOA of unknown cause; 45.9% (34/74) of patients with Klinefelter's syndrome; 50.0% (5/10) of patients with spinal cord injury; 77.8% (7/9) of patients with cryptorchidism; and 45.5% (5/11) of patients after exposure to chemotherapy.

Klinefelter's syndrome

Klinefelter's syndrome is the most common chromosome disorder among chromosome disorders associated with male infertility. At our clinic, ICSI or frozen-thawed embryo transfer was performed in 33 cases (77 cycles), in which viable sperm could be detected by MD-TESE, and 32 babies (18 male and 14 female babies) were born to 20 couples (25 cycles), and one woman is now in pregnancy. The fertility (live birth) rate for couples with Klinefelter's syndrome in whom sperm was detected was 60.6% (20/33).

Cryptozoospermia

When semen tests are performed on separate occasions, no sperm is detected on one test (suggestive of azoospermia), and several spermatozoa are detected in semen on another test. Such case is referred to as cryptozoospermia, implying that sperm lies hidden (= crypto) in semen. In the case of cryptozoospermia, any good embryo may not be obtained by ICSI using ejaculated sperm, or any good sperm may not be obtained from ejaculated semen. In such cases, testicular sperm extraction may be performed in order to find better sperm, or in an emergency on the day of egg collection. In addition, instead of conventional TESE performed for OA, MD-TESE is usually performed to collect spermatozoa present in large, white seminiferous tubules, and ICSI is then performed using the sperm thus collected. At our clinic, the sperm retrieval rate for men with cryptozoospermia was 83.3% (5/6).

Conclusion

MD-TESE-ICSI is an infertility treatment that relies heavily on the skills of physicians (reproductive specialists: urologists, obstetricians, and gynecologists) and the skills of embryologists, that is, the total strength of a fertility clinic.

◆Biosketch

Mar. 1986	Graduated from Ehime University School of Medicine
May 1986	Department of Obstetrics and Gynecology, Tokyo Metropolitan Police Hospital
Apr. 1992	Japan Society of Obstetrics and Gynecology-certified specialist
Oct. 1993	Adjunct physician at Central Clinic (Tokyo)
Jul. 1994	Postgraduate researcher at the First Department of Urology, Toho University School of Medicine
Nov. 1997	Received the degree of M.D. (Toho University)
Jan. 1999	President of Kiba Park Clinic
Apr. 2007	Certified as a supervisory doctor of reproductive medicine
Oct. 2009	Certified as a specialist of clinical genetics
Mar. 2011	Received the degree of Master of Business Administration (MBA) (Nihon University)

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IMSI, already 8 years in ART practice. Where do we stand?

P. Vanderzwalmen[1,2], G. Cassuto[3], T. Neyer[1], A. Stecher[1], M. Zintz[1], N. Zech[1]
[1]IVF Centers Prof. Zech - Bregenz, Austria
[2]Centre Hospitalier Inter Régional Cavell (CHIREC), Braine l'Alleud, Belgium
[3] Laboratoire Drouot, Paris, France

The implementation of Intracytoplasmic Morphologically Selected Sperm Injection (IMSI) was reported to be associated with higher blastocyst, implantation and pregnancy rates and lower miscarriage rates *(Bartoov, Berkowitz, Antinori, Cassuto, Vanderzwalmen).*

However, almost ten years after the introduction of IMSI, this approach of sperm selection still receives fierce criticisms and scepticisms.

The selection of a normal sperm head morphology at high magnification prior to ICSI permits to select not only normal spermatozoa based on shape and size criteria but in addition spermatozoa devoid of sperm head large vacuoles (LV)

Are there justified reasons not to select spermatozoa, especially such showing no LV?

1. Effectiveness of selecting a normal spermatozoon.

Selection of spermatozoa without vacuoles by IMSI was shown to be effective in case of:

- repeated failure of implantation *(Bartoov, Berkowitz, Antinori)*
- male infertile patient, *(Bartoov, Berkowitz, Balaban)*
- high degree of DNA fragmentation *(Hazout)*
- No blastocyst formation or low rates of blastocysts after ICSI *(Vanderzwalmen, Cassuto).*

2. Pathological character of nuclear vacuoles:

The formation and presence of large sperm head vacuoles is not a physiological process and thus needs attention.

In most cases (60%), LV represent a nuclear thumbprint-like concavity associated with a complete or partial failure of chromatin condensation predisposed to DNA damage secondary to a possible lack of DNA methylation or abnormal histone acetylation and methylation *(Garolla, Perdrix,Cassuto).*

This concavity isischaracterized as a very thin area where the plasma membrane is intact but sunken *(Boitrelle).*

3. Their origin:

Abnormal sperm morphology is an indicator for an impaired progression of spermatids through spermiogenesis *(Franken)*. Meiosis abnormalities may affect the various steps in spermiogenesis that could lead to insufficient chromatin condensation and perhaps immaturity of the sperm plasma membrane *(Ovari, Perdrix, Boitrelle, Cassuto) leading to* the formation of LV *(Miller).*

4. Effects on embryo development and the health of the progenies

As nuclear defects such as LV negatively influence the outcome of embryo development due to their implicated late paternal genome effect, the pending question relates to their possible negative effects on the health of the progenies.

Recently Cassuto communicated the results of the first prospective study including 450 children born after IMSI compared with the outcome of 578 children born after ICSI in a group of women younger than 39 years.

The number of major congenital malformations and genetic disorders, mainly affecting the genitourinary system, was 24 from 578 ICSI (4.15%) versus 8 from 450 IMSI (1.77%) (X2 = *0.031)* thus emphasizing the impact of the sperm head morphology defects on congenital abnormalities.

5. How to appreciate the benefit of IMSI?

Additional to the former mentioned increase in implantation and pregnancy rates and probably be of benefit for the health of offspring, IMSI was reported to increase the blastocyst rates. With the advanced vitrification techniques nowadays surplus blastocysts are cryopreserved for later cycles and IMSI thereby contributes to increase cumulative pregnancy rates (*Vanderzwalmen)*

6. Technical aspect

Some are reluctant to apply IMSI because as compared to ICSI it is more time consuming and more expensive. Such arguments are not sustainable because our final aim is to help patients who give all their hopes.

◆Biosketch

Pierre Vanderzwalmen, embryologist, is currently working as scientific coordinator at the IVF center of Prof Zech in Austria and in the CHIREC IVF institute of Prof Lejeune in Brussel.

His scientific investigations focused: on the relationship between selection of spermatozoa and embryo selection criteria on the outcome of embryo development, and on vitrification of oocytes and embryos.

Clinical application of AMH in the reproductive medicine

Yoshimasa Asada[1,2,3]
[1]Asada Ladies NAGOYA Clinic, Nagoya, Japan,
[2]Asada Ladies KACHIGAWA Clinic, Aichi, Japan,
[3]Asada Institute for Reproductive Medicine

Introduction: Japanese people are marrying later in life. Ovarian reserve is now a important competent of evaluation for patients. Because of FSH levels insufficient to evaluate their ovarian reserve, AMH has recently been seen as an alternative marker for such patients. Three years ago, I began measuring the serum AMH levels in my patients and have reported on the importance of this hormone as a marker of ovarian reserve in reproductive medicine.

1) What is AMH? AMH is characteristically not modified through the menstrual cycle. Large amounts of AMH are secreted from the granulosa cells of the preantral and antral follicles. Its secretions are decreased by the maturing procedures of follicles. By such characters, the hormone is a quite useful marker for infertile treatment. All patients visiting in my clinics are checked serum AMH level. The method for infertility treatment is decided by this result. Although there are standard and mean AMH levels, there is no range seems to be normal. Mean levels in women in their early 20s about 7 ng/ml, however its levels linearly go down 0 in women in their late 40s. The ages of our infertile patients are not statistically correlated with AMH levels. AMH levels, moreover, show irregular distributions not only in infertile patients but also in pregnant women and fertile women.

2) AMH and ART: Previously in my Clinic, the ovarian reserve is evaluated by basal FSH, the number of antral follicle, age, the patient history and outcomes of prior treatment. However, AMH levels are particularly well correlated with the number of eggs can be collected. Convincingly, AMH is an excellent indicator of the number of collectable eggs in ART procedures. In my opinion, the AMH levels are most important to select appropriate procedures of controlled ovarian stimulation for my patients. Although I also consider age, I have found that patients with AMH levels of below 1 ng/ml, are candidates for simple ovarian stimulation because they do not respond well to the short protocol. The patients with beyond 3 ng/ml of the AMH level seem to respond well to the antagonist protocol. A high percentage of quality ova are required for a high conception rate. High AMH levels and a large number of ova increase the quality of eggs can be available in the operations. While high AMH levels facilitate conception, pregnancy quality is largely dependent on age, meaning that AMH cannot be used to predict conception rates. Moreover, AMH is a very effective predictor of ovarian hyperstimulation syndrome and should be measured to prevent this side effect.

3) Current infertility treatments: I emphasize that mature egg can be collected in the operation by checking AMH levels and the patient age, not by FSH. The conventional by-the-book decisions are no longer effective because of this AMH method.

Conclusions: By measuring AMH levels in many patients, I realize that there is no mean range consistent enough to indicate ovarian age. Women cannot foresee their reproductive potential by their age. AMH is the only true indicator. It can be concluded that the range of reproductive years are different in person. I can advice women, whether married or unmarried, is to check her AMH level to know their true reproductive age and obtain data to decide when she should be married and pregnant.

◆Biosketch

Current position:

Executive Director, Asada Ladies'ClinicDirector,
Asada Ladies Nagoya Clinic Director,
Asada Ladies Kachigawa Clinic

Education and professional experience:

March 1982	Graduated from Nagoya University School of Medicine
May 1991	Worked as a staff member in the Department of Obstetrics and Gynecology, Nagoya University School of Medicine
January 1993	Studied abroad at the Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, Virginia, where I performed basic research on ICSI
March 1995	Began treating patients with ICSI at Nagoya University School of Medicine, Branch Hospital
May 1995	Published a paper reporting Japan's first pregnancy with ICSI using testicular spermatozoa
April 1998	Served as Director of the Infertility Center at Nakajima Clinic
April 2004	Founded Asada Ladies Clinic
April 2006	Received certification as Board Certified Member of Japan Society for Reproductive Medicine
August 2010	Founded Asada Ladies NAGOYA Clinic

Chronological Aging vs Biological Aging: AMH as an early marker of ovarian aging

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Kanadi Sumapraja, Muharam Natadisastra
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Universitas Indonesia, Jakarta, Indonesia

Background
As women aged, her ability to produce ovum with good quality and quantity will be decreased. This has been related to chronological age with the ovarian biological age, representing the ovarian reserve and its response to ovarian stimulation. Therefore this study was conducted to evaluate the correlation between the chronological age and ovarian biological age with a graph model and normogram of AFC, AMH, and FSH, and to see the decreasing pattern of each variable based on age of the women.

Method
A retrospective cohort study with AFC, FSH, and AMH serum level data taken from medical records of IVF patients at Yasmin Clinic, dr.Cipto Mangunkusumo Hospital, Jakarta, Indonesia between January 2008 to December 2010.

Result
Correlation between 3 variables, AFC, AMH, and FSH, related to the age is statistically significant. AFC and AMH serum level in graph with percentile 3, 10, 25, 50, 75, 90, and 97 has decreased following age, whereas FSH increased following age. There is relatively lower sloping degree of FSH showed that it is increased in older age compared to AFC and AMH, therefore FSH is observed to be a later predictor for evaluating ovarian reserve, whilst AMH is an earlier predictor.

Conclusion
Age-related normograms in infertile women demonstrate a biphasic pattern of decreased antral follicles while AMH and FSH transformed with a linear pattern. AMH found to be an earlier predictor for ovarian biological age assessment. These curve models and normograms could provide a reference guide for physicists to counsel infertile women. However, future validation with longitudinal data is still needed.

◆Biosketch
Budi Wiweko is an OBGYN Consultant on Fertility currently working at dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia and involved in Yasmin Reproductive Clinic dealing with patients with menstrual and fertility problem since 2006. He finished his study at Universitas Indonesia as an OBGYN in 2005 and had been studying as a research student for 3 months at Hyogo College of Medicine, Nishinomiya, Japan back in 2006. He continued his study as a consultant, and finished in 2008. He is interested in doing research focusing on reproductive endocrinology and already wrote many papers.

Premature Ovarian Failure (POF) and Anti-Mullerian Hormone (AMH)

Bunpei Ishizuka, M.D.
Dept. Ob/Gyn, St. Marianna University School of Medicine

It has been thought that, once the diagnosis of POF is established, it is impossible to induce ovulation except for rare, occasional spontaneous remission. We have been attempted ovulation induction by giving estrogen- progestin replacement and rFSH/ HMG in POF patients. AMH has often been reported as a predictor of ovarian reserve in infertility treatment. In the present study, we examined the usefulness of AMH for predicting possibility of ovarian follicle growth and/or oocyte retrieval in POF patients.

AMH levels were measured in 270 POF patients who started ovulation induction in 2010. Their AMH levels ranged from 0 to 13.8 pmol/ml (mean 1.51±1.92 pmol/ml). We divided the patients into 4 groups; Group A, patients in whom follicular growth was not observed by ultrasound or follicles did not reach the size that we could attempt oocyte retrieval during the course of treatment; Group B, patients in whom follicular growth was observed and oocytes were confirmed at oocyte retrieval but could not get fertilization; Group C, patients in whom follicular growth was observed and oocytes were confirmed at oocyte retrieval and embryo was obtained but could not get frozen embryo; Group D, patients in whom, frozen embryo was obtained. Group E; patients in whom, no follicle growth was observed. We analyzed the AMH levels in these 5 groups of POF patients to examine whether measuring AMH levels in POF patients could predict outcome of controlled ovarian stimulation- IVF-ET.

◆Biosketch

1971	M.D.: Showa University School of Medicine
1979	Doctor of Medical Science: Keio University
1979-81	Research Fellow: Dept. Reproductive Medicine, University of California, San Diego, U.S.A.
1982-	Assistant Professor, Dept. Ob/Gyn, St. Marianna University School of Medicine
1993-	Associate Professor, Dept. Ob/Gyn, St. Marianna University School of Medicine
2000-present	Professor and Chair, Dept. Ob/Gyn, St. Marianna University School of Medicine

Dynamic serum AMH levels and AMH-related gene expression in human ovarian follicles

Hsin-Fu Chen, Hong-Nerng Ho
National Taiwan University Hospital, Dept. of OB/GYN, Taipei, Taiwan

Introduction: The predictions of ovarian reserve and probability of pregnancy after in IVF or ICSI are significantly relevant in clinical practice. An accurate prediction can efficiently improve counseling for patients, selecting optimal ovarian stimulation protocols, preventing unfavorable outcomes (including ovarian hyperstimulation syndrome or cancellation of treatment), and predicting the pregnancy outcome. Commonly used methods for predicting ovarian reserve include baseline FSH and inhibin B levels, antral follicle count (AFC), and in recent days serum AMH levels. It was reported that although FSH, inhibin B and AFC all could predict the ovarian response after gonadotropin stimulation, only AMH might predict the pregnancy outcome. However this assumption remains to be tested and the AMH-related gene expression in follicular compartment has been less well explored. The development of mature eggs in the ovarian follicles is a complex and ill-defined process which involves somatic cells in the follicles to nurture oogenesis. Recent studies also revealed critical roles of the oocyte itself in folliculogenesis and showed the intimate bidirectional communication between the oocyte and somatic cells. It has been presumed that some factors may participate in this communication and these may include, though not restricted to, KL, AMH, Activins, GDF9, FSH, BMP15 and LH. Previous study has shown that both thecal and granulosa cell dysregulation may occur in patients with PCOS, which lead to the extreme ovarian response during ovarian stimulation. It showed that AMH, AMH-receptor II, FSHR, and AR genes were over-expressed by granulosa cells from women with PCOS. Recent report also showed that level of AMH in the follicular fluid, but not in the serum was a more useful marker of subsequent embryo implantation.

Materials and Methods: We therefore search the literatures for this issue. In addition we examined the serial serum AMH levels of patients undergoing IVF program and collected the follicular fluid for AMH and steroid hormone levels. The luteinized granulosa cells from smaller and dominant follicles were also separatively collected for the examination of the AMH, AMH receptor, AR, FSH receptor, and LH receptor transcript levels by real-time RT-PCR. This talk therefore aims to clarify the role of AMH-related gene expression in relation to human reproduction by analyzing the above mentioned data.

Results: Our data showed that the serum levels of AMH showed a trend toward decrease at an insignificantly manner during the course of ovarian stimulation in patients undergoing IVF. A nadir could be identified around the time of hCG injection and started to return gradually to the baseline level at the time of pregnancy checkup. Subsequently we will also show the data comparing patients with different ovarian responses to the ovarian hyperstimulation.

Conclusions: Intrafollicular factors including AMH significantly modulate the ovarian responses during ovarian hyperstimulation. It is likely that identification of the signals originating from these factors may improve our clinical practice in ART.

◆Biosketch

Dr. Chen obtained his M.D. degree from National Taiwan University (NTU) and received resident training in the Department of OB/GYN, NTU Hospital (NTUH) (1988-1992). He received fellowship training for Reproductive Endocrinology and Infertility in NTUH (1992-1994), did postdoctoral research in the Department of OB/GYN, University of British Columbia, Canada (1996-1997), and received short-term study for infertility treatment in Assistance Hopitaux Publique De Paris, Cochin (Universite De Paris V). Dr. Chen majors in the treatment of diseases related to reproductive endocrinology and infertility. His research interests are focused on embryo growth and the development of pluripotent stem cells to germ cells. He is now the associate professor/visiting staff in the Department of Ob/Gyn and Graduate Institute of Clinical Genomics, NTUH. Dr. Chen is also the President of Taiwanese Society for Reproductive Medicine (TSRM).

Implementation of the fertility assistance program after the Wenchuan earthquake in China

Shangwei Li, Xiaohong Li, Lang Qin, Han Hu, Shan Luo, Lei Li
West China Second Hospital, Sichuan University, Chengdu Sichuan, China

Objective: To report the implementation of the fertility assistance program after the Wenchuan earthquake in Sichuan, China.

Methods: Fertility assistance program has been carrying out for these parents who lost children in the Wenchuan earthquake since 8, 2008, 3 months after the quake in China. This program included 3 parts: sociomedical fertility health support; psychological counseling and intervention in reproductive health; and an assisted reproductive technology (ART) service.

Results: There were over 8000 couples who lost their only child in the earthquake and planned to have another one, but their reproductive health and psychological well-being were not well in the following several months. Our observation study found that about 30% (59/197) of the surviving women with a high degree of loss in the earthquake reported menstrual irregularity within 6 months after the earthquake. Association analyses showed that some stressors of the Wenchuan earthquake were strongly associated with self-reports of menstrual irregularity, including the loss of children (RR: 1.58; 95% CI: 1.09, 2.28), large amounts of property (RR: 1.49; 95% CI: 1.03, 2.15), and social resources (RR: 1.34; 95% CI: 1.00, 1.80). Our study also reported that 44.7% (88/197) women were in the status of anxiety and 91.9% (181/197) women had depression within 6 months after the earthquake, which were significantly higher than those in the control groups (P&lt0.05). With the help of the fertility assistance program, majority of the surviving women restored good reproductive health and psychological well-being. There were 3,761 women achieved successful pregnancies and 2,864 healthy babies were born up to 3, 2011. For those couples with fertility problems, we gave medical interventions such as medcines, surgeries, and ART. The major reproductive problems of surviving women older than 40 years old were low pregnancy rate and high spontaneous abortion rate, which were very challenging for our post-earthquake fertility assistance program.

Conclusion: The post-earthquake fertility assistance program played a great role in helping surviving women reconstruct their lives and families.

◆Biosketch

ShangWei Li, professor of medical science and doctoral supervisor, is the chief of Assisted Reproductive center of West China Second Hospital, Sichuan University, China. Her academic field involves assisted reproduction technology, Family Planning and infertility. Her professional affiliations includes the member of standing committee and the vice chief of management and ethics group of the Chinese Society of Reproductive Medicine, Chinese Medical Association; vice director of Sichuan Family Planning Policy Association; academic and technical leader in Sichuan province, etc. She honored 38 red-banner pacesetter of Sichuan province and the national medical health system advanced individual in 2010. She rewarded 2 second prize for technology progress of Sichuan province and the national patent for utility model. She undertakes more than ten national and province based scientific research programs and has already tutored 20 post-graduate master students and 20 doctoral students.

Cross-border Cryo-shipping of Vitrified Embryos(CCVE): The newest ART success story

Goral N Gandhi, MSc[1], Gautam N Allahbadia, MD, DNB, FNAMS[2], Aaisha Khatoon, MSc[3], Sakina Kagalwala, MSc[4], Gouri Gupta, MD, DNB[5], Rubina Merchant, PhD[6]

[1]Laboratory Director, Rotunda- Center for Human Reproduction, Mumbai, India
[2]Medical Director, Rotunda- Center for Human Reproduction, Mumbai, India
[3]Embryologist, Rotunda- Center for Human Reproduction, Mumbai, India
[4]Embryologist, Rotunda- Center for Human Reproduction, Mumbai, India
[5]Consultant Fertility Specialist, Rotunda- Center for Human Reproduction, Mumbai, India
[6]Embryologist, Rotunda- Center for Human Reproduction, Mumbai, India

Objective: To compare survival rates, implantation rates and pregnancy rates of locally vitrified human embryos versus cross-border cryo-shipped vitrified embryos transferred into Indian gestational surrogates.
Design: Prospective clinical study
Materials and Methods: A total of 82 frozen-thaw embryo transfer cycles using gestational surrogacy performed in our private clinic during a one year period from January 2010 to December 2010 were analyzed. Group A included the frozen embryo transfers (FET) using locally vitrified embryos (n = 28) and Group B included FETs using cross-border cryo-shipped vitrified embryos (n = 54). The KitazatoTM vitrification kit (Kitazato India, Mumbai, India) and CryotopTM device (Kitazato India, Mumbai, India) were used to cryopreserve all the locally vitrified embryos. The cryo-shipped embryos were vitrified using different media and devices.
The embryos were cryo-shipped to our center using the Rotunda CryoShipTM Global service, our own cryo-shipping program, using a 21 day dry shipper. The shipper was exempt from X-ray screening at all international cross-border custom points. The shipper was tracked on the internet throughout its journey across the globe, not just by the courier company but also by our embryologists to ensure successful, timely deliveries. The infectious diseases screen of the commissioning parents at the time of embryo freezing & the gestational surrogate prior to Embryo Transfer were reviewed. All the embryo transfers were performed by the same clinician in screened gestational surrogates aged 21-35 years.
Results: 94.51% of locally vitrified embryos survived as compared to 92.37% of cryo-shipped vitrified embryos ($p > 0.05$). The locally vitrified embryos had 43.49% implantation rate and 53.85% clinical pregnancy rate following transfer into gestational surrogates, as compared with 41.28% implantation rate and 50.0% clinical pregnancy rate following transfer of cryo-shipped vitrified embryos into gestational surrogates ($p > 0.05$). There were no significant differences in all corresponding values. The Student's t-test, Mann-Whitney test and Fisher's exact test were used where appropriate.
Conclusion: No significant difference was found between the viability of locally vitrified embryos and the cross-border cryo-shipped vitrified embryos. Hence we conclude that cross-border cryo-shipping of embryos, when done appropriately, is a very efficient way to achieve a gestational surrogate pregnancy without compromising the implantation and pregnancy rates.

◆Biosketch
Dr. Gautam Allahbadia

Gautam N Allahbadia is a noted world authority on ultrasound guided embryo transfers and one of the pioneers in third party reproduction in South-East Asia. He has over 100 peer-reviewed publications to his credit and is on the Editorial Board of several International Journals. Dr Allahbadia was responsible for India's first trans-ethnic surrogate pregnancy involving a Chinese couple's baby delivered by an unrelated Indian surrogate mother. He also has to his credit India's first Same-Sex Couple pregnancy and delivery of twins. Dr Allahbadia has had quite a prolific career -- he has authored over 100 scientific papers, 60 book chapters and 13 textbooks, the latest of which is a comprehensive text, entitled "Embryo Transfer" available from Jaypee Medical Publishers. He was awarded the AOFOG-Young Gynecologist Award for the year 1998. He was recently made a visiting lecturer to the University of Tel Aviv- Sackler School of Medicine - the first Indian to be given such a position.

Cross-border Reproductive Care: Exploitation or Opportunity in the Global Quest for a Baby

Daniel S. Seidman MD
Department of Obstetrics and Gynecology of the Chaim Sheba Medical Center, affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Cross-border Reproductive Care indicates, according to the ESHRE Task Force on Ethics and Law, the movements by patients looking for infertility treatment from one country or jurisdiction where treatment is unavailable for them to another country or jurisdiction where they can obtain the treatment they need. It was suggested that the terms 'reproductive' or 'procreative tourism' should be avoided because of their negative connotations and that the neutral term 'cross-border reproductive care' should be used instead. Multiple authors have recently pointed out in the medical literature that cross-border reproductive care represents an urgent and challenging issue to tackle from medical, legal, psychological and ethical perspectives.

The main reasons patients seek cross-border reproductive care include the pursuit of high quality care, absence of local availability, differences in laws and regulations, cost, social support concerns and ethnic needs.

The most important motivators behind the recent rapid expansion in the demand for Cross-border Reproductive Care include: 1. New information technology allows easy dissemination of information on reproductive procedures in foreign countries; 2. International travel currently more comfortable and affordable; 3. IVF clinics in certain countries are now actively seeking patients from foreign countries through advertisements in airline magazines or international satellite TV channels; 4. Fertility centers now offer convenient all-inclusive packages that include not only the reproductive procedures, but also flight tickets, escorted transfer, hotels, interpreters and local recreational tours; 5. The internet allows a very cost-effective and efficient way for patients to compare the foreign clinic's services and unique merits in terms of cost, expertise in reproductive technologies and local policies; 6. More patients seeking treatment forbidden by law in the couple's own country or is inaccessible to the couple because of their demographic or social characteristics. As this relatively new phenomena seems unstoppable, growing concerns remain in regard to the lack of international regulations and quality assurance.

◆Biosketch

Daniel S. Seidman, MD, MCSc

- Co-director, IVF Unit, Assuta Medcial Center, Tel-Aviv (one of the largest IVF Units in the world with over 7,000 cycles per annum).
- Senior physician at the IVF unit of the Division of Obstetrics and Gynecology at the Sheba Medical Center, Tel-Hashomer, Tel-Aviv.
- Associate Professor at the Sackler School of Medicine, Tel-Aviv University.
- President of the *Israeli Society of Contraception and Sexual Health*.
- Board member and member of the International Scientific Committee of the *European Society of Contraception and Reproductive Health* (***ESC***).
- Director of the Annual Course on *Human Reproduction*, School for Advanced Degrees of the Sackler School of Medicine, Tel-Aviv University.
- Editor, Update Journal of the Israeli Obstetrics and Gynecology.
- Editorial board member, Israeli Journal of Medical Sciences (IMAJ) and Journal of Pregnancy.

- Trained in Advanced Endoscopic Surgery for three years at the Stanford Endoscopy Center, under the supervision of Prof. Camran Nezhat.
- Completed a fellowship in *Reproductive Surgery* at the Stanford Medical Center, Stanford, California.
- Two times winner of First Prize in the American Association of Gynecological Laparoscopists (AAGL) "Golden Hysteroscope Competition".
- Published over 200 research papers and several books mainly in the field of human reproduction and advanced endoscopic surgery.
- Member of the Examination Committee of the Israeli Ob/Gyn Review Board.

Oocyte donors: prevalence of genetic, infectious diseases and others

Coll O; Guillen J, Garcia D, Colodron M; Rodriguez A; Vernaeve V
Clínica EUGIN**,** Barcelona, Spain

Oocyte donation (OD) is a well established treatment for ovarian failure and for some genetic diseases. Pregnancy rates achieved by OD are above 50% per completed cycle. Despite its effectiveness, this technique cannot be offered in all countries due to legal constrains. In some other countries donor availability is limited for several reasons such as donor non-anonymity, lack of donors or to very limiting requirements. Different laws, guidelines, protocols apply in different countries in the world.

In the last decade, the Spanish donation model has become one of the reference models and certainly is the most effective in numbers. Spain achieves more than half of all oocyte donations performed in Europe. Several reasons explain this phenomenon: Spain has always been the world leading country in solid organ donation and has a long lasting tradition, its laws are well defined and are oriented to facilitate OD programs and donors may be compensated according to the European donation law.

Despite this favorable scenario, large numbers of donors have to be recruited in order to properly select them according to laws and guidelines. In Spain, donors should be 18 to 35 years of age, should not carry genetic disorders or infectious diseases and be in good health.

Screening of inherited disorders such as known family history, an abnormal caryotype, cystic fibrosis carriers (etc.), infectious diseases such as HIV, HBV, HCV, sexually transmitted diseases, gynecological or psychiatric disorders among others is performed.

There is no published information regarding the prevalence of such problems among a “normal” young population of women.

Data will presented on the prevalence of these diseases of abnormal screening tests in a normal population that are evaluated in Clinica Eugin. Our center is one of the largest OD centers in the world with thousands of donation cycles completed per year.

These data are of interest for general in order to establish the prevalence of these disorders in an “unselected young female population”, and to establish the performance of each single screening test. But the percentage and the reasons for excluding donors is as well an information of great value for fertility centers. It will allow them to plan in advance the real number of donors and costs, required to achieve a certain number of OD. Furthermore, once a disease or a carrying state is identified in a donor, the workload to complete confirmation tests, and to treat or refer patients to another institution or physician (general practitioner etc.), may as well be established.

◆Biosketch

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Current work position: Clinica Eugin. Founder
Adress: Entença 293 baixos, 08029 Barcelona. Spain
Academic and Societies positions achieved:
MD and PhD: University of Barcelona
Obstetrics and Gynecology: Hospital Clinic Barcelona
Executive Education: General Management. IESE Business School.
Fellowship in Reproductive immunology and Infectious Diseases. University of California at San Francisco

(1988-1990)
Prior positions:
Director of Clinica Eugin 1999-2010
Former head of High Risk Obstetrics at the Hospital Clinic, University of Barcelona (2003-2010)
Professor at the Universty of Barcelona (1990-2010)
Areas of interest:
Assisted Reproduction
Perinatal Medicine
Infectious diseases
Indexed scientific publications: 80

Vouchers for health: A demand side output-based aid approach to reproductive health services. A model to pay for infertility services?

Claus P. Janisch
Health Policy Department, IGES Institut

Reaching the United Nations Millennium Development Goals has been a focus for many countries and development partners. In many developing countries with low levels of development, access to and equity of basic quality health services is limited, especially for the very poor. Among poor populations, maternal mortality is high as access to medical care and financial means are lacking. Infertility represents a national health problem in many African- and also poorer Asian countries. Limited financial health resources in developing countries are a major obstacle facing infertility management. Affordable IVF treatment for financially needy couples is, nevertheless, supported by many medical experts, human right groups, and religious leaders A new approach, herein referred to as Vouchers for Health, is proposed as a possible way to organize and finance infertility services for the poor. The way that these programs are being implemented in a number of countries for women's health services demonstrates, that the voucher based approach comprises a variety of key structural elements of a national health insurance scheme: accreditation; quality assurance; reimbursement system; claims processing; integrating the public and the private sector; client choice; provider competition; and access to and equity of services provided. Voucher Schemes ma be a way to finance IVF services.

◆Biosketch

Born 1948 in Celle in the north of Germany Claus Janisch studied medicine from 1969 till 1975 at the free University of Berlin in West Germany. He specialized in the field of Obstetric and Gynecology at the Academic hospital in Berlin Neukölln (Professor Erich Saling), at the University Hospital Vienna (Professor Eduard Gitsch), and at the University Hospital Mainz (Professor Friedmann). He then worked for a couple of years as senior consultant (Oberarzt at various major hospitals. In 1984 Dr. Janisch joint the 'Deutsche Gesellschaft für Technische Zusammenarbeit' an organization of the German Government to implement the support of the German Government with developing countries. Till 189 he was Director of three Ministry of Health Hospitals in Tabuk, Hail and Najeran in Saudi Arabia. In 1989 Claus Janisch became the first Medical Advisor of the German Development Bank (KfW Banking Group). He was responsible for the financing of health projects in Asia, Africa and South America.

Global Challenges and Perspectives

Sheryl Vanderpoel
World Health Organization, Headquarters, Geneva, Switzerland

Introduction: No single country in the European Union and, few transitional and developed Asian countries had a total fertility rate above replacement level in 2009. Many are coming close to becoming, or already defined as, low fertility countries. Women in developed countries are having fewer children, but this trend is expanding into countries in transition and urban centres in developing countries. In some countries, with burgeoning youth populations, this can be viewed as a positive trend. However, a healthy balance is required, as prolonged delays in childbearing can skew population compositions if a large proportion of women begin having their children significantly later in life. Although initial population decline can provide an economic boost, the ultimate long term sustainability of political, social and economic national models can become constrained if low fertility population trends continue to stagnate and do not reverse. The general term, "infertility" is defined as *a disease of the reproductive system* which results in an inability to become pregnant or bring a pregnancy to term. Primary infertility (no prior pregnancies) is predominantly a result of delayed childbearing, complications of childbearing and/or congenital disorders. Secondary infertility (infertility following a previous pregnancy or live birth) is often a result of maternal sepsis (infection following delivery) or of complications associated with R/STIs (including HIV/AIDS) and unsafe abortions.

Results: The joint WHO/World Bank World Report on Disability (June 2011): *Infertility* following maternal sepsis and unsafe abortion was ranked 8th highest in prevalence *as a disability* across all age groups. Among populations under the age of 60, infertility ranked 5th highest in prevalence as a major global disability. Within public health documents and strategies, "family planning" is often interpreted as "contraception" and emphasizes the prevention of unwanted pregnancies. Within other public health strategies, critical "maternal and child health" care issues are emphasized and addressed - *only* when a woman presents, often far into, her pregnancy. Peri-conception care, which includes infertility care, is the critical link between these two areas of reproductive and maternal health care services. Attempting to rectify sub-standard or poor "beginnings" which may occur at the time of conception by providing later enhanced antenatal care may be "*too much, too late*." Peri-conception services together with evidence-based reproductive medicine clinical management and interventions can better ensure safe pregnancy and birth and decrease use of socio-behavioural methods to solve infertility. Traditional methods, in the absence of quality infertility care, are problematic on a global level within high HIV endemic areas; they exacerbate not only the problem of horizontal (and vertical) transmission of infectious diseases such as HIV/AIDS and syphilis, but can result in a further increase in infertility within the community.

Conclusion: Access to simple, available and affordable solutions for peri-conception and infertility care services represents a highly neglected component of global public health care. Revitalization of the field of infertility and peri-conception care is critical in order to fully and adequately address the Secretary General's Global Strategy for Women and Children's Health to ensure healthy mothers, healthy children and healthy family life.

◆Biosketch

Dr. Sheryl Vanderpoel,

Current: Scientist and Medical Officer in the Director's Office of the Department of Reproductive Health and Research, World Health Organization, WHO-RHR; UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Training in Human Reproduction (HRP). Primary responsibilities: scientific, technical, financial and ethical peer review of research proposals submitted to HRP; RHR/HRP's research and normative work in peri- conception concentrating on issues associated with infertility; and activities in ethics in sexual and reproductive health and assisted reproduction; and representing WHO-HQ at inter-agency/international consultations in these areas of focus.

Affordable IVF – Low cost IVF: The Arusha project

Willem Ombelet
Department of Obstetrics and Gynecology, Genk Institute for Fertility Technology, Schiepse Bos 6, 3600 Genk, Belgium (coordinator of the ESHRE Special Task Force on Developing Countries and Infertility)

INTRODUCTION: The majority of childless couples are residents of developing countries. When compared to Western societies, negative consequences of childlessness are experienced to a greater degree. Residents of developing countries encounter a lack of facilities at all levels of health care but especially infertility diagnosis and treatment. Tubal infertility due to sexually transmitted diseases, unsafe abortion and postpartum pelvic infections are the main causes of infertility in developing countries. This means that most cases of infertility are only treatable with assisted reproductive technologies (ART) which are either unavailable or very costly.

METHODOLOGY: December 2007 an expert meeting was organized by ESHRE in Arusha, Tanzania. The meeting was the start of a global project aiming at increasing the diagnostic and therapeutic options for childless couples in developing countries, emphasizing the need for reproductive health care education. The final objective is the implementation of infertility services in many developing countries if possible linked to existing family planning and mother care services.

RESULTS: Pilot studies will be organized to study the results of the combination of a one-day diagnostic phase, the use of clomiphene citrate for ovarian preparation and the use of an unexpensive high quality IVF laboratory phase.

CONCLUSION: Although prevention is better than cure, we believe it is justified and possible to implement simplified, safe and effective methods of ART in resource-poor countries. We propose a special designed infertility care program leading to a cost effective simplified ART program as a valid treatment protocol in developing countries when prevention has failed.

◆Biosketch

Willem Ombelet started his career researching infertility and IVF in 1984 in Pretoria, South Africa. Since 1987, he has been working in the Department of Obstetrics and Gynaecology at the St. Jans Hospital in Genk, Belgium. In 1998, he obtained his PhD degree at the University of Leuven on 'The value of sperm morphology and other semen parameters in diagnosis and treatment of human subfertility. He became the Head of the Department of Obstetrics and Gynaecology at the St. Jans Hospital in Genk, in 1999. From 2001 until 2004 he was the President of the Flemish Society of Obstetrics and Gynaecology.

Willem Ombelet (co-)authored 89 international peer-reviewed articles and was a (co-)-editor of 18 books. He received 2 international awards.

Dr Ombelet is also the founder of the Genk Institute for Fertility Technology.

He organised nine "Andrology in the Nineties" meetings and different workshops on assisted reproduction.

He is a consultant and Professor (Reproductive Medicine) at the University of Hasselt and the University of Ghent.

Since 2006 he is the coordinator of the ESHRE Special Task Force on "Developing countries and infertility". Presently he is the chairman of the Workgroup "Reproductive Medicine" of the Flemish Society of Obstetrics and Gynaecology since 2007 and a board member of the Belgian Society of Obstetrics and Gynaecology (BSRM) and the Belgian College of Reproductive Medicine.

Willem Ombelet is also the editor-in-Chief of the international scientific journal "Facts, Views & Vision in ObGyn".

How to improve implantation in ART: novel approaches.

Yael Gonen, MD
Infertility Clinic, Lin Medical Center, Haifa, Israel

Recurrent implantation failure is a distressing phenomenon, both for the infertile couple and for physician responsible for their treatment. Aetiology is often not clear and treatment options are vague. Particularly when transferred embryos are of good quality, recurrent implantation failure may be attributed to decreased endometrial receptivity, molecular abnormalities at the endometrial level and abnormal embryo – endometrium dialogue. Furthermore, there may be over or under expressed genes that may be related to successful implantation or less than optimal embryo transfer technique and factors with combined effect.
At present, the diagnosis of recurrent implantation failure is not sufficiently specific and we do not have the tools to diagnose in each case the exact cause for the repeated failure. The expense, time and stress has led to a search for new technologies and drugs that will increase success rates, however progress has been limited. Novel approaches to treat this frustrating and limiting step in the success of ART will be presented.

◆Biosketch

Dr. Yael Gonen received her MD degree from the Sackler School of Medicine at the Tel Aviv University in Israel and completed residency in OB/GYN at Lady Davis Medical Center in Haifa.
After two years of a clinical and research fellowship in the field of Infertility and Reproductive Endocrinology with Prof. Robert Casper at Toronto General Hospital, University of Toronto in Canada (1987–1989) she returned to Haifa to the OB/GYN department at Lady Davis Medical Center and performed as Senior Physician in IVF Unit.
In 1996 Dr. Gonen founded the new IVF Unit at Herzliya Medical Center in Haifa, while maintaining the position of Medical Director for over three years.
Presently Dr. Gonen holds the position as Head of Infertility Clinic at Lin Medical Center in Haifa in addition to maintaining a private practice.
Her abundant experience, dedication and avid participation in the field of infertility has earned her awards, recognition for both clinical and research accomplishments. Her scientific studies are quoted in leading journals and international meetings.
Dr. Gonen pioneered the use of ultrasound for endometrial quality (pattern and thickness) related to ART pregnancy, she was the first to publish in 1990, the use of GnRH agonist in place of HCG as a means to trigger the gonadotrophic surge and also pioneered the use of Oral Contraceptives for follicle synchronization and cycle scheduling in IVF.

Studies on the efficacy of immunotherapy using paternal mononuclear cells for patients with infertility

Koichi Takakuwa
General Center for Perinatal, Maternal and Neonatal Medicine,
Niigata University Medical and Dental Hospital, Niigata, Japan

Although the etiology of recurrent spontaneous abortion (RSA), defined as three or more consecutive early pregnancy losses, is often unclear, several investigators have reported the occurrence of immunologically explainable RSA. Immunotherapy for patients with RSA using the husband's mononuclear cells has been reported for the past three decades. Although the efficacy of this modality has been controversial, the randomized trial revealed the efficacy for patients with RSA, especially for those who were negative for blocking antibodies evaluated by a mixed lymphocyte culture reaction between spouses (MLR-BAbs). In this presentation, I show the results of immunotherapy for unexplained primary RSA using freshly prepared mononuclear cells from the husband and the efficacy of the therapy, especially in patients negative for MLR-BAbs. Moreover, there is possibility that the immunotherapy could be effective for patients with recurrent IVF-ET failure, as the recurrent IVF-ET failure is considered to be the similar condition as the recurrent early miscarriages. We previously tried to evaluate the efficacy of the immunotherapy for patients with recurrent IVF-ET failure. So, in this presentation, the results of immunotherapy for recurrent IVF-ET failure are shown, in addition to the results of immunotherapy for RSA.

Two hundred and twenty-eight unexplained RSA were prospectively followed up by means of non-randomized study regarding the immunotherapy. Of 228 patients, 165 cases underwent the immunotherapy (group I). No MLR-BAbs were observed in these patients prior to vaccination. Pregnancy outcome was also analyzed in RSA who revealed positive MLR-BAbs without immunotherapy (group II). Furthermore, the outcome of 20 pregnancies in 18 unexplained RSA was analyzed who were negative for MLR-BAbs and had become pregnant without immunotherapy (group III). In group I, of 140 newly pregnant patients after vaccinations, pregnancy successfully continued in 110; thus, the success rate of the therapy was 78.6%. Regarding group II, in 24 of 32 patients (75.0%), the pregnancy continued successfully. The rate of continuation of pregnancy was not significantly different between groups I and II. Pregnancy was successfully continued in 6 cases (30.0%) in group III. The rate of successful pregnancy in experimental groups I and II was significantly higher compared with that in group III.

Fourteen patients underwent the immunotherapy concerning the immunotherapy for recurrent IVF-ET failure. The selection criteria for the immunotherapy were as follows; 1) the patients experienced 3 or more IVF-ET failures in spite of transfer of good quality embryos, 2) they were negative for autoimmune examinations such as anti-phospholipid antibodies, 3) they were negative for MLR-BAbs. Of 14 immunotherapy patients, ET was performed at 30 cycles, and successful pregnancy was achieved in 6 cycles (20.0%). As control, 20 cases with same condition as immunotherapy group, and did not undergo immunotherapy, were analyzed. Of 20 cases, ET was performed at 48 cycles and pregnancy was achieved in 3 cycles (6.3%). The tendency of improvement of pregnancy rate was observed in immunization group (20.0% vs. 6.3%, p=0.07).

In conclusion, the immunotherapy using the husband's mononuclear cells on RSA negative for MLR-BAbs is effective, and there is possibility that the immunotherapy might be effective for patients with recurrent IVF-ET failure.

◆Biosketch

2009-present: Professor and Director, General Center for Perinatal, Maternal and Neonatal Medicine, Niigata University Medical and Dental Hospital

1998-2009: Associate Professor, Department of Obstetrics and Gynecology,Niigata University School of Medicine
1994-1998: Assistant Professor, Department of Obstetrics and Gynecology,Niigata University School of Medicine
1986-1994: Instructor, Department of Obstetrics and Gynecology, Niigata University School of Medicine
1979: Passed the Examination of National Board of Medical Doctor

Societies:
Executive Board Member, Japan Society for Immunology of Reprodcution
Councilor, Japan Society of Obstetrics and Gynecology
Councilor, Japan Society of Perinatal and Neonatal Medicine
Councilor, Japan Society of Reproductive Medicine
Councilor, Japan Society for the Study of Hypertension in Pregnancy
Councilor, Japanese Society for the Sexually Transmitted Disease

Improvement of implantation rates using autologous peripheral blood mononuclear cells

Hiroshi Fujiwara, Yukiyasu Sato, Akihito Horie, Ko Suginami, Hisanori Matsumoto, Ikuo Konishi
Department of Gynecology and Obstetrics, Graduate School of Medicine, Kyoto University

Introduction: In humans, the implanting embryo secretes human chorionic gonadotropin (HCG) and stimulates the maternal corpus luteum of pregnancy via blood circulation to produce progesterone. Progesterone in turn maintains embryo implantation in the uterus during early pregnancy. Along with this embryo-maternal crosstalk by endocrine system, accumulating evidence suggests that circulating immune cells also play an important role in embryo implantation.
Material & Method: The effects of human peripheral blood mononuclear cells (PBMC) and murine spleen cells on embryo implantation were accessed by human luteal cell culture, human trophoblast invasion assay and murine implantation experiments. The effects of human PBMC were also evaluated by clinical trials.
Results: PBMC derived from pregnant women increased the progesterone production by human luteal cells and promoted the invasion of embryos in vitro. In mice, spleen cells derived from early pregnant mice could promote endometrial differentiation and embryo implantation in blastocyst-transferred pseudopregnant mice in vivo, suggesting that the maternal immune system undergoes functional changes by recognizing developing embryos from the very early stages of pregnancy. In addition, recombinant-HCG increased chemokine production by human PBMC through lectin-glycan interaction, promoting trophoblast invasion. Furthermore, intrauterine administration of autologous PBMC that were treated with HCG effectively improved pregnancy and implantation rates in patients with repeated implantation failure during in vitro fertilization therapy.
Conclusion: These findings suggest that circulating immune cells are useful materials to improve implantation rates in ART.

◆Biosketch

I. Academic and Professional Backgrounds:
1983 Graduated from Kyoto University, Faculty of Medicine 1985 Medical Staff, Dept of OB/GYN, Hikone City Hospital
1986 Director, Dept of OB/GYN, Otowa Hospital
1988 Medical Staff, Dept of OB/GYN, Wakayama Red Cross Hospital
1993 Assistant Prof, Dept of GYN/OB, Faculty of Medicine, Kyoto University
1995 Lecturer, Dept of GYN/OB, Faculty of Medicine, Kyoto University
2009- Associate Prof, Dept of GYN/OB, Faculty of Medicine, Kyoto University

II. Academic Degree Medical Doctor:
1983 Kyoto University Doctor of Medical Science
1994 Kyoto University

III. Academic Awards: Research Promoting Award of Japan Endocrinology Society (1997)

IV. Editorial Services
1. Human Reproduction (Associate Editor, 2001-2003)
2. Reproductive Biology and Medicine (Executive Editor, 2003-)
3. Journal of Obstetrics and Gynaecology Research (Associate Editor, 2008-)
4. American Journal of Reproductive Immunology (Editorial Board, 2010-)

Prevention of infertility in patients for the antiphospholipid syndrome and deficiencies of natural anticoagulants

Moritoshi Seki
Sekiel Ladies Clinic

Antiphospholipid syndrome is a multisystem disease with the major elements of venous and arterial thrombosis, recurrent pregnancy loss, fetal death and infertility. New mechanisms are described by which antiphospholipid antibodies could cause placental thrombosis and infarction, acting directly on the surface anticoagulant expressed on trophoblastic cells. The treatments have been proposed to treat with low doses of aspirin and preventive or effective dose of heparin.
Oocyte retrievals of 1992 cases (736 patients) and embryo transfer of cryopreservation of 841cases (460 patients) from 2008 to 2009 years in Sekiel Ladies Clinic were analyzed for the estimation of effectiveness heparin and aspirin in recurrent pregnancy loss.
Out of the 736 patients examined, 6 patients resulted positive for abeta2GPI, 2 patients for lupus anticoagulant antibodies, 43 patients for antiphosphatidylserine IgG, 131 patients for antiphosphatidylethanolamine and 31 patients positive for anti-phosphatidylinositol antibody.
One patient for deficiencies of natural anticoagulants protein C, 2 patients for protein S and125 patients for the decrease in factor XII activity.
There is increasing evidence that heparin exerts its effect by inhibiting complement activation rather than by its anticoagulation capacity. We aim for improve the outcome of pregnancies in APS by the invention of therapies using combinations of aspirin, unfractionated heparin and/or low molecular weight heparin.
We infuse unfractionated heparin with Coopdech Syrinjector (Daikenika CO., LT. Japan) that is disposable infusion pump operated by the use of atmospheric pressure. Patients can take the infusion of unfractionated heparin dealing 24 hours at home.
We analyzed 97 cases (35 patients) of recurrent miscarriages patients in 1992 cases (736 patients) of IVF-ET. Embryo transfer was performed in 73 cases. Forty nine cases (29 patients) were treated by using combinations of aspirin and unfractionated heparin (UFH). Eighteen cases were pregnant and 4 patients delivered. Twenty five cases (11 patients) were treated by using combinations of aspirin and low molecular weight heparin. Thirteen cases were pregnant and 5 patients delivered.
We analyzed 66 cases (24 patients) of recurrent miscarriages patients in 841cases (460 patients) of embryo transfer of cryopreservation. Thirty four cases (15 patients) were treated by using combinations of aspirin and unfractionated heparin, 17 cases were pregnant and 5 patients delivered 6 babies. Twenty five cases (11 patients) were treated by using combinations of aspirin and low molecular weight heparin, 18 cases were pregnant and 7 patients delivered.
The chromosomal abnormalities were 88.9% in products of conception (POC) chromosome analysis. The average age of patients of recurrent miscarriage is 38.6 years old. The early treatment is recommended to prevent the chromosomal abnormalities.
We stop the heparin treatment by 8-10 weeks gestation for the introduction of patients to birthing hospital. Some of the patients should have been cared by keeping heparin even after 10 weeks gestation by following up the subsequent history of the patients.
The women with recurrent pregnancy loss should receive the prophylactic-dose heparin and aspirin.

◆Biosketch

1974	graduate from the medical department of Toho University
1974-1976	Intern of Toho University
1978	Assistant of Department of Obstetrics & Gynecology in Akita University
1979-1981	Scholarship of Humboldt foundation, Department of Obstetrics & Gynecology in Kiel University
1992	Assistant professor Department of Obstetrics & Gynecology in Gunma University
2001	The director of Sekiel Ladies Clinic

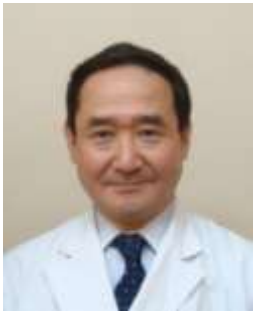
PGD for recurrent pregnancy loss

Atsushi Tanaka
Saint Mother Hospital, Fukuoka, Japan

It has been commonly assumed that preimplantation genetic diagnosis (PGD) for translocations in couples with recurrent miscarriages has little advantage over non-PGD intervention. We did PGD for translocation in couples with recurrent miscarriages and compared the results to natural conception. One blastomere from 8 cell embryos following IVF in 55 cycles (33 patients) was biopsied by the extrusion method and normal type (normal chromosomal or balanced translocation) was distinguished from the abnormal type (unbalanced translocation). A single normal-typed embryo was transformed. Fifteen couples suffering translocation with recurrent miscarriages and not using PGD were followed up for 2 years. Of 64 cycles of PGD (36 patients), 22 subsequently conceived (pregnancy rate: 37.5% [24/64]) per cycle, 66.7% [24/36] per patient), resulted in 18 live births with a miscarriage rate of 20.8% (5/24). Of 24 pregnancies, 15 conceived at the first PGD (62.5% [15/24]). Seven conceived at the second trial (29.2% [7/24]) and 2 at the third trial (8.3% [2/24]). All 24 cases achieved conception by the 3rd trials. The average maternal age and the collected eggs number from 32 unsuccessful cycles which had been performed more than three times were 39.2 yrs and 3.4 eggs. These figures were significantly lower than that of 24 successful cases with an average maternal age of 33.2 yrs and an average collected egg sum of 9.8 eggs. Of 15 cases, 10 were conceived without PGD, resulting in 6 live births (birth rate: 40.0%) with 3 miscarriages (miscarriage rate: 30.0%). The six cases had an average 2.7 miscarriages and took on average 12.3 months until the live birth. In conclusion PGD is clearly advantageous for couples with recurrent miscarriages suffering translocation when they are younger than 35 years of age and the collected oocytes are expected to be over 10 in total. The next concern is whether it is possible to distinct between a balanced translocation and a normal karyotype in order to select the normal one correctly. After providing informed consent, morphologically excellent 3-day-old human embryos (8-cell stage after IVF), along with immature GV oocytes, were donated by our clinic's patients for this study. The immature GV oocytes were cultured overnight until the first polarbody was extruded. The 3-day-old embryo and IVM oocytes were freed from the zona-pellucida with a 0.3% tripsin solution. The embryos were then placed in PBS (without Ca and Mg ions) to separate the blastomeres. Then electrically stimulated (AC 15V, 2 sec and DC 1000V/cm, 50 μsec) in 295mM D-mannitol containing PVP, Ca^{2+} and Mg^{2+}. Each hybrid cell was treated with hypotonic solution (30% fetal bovine serum) for 20 min, and then affixed to a chromosome slide using the gradual fixation-air drying method. 22 human blastomeres and 22 IVM oocytes successfully combined through electrical stimulation to create 20 hybrid cells. Of these 20 hybrid cells 3 were unsuccessful, 13 contained 1 complete set of clearly arranged meiotic chromosomes (n=23) that were derived from the oocyte. In conclusion this assay system has the advantage that it only takes 2 hours from biopsy to chromosome analysis. In this study, the success rate for chromosome analysis was as high as 65.0% (13/20) when the hybrid cells were prepared for chromosome slides one hour after electro-fusion.

◆Biosketch

(For Dr. Tanaka's Biosketch, please refer to his abstract for Concurrent Symposium C-17.3.)



PGD outcome and alternatives for single gene disorder

Kou Sueoka, Yasunori Sueoka
Keio University School of Medicine, Tokyo, Japan

Preimplantation genetic diagnosis (PGD) for single gene disorders is the principle of original PGD aimed at preventing outbreak of a child for the client who is a genetic carrier of the diseases. However, as for the PGD, always tailor-maid diagnostic chemicals and procedures are required for various genotypes to be present in each individual disorder family. However, the methods of genetic diagnosis are going to the direction of common and stable analysis from tailor-maid diagnostic method for individual genotype. On the aspect of the material collection for diagnosis, biopsy method is also changing to be less invasive and according to the methods of genetic analysis. Especially blastocyst biopsy has been performed for more accurate and with a higher potential diagnosis by obtaining many cells, which can afford to apply for wide variety of genetic diseases, and also to collect enough DNA specimen of embryonic cells for genome wide analysis. The amplification efficiency by WGA from single cell is not yet stable and enough for analysis to the present. As for our PGD cases to date, there were structural chromosome aberration, deletion type mutation, point mutation, triplet repeat diseases in Mendelian disorders as single gene disorders, and mitochondria disease as non-Mendelian disorders. The gene amplification is inevitable for a diagnosis of single gene from single cell in the technical aspect. Nested PCR has been the basic procedure to amplify single copy DNA up to enough level for analysis. However, it yield to amplify only a few target genes and unable to avoid amplification failure or DNA contamination. Whole genome amplification (WGA) has been developed for genome wide analysis. WGA has several options with both gene amplification types of PCR base and non-PCR base methods, which would be selected for the analytical method of amplified products. WGA yields the difficulty of conditional setting of nested PCR, and enables a repetition diagnosis for an amplification product. As a result to the present, WGA expands diagnostic width of gene genotypes and improves to decrease dismissed embryos that the diagnosis is unable to finalize, whereas amplification efficiency from single cell has not been yet more than that of nested PCR. In addition, WGA makes possible to analyze both genes of compound heterozygote mutation stably. We are approaching PGD using WGA method to the disorder gene carriers of compound heterozygote mutation gene such as Fukuyama congenital muscular dystrophy (FCMD), and epidermolysis bullosa-muscular dystrophy syndrome, and also to myotonic dystrophy (DM1) which is not easy to diagnose by PCR amplification on the expansion of CTG repeat length. It is also a large benefit that array technology is available to apply for the diagnosis of this amplification product. There is a new direction of PGS shifting to a diagnosis using comparative genomic hybridization (CGH) instead of uncertain florescence in situ hybridization (FISH). However, applying CGH or the other array analysis for PGD can give genome wide information from embryo, which might be including sporadic mutations or chromosomal aberrations. Although genome wide technologies must be strong tools, the analysis is not always only to the required genetic information. For this directionality, we need to consider and argue on the aspect of genetic information handling in PGD.

◆Biosketch

(For Dr. Sueoka's biography, please refer to his abstract for the Pre-Congress Workshop: PGD 1.)

Preimplantation genetic diagnosis for aneuploidy using whole genome amplification and microarray comparative genomic hybridisation

Alan Handyside
London Bridge Fertility, Gynaecology and Genetics Centre

The development of methods for whole genome amplification from single cells and microarray comparative genomic hybridisation (array CGH) have revolutionised our ability to detect copy number abnormalities for all 23 pairs of chromosomes. Accurate analysis is now possible with polar bodies, blastomeres and trophectoderm biopsied from cleavage and blastocyst stages. Analysis of both the first and second polar bodies and corresponding zygotes has revealed multiple errors caused by premature predivision of chromatids in women of advanced maternal age. This is further evidence that as women reach the menopause but before their ovarian reserve is exhausted, many and eventually all oocytes have multiple aneusomies so that the resulting embryos are highly unlikely to be viable. Recent advances in the technology will be reviewed which allow accurate detection of structural chromosome imbalance, particularly in carriers of balanced reciprocal and Robertsonian translocations.

◆Biosketch

Background in mouse and human embryology and genetics with over 200 publications. Created one of the first mouse transgenic mice using embryonic stem cells. With Prof Lord Robert Winston, achieved the first pregnancies following IVF and PGD worldwide in 1990. First Chairman ESHRE Special Interest Group in Reproductive Genetics and founder and first Chairman of the ESHRE PGD Consortium.

Human ES cell and iPS cell derivation: Clinical applications and biological characterization

Hidenori Akutsu
Chief, Division of Stem Cell and Reproduction, Department of Reproductive Biology,
National Research Institute for Child Health and Development

The potential to generate and expand human pluripotent stem cells in vitro has great implications for basic research, drug discovery, toxicology, to study diseases and can potentially serve as a resource for cell replacement therapies. The U.S. Food and Drug Administration (FDA) has recently approved the first embryonic stem cell-based clinical trial, indicating that human application of stem cell research is being realized. Regenerative medicine and cell therapy are based on the premise that large numbers of normal cells and that the cells will migrate, integrate and survive for a sufficient length of time as to be clinically useful. An important issue facing investigators irrespective of their cell of choice or target for therapy is growing cells in sufficient numbers for treating patients in an aseptic environment with adequate safeguards, sterility and traceability. As research translates to clinical therapies, one of the primary concerns is that therapeutic cells may carry foreign pathogens or antigens that induce disease or immune response by the patient. These undesirable byproducts may come from the use of murine-derived feeder cells, xenogeneic matrices, or from animal-derived components found in the cell culture medium used to isolate or expand the stem cells. One of the major challenges for the clinical use of stem cells is the exposure to undefined animal-derived products during derivation and expansion of the cells. In this study, we have developed a xeno-free cell culture system that will support both hESC and hiPS cell systems. We have recently reported three new hESC lines (SEES1-3) which were derived and maintained on irradiated human mesenchymal stromal cells grown in xeno-free culture media and substrate. SEES1-3 lines did not express non-human sialic acid reported as an association with increased immunogenicity. Here we describe a xeno-free cell culture system that will support both hESC and hiPS cell systems. In this presentation we will discuss our experiences in maintaining pluripotent stems cells using these xeno-free media systems. Our center is officially approved hESC derivation study by the Minister of Education and Science of Japan, and has built up the stem cell technology for stem cell based therapy.

◆Biosketch

Hidenori Akutsu, *MD/PhD*
Chief, Division of Stem Cell and Reproduction, Department of Reproductive Biology,
National Research Institute for Child Health and Development

Dr Hidenori Akutsu became interested in nuclear reprogramming in mammalian species when he was a research fellow at University of Hawaii under Dr Ryuzo Yanagimachi. This interest endured and motivated him to undertake further research for epigenetic and nuclear reprogramming under Dr Minoru Ko at NIA/NIH and Dr Kevin Eggan at Harvard University. While at Harvard University he also became an important part of Dr Douglas Melton's team, deriving human embryonic stem cell lines which were later offered freely to the scientific community to facilitate the efforts of other scientists. His special interests are deriving human embryonic stem cells for applying to clinical use, and egg development and epigenetic reprogramming.

Selected Publications:

1. X-Chromosome inactivation in cloned mouse embryos. Eggan K, Akutsu H, Hochedlinger K, Rideout W

3rd, Yanagimachi R, Jaenisch R. ***Science*** 290(5496):1578-1581, 2000

2. Maintenance of pluripotency and self-renewal ability of mouse embryonic stem cells in the absence of tetraspanin CD9. Akutsu H, Miura T, Machida M, Birumachi JI, Hamada A, Yamada M, Sullivan S, Miyado K,Umezawa A. ***Differentiation*** 78(2-3):137-142, 2009

3. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. Stadtfeld M, Apostolou E, Akutsu H, Fukuda A, Follett P, Natesan S, Kono T, Shioda T, Hochedlinger K. ***Nature*** 465(7295):175-181, 2010

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Development of pluripotent stem cells to female germ cells

Hsin-Fu Chen, Hong-Nerng Ho
National Taiwan University Hospital, Dept. of OB/GYN, Taipei, Taiwan

Introduction: Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) are unique stem cells characterized by the great capability of unlimited self-renewal and under appropriate environments can develop into all cell lineages including germ cells in human. These cells therefore will be useful as models for toxicology and pharmacology and also in clinical practice as sources of cell-based therapy for those patients suffering infertility, premature ovarian failure (POF) or recurrent abortion, due to defects in germ cell quality and quantity. Recent data suggested that ESCs can develop to early germ cells, but mature female germ cells remains a big challenge. Our Lab has previously showed that hESCs were capable of differentiating spontaneously in vitro into cells expressing germ cell markers and ovarian follicle-like structures. Recently we also derived a POF patient-specific hiPS cells line. Therefore by using these stem cells as starting materials, germ cells hopefully may be produced for future application.

Materials and Methods: We have created a reporter Oct4-eGFP hESCs line for tracking and enrichment of germ cells during in vivo and in vitro development. We differentiated NTU1 hESCs and H9 Oct4-eGFP hESCs in vitro using a number of niche environments, including laminin-coated dishes, human granulosa cell co-culture or conditioned medium (CM), mouse ovarian stromal cell co-culture or CM, and addition of growth factors (retinoid acid, SCF and BMP4).

Results: We showed that granulosa cells co-culture and its CM increased the percentages of cells expressing early germ cell marker SSEA1 (SSEA1+)(up to 40%) and/or Oct4-eGFP (Oct4-eGFP+) compared to laminin alone (18%). Though the use of Oct4-eGFP line was not absolutely necessary, manual collected Oct4-eGFP+ cells on day 14 of differentiation significantly up-regulated specific germ cell gene VASA and late marker GDF9 expression, although meiosis markers SCP1 and SCP3 and mature germ cell marker ZP3 were not. These Oct4-eGFP+ cells developed into ovarian follicle-like structures after extended culture for 28 days. In addition, supplement of retinoid acid also enhanced VASA and GDF9 expression in the differentiated cells, whereas SCF and BMP4 did not. In addition, the injection of undifferentiated OCT4-eGFP hESCs into SCID mice produced teratomas containing differentiated Oct4-eGFP+ cells. These eGFP+ cells and/or their surrounding cells also expressed early germ cell markers. Taken together, early human germ cells can be identified and isolated through the use of pluripotent stem cells but can be enhanced by the use of transgenic OCT4-eGFP hESCs reporter.

Conclusions: It is concluded that granulosa cells co-culture and CM may increase the percentages of cells showing germ cell potential. SSEA1 alone may not be a good marker for isolating primordial germ cells from differentiated hESCs, except perhaps at an early stage of differentiation. On the contrary, GDF9 may become a useful marker for selection at later stage (after day 21). We propose that the protocol of differentiating hES cells in granulosa cell co-culture/conditioned medium and/or retinoid acid supplement, followed by early manual selection of Oct4-eGFP+ cells, and finally by selection with VASA and/or GDF9 (especially) markers can become an effective strategy to enrich the cells with greatest potential for germ cell formation.

◆Biosketch

(For Dr. Chen's Biosketch, please refer to his abstract for Concurrent Symposium C-19.4.)

Testicular stem cells as sources of fertility preservation and cell therapy

Kwang Yul Cha
CHA Health Systems and CHA University of College of Medicine, Seoul, Korea

It is well known that many types of stem cells and somatic cells exist and are sustained in testis tissue for the life of a male. At first, spermatogonial stem cells (male germ-line stem cells, GSCs) self-renew and produce large numbers of differentiating germ cells that become spermatozoa throughout the adult life. Specific models for in vitro proliferation and expansion of GSCs from neonatal or adult testis have been developed by several researchers, and that has become a useful tool for studying spermatogenesis mechanisms, and has important implications for the development of new technologies in transgenesis or medicine.
Recently, multipotent SSCs were derived from SSC *in vitro* and were proposed as a potentially useful source of pluripotent cells for cell-based therapy. This type of human cell may provide simple and non-controversial access to individual cell based therapies without any ethical issues, but their efficiency for establishing heterogeneous testicular cell population was still extremely low and not well characterized. Last one is testicular mesenchymal stem cells (MSCs) with high proliferative activity. The MSCs were easily acquired by simple culture and MACS sorting from male donors with either normal or abnormal testis physiology and have shown similar growth kinetics, expansion rates, clonogenic capacity and differentiation potential as MSCs. Further research and application of these types of testicular stem cells may allow individual cell-based therapy without the ethical and immunological problems as well as infertility treatment.

◆Biosketch

Dr. Kwang Yul Cha is the Founder and Chancellor of CHA HEALTH SYSTEMS and CHA University in Seoul, Korea. Dr. Cha received his medical degree from the prestigious Yonsei University graduating summa cum laude and performed his postdoctoral fellowship in endocrinology and infertility at the University of Southern California. Dr. Cha also served as a visiting professor at Columbia University where he established and operated CHA Columbia Fertility Center in collaboration with Columbia Presbyterian Hospital.
An internationally renowned medical expert and one of the most recognized authorities on reproductive endocrinology and infertility, Dr. Cha pioneered the world's first successful pregnancy and birth through in vitro maturation from immature oocytes and also the world's first successful birth with frozen oocytes utilizing the technique of oocyte vitrification using grid.

In vitro production of functional sperm in cultured neonatal mouse testes

Takehiko Ogawa
Yokohama City University

The whole process of spermatogenesis, which produce sperm from spermatogonia through meiosis, has never been reproduced in vitro in mammals, nor in any other species with a very few exceptions in some particular types of fish. In our effort to develop in vitro system for spermatogenesis, we evaluated organ culture method to induce spermatogenesis in the testes of immature mice. To make evaluation simple and easy, we exploited 2 lines of transgenic mice, Gsg2/Haspin-GFP and Acr-GFP, which carry marker GFPs specific for meiosis and haploid cells. In our culture condition using serum-free media, both Acr-GFP and Gsg2-GFP showed their expression mostly at the same time as they did in vivo and spermatids and sperm were produced from gonocytes or primitive spermatogonia. The spermatogenesis was maintained over 2 months in tissue fragments positioned at the gas-liquid interphase. The obtained spermatids and sperm resulted in healthy offspring through microinsemination. In addition, neonatal testis tissues were cryopreserved and, after thawing, showed complete spermatogenesis in vitro. Our organ culture method could be applicable through further refinements to a variety of mammalian species, which will serve as platform for future clinical application as well as mechanistic understanding of spermatogenesis.

◆Biosketch

Takehiko Ogawa, MD, PhD

1989: Yokohama City University Graduate School of Medicine, finished MD course majoring at Pathology.

1989-1995: Working as urologist at several hospitals including Yokohama City University Hospital.

1995-1998: Post-doctoral fellow at University of Pennsylvania, Veterinary School of Medicine (Ralph L. Brinster's lab).

1998-present: Department of Urology, Yokohama City University School of Medicine.

STGO SESSION
ISF SESSION
APART SESSION

How to manage hyper- response to COS in IVF

Youssef Boutaleb
Morocco

Ovarian hyper stimulation is the most serious complication of ovulation inductors from a medical and medico-legal standpoint.
These complications present themselves as different clinical aspects.
There may be a pelvic accident such as a very high increase in the volume of the ovaries and the twisting or rupture thereof. Or there might be phenomena altering several vital functions classified by Golan into five stages:

1- Severe abdominal distension
2- Nausea, vomiting, diarrhea, ovary 5 to 12 cm
3- As cites and/or hydrothorax with respiratory difficulties
4- Disturbance Coagulation and/or of the kidney function.
5- Abnormalities of coagulation and/or renal function .

The condition of these patients may become complicated and show evidence of:
a state of shock, respiratory distress, disturbance of liver function, thromboembolic accidents, sometimes leading to death. In certain instances sequels can occur: hemiplegic, amputation of members, ovariectomy.
Prevention remains the most effective treatment.
In other instances, the ovarian stimulation must be stopped. Sometimes just rest is sufficient. At other times hospitalization is necessary in order to provide the patient with vascular expansion, punction of peritoneal or pleural effusions to give relief to the patient, puncture of ovarian cysts - but rarely surgery. The use of anti coagulants should be considered with care, as well as the use of anti-inflammatory substances, antihistamines, inhibitors or conversion enzymes. Termination of pregnancy is considered a final recourse

◆Biosketch

Doctor Youssef Boutaleb

Professor of Gynecology and Obstetrics
Formerly Medical Chief of Staff in Gynecology and Obstetrics of University Hospital Ibnou Rochd, Casablanca, Morocco
Former President, Moroccan Foundation of Medical Research
Former President, Moroccan Association for the Fight Against Cancer
Former President, Royal Moroccan Society of Gynecology and Obstetrics

Founder and Former President of the Moroccan Society of Fertility - Contraception
Former President of the World Congress I.F.F.S, 1989 Marrakech, Morocco
Author of Sterility of the Couple in the Arabo-Muslim Context
Royal Decoration as "Chevalier"

How to manage poor responders?

R. Frydman[1,4], F. Lamazou[1,4], V. Gallot[1,4], M. Grynberg[1,4], R. Fanchin[1,4], L. Hesters[2,4], N. Frydman[2,4], J. Taieb[3,4]

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AMH is considered like a major prognostic factor of ovarian response during controlled ovarian hyperstimulation. IVF in modified natural cycle has gained attention recently, even for both normal and poor responder. We conducted a retrospective study on 415 cycles of IVF in modified cycles with an objective to evaluate the impact of serum AMH concentration on day 3 on the outcomes. Patients of less than 37 years of age have been regrouped by AMH concentration: 0.04-0.49 ng/ml, 0.5-0.99 ng/ml, 1-1.49 ng/ml, 1.5-1.99 ng/ml, 2-2.249 ng/ml and 1.5-5 ng/ml. No statistically differences have been observed on cancellation rate, failure of oocyte retrievals, embryo transfers rate, pregnancy or implantation rate. The embryo quality based on the morphology on day 2 was similar in all the groups. In conclusion serum AMH concentration in day 3 does not seem to be correlated to pregnancy rate nor implantation rate, nor embryo morphology in IVF in modified cycle for patients of less than 37 years of age. IVF in modified cycle should be proposed in first intention to the patients with a poor prognosis characterized by a low AMH concentration inferior to 0.5 ng/ml with an implantation rate of 27.18%.

◆Biosketch

Name: Rene FRYDMAN
Birth date: November 3, 1943
Birth place: Soumoulou, France
Nationality: French
Current Positions and Rank: Professor, Faculty of Medicine, University Paris XI and Head of the Department of Gynecology and Obstetrics of Antoine Beclere Hospital, since 1990 to 2010 – Head of Mother Child Department 2000-2010.
Awards of Distinction:
Officer of the French Legion d'Honneur
Areas of Special Interest and Accomplishments:
My special areas of interest in Gynecology and Obstetrics include **infertility** and **high- risk pregnancy.** My work in infertility led the first baby born in France as a result of ***in vitro fertilization*** in 1982, after embryo freezing in 1986, after PGD in 2000, after IVM in 2003, after oocyte cryopreservation 2010, after PGD with HLA matching in order to bring about the birth of a saviour sibling in January 2011.

My other area of interest has been **biomedical ethics**. My work in this realm led to many invitations to debate the moral issues created by the use of the **techniques of artificial procreation.** I have had an active participation in the preparation on the **law on bioethics.**

The thin endometrium in ART: What to do?

Amina Oumeziane
TIZIRI: IVF center, Algiers, Algeria

The thin endometrium stills an unresolved problem in infertility. The negative impact of thin endometrium in the pregnancy rate and live birth rate is still controversial. In addition to the thicknesses of the endometrium, different studies show that other parameters must be considered to evaluate the unresponsiveness of the the endometrium; these include the following:

- The volume of endometrium by 3D sonography the day of HCG.
- The endometrium vascular pattern
- The immunological factors
- The genomic analysis of the endometrium responsiveness

Different treatments have been suggested for improving IVF outcome. Oestradiol at luteal phase, Triptoreline at luteal phase, Vitamin E, L.Arginine, Sildenafil, Low dose Aspirin
And recently, advance point to the role of cytokines in the proliferation of the endometrium by the use of intrauterine infusion of G-CSF which may be considered as a new way of managing thin endometrium

◆Biosketch
Dr AMINA OUMEZIANE: Gynecologist specializing in reproductive medicine. Currently: Medical Director of TIZIRI: IVF center at Algiers
Fellowship at Cochin Hospital University Paris V France in 2000

How to treat the female causes of repeated implantation failure?

Khaled Terras
Centre of Reproductive Medicine and Prenatal Diagnosis, Tunis, Tunisia

Implantation is a complex initial step in the establishment of a successful pregnancy. Although embryo quality is an important determinant of implantation, temporally coordinated differentiation of endometrial cells to attain uterine receptivity and a synchronized dialog between maternal and embryonic tissues are crucial. The exact mechanism of implantation failure is still poorly understood.

The causes for repeated implantation failure (RIF) may be because of reduced endometrial receptivity, embryonic defects or multifactorial causes.

Various uterine pathologies, such as thin endometrium, altered expression of adhesive molecules and immunological factors, may decrease endometrial receptivity, whereas genetic abnormalities of the male or female, sperm defects, embryonic aneuploidy or zona hardening are among the embryonic reasons for failure of implantation.

In this mini review, we discuss the suggested methods for evaluation and treatment of RIF: repeated hysteroscopy, myomectomy, endometrial stimulation, immunotherapy, preimplantation genetic screening (PGS), assisted hatching, zygote intra-Fallopian transfer (ZIFT), co-culture, blastocyst transfer, cytoplasmic transfer, tailoring stimulation protocols and salpingectomy for hydrosalpinges.

Optimizing endometrial receptivity in fertility treatment will improve success rates. Evaluation of implantation markers may help to predict pregnancy outcome and detect occult implantation deficiency. Treating the underlying gynecological disease with medical or surgical interventions is the optimal current therapy. Manipulating the expression of key endometrial genes with gene or stem cell-based therapies may some day be used to further improve implantation rates.

◆Biosketch

Pr Khaled Terras is born in Bizerte (Tunisia) in January 1st, 1964
Medical studies, gynaecology and obstetrics degrees: faculty of medicine, Tunis
Diploma in the reproductive health, in foetal medicine and ultrasound in Paris universities (V &XIII). Training in these fields was achieved in IVF unit of Baudeloque clinic in Paris and Harris birth right unit of foetal medicine (Pr K. Nicolaides, London). He was a senior consultant at Charles Nicolle Hospital in Tunis.
Dr Terras is now member of the team of the “centre of reproductive medicine and prenatal diagnosis” in Tunis. He is the former general secretary of the Tunisian society of gynaecology and obstetrics, and the former president of the scientific committee of the Arab Association of gynaecology and obstetric

How to manage sperm investigation after ICSI Failure

Moncef Benkhalifa[1,2], Paul Cohen Bacrie[2], Alain dalleac[2], Yves Menezo[3]
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Cytogenetics studies of infertile male karyotype revealed between 2 to 15% of abnormalites. In azoospermia cases nearly 15 % are carrier of unbalanced karyotypes (mainly 47, XXY) but in oligospermia cases only 2 to 5% of chromosomes disorders were observed. More than numerical abnormalites translocations including gonosomes are detected also. In the litterature its clearly reported that the male genome abnormalities can be involved in oocyte activation failure, early embryo development blocking, implantation failure and pregnancy loss.

In male infertility several Y and autosomal linked genes are involved not only in testicular but also in ovarian development. Others mutations in X chromosome (Kalleman gene, androgen receptor) or autosomal: (mytonic dystrophy, CFTR, 5-alpha -reductase Deficiency) are Known to interfere with normal spermatogenesis . For male patient with abnormal caryotype the assessment of aneuploidy or chromosome segregation in sperm is recommended prior IVF for genetics counselling.

During the spermatogenesis meiosis all chromosomes are equally submitted to the non disjunction with the nearly 10% of aneuploidy risk. Regarding reciprocal and robertsonian translocation the profile of segration is variable from one translocation to another and between patient carrying the same translocation. For this reason FISH analysis of sperm in such kind of cases is very informative to set up the strategy of couple management (IVF, ICSI, Blastocyt, PGD).

Based on the profile of chromosome disjunction we will get an idea about the risk of embryo blocking, implantation failure and abortion. Another factor can contribute in ART failure more than chromosome abnormalities is the DNA integrity and compaction of the entire male genome. An abortive spermatogenesis or an oxidative stress can affect the DNA integrity by producing hight level of sperm DNA frangmentation. We reported earlier that there is a relation between sperm parameter, the level of DFI and IVF outcomes.

During the spermiogenenis and germ cell final maturation, the efficiency of proteines replacement of histones by protamines is important to keep the compaction of the genome and to protect the paternal transcripts which involved in oocyte activation after fertilisation. Any unbalance of proteines replacement and /or between the ration of protamine 1 and 2 can affect the competency of the spermatozoa for oocyte acticvation, embryo development and implantation.

It's now established that mature spermatozoa contain a complex panel of m RNAS, studies performed earlier showed that these mRNA have an important role in normal spermatogenesis and early embryo development . From our experience using microarrays we showed that germ cells of oligospermic men have a major alteration of gene expression profile when compared to normospermic individuals

In male to assure the final biological function of the spermatozoa any defect of the spermatogenesis (chromosome non disjunction, apoptosis and DNA fragmentation) and the spermiogenesis (proteines replacement, compaction defect by denaturation, mitochondrial function or transcripts alterations) will play a key role in IVF success mainly at embryo development and implantation failure levels.

◆Biosketch

(For Dr, Benkhalifa's Biosketch please refer to his abstract for Concurrent Symposium C-16.2)

How to improve laboratory procedures to enhance implantation?

Ghaya Merdassi
IVF Center, Hospital of Aziza Othmana, Tunis, Tunisia

The expansion of indications for ART has not only increased the number of patients attending IVF clinics, but also the number of techniques currently in use. The embryologist has a responsibility for the correct and justified application of ART in the laboratory. The purpose of this report is to review the indicators and guidelines of good practice for each center of the ART to enhance the implantation rate. In fact, current methods of evaluation and quality measurement are reported as the optimization of culture conditions, the operation of equipment and consumables. The application of system quality management allows a high level of operation of the laboratory and the improvement of the implantation rate through a better selection of gametes, zygotes and embryos.

◆Biosketch

Dr. Ghaya Merdassi
Date of Birth: 01/08/1976. Tunis
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PRESENT ADDRESS:
IVF Center, Hopsital of Aziza Othmana
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EDUCATION AND TRAINING:
Inter University Degree: University of Versailles, Paris, France 2010
Master Degree: University of Claude Bernard Lyon, France 2
Biology Degree: University of pharmacy, Tunisia 2005
Pharma.D: University of pharmacy, Tunisia 2000

RESEARCH AREAS:
Cryopreservation of ovarian tissue
Cytogenetic analysis in teratozoospermia
Aneuploidy screening and polar body biops

Gonadotropin-releasing hormone agonist trigger: The way to eliminate OHSS

Arye Hurwitz
Professor, Head of the IVF Unit, Hadassah University Hospital, Mount. Scopus, Jerusalem, Israel

After more than thirty years of IVF ovarian hyperstimulation syndrome (OHSS) still remains a major threat to our patients in both long agonist and short antagonist cycles. It was first suggested by Itskovitz et al in 1988 that GnRH agonist (Buserilin) can induce the LH surge and oocyte maturation while eliminating OHSS in 2 women who previously developed severe OHSS. Only in the last 5-6 years many centers have started using GnRH agonist to trigger ovulation in high risk and donor patients in order to avoid OHSS. It was found that in 16 publications between 2006 - 2010 comparing hCG to GnRH agonists there was not a single women who developed OHSS out of 2005 patients after GnRH agonist triggering, while 92 out of 1810 (5.1%) developed OHSS after hCG. Even in GnRH antagonist cycles there was an incidence of 2% of severe OHSS. It is suspected that GnRH agonists prevents OHSS by inducing luteolysis of the corpus Luteum. This assumption is based on the studies by Nevo et al who demonstrated a significant reduction of Estradiol and Progesterone as well as Inhibin A and Pro alphaC, all markers of luteal demise. Since triggering ovulation with GnRH agonist causes a quick and irreversible luteolysis adequate luteal support is crucial. When conventional luteal support was given (Uterogestan 200mg tid and Estrofem 2mg bid) the pregnancy rates were extremely low. However when the luteal phase was inforced by either 50 mg Progesterone I.M and 2mg Estrofem tid, or the 1500 IU hCG (one dose), from the day of oocyte retrieval the pregnancy rates improved significantly and were similar to those obtained after hCG triggering. In conclusion, we believe that OHSS should be a disease of the past and that GnRH agonist triggering should be administered in high responders to totally abolish its occurrence.

◆Biosketch

Graduate of the Tel Aviv University Medical School.
Completed his residency in Obstetrics and Gynecology at Hadassah University Hospital at Mount Scopus, Jerusalem.
Did his subspecialty in Reproductive Medicine at the University of Maryland in Baltimore, Maryland, USA.
Is currently the Director of the IVF unit at Hadassah University Hospital, Mount Scopus, Jerusalem.
Published over 100 articles in leading international journals.

From endometrial injury to IMSI: New approaches for repeated IVF failure

Martha Dirnfeld
Reproductive Endocrinology and IVF at the Carmel Medical Centre, Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel

In vitro fertilization (IVF) has become a widely accepted clinical practice to assist reproduction. However, despite improved technologies and better results in recent years, the majority of patients do not have a successful pregnancy following a single IVF treatment.

The availability of assisted reproductive technology (ART) and delivery rates per oocyte retrieval vary greatly between countries. Per aspiration, pregnancy rates ranges are 17% to 42% and delivery rates 8% to 34%

Repeated treatments and failures have negative impact on quality of life. Each failed cycle incurs substantial financial costs. The expense, time, stress, and frustration felt by couples and physicians has led to a search for new drugs and technologies that will increase success rates. The aim of this presentation is to establish the role of various therapies for patients with repeated implantation failures.

Although some techniques have shown to increase pregnancy rates in women with poor prognosis due to specific conditions such as Hydrosalpinx, intrauterine polyps or adhesions, specific endocrine diseases or long-term gonadotrophin releasing hormone agonists for women with endometriosis, many infertile women still fail to conceive, despite repeated transfers of high-quality embryos.

A number of strategies have been suggested to improve unexplained repeated implantation failures. Various techniques in the IVF laboratory have been used on sperm oocytes and embryos in conjunction with IVF to increase the pregnancy rate. Empiric drugs before after or adjacent to ovarian stimulation have been used and various procedures have been advised to patients with repeated implantation failures .

Although mechanisms of actions have been proposed, justification for the use of some therapies is usually empirical, and based on physicians' personal views. Controlled randomized studies of adjuvant therapies in women who have had repeated IVF failure are often difficult to perform, due to the great variation between patients and the large sample size required.

The main factors found to influence the outcome of IVF and intracytoplasmic sperm injection (ICSI) include maternal age, the number of oocytes retrieved, sperm quality, the number and quality of the embryos transferred, the technique and ease of embryo transfer (ET), and endometrial receptivity. All the above should be addressed in patients with repeated failures.

Individual review of each implantation failure and the use of some adjuvant therapy may improve the outcome of IVF.

Review of the literature shows that none of the available adjuvant therapies has a clear advantage to all patients. Notably, adjuvant therapies have been administered without a diagnosis as to whether the failure to conceive is due to a maternal or fetal factor. Salpingetomy in cases of Hydersalpinx and conservative Myomectomy of fibroids encroaching the cavity may improve implantation in a subgroup of patients. High magnification (over ×6000) provides the identification of spermatozoa with a normal nucleus and nuclear content. Injection of spermatozoa selected according to fine nuclear morphology under high magnification may improve the clinical outcome in cases with severe male factor infertility. However, Intracytoplasmic morphologically selected sperm injection (**IMSI**) did not provide a significant improvement in the clinical outcome compared with ICSI.

If the embryos are genetically abnormal, no maternal adjuvant treatment will improve the pregnancy rate, and the genetic aberration will confound the results. Similarly, PGS will not be effective if implantation failure

is due to a maternal factor. Therefore, some of the therapies that have not been confirmed may prove efficacious in subgroups of patients.
Even when failure to conceive originates in the woman, certain adjuvant therapies may benefit only women with particular characteristics. As examples, endometrial biopsy or minor injury to the endometrium (Pipel) may benefit patients with a thin or nonresponsive endometrium
Uncertainty of the effectiveness of sildenafil may be due to the confounding
of fetal factors. Similarly, heparin may be effective against antiphospholipid antibodies other than lupus anticoagulant or anticardiolipin antibody. Profilaxis antibiotics may be suitable for some patients.
Many patients with repeated failure are also poor responders. Treatments such as Steroids , Growth Hormone and DHEA will be discussed. Presently, the diagnosis of IVF failure is not sufficiently specific to indicate definite adjuvant therapy.
In light of patients' easy access to updated articles, physicians need to be especially prepared to answer questions raised by couples that have repeatedly failed to conceive. Physicians endeavor to provide treatment that may be beneficial; as their role is not only to withhold treatment until sufficient randomized trials are conducted. Treatment often needs to be "tailor-made" to suit the individual patient.

◆Biosketch

Prof Martha Dirnfeld

Graduated medicine at the Technion, Israel, Institute of Technology, Haifa and Board certified in OB/GYN at the Carmel medical centre.
Since 1986 Head of Division - Reproductive Endocrinology and IVF at The Carmel Medical Centre, Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel.

President of the Israeli Fertility Society (IFS) and Israeli representative to ESHRE – National Committee Representative, (NRC) .

Worked in leading IVF Centres in Europe.
During 3 years (1982-1984) in Belgium with IVF team at the Free University (ULB) and Honorary Senior Clinical Research fellow at Bart's and the London Queen Mary's School of Medicine and Dentistry, London
In 2002-2004 invited to be General Manager at London Bridge Fertility-IVF Gynecology and Genetic Centre. UK Leading center in ART and PGD and since then regularly visits for consultations at the same centre.

Prof M.Dirnfeld Research interests include:

- Various research projects in Reproductive endocrinology and assisted reproductive technologies
- Oocyte and Embryonic microenvironment
- Factors involved in Implantation mechanisms and implantation. .

Published more than 150 papers, reviews and Abstracts at international conferences and Chapters in 6 books.
Invited speaker at national and International Conferences.

High ART success rates when financial constraints are completely lifted

Seidman DS, Lande Y, Maman E, Baum M, Dor J, Hourvitz A.
IVF Unit, Department of Obstetrics and Gynecology of the Chaim Sheba Medical Center, affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Israel has a unique situation because IVF treatments are fully covered by the national health insurance and thus are provided free of charge to all women until their first two children are born. Israeli patients are offered easy access to IVF clinics where they can immediately undergo, when medically indicated, an unlimited number of IVF treatment cycles. We assumed that for infertile patients the chances of becoming pregnant and having a live child should be greater in Israel than in most other countries because the financial cost does not play a role in the patient's decision to obtain treatment.

We undertook an historical prospective cohort study that included all couples referred to the Sheba Medical Center IVF unit who met the following inclusion criteria: [1] women aged <35 years at the time of referral, [2] women with primary infertility, [3] women with no IVF treatments before referral, [4] first IVF cycle performed between 2001 and 2002, and [5] a minimum of 12 months' infertility before initiation of IVF treatment.

During the 5- to 7-year follow-up period, 95.5% of couples conceived, and 89.6% of couples gave birth to a live infant. Of these couples, 81.3% achieved a live birth within the first 4 years of the follow-up period, and 85.1% within eight treatment cycles. Of the 14 couples (10.4%) who did not give birth to a live infant, five adopted, two divorced, four are still undergoing IVF treatments, and three (1.8%) decided not to become parents.

We concluded that young couples beginning IVF treatment in an environment free of economic hurdles can be reassured that they have an excellent chance (~90%) of achieving a live birth within 4 years. When IVF is provided free of cost, very few couples discontinue treatment before a live birth is achieved.

◆Biosketch

Daniel S. Seidman, MD, MCSc

(For Prof. Seidman's Biosketch please refer to his abstract for Concurrent Symposium C-20.3)

Autologous transplantation of very thin ovarian fragments which preserve the ovary's main cortex structure lead to successful pregnancy

Ariel Revel[1], Meital Lebovich[1], Alex Simon[1], Neri Laufer[1], Einat Eizenmann[1], Eduard Mitrani[2]
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[2]The Alexander Silberman Institute of Life Sciences, Hebrew university, Jerusalem, Israel

The improved survival rates among patients with hematological malignancies, such as lymphoma and leukemia, are shifting areas of focus towards understanding and preventing treatment-induced sequel. Of these, infertility is one of the most devastating consequences for patients with reproductive potential. The degree of treatment-induced gonadal dysfunction depends on age and gender-related differences, the type and dosage of chemotherapy used and the field and cumulative dose of abdomino-pelvic irradiation. There is also the interesting phenomenon of reduced pre-treatment fertility among male lymphoma patients. At present, the only established methods of fertility preservation are cryopreservation of sperm, oocytes and embryos, as well as gonadal shielding and transposition of ovaries during irradiation. Several other methods, such as cryopreservation and subsequent transplantation of gonadal tissue and the gonadoprotective role of hormonal suppression, are under investigation. Pre-pubertal patients present a unique constellation of fertility considerations, especially as embryo and sperm cryopreservation are not applicable to this age group. We will present our data on ovarian cryopreservation for the past decade for various indications. We will detail our 19-year-old thalassemic patient who had tissue from one of her ovaries cryopreserved prior to bone marrow transplantation, total body irradiation and sterilizing chemotherapy. As expected, premature ovarian failure resulted from this treatment. Transplantation of her thawed ovarian tissue resulted in return of menstrual cycling and the patient then underwent several IVF cycles. The patient, however, had poor ovarian response to hyperstimulation. We thus considered an alternative approach based on the observation that very thin ovarian fragments that preserve the basic ovarian structure [ovarian micro-organs (MOs)] induce angiogenesis and remained viable after autologous transplantation in animals. Preparation of autologous tiny ovarian fragments (MO)s and re-implantation into our patient resulted in IVF pregnancy and delivery of a healthy baby. The presentations will discuss the state of the art in the field of fertility preservation before gonadotoxic chemotherapy.

◆Biosketch

Born in Strasbourg, France (1961), Married (Nili, 1984) and father of four. MD from Hebrew University (1988). Israel board certified in Ob/Gyn following residency at Hadassah Mt. Scopus (1996). Completed fellowship in reproductive medicine at the Department of Obstetrics and Gynecology, University of Toronto, Ontario, Canada (1998-2000). Currently, staff physician, in vitro fertilization (IVF) unit and Department of Obstetrics and Gynecology, Hadassah. Member, Endocrinology circle, Hebrew University Medical School. Manager of the oocyte donation (OD) program. Leader of the fertility preservation program. Clinical and research fields include IVF, embryo implantation, endometriosis, polycystic ovarian syndrome (PCOS), laparoscopic and hysteroscopic surgery and ovarian cryopreservation. Served as treasurer of the Israel Fertility Association (IFA) executive committee (2004-7). Board member Special Interest Group for Endometriosis & Endometrium (SIGEE) of the European Society for Human Reproduction and Embryology (ESHRE), Board of directors for the cancer institute for fertility preservation. Hobbies include snowboarding, tennis and movie scriptwriting.

Specialties: infertility, oocyte donation, ovarian cryopreservation and transplantation, in vitro fertilization, endometriosis, polycystic ovarian syndrome, fibroids, operative hysteroscopy, laparoscopic surgery

How should we consult candidates for fertility preservation due to a malignant disease regarding their expected ovarian response to COH?

Shevach Friedler
Sasckler Faculty of Medicine, Tel Aviv University, Israel
The Infertility and In-Vitro Fertilization Unit, at Assaf-Harofeh Medical Center, Israel

Evidence based consultation is crucial for candidates for fertility preservation facing chemo/radiotherapy due to a malignant disease, regarding their expected ovarian performance after COH. A systematic MEDLINE (Pub-Med) search revealed a total of only seven retrospective, case control studies matching our inclusion criteria. A meta-analysis was performed based only on published results in which the outcome of these patients was compared to age matched healthy patients undergoing COH for IVF/ICSI. The available literature so far includes patients with variable age groups and the outcome is not stratified according to the specific malignant diseases. COH protocols used are variable, the majority using GnRH antagonist. However, it is evident from this meta-analysis that candidates for fertility preservation with a malignant disease, reached a significantly lower ovarian response compared with their healthy controls. Their fertilization rates were comparable. The number of cryopreserved oocytes or embryos is not stated systematically in the studies included in this review. Since only a minority of the embryos underwent thawing and transfer the data available does not allow to draw conclusions regarding the true reproductive potential of fertility preservation in these patients.

◆Biosketch

Prof. Shevach Friedler is an associate professor at the Sackler School of Medicine, Tel-Aviv University and a senior physician at the Infertility and In-Vitro Fertilization Unit, at Assaf-Harofeh Medical Center, Israel. He is also the director of the male and female infertility clinic of Kupat Cholim Meuchedet, Tel-Aviv. He obtained his M.D.degree at the Medical faculty of the Hebrew University of Jerusalem. Following specialization in Obstetrics and Gynecology in Jerusalem, he also completed a fellowship in Reproductive Endocrinology at Stanford University, California, USA. For the last twenty six years he works in the field of infertility and in vitro fertilization, currently in a university affiliated IVF unit, treating over 1000 IVF/ICSI cycles per year. He has authored over 80 publications including original articles, case reports, chapters in books and more, dealing with various aspects of infertility treatments, hysteroscopy, several pioneering papers concerning surgical sperm retrieval in azoospermic patients as well as different treatment modalities in in-vitro fertilization.

Clinical efficacy of adipose MSCs in human

Byung-Rok Do
HurimBioCell Inc., Seoul, Korea

Stem cells are unique cell populations with the ability to self-renewal and differentiation, and categorized into embryonic stem cells (ESCs), adult stem cells and induced pluripotent stem (iPS) cells. Adult stem cells existed in most of functionally mature tissues, such as epithelia, blood, germ line, brain, muscle and pancreas and are known to contribute replenishment of tissues after normal cellular senescence or injury. Mesenchymal stem cells (MSCs), one of the adult stem cells, are excellent sources for developing a practical use in cellular therapeutics. They have no ethical problem and many advantages which is multipotency, auto-transplantation and safety from carcinogenesis. However, heterogeneous populations of MSCs are inevitable consequence of isolation methods. It makes difficult to define stem cells and elucidate therapeutic mechanisms. Recently, MSCs are defined as an ability of plastic adherence, capacity of multipotency, and characteristic phenotype at the 2007 conference of the International Society for Cellular Therapy (ISCT). The phenotype expression is CD73, CD90, and CD105 positive and CD34, CD45, CD14 or CD11b, CD19 or CD79a negative, and HLA-DR low.Adipose tissues, originating from embryonic mesodermal tissues, contain MSCs named as adipose derived stem cells (ASCs) with the highest frequency among mesenchymal stem cells about 3 x 104 ~ 2 x 106 cells/ml aspirated fat tissue. ASCs show similar characteristics with bone marrow derived MSCs. They can be differentiate into adipocytes, chondrocytes, osteocytes in vitro, and can also express of phenotypic characteristics of endothelial cells, neural cells, smooth muscle cells, skeletal myoblasts, and cardiac myocytes after differentiation induction. ASCs have regarded as a valuable source for regenerative medicine, aesthetic plastic surgery and oncological disorders. Many researchers have investigated that ASCs promote regeneration of damaged tissues by angiogenic effect, paracrine effect and immune modulation through animal models such as stroke, myocardial infarction, liver disease and diabetes. Especially, abilities of various cytokine secretions and immune modulation of ASCs have expected a various application in pharmaceutical or cosmetic. Moreover, ASCs have provided the important cues for cell survival in the damaged tissues during long term tissue repair, so they may become major cellular candidates in attempts to clinical applications. Recently, attractive investigations are in progress for functional improvement of genital glands and differentiation into germ cell lines. Especially, germ cell differentiation of ESCs, iPS cells and oocyte differentiation of MSCs have gradually extended into clinical field. In Summary, development of effective cell therapy using human ASCs should be accomplished technical point such as ex vivo expansion, controlled differentiation, stable phenotype, genetic stability, optimal microenvironment conditions, and functional conservation after transplantation.

◆Biosketch

Name: Byung-Rok Do, Ph.D.
Date of Birth: Jan. 26. 1962.
EDUCATION
1995 - 1998 Department of Biology, Doctor Course of Graduate School, Hanyang University, Seoul, Korea
PROFESSIONAL POSITION
2005 - Director Research Institute, Hurim BioCell Inc. Plural Professor in Hanyang University
1997 - 2003 Infertility research center, KangSeo MizMedi Hospital.
1990 - 1997 Infertility medical center, CHA General Hospital
RESEARCH INTEREST: Stem cell isolation, cryopreservation and guided differentiation Growth, development of human fetal ovarian tissues and follicles in vitro Human oocyte maturation in vitro
BIBLIOGRAPHY International Journal (12), Korean Journal (15) Proceedings and Poster Presentation; International (62), Local (40) PATENT and PATENT ON A NEW DEVICE; Patent - 15

Optimized liposuction method for mesenchymal stem cell extraction and subsequent culture in lean female patients

Ken Nakama
Coeur clinic

Objective: It has been recently found that compared to other sources, stromal-vascular cells = the progenitors of human fat tissue - are relatively easy to extract and can be efficiently cultured into mesenchymal stem cells (MSC).This could become a valuable source of MSC with potential applications in the field of gynecology. The present study evaluated the efficiency of an optimized liposuction method using Ringer's lactate solution for cell extraction.

Materials and methods:
Conventionally when liposuction is applied to the lower abdomen the "tumescent" method is used. This method developed in 1980 requires the subcutaneous injection of a large amount of isotonic saline solution. Although, currently this is the established method for weight reduction purposes it might be suboptimal for MSCextraction. It has been found previously that compared to the currently used saline solution survival rates were significantly higher when extracted stem cells were placed into Ringer's lactate solution. Another potential problem related with MSC extraction is that in lean patients, sufficient subcutaneous fat tissue is often lacking in the lower abdomen and the procedure has to be performed in other body areas.

Results:
Randomly selected gynecological outpatients who were candidates for liposuction and MSC extraction and culture had a mean age of 43.5 years and an average BMI of 20.9. In contrast, in a control sample of two-hundred patients who were scheduled for a weight-reduction liposuction intervention, the mean age was 32.2 years and the average BMI reached 24.7 (statistically significantly different). Compared to isotonic saline solution MSCrecovery rates were not significantly different when Ringer's lactate was used. The differences in MSCrecovery rates from different body areas have to be evaluated in a future study.

Conclusions:
Compared to the general liposuction patient population, gynecological outpatients who were candidates for liposuction and MSC extraction and culture were older. They had a lower BMI and diminished body fat tissue. Optimized liposuction by modifying the currently established "tumescent" method with the use of Ringer's lactate and using alternative body areas in lean patients might improve the results of subsequent MSC culture.

◆Biosketch
M.D.degree University of CungUng, Seoul 1987
Assistant Resident in *Surgery, University of Catholic HospitalGent, Seoul, May1987-Aug1991*
ICRC, République du Burundi, Sep1991-Aug1992
Fellow in the Department *of Transplant Surgery,* University Hospital, May1993-May1995
Fellow in the Department *of* Anesthesiology, Tokyo Med. University Hospital, Tokyo, May1995-May1999
Coeur clinic, Dec2009-present

Vitrification of adipose MSCs

Noriko Kagawa
Advanced Medical Research Institute of Fertility, Kato Ladies Clinic, Tokyo, Japan

Mesenchymal stem cells (MSCs) are capable of differentiation into a variety of cell types including osteoblasts, chondrocytes and adipocytes and are a promising source for cell-based therapy in regenerative medicine. Stem cell population isolated from human adipose tissue contains MSCs and their biological characteristics are similar to those derived from bone marrow. Since human adipose tissue can be harvested in large quantity by a minimally invasive liposuction procedure, tissue cryopreservation system is required to keep their freshness. Here, we developed successful vitrification method for cryopreservation of human adipose tissue. Human discarded adipose tissues were donated from anonymous patients who underwent a liposuction procedure and signed our informed consent. Human adipose tissue were vitrified by using human ovarian vitrification method (Cryotissue R: Kitazato BioPharma, Kagawa et. al., 2007) with modification. After thawing vitrified tissue, MSCs were isolated and survival rate of MSCs were examined. Survival rate of MSCs isolated from vitrified adipose tissue was 78.9% and there was no significant difference compared with MSCs from fresh adipose tissue (83.4%). These results indicate that our new vitrification method is safe and effective for cryopreservation of human adipose tissue. We speculate that our vitrification method will open the door for feasibility to access samples to promote stem cell research and translational medicine.

◆Biosketch

Dr. Noriko Kagawa obtained her Ph.D. in 2005 from the Kyoto University, Japan. She is currently the senior scientist at the Advanced Medical Research Institute of Fertility in Kato Ladies' Clinic, Tokyo, Japan, the world's largest human IVF unit. In 2000, she started animal embryology and studied Assisted Reproductive Technologies, for example, IVM, IVF, vitrification, embryo culture in pig, and established the porcine follicular growth system using SCID mice. She moved to the human IVF field in 2004, and has developed vitrification methods for mammalian ovaries and ovarian tissues in mice, cat, dog, lion, giraffe, bovine, human. Her major research interests have focused on in vitro growth and in vitro maturation for preantral follicles of adult mammals as well as on vitrification of Germinal Vesicle stage oocytes and various organs for unlucky patients.

Preparation of Adipose-Derived Stem Cells

Rie Yamadera, Yuji Takehara, Chiaki Sano, Kazumi Kawashima, Yoko Ito, Osamu Kato
Kato Ladies Clinic

Adipose-derived stem cells (ASCs) are increasingly drawing attention as a tool in regenerative medicine, because large volumes of adipose tissue can be collected from patients using a minimally invasive procedure.The general method for separating cells from adipose tissue involves the use of enzymes. The cells separated from adipose tissue belong to heterogeneous cell populations, including blood-derived cells, vascular endothelial cells, adipose precursor cells, and other cells. Recently, some research groups have reported the existence also of a very small number of stem cells, similar to mesenchymal stem cells (MSCs), in the cell populations separated from adipose tissue. These cells are called adipose-derived stem cells (ASCs). We performed flow-cytometric analysis and CFU-F (colony forming units-fibroblast) assay to confirm the number of ASCs included in this adipose-derived cell populations, and obtained results that were consistent with previous reports.Furthermore, it is possible to isolate and expand the stem cells by adherent culture of the cells separated from adipose tissue; this represents purification of ASCs. We confirmed a monogenous population of the cells under culture and also that the characteristics of the cells did not change under post-culture conditions.In this session, we shall introduce a general protocol to separate the cells from adipose tissue, and also the approach to culture that we employed to isolate the ASCs.

◆Biosketch

2007: Graduated from University of Tsukuba, Ibaraki, Japan, with B.Sc.
2007-Present: Kato Ladies Clinic
2009-2010: Visiting scientist, physiology, University of Keio

Laboratory medical technologist
Genetic counselor

Discussion about clinical applications of adipose MSCs

Yuji Takehara
Kato Ladies Clinic in Japan

Mesenchymal Stem Cells (MSCs) have recently received attention in clinical fields to cure serious illnesses for which no effective treatments exist, such as brain diseases, diabetes, hypertension, joint pain due to cartilage erosion, and myocardial infarction. While there is hope for regenerative medicine using stem cells, on the other hand, there is also anxiety about its effectiveness and side effects. Because we have very limited knowledge about stem cells and its regulatory pathways, new reliable reports are awaited. There are three types of stem cells that are potentially useful in the field of regenerative medicine. Embryonic Stem (ES) cells were established by Dr. Martin Evans in 1981, and accumulated data has given humans bright hope for cure of diseases, especially after the establishment of human ES cells by James Thomson in 1998. Although scientists have clarified in detail the mechanisms of actions of ES cells and also their efficacy, establishment of human ES cells involves sacrifice of blastocysts which have the potential to develop into human beings, as the inner cell mass of certain blastocysts is the source of ES cells. Once an ES cell line is established, it can be used permanently. This is an attractive idea, however, we need to apprise the couple donating their embryos to the basic scientist who will handle their precious blastocysts about this ethical issue. Another problem of use of ES cells in therapeutics is that it may evoke a rejection response when administered to the patients, because the ES cells originate from a different human and the chance of HLA type matching is very low. As a result, an ES cell banking system that will cover different types of HLAs will be required, and many ES cell lines are needed, which would need many healthy blastocysts to be destroyed. This ethical issue prompted scientists to establish iPS (induced Pluripotent Stem) cells, which can be obtained from mature adult cells, such as skin. In this regard, the greatest success in the establishment of iPS cells was reported by Dr. Yamanaka in 2006 and 2007, which again raised enthusiasm about curing difficult-to-cure diseases. iPS cells are not derived from blastocysts, which can develop into human beings, but from mature cells which are destined to be eliminated by cell death. No ethical disputes would be provoked when these cells are collected from the skin of the patient himself/herself. Another advantage of iPS cells is that administration of iPS cells is not associated with the risk of a rejection response, because these cells originate from the patients themselves and the HLA type is exactly matched with that of the patients. Despite these miraculous advantages, iPS cells have the potential to induce tumors as a result of induction of viruses. This possibility raised fear in respect of the clinical application of these cells. At the time of discovery of iPS cells, it was suggested that a two-year period would be needed to get safe iPS cells. This was not related to the production time, but the time needed to check whether the iPS cells will induce production of tumors. Recent competition to avoid virus use in the production of iPS cells again raises hope of obtaining safe iPS cells, but they still remain to be established. Perhaps iPS cells will also be useful for checking the effectiveness or side effects of newly produced drugs before clinical application. On the other hand, adipose tissue-derived MSCs (ADMSCs) are easy to obtain from abdominal fat tissue, and this liposuction procedure is applied in the cosmetic field for the purpose of weight loss. MSCs have the potential to differentiate into any type of cells required at the site of damage. There are some reports that the administration of MSCs by drip infusion may sometimes be dangerous as they may be associated with the risk of pulmonary embolism. Because cultured MSCs are larger than the original parent cells and there is a surface antigen whose function is to interfere adhesion, and number of this antigen decreased after culturing MSCs, decrease in the expression of surface antigens interfering with cell adhesion after culture of MSCs may increase cell-to-cell adhesion, resulting in the formation of bigger cells. We submitted a clinical research proposal to the Japanese Health and Labour ministry in September in 2010, because clinical research using large amounts of stem cells

requires approval of the Japanese government. The government recommended that we perform animal experiments and establish our own animal laboratory to conduct the experiments. Therefore, it is not easy to start a clinical study in Japan because of the Japanese Government's regulations. We believe that MSCs are the best candidate for regenerative medicine, because there are no related ethical issues and these cells are easy to obtain and also easy to handle.

◆Biosketch

Yuji Takehara MD., Ph.D.

1983 Graduated from Keio University, School of Medicine, Tokyo, Japan
Received M.D.
1991 Assistant Professor, Department of Obstetrics and Gynecology, Tokyo Dental College, Chiba, Japan
1991 Research Fellow at Johns Hopkins University, School of Medicine, Baltimore, MD, U.S.A.
1992 Instructor at Johns Hopkins University, School of Medicine, Baltimore, MD, U.S.A.
2008 Vice director of Kato Ladies Clinic
2011 Visiting Professor of Kinki University
Director of KLC Regenerative Medical Laboratory

ORAL COMMUNICATIONS

O-001 Does male reproductive tract CD52 (mrt-CD52) prevent complement activation via binding C1q?

○L. Hardiyanto, A. Hasegawa, S. Komori
Hyogo College of Medicine, Kobe, Japan

[Introduction] Human CD52 is a glycosylphosphatidylinositol (GPI) anchored peptide expressed in lymphocytes as well as the male reproductive tracts (mrt). mrt-CD52 is known to be a pathogenic antigen for immunological infertility. A human monoclonal antibody (Mab H6-3C4) generated from an infertile woman has strong sperm immobilizing activity with complement and specifically recognizes the N-linked carbohydrate of mrt-CD52 but not lymphocyte CD52. In addition, mrt-CD52 interfered with the classical pathway but not lectin binding or alternative pathways of the complement system. In this study we focused on the C1q molecule as a binding component to mrt-CD52. [Material and methods] mrt-CD52 was purified from human seminal plasma or sperm membrane by extraction with a mixture of chloroform: methanol: water. For examination of mrt-CD52 binding to C1q, we carried out immunoprecipitation analysis by incubating a reaction mixture of mrt-CD52, human serum for C1q source, and Mab H6-3C4. N-glycosidase F (NGF) and phosphatidylinositol-phospholipase C (PI-PLC) were used to release the N-linked carbohydrate and the GPI-anchor from mrt-CD52, respectively. When NGF treated mrt-CD52, the carbohydrate moiety was isolated by ConA sepharose resulting unbound fraction comprised GPI-peptide portion and bound fraction comprised carbohydrate moiety, respectively. Those fractions then reacted with human serum and Mab H6-3C4. The formed immunoprecipitate was analyzed by a western blotting with the ECL method probed with anti C1q antibody. Sperm immobilization test (SIT) was carried to determine the effect of purified-CD52. [Results] Immunoprecipitate formed from the reaction mixture of mrt-CD52, human serum and Mab H6-3C4. After treatment of mrt-CD52 with NGF, immunoprecipitate formed from the reaction mixture of mrt-CD52, human serum and Mab H6-3C4. The immunoprecipitate was formed by the carbohydrate moiety reaction with human serum and Mab H6-3C4 while GPI-anchor peptide was not. mrt-CD52 treated with PI-PLC also formed an immunoprecipitate with human serum and Mab H6-3C4. The C1q molecule (29 kDa) was detected in the formed immunoprecipitates. In SIT, purified-CD52 showed an inhibitory effect on sperm immobilization value (SIV). [Conclusion] These results indicate that the carbohydrate moiety of mrt-CD52 binds to C1q but not the GPI-linked peptide. mrt-CD52 may inhibit the complement activity induced by the sperm antigen-antibody complex in female genital tracts.

O-002 Technology Research of Prostatic Urethral Irrigation and Drainage Catheter Injection

○Weidong Huang
Xinjiang Jiayin Hospital Center for Reproductive Medicine, Urumqi, China

Chronic prostatitis is a common and frequently-occurring male disease, it can seriously affect the semen quality. Cause of its complexity, longer duration and easy to relapse, so the clinical efficacy is uncertain. On the basis of the two-purse three-cavity prostatic urethra injection irrigation and drainage catheter, Professor Huang Weidong developed the catheter patents technology: it directly against because of urinary reflux, urinary prostate smooth muscle spasm, immune response, bacterial infection caused by factors such as the prostate duct obstruction and inflammatory exudate within the lumen of the prostate is difficult to discharge these two clinical problems, through the involvement of the prostatic urethra characteristics of catheter positioning section and thrombolytic drugs containing antibiotics and mixed liquid composed of the formation of pressure washing simulation prostatitis occur when the "urine reflux" type of "liquid reflux" to clear the prostate duct openings clear of inflammatory exudate and the prostate gland to relieve the obstruction. Because of its direct, safe, effective, simple, painless, repeatable and non-injury characteristics, making the cure rate of chronic prostatitis has been significantly improved.

O-003 Measurement of reactive oxygen species in neat and washed sperm, is there any difference?

○Mohammadreza Moein, Nasim Tabibnejad, Jalal Ghasemzadeh
Yazd Research and Clinical Center for Infertility, Iran

Objectives: Reactive oxygen species (ROS) are regarded as a major cause of infertility especially in idiopathic male infertility. ROS level have been measured both in washed and neat sperm. So in this study we determined the level of ROS in neat and washed samples of infertile men to see which sample could show more reliably the overproduction of ROS and oxidative stress.Materials and Methods: we collected semen sample from 35 infertile men referred to our andrology clinic. Patients with pyospermia and patients who had history of antioxidant treatment were excluded from study. The ROS levels were assessed by a chemiluminescense assay, using Luminol as a probe, with an Autolamat LB 935 luminometer (Berth Hold Technologies, Germany) before and after washing. Semen parameters and ROS level were compared in neat and washed samples.Results: The mean age of subjects were 31.43 years ranging from 23 to 60 years. Mean total sperm concentration in neat samples was 95.94 million/ml and mean quick and slow progressive motility was 17.82% and 31.14% respectively. Mean ROS level in this samples was 216.34 RLU. The mean sperm concentration in washed samples was 57.57 million/ml which was significantly lower than neat samples (p=0.000). The mean quick and slow progressive motility was 49.37% and 28.91% respectively. The quick motility was significantly higher than neat samples with (p= 0.000), but slow motility has not significant difference with neat samples (p=0.494).The mean ROS level was 62.06 RLU in washed samples. The mean ROS level was significantly lower in washed sperm (p=0.01). Conclusion: washing sperm can affect ROS level in semen samples of infertile me. Although sperm processing seems to increase the level of ROS in semen samples, but removal of immature germ cell and white blood cells will decrease ROS level in seminal plasma. Which one is more accurate for predicting oxidative stress and fertility must be determined in future studies.

O-004 Whole Genome Amplification is efficient for CTG repeats length detection of PGD for DM1

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[1]Saitama municipal Hospital Department of Obstetrics and Gynecology, Department of Obstetrics and Gynecology, Saiatama, Japan
[2]Keio University School of Medicine, Tokyo, Japan

Introduction: Myotonic dystrophy type1 (DM1) is an autosomal dominant disorder, with an incidence of 1/8000. The gene is located on chromosome 19q13.3 and unstable expansion of the CTG repeats. This explains anticipation, that is increase in severity PGD for DM1. PGD of DM1 has been performed by Nested PCR with linked polymorphic markers. However, carrying out PGD for DM1 is difficult for multiple reasons. First, the large expansion of CTG repeats is refractory to PCR because of the high GC contents. Direct diagnosis exposes also risk of allele drop-out (ADO). The first single cell assay developed for PGD for DM1 allowed selection of "unaffected" embryos based on the presence in the biopsied cell of the 'normal CTG repeat' of the affected partner and on the "normal CTG repeat" of the unaffected partner. Then it is necessary that the healthy allele of the affected parent differs from the two short alleles (within 37 repeats) of the healthy parent. Multiple Displacement Amplification (MDA) has been reported to yield large amounts of DNA from single cells. We improved PGD for DM1 to using MDA for purpose of detect of two normal alleles. Material & methods: (Genetic test) CTG repeats numbers of normal and affected alleles in both partners were analyzed before PGD. We analyzed the number of CTG repeat of the client family using Gene Scan. Whole Genome Amplification(WGA) with MDA method and Gene Scan were carried out for clients of PGD for DM1. MDA was performed using the GenomiPhi® Kit on lymphocytes. Gene Scan analysis was performed on the MDA product (5µl). This study aims to analyze the amplification rate and size of the expanded CTG repeat number after MDA methods. Results: Single lymphocytes gave very high amplification efficiencies for both protocols. Amplification rate by MDA was increased by this technique from 40% (4/10) to 60% (6/10). We performed gene amplification by using MDA methods, the number of CTG repeat showed no change. Conclusion: A repetition diagnosis of PGD for DM1 was enabled by using the MDA method which was non-PCR-based WGA. In addition, we can expect possibility of a rise of the diagnosis efficiency and the reuse of the rejection embryo was shown by using MDA. The MDA method showed the diagnosis efficiency that was higher than the Nested PCR method. WGA of MDA was shown to be useful PGD for DM1.

O-005 Reducing multiple pregnancies by selection of a single chromosomally normal blastocyst for transfer using array CGH (aCGH) analysis of 24 chromosomes within 24 hours

○Zhihong Yang[1], Xiaohong Liu[2], Shala Salem[1], Jiaen Liu[2], Rifaat Salem[1]
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Introduction: Selection of a single viable blastocyst for transfer is the solution for reducing multiple pregnancies in the stimulated IVF cycles. The objective of the present study was to evaluate the effect of selection of a single euploidy blastocyst for transfer by using aCGH analysis of 24 chromosomes on clinical pregnancy outcomes. Materials and methods: Two phases of studies were performed with an informed consent. In phase I, the efficacy of aCGH screening of 24 chromosomes of trophectoderm cells derived from blastocyst biopsy within 24 hours was evaluated. The trophectoderm cells were analyzed using the 24sure aCGH analysis of 24 chromosomes within 24 hours (BlueGnome, UK). The hatching rates were compared between the euploidy blastocysts and the anuploidy blastocysts after blastocyst biopsy. In Phase II, the effect of transfer of a single euploidy blastocyst following aCGH analysis on the clinical pregnancy outcomes was investigated. All patients were under 35 years old with normal FSH levels on their first IVF cycles. After biopsy and aCGH analysis, a single euploidy blastocyst was selected and transferred to each patient on day 6. A clinical pregnancy was defined as the presence of gestational sac(s) and fetal heart beat(s) detected by ultrasound. IVF patients with similar demographic and clinical characteristics were used as control. Significance of data was determined by Chi-square and t-tests. Results: 70.8% of the blastocysts were euploidy while 29.2% were aneuploidy. There was no significant difference in hatching blastocyst rates between the euploid blastocysts and the aneuploid blastocysts (P>gt0.05). 91.2% (31/34) of the patients had normal embryo(s) for transfer after the aCGH testing. 76.5% (26/34) of the patients became clinically pregnant, 96.2% (25/26) of them was confirmed with ultrasound as singleton pregnancy while 3.8% (1/26) was twin pregnancies.There was a significant difference in clinical pregnancy rates between the aCGH tested group and the non-tested IVF control group (76.5% vs. 40.3%, P<lt0.05). Conclusion: Our data suggests that the aCHG analysis of 24 chromosomes enables efficient differentiation of the euploidy blastocysts from the aneuploidy blastocysts. Moreover, selection of a single chromosomally normal blastocyst for transfer by using the aCGH screening of 24 chromosomes in fresh cycles may increase clinical pregnancy rates while reducing multiple pregnancies.

O-006 Preimplantation genetic screening of eggs and blastocysts with CGH-microarray: clinical experience with the first 51 cases

○Stuart Lavery[1], Ben Lavender[1], Paul Knaggs[1], Anastasia Mania[1], Geoffrey Trew[1], Dagan Wells[2]
[1]IVF Hammersmith, Hammersmith Hospital, London UK, [2]Reprogenetics Oxford UK

Introduction: The efficacy of preimplantation genetic screening has been one of the most keenly debated and controversial topics in assisted conception. Current evidence suggests that preimplantation screening of embryos at the cleavage stage using multicolour FISH does not improve outcome. Recently the ESHRE PGS taskforce has reported in a proof of principle study that screening of polar bodies using whole genome amplification and comparative genomic hybridisation on micro-arrays is a reliable and robust method for determining the chromosomal status of the egg. Materials and methods: Polar body micro-array CGH is likely to be able to detect where errors have a meiotic origin, but would not detect post zygotic errors leading to aneuploidy. Polar body biopsy and micro-array analysis offers the advantages of more time to study the chromosomes, a more complete karyotype, and may be ethically more acceptable than embryo diagnosis. Biopsy of the blastocyst should be able to detect aneuploidies with maternal, paternal and post-zygotic origin. 53 patients with more than three failed cycles each were eligible for our pilot study and all proceeded to egg collection. Results: 14 patients did not proceed to genetic analysis (less than three embryos). 7 patients had all aneuploid embryos and did not have a transfer. 4 patients had no CGH result but proceed to transfer of undiagnosed embryos, one conceived. 34 patients had embryo transfer following euploid results and 10 have a clinical pregnancy (29% pregnancy rate/ET). Conclusions: True clinical validation of this technique requires a large multicentre prospective randomised clinical trial. Such a trial is proposed by ESHRE with a completion date in 2013. Our initial pilot experience contributes some promising data to this hotly contested area.

O-007 Most Motile Sperm Can Be Separated at the Bottom of the Microfluidic Channel of a Plastic Microfluidic Sperm Sorter

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Objective: A microfluidic sperm sorter (MFSS) is a simple chip device in which motile sperm are selected by two gravity driven laminar flows within the microfluidic channel. Fluid mechanics simulation suggests that fluid velocity is the highest at the center of the microfluidic channel and that it is difficult for sperm to swim across the interface at the center. Because the observation of these phenomena using a conventional epifluorescence microscope is difficult, we employed a confocal microscope with a high-speed scanner. Here we investigated the number of sperm swimming across the interface in the 0.2 mm deep microfluidic channel of a plastic MFSS. Materials and methods: Separation Procedures: Semen was left for at least 30 min to liquefy before processing and was mixed with 2 μM Fluo-4 AM solution. Human Tubral Fluid (Irvine Scientific Inc., USA) was pipetted into the two outlets and one inlet of MFSS (Strex Inc., Japan), while the diluted semen was pipetted into the adjacent inlet. Fluorescent Imaging: The intracellular concentration of Ca^{2+} was measured in stained sperm obtained from MFSS, using a fluorescence microscope attached to a CSU10 confocal scanner (Yokogawa Electric Co., Tokyo, Japan). The time resolution of each frame was approximately 50 ms. Twenty minutes after loading the semen sample, the number of sperm that swam across the field of view and the maximum fluid velocity during 1 min recording at the bottom, middle, and top of the channel were determined. Results: The number of motile sperm was highest at the bottom of the adjacent inlet. At the top and bottom of the MFSS channel, the fluid velocity had decreased and the sperm readily swam across the interface. These results suggest that the number of sorted motile sperm obtained depends on the fluid velocity and the concentration of motile sperm at each level in the MFSS channel. Conclusions: Motile sperm could be separated most efficiently at the bottom of the MFSS channel because the fluid velocity was sufficiently low to allow sperm to swim across the interface. Furthermore, the motile sperm were concentrated by gravity and sperm/geometry interactions. Human sperm concentrate at the bottom of the microfluidic channel with decreased fluid velocity. This characteristic is an important consideration when attempting to increase the number of sorted motile sperm.

O-008 Polymerization of Insulin-Like Growth Factor-Binding Protein-1 (IGFBP-1) Potentiates IGF-I Actions in Placenta

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Background: Insulin-like growth factor (IGF)-binding protein-1 (IGFBP-1), the main secretory protein of decidua that binds to IGFs and has been shown to inhibit or stimulate IGFs bioactivities. Polymerization, one of the posttranslational modifications of IGFBP-1, has been shown to lead to loss of inhibiting effect of IGFBP-1 on IGF-I actions. The current studies were undertaken to elucidate the effects of steroid hormones on IGFBP-1 polymerization in trophoblast cell cultures. Materials and Methods: Placental tissues were obtained during legal, elective procedures of termination of pregnancy performed between 7 and 10 weeks of gestation, and primary trophoblast cells were separated. IGFBP-1 polymerization was analyzed by SDS PAGE and immunoblotting. IGFBP-1 was polymerized when IGFBP-1 was added to trophoblast cell cultures. Polymerization of IGFBP-1 was inhibited by the addition of anti-tissue transglutaminase antibody into the culture media. Results: There was an increase in the intensity of polymerized IGFBP-1 bands with the addition of medroxyprogesterone acetate (MPA), while no such difference was observed upon treatment with estradiol. MPA also increased the expression of tissue transglutaminase on trophoblast cell membranes. IGF-I stimulated trophoblast cell migration, while IGFBP-1 inhibited this IGF-I-induced trophoblast response. Addition of MPA attenuated the inhibitory effects of IGFBP-1 on IGF-I-induced trophoblast cell migration. IGFBP-1 was polymerized by tissue transglutaminase on the cell surface of trophoblasts, and MPA increased tissue transglutaminase expression on the cell surface and facilitated IGFBP-1 polymerization. Conclusion: These results suggest that progesterone might facilitate polymerization of decidua-secreted IGFBP-1 and increase IGF-I actions at feto-maternal interface, thereby stimulating trophoblast invasion of maternal uterus.

O-009 MicroRNAs associated with human embryo implantation defects

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Background: Repeated implantation failure (RIF) is a major problem encountered in in-vitro fertilization (IVF). We have previously reported that RIF-IVF patients have a different endometrial gene expression profile during the window of implantation. Considering microRNA (miRNA) function in post-transcriptional regulation of gene expression, the aim of the study was to confirm the involvement of miRNA in the defects of endometrial receptivity. Methods: We used TaqMan miRNA array cards to identify the miRNAs differentially expressed in the secretory endometrium of RIF-IVF patients as compared to fertile women and bioinformatics tools to identify their predicted targets and the molecular networks they may affect. Results: Comparing miRNA expression profiles, we identified 13 miRNAs differentially expressed in RIF endometrial samples that putatively regulate the expression of 3800 genes. We found that 10 miRNAs were overexpressed (including miR 145, 23b and 99a) and 3 were underexpressed. Using our previous gene expression analysis, we paralleled miRNA 8211 mRNA expression profiling. By this mean, we identified novel and previously characterized miRNA-regulated molecular pathways such as adherens junctions, Wnt-signaling and cell cycle pathways. Consistent with the miRNA data, mRNA levels of N-cadherin, H2AFX, netrin-4 and sFRP-4, belonging to the Wnt signaling and CAMs pathways were lower in RIF-IVF patients. Conclusion: To our knowledge, this is the first study to evaluate the differential expression of miRNAs in the secretory endometrium of RIF-IVF patients. We suggest that the RIF-associated miRNAs should be exploited as new candidates for diagnosis and treatment of embryo implantation failures.

O-010 Pre-ovulation leptin serum as a marker for endometrial receptivity

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Introduction: Having marker for predicting the endometrial receptivity would be the crucial point in calculating the time for embryo transfer. Leptin is a peptide that play an important role in conducting and maintaining the good communication between oocyte, embryo and endometrium. We are trying to find whether pre-ovulation leptin serum would be the useful marker for predicting the endometrial receptivity.Material and Methods: This study was conducted at Yasmin Reproductive and Fertility Clinic, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia. Thirty-three infertile women were recruited for this study. Leptin serum levels were measured twice on day 11-13th, and on day 20-22th of menstrual cycle. Endometrial biopsy was taken in mid luteal phase for assessing endometrial integrin αvβ3 immunohistochemically. Results: Positive correlation was observed between pre-ovulation leptin serum and mid luteal phase leptin serum ($r = 0.621$). More over, pre ovulation leptin serum showed a strong correlation with endometrial receptivity ($p< 0.001$). The best pattern of relationship between endometrial receptivity and leptin level in this study is linear with the formula of endometrial receptivity score = 1.813 x log (pre-ovulation leptin serum) -1.813. The leptin level that gave the best endometrial receptivity was 24.85ng/ml with 50.65 ng/ml as the maximal threshold. The multivariate analysis showed that body mass index, endometrial thickness, and leptin serum level are the variables that have correlation to endometrial receptivity. However, the only variable that showed significant corelation ($p < 0.05$) was pre-ovulation leptin serum (OR= 1.08; CI 95%= 1.01-1.16). During this study, there were four patients who got pregnant with the integrin αvβ3 expression ranged between score 1.5 to 4.0. Conclusion: Pre-ovulation leptin serum can be widely used as a marker to assess endometrial receptivity because it has a good predictive value. Moreover, it is easier to do and relatively cheaper than integrin αvβ3 expression test.

O-011 Application of Mechanical Stimuli Using a Microfluidic Air Actuation System: Dynamic Embryo Culture

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Objective: Mammalian embryos experience not only hormonal but also mechanical stimuli (MS), such as shear stress (SS), compression, and friction force, in the fallopian tube before nidation. Previous in vitro dynamic culture systems have significantly improved embryo development compared to conventional static culture systems. However, a demerit of these systems is the need to use electric devices inside an incubator under humified conditions. In addition, we intend to apply MS larger than those applied using these devices. To address these issues, we developed a dynamic embryo culture system using air actuation and evaluated the applied MS and embryo culture results. Materials and methods: We developed an air actuation system with microfluidic channels to apply MS by deforming a 0.1 mm thick polydimethylsiloxiane membrane and evaluated SS applied to mouse embryos inside the microfluidic channel. Frozen 2 cell stage embryos of the ICR mouse (Arc Resources Inc., Japan) were thawed and cultured in approximately 200 µl of the potassium simplex optimized embryo culture medium for 3 days at 37 C under a humidified atmosphere of 5% CO_2. The number of cultured embryos was from 10 to 13. Results: When syringe velocity (V_s) was 0.5 mm over seconds, the observed embryos rotated and did not slide. When V_s and fluid velocity increased, the embryos slipped, did not come in contact with the floor, and were not compressed because of the fast translation. We conclude that different types and amounts of MS can be applied to the embryos by changing V_s. Maximum SS applied using this system was approximately 10 times higher than that using previous dynamic culture systems such as the tilting embryo culture system and microfunnel. We compared embryo development from the 2 cell stage to blastocyst stage between the static and dynamic cultures in the medium channel. Dynamic cultures significantly improved the blastocyst development rate [dynamic 74% (n = 126); static 62% (n = 118); $P < 0.05$]. The average number of cells in the blastocysts cultured under dynamic conditions was 83 cells (n = 54, ± 3), while that under static conditions was 76 cells (n = 51, ± 3). A significant difference was observed in the average cell number between the two groups ($P < 0.05$). Conclusions: We demonstrated that this device could be used for dynamic embryo culture in a microfluidic channel. MSs applied using this system were similar to those generated in the physiological environment of the oviduct.

O-012 Caffeine treatment influences in vivo and in vitro fertilization, embryo development and morphology in mice

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Introduction: We previously reported that pregnancy rates and testosterone levels in the follicle were significantly lower in patients that habitually drink coffee. Caffeine thus appears to affect regulation mechanisms related to testosterone release and oocytogenesis. Therefore, in the present study, we evaluated effect to produce oocytes by caffeine in vivo and embryo growth features after in vitro fertilization (IVF) using oocytes after long-term caffeine treatment in mice, and we evaluated the direct influence on embryo growth using culture media containing different levels of caffeine.Material and Methods: Mature, female ICR mice were divided into three groups and were treated with intraperitoneal injection of caffeine for one month at 0, 0.1 or 1 mg/day equivalent. Oocytes were collected from oviducts at 14 h after superovulation with 10IU PMSG and 5IU hCG, and used these for IVF, and then observed embryo development. In an independent experiment, after IVF, we evaluated the development of embryos to blastocyst formation and morphology using media that directly included different caffeine concentrations at 0, 0.05 and 1.0 mg/ml. Results: Number of oocytes dose-dependently decreased after caffeine treatment. Mean oocytes per mouse in the 1 mg/day group was significantly lower (17.7) than in the 0 mg/day control group (39.0) ($P<0.05$).In vitro fertilization rate did not significantly differ from 0 to 1.0 mg/ml; however, the formation of blastocysts at 120 h after insemination was significantly lower with higher caffeine levels; 49.8% in the 0.1 mg/day group and 38.2% in the 1.0 mg/day group (vs. 64.0% in controls) ($P<0.05$). When caffeine was directly added to culture media, blastocyst formation rate was significantly lower (0%; 0/81) at 1.0 mg/ml caffeine, as compared with 98.8% (80/81) in the 0.05 mg/ml group ($P<0.05$) and 97.4% (75/77) in the control group ($P<0.05$). Conclusions: Caffeine treatment prior to retrieving oocyte influences oocytogenesis, and reduces the number of oocytes and blastocyst formation rate using picked up oocytes in vitro. The results also show that caffeine in media has direct negative effects on embryo development to blastocyst formation in vitro. Thus, it suggests that caffeine affects embryo development as well as producing oocytes in the body. Further studies are needed in order to understand the mechanisms responsible for these effects.

O-013 Dynamic analysis of compaction initiation in human embryos using time-lapse cinematography

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Objective: Compaction is the change in cellular adherence that indicates the first morphological event in embryonic differentiation. Although it was reported that it occurs at the late eight-cell stage in mouse embryos, it is still unknown in human. We used time-lapse cinematography to examine the timing of initiation of this event in cultured human embryos. This study analyzed the etiology as well as the fate of those embryos showing early initiation of compaction in human embryos obtained from TLC. Materials and Methods: We developed a new system for time-lapse cinematography (TLC), which uses an inverted microscope with differential interference contrast (DIC) optics and a micromanipulator covered with a handcrafted chamber of acrylic resin. The temperature was maintained at 37±0.2°C and pH at 7.37±0.05 by controlling CO_2 flow. Digital images of the cultured embryos were acquired for 5 days with an exposure time of 1/20 second. Of 119 donated embryos frozen at an early developmental stage (pronuclear stage to 4-cell stage), 109 successfully developed to the blastocyst stage and could be analyzed. Result: Our TLC analysis revealed that compaction occurred in 115 embryos. Initiation of compaction occurred from the 4- to 16-cell stage, with the 8-cell stage the most common (26/115; 22.6%). Of those embryos, 99 (86.1%) embryos initiated compaction after the 8-cell stage and 49 (49.5%) developed into good-quality blastocysts. On the other hand, of the 16 (13.9%) embryos in which compaction was initiated before the 8-cell stage, 15 (93.8%) presented multinucleated blastomeres, and only 3 (18.8%) developed into good-quality blastocysts. The remaining 13 (81.3%) developed into poor-quality embryos. There was a significant difference in the rate of development into good-quality embryos between embryos that initiated compaction after the 8-cell stage and before the 8-cell stage (P<0.05). Ten of the embryos that initiated compaction before the 8-cell stage (62.5%) showed interruption of blastomere cleavage after forming of the cleavage furrow, followed by multinucleation in the blastomere. There was no difference in time required from embryo thawing to the initiation of compaction between the embryos where compaction started before the 8-cell stage and those that initiated compaction after the 8-cell stage. Conclusions: Our study suggested that the initiation of compaction is more likely to occur in human embryos at the 8-cell stage or later and that early initiation of compaction is strongly associated with poor quality in human embryonic development. Because most of the multinucleated blastomeres observed here failed to complete normal cytokinesis, early initiation of compaction might occur due to aberrant cytokinesis followed by karyokinesis.

O-014 Chromosomal analysis of cleavage embryos and blastocysts derived from tripronuclear zygotes after ICSI.

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Introduction: In IVF and ICSI, normal fertilization is determined by the presence of two distinct pronuclei (2PN) at 16-20 hours after insemination (Day-1). Occasionally, three pronuclear zygotes (3PN) are observed. 3PN after IVF, in most cases, is treated dispermic fertilization with the chromosomal abnormality, triploid. It has been reported that 70% of ICSI 3PN cleavage embryos were polyploid. However some of them have been showed diploid. If these embryos can be estimated diploid morphologically, they might be useful for embryo transfer for the patient that does not have a lot of oocytes or embryos. In this study, we analyzed the chromosomes of ICSI 3PN cleavage embryos and blastocysts. Materials and Methods: In cleavage embryos, among 4016 oocytes after ICSI from July 2008 through May 2009, we conducted chromosomal analyses of Day-3 37 embryos defined as 3PN at Day-1after ICSI. In blastocysts, 112 3PN of 5171 oocytes were observed at Day-1after ICSI from July 2008 through May 2010. 102 of 112 embryos were cultured to Day-5 or 6, and then 23 blastocysts were examined for chromosome. The chromosomes of cleavage embryos and blastocysts were examined by fluorescence in situ hybridization technique with 18, X and Y probes. Results: One-hundred-twelve of 5171 oocytes (2.2%) after ICSI were found 3PN. The percentage of BLs formed from 3PN was 24.5% (25/102). The chromosome analysis showed that, 8.1% (3/37), 29.7% (11/37), 32.4% (12/37), 2.7% (1/37), 21.6% (8/37) and 2.7% (1/37) of the ICSI 3PN cleavage embryos were 2n, 3n, mosaic included 3n, mosaic included 4n, n, chaotic mosaic and other abnormality, respectively. The polyploidy embryo rate was 67.6% (25/37). Y chromosome was detected in 45.9% (17/37) of the ICSI 3PN cleavage embryos. 4.3% (1/23), 52.2% (12/23), 26.1% (6/23), 13.0% (3/23), and 4.3% (1/23) of the ICSI 3PN blastocysts were 2n, 3n, mosaic included 3n, chaotic mosaic, and other abnormality, respectively. The polyploidy blastocyst rate was 78.3% (18/23). Y chromosome was detected in 56.5% (13/23) of the ICSI 3PN blastocysts. Conclusions: In the ICSI 3PN cleavage embryos, the prevalence of 2n was 8.1% and the chromosomal abnormality was 91.9%. In the ICSI 3PN blastocysts, 2n was 4.3% and the chromosomal abnormality was 95.7%. ICSI 3PN was confirmed to have a high chromosomal abnormality rate in both of cleavage embryos and the blastocysts. Therefore, even if ICSI 3PN zygote develops to the blastocyst, it should not be useful for embryo transfer.

O-015 THE DYNAMIC PROCESS OF SPERM PENETRATION OF THE HUMAN OOCYTE ANALYZED USING TIME-LAPSE CINEMATOGRAPHY

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Objective: Using time-lapse cinematography (TLC), we analyzed the dynamic process of human sperm penetration to investigate the mechanisms blocking polyspermy after sperm attachment. Materials and Methods: Between July 2004 and April 2008, TLC was performed on single, randomly selected oocytes after insemination (Mio, 2008). The study focused on oocytes where the movements of the leading sperm (the one that penetrated the zona pellucida (ZP) most deeply) and the following sperm were captured in the same field. Sperm movements were then analyzed from the time of entry into the ZP to the incorporation of the leading sperm. Results: We observed the process of sperm penetration and the sperm entry point in 22 embryos. In 3 of these embryos (target embryos), we were able to capture the movements of the leading and following sperm. Sperm attached to the oocyte membrane approx 96 minutes after insemination and 37 minutes later, the sperm head decondensed and disappeared. There was no difference in the time-course of the fertilization process of the target embryos and the other embryos observed. In the 3 target embryos, both leading and following sperm proceeded forward until the leading sperm attached on the ooplasmic membrane. The following sperm penetrated 12.9μm of the ZP on average and, despite their active tail movements, stopped their progression within 10 seconds of the leading sperm attaching on the membrane. Conclusions: To date, the primary block to polyspermy in the mammalian oocyte is described as a zona reaction induced approximately 5 to 8 minutes after oocyte activation by sperm incorporation. In this study, 10 seconds after sperm-oocyte attachment, the following sperm were unable to continue further despite their sustained tail motility. This suggests that an unknown process has prevented the progression of the following sperm. Further investigations are now on going.

O-016 Depletion of Plk1 delays cell cycle progression in endometrial cells derived from endometriosis

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The ectopic (Ect) and eutopic (Eut) endometrial cells (ECs) derived from Endometriosis (EMS) displayed similar cellular biological behaviors to malignant cells, but their specific cellular pathogenesis remains to be elucidated. To investigate the pathological mechanism of endometriotic cells, firstly, we corroborated a higher polo-like kinase 1 (Plk1) expression in ECs from women with EMS, either in Ect or Eut emdometrium. Furthermore, the roles of endogenous Plk1 in ECs growth and proliferative regulation were investigated. Three kinds of ECs (Ect and Eut ECs from women with EMS and Eut ECs from normal women) were stably cultured and transfected with small RNA interference (siRNA) targeting Plk1. We observed retarded growth rate and reduced proliferation in depletion of Plk1 ECs as compared with control mock-transfected ECs. Cell cycle analysis displayed that ECs expressing Plk1-specific siRNA accumulated during G2/M phase of the cell cycle. Further analysis demonstrated that, compared with normal Plk1-depleted ECs, endometriotic Plk1-depleted ECs showed a significantly delayed induction of cyclin B1. These results suggest that knockdown of Plk1 in ECs from EMS may disturb cell proliferation and delay cell cycle progression by inhibiting cyclin B1 expression.

O-017 Male fetal DNA detection in maternal serum from pregnant cynomolgus monkeys (Macaca fascicularis) in an established breeding colony

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Introduction: Determination of fetal sex at an early stage of gestation is essential for the prenatal diagnosis of certain inherited dominant genetic diseases such as the male-specific disorders Duchenne muscular dystrophy and aneuploidy. As human and nonhuman primates are similar in their development, monkeys are often used as a model for fetal development in biomedical research. There has been no information about the presence of fetal (f) DNA in maternal serum during gestation for the most widely used model in biomedical research, the cynomolgus monkey. Breeding of the cynomolgus monkey is not controlled seasonally as it is in the rhesus monkey. The aim of this study was to develop an efficient method for detecting fDNA in monkey serum at an early stage of gestation to diagnose gender-specific genetic disorders. Material & Methods: We used quantitative real-time PCR with the TaqMan system to analyze maternal serum obtained from pregnant cynomolgus monkeys in an established monkey breeding colony. Primers and probes for the cynomolgus monkey-specific *SRY* gene and the Y-chromosome-specific multicopy marker sequences DYS14 were designed by using Beacon designer 7.9 software. The presence of *SRY* and DYS14 was determined in five cynomolgus monkeys at the 5th, 12th and 22nd weeks of gestation. All the animals were examined by ultrasonography, and sex was confirmed at delivery. Results: A real-time quantitative generic multiplex PCR assay was developed that is capable of detecting the specific region of *SRY* and DYS14, making it possible to quantify the number of gene copies in each multiplex reaction. We report the 100% specificity and 100% sensitivity of the probe for detecting the Y-chromosome sex-determining region at the 12th and 22nd gestational weeks in the monkeys studied. At the 5th gestational week, we observed <100% sensitivity for detecting male fDNA. Conclusions: We present a specific, highly sensitive method for determining and quantifying male specific fDNA in pregnant cynomolgus monkeys. The lower sensitivity at the 5th week of gestation might be due to a lesser amount of fDNA during early pregnancy.

O-018 Clinical outcome of 852 tubal factor infertility patients without ART after Falloposcopic tuboplasty.

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Introduction: Falloposcopic tuboplasty (FT) has been recognized as a highly useful instrument of both an assessment of tubal luminal lesion and a surgical technique of recanalization of tubal occlusion and stenosis. We have used FT as a diagnosis and a treatment for about 1500 over tubal infertility patients since 2003 at our clinic. The aim of this study was to evaluate the pregnancy outcome without ART after FT and the effectiveness and safety of FT. Materials and Methods. FT was performed for the 1468 patients with bilateral or unilateral occlusion and stenosis from January 2005 through December 2010. These patients had been diagnosed by hysterosalpingography, hysteroscope and hydrotubiation. All FT was performed with the procedure of transcervical balloon tuboplasty by intaravenous anesthesia in outpatient base. Lesions in the tubal lumen were observed falloposcopically during retrograde imaging after complete recannulation. 852 patients were able to follow up, and 253 of them have been pregnant by natural intercourse or artificial insemination. Patients with hydrosalpings, severe male factor and ART followed FT were excluded from this study. Results: Mean age of the patients was 33.5 years old (ranged from 22 to 49 years old) Clinical pregnancies occurred in 253/852 patients (29%). All pregnancies were singletons and the miscarriage was 7.5%. The pregnancy rate decreased with the age. The highest, 50% (3/6), pregnancy rate was seen for the 25 year old cases. Under 38 years old, the pregnancy rate was over 22 %. The oldest case of conception was 41 years old, but she had a miscarriage. The mean pregnancy period after FT was 3.5 months. The pregnancy cases occurred within the first month after FT. Within 6 months after FT 85.7% (216/252) of the women became pregnant. Ectopic pregnancy was 1.6% (4/253). The 250 out of 599 cases that had not conceived were conducted to ART and at present about 82.4% have become pregnant. Conclusion: Although ART has been used for women with tubal lesion as a standard treatment, we apply FT as an initial treatment before ART. Our results show that this FT technique is useful for the patients with tubal infertility and does not cause an increase of ectopic pregnancy. FT might be recommended to the selected patients with tubal infertility under 40 years old before conducting IVF. And it is also recommended to reconsider the therapeutic plan for these patients about 6 months over after FT.

O-019 Outcomes of vitrified-warmed embryo transfer (FET) cycles: single blastocyst transfer versus double blastocyst transfer

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Introduction: Vitrification, an ultra-rapid freezing technique, is an optimal cryopreservation procedure for embryos. Since 2008 while we've been using the Cryotop method for human embryo cryopreservation we achieved high clinical pregnancy rate after transferring two vitrified-warmed blastocysts (double FET). But these optimistic results came across with the high rate of twin pregnancies that has a negative impact on the perinatal outcome of the IVF programs. In order to reduce the multiple pregnancies rate we began to perform single vitrified-warmed embryo transfer (single FET). The purpose of the present retrospective study was to compare the clinical outcomes of single versus double FET cycles. Materials and methods: 237 patients were included in the study. All good quality supernumerary day 5-6 blastocysts were vitrified by the Cryotop method and then warmed for carrying out embryo transfer. We analyzed 269 FET cycles performed from January 2010 to January 2011. Assisted hatching was used to remove ¼ part of zona pellucida after blastocyst warming. No significant differences were observed between the survival rate in single and double FET (100% and 98.8%). Endometrium for embryo implantation was prepared by the hormonal replacement therapy or in natural cycle. The main outcome measures were clinical pregnancy rate, ongoing pregnancy rate and implantation rate. Results: 225 and 44 ET were carried out in double and single FET cycles respectively. The differences in the endometrium method preparation between groups were not significant. The clinical pregnancy and ongoing pregnancy rates were higher in double versus single FET (62.7% vs 52.3% and 53.3% vs 45.5%, respectively). Implantation rate was 52.3% in single FET cycles and 41.1% in double FET cycles. The multiple pregnancy rate was significantly lower in single compared to double FET group (4.3%: 1 case of monozygotic twins vs 31.2%: 43 dizygotic and 1 monozygotic twins, respectively). Conclusion: The Cryotop method keeps high implantation potential of blastocyst. The transfer of one vitrified-warmed blastocyst gives us an opportunity to achieve high pregnancy rates while reducing the multiple pregnancy rate making IVF-procedures rather more patient friendly.

O-020 Are Mild ART-derived blastocysts more favorable than conventional COH-derived ones?

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Introduction: To evaluate the efficacy of assisted reproduction with mild stimulation using clomiphene citrate (CC-ART), we compared the outcome of frozen-thawed transfer of blastocysts obtained by CC-ART with those by conventional controlled ovarian hyperstimulation protocol (COH-ART). Materials and methods: CC-ART was conducted exclusively for patients with COH-ART failure or poor ovarian reserve. In our CC-ART protocol, daily 50 mg clomiphene citrate was started on the 3rd day of menstrual cycle and a couple of low dose gonadotropin were administered after the 8th day. Blastocysts with Gardner's grade 3BB or better were cryopreserved without fresh embryo transfer, thawed and transferred in natural cycle or HRT regimen. Results: In 532 CC-ART cycles, blastocysts were cryopreserved in 184 cycles (34.6%). Pregnancy rate (PR) of single frozen-thawed blastocyst transfer (SBT) after CC-ART (27.8%; 44 in 158 cycles) was significantly lower than those after COH-ART (35.4%; 336 in 950 cycles), although patients who had SBT after CC-ART (38.6±3.3 yo) were significantly older than those after COH-ART (35.1±3.8 yo). When PR was compared in age-matched condition, however, no significant diffirence was detected (45.5% vs 38.3% in 30-34 yo, 28.8% vs 33.1% in 35-39 yo, 21.1% vs 31.7% in 40- yo, for CC-ART vs COH-ART, respectively). Conslusions: PR of blastocysts obtained by CC-ART was comparable with those by COH-ART, even when CC-ART was limited for patients with COH-ART failure or poor ovarian reserve. Age- and AMH-matched RCT may remain to be conducted.

O-021 Progress towards a universal warming method after vitrification

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Introduction: Establishment of various vitrification techniques with diverse carrier tools and solutions may create serious problems when cryopreserved samples are transported from one clinic to the other, and routinely used vitrification techniques differ between the two institutions. The purpose of our work was to test the possibility to establish a universal warming method that can be used after vitrification performed with various techniques. Material and Methods. In Experiment 1, D5 blastocysts and MII phase oocytes were exposed to in-house media, 7.5-7.5% ethylene glycol (EG) and dimethylsulphoxide (DMSO) for 15 min, then 16-16% EG-DMSO and 0.5 M sucrose for 1 min before loading into open pulled straws (OPS) and cooling. Warming was performed in 1M sucrose for 1 min, then in 0.5 M sucrose for 3 and in holding medium for 2x5 min, respectively. Samples were transferred in large amount (20 µl) medium from the concentrated to the diluted solution.In Experiment 2, D3 embryos and blastocysts vitrified with the MediCult Vitrification kit and parameters were warmed either by using the original MediCult solutions and parameters, or in-house media and parameters as described above. The age of patients did not differ significantly between the two groups. Results. In Experiment 1, the re-expansion rate of blastocysts was 100% (7/7). Survival and developmental rates to morula/blastocysts stage after vitrification of oocytes were 100% (9/9) and 30% (3/9). In Experiment 2. survival of morulae and re-expansion rates of blastocyst warmed in Medicult versus in-house media were 92% (55/60) and 66% (45/68) versus 98% (47/50) and 64% (27/42) respectively Pregnancy rates achieved with transfers of embryos warmed in Medicult versus in-house media were 21% (12/41) versus 37% (11/30), respectively. Discussion Results of Experiment 1 have demonstrated that in-house media could be successfully applied for vitrification and warming of both MII oocytes and blastocysts. In Experiment 2, warming with in-house media resulted identical survival rates than those achieved with the original Medicult warming media, and pregnancy rates tended to be higher. As the Medicult vitrification kit uses different cryoprotectants (propylene glycol instead of DMSO) and equilibration parameters different from our in-house media, these results indicate that establishment of an universal warming method is a realistic perspective and research should be conducted towards this direction.

O-022 Failure of pronucleus formation in in vitro matured and fertilized oocytes from SOD1-deficient mice

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Introduction: Higher levels of intracellular reactive oxygen species (ROS) are detected in cleavage-stage embryos produced *in vitro*, compared with *in vivo*, and are related to early developmental arrest or cell death. We have previously shown total two-cell arrest of *in vitro*-fertilized embryos from superoxide dismutase 1-deficient mouse (SOD1KO) oocytes without mitochondrial malfunction under culture with 20% O_2, indicating that the mechanism of cell cycle regulation might be a target of elevated ROS. This study was conducted to investigate effects of a SOD1 deficiency during IVM on fertilization and the following development. Material and Methods: Immature cumulus-oocyte complexes (COCs) were retrieved from ICR SOD1KO and wild-type mice (WT) after eCG administration. The COCs underwent IVM for 18 h under several culture conditions, followed by IVF with WT sperms. In parallel, matured oocytes were treated with 10 mM $SrCl_2$ for parthenogenetic activation (PA). The oocytes were assessed the nucleus stage at 6 h after insemination or PA. To observe the early events of egg activation, cortical granules (CGs) were detected by lectin staining. Expressions of CaMK II were also detected by immnofluorecence staining. Results: Under culture with 20% O_2, SOD1KO oocytes were completely prevented from attaining to two-cell stage. Most of the SOD1KO oocytes were arrested at Met II to Telo II at 6 h after insemination regardless of the high frequency of sperm penetration. Culture with antioxidants or hypoxia slightly improved cleavage. PA did not remove impairment of pronucleous formation in SOD1KO oocytes, indicating that cascades through sperm factors were not related to this impairment. The CGs distributed on the peri-membrane of the Met II oocytes, apart from over-the-spindle, were slightly weakened after egg activation. Phospho-CaMK IIαβ was localized in the cytoplasm with a strong presence on the Met plate. CaMK IIγ was detected on the peri-membrane of the Met II oocytes, apart from over-the-spindle, and was present spottily in the cytoplasm after egg activation. The localizations of CGs, phospho-CaMK IIαβ and CaMK IIγ were similar between SOD1KO and WT oocytes. Conclusions: The oxidative stress caused by a SOD1 deficiency during oocyte maturation causes the failure of pronucleus formation after fertilization. This impairment seems to be irrelevant to the abnormality of egg activation events, implying an injury to the cell cycle regulation of the meiosis II exit.

O-023 A randomized double-blind comparative study between two different laser assisted hatching in the frozen-thawed embryo transfer at the cleavage stage

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BACKGROUND: Laser Assisted Hatching (LAH) in the frozen-thawed embryo transfer (FET) cycles increases the implantation and pregnancy rates. However, Little information exists in the literature regarding the efficacy of different LAH. The object of this study was to evaluate the efficacy of two different LAH in the frozen-thawed embryo transfer at the cleavage stage. METHODS: A total of 554 FET cycles using LAH was undertaken in our centre between October 2009 and June 2010. Embryos were cryopreserved at the cleavage stage. On the day of FET, 554 cycles were randomized according to a computer-generated randomization list into the zona drilling group and zona thinning group. The patients and the clinicians were blinded to the group assigned. In the zona drilling group, the drilled hole was 1.5 times the thickness of zona pellucida; In the zona thinning group, the outer half of the zona pellucida over a quarter of the diameter of zona was removed using a non-contact laser. RESULTS: In the zona drilling group, the clinical pregnancy rate of 39.4% with an implantation rate of 22.4%. In the zona thinning group, the clinical pregnancy rate of 42.1% with an implantation rate of 24.9%. All data of pregnancy rate and implantation rate in the zona thinning group were slightly higher in comparison with the zona drilling group, although there was a non-significant difference of pregnancy rate and implantation rate between the zona drilling group and the zona thinning group. CONCLUSIONS: In order to avoid the damage of the embryos in the LAH, the zona thinning should be performed routinely to replace the zona drilling in the FET cycles at the cleavage stage.

O-024 A trial for observation of chromosome dynamics in human embryos using a live-cell imaging system

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(Introduction) Chromosome analysis is generally performed using fixed samples. Recently, the development of a confocal imaging system that includes an embryo culture system has made it possible to obtain time-lapse images of chromosome dynamics in mouse (Yamagata et al. 2010). In this study, we attempted to observe chromosome dynamics and cytokinesis in human embryos using an all-in-one confocal imaging system. (Material & Methods) Eighteen pronuclear embryos intended for disposal were used after obtaining the informed consent of the patients and the approvals of the IVF JAPAN and Japan Society of Obstetrics and Gynecology research ethics committees. A mixture of mRNAs encoding enhanced green fluorescent protein coupled with α-tubulin and monomeric red fluorescent protein I fused with histone H2B was injected into the cytoplasm of the pronuclear embryos using a Piezo drive manipulator. The pronuclear embryos were cultured under the atmosphere of 5% O_2, 5% CO_2 and 90% N_2. Time-lapse images were captured at 15-minute intervals until the day 3 stage using an all-in-one confocal imaging system (CV1000, Yokogawa Electric Corp.). (Results) Fluorescent signals from tubulin and histone were detected in all RNA-injected embryos around first mitosis. However, signals at first mitosis of some embryos were not so clear because the fluorescent protein had not yet been translated from mRNA. (Conclusions) We succeeded in observing chromosome dynamics using an all-in-one confocal imaging system in human live embryos. The observation of chromosome dynamics in human embryos using a live-cell imaging system may help to elucidate the mechanisms and incidence of chromosome aberrations such as aneuploidy and polyploidy.

O-025 Autologous transplantation of very thin ovarian fragments which preserve the ovary's main cortex structure lead to successful pregnancy

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Introduction: Ovarian cortex was cryopreserved in a young thalassemic patient prior to premature ovarian failure associated with stem cell transplantation. Aim: to restore fertility using frozen thawed ovarian cortex. Materials: Eleven in vitro fertilization (IVF) cycles were then performed following conventional ovarian-strip transplantation; however, the ovarian response was poor. In parallel, we explored, in a rat model, the use of micro-organs (MOs), i.e. microscopic ovarian fragments, which preserve ovarian cortex architecture allowing for sufficient diffusion of gases and nutrients. Results: We found that rodent ovarian MOs induced angiogenesis and remained viable after autologous transplantation. Subsequently, we reimplanted MOs prepared from autologous thawed ovarian strips into our patient, resulting in a successful delivery. Conclusions: Precision cutting is important in order to preserve ovarian cortex architecture. The facts that the resulting fragments are of uniform thickness and only a few hundred microns thick, ensure reproducibility and appropriate and homogeneous diffusion of gases and nutrients to all cell components. Furthermore, very thin (350μ) ovarian fragments secrete a whole range of angiogenic factors which ensure that a vascular network is rapidly formed.

O-026 Important implications of advanced glycation end-products (AGE) in poor ART outcomes and a novel successful therapy for very severe ART patients with sitagliptin possibly by decreasing AGE.

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Objective: Advanced glycation end-products (AGEs) are accumulated with ageing, diabetes mellitus and polycystic ovary syndrome, playing a pathogenic role. We showed adverse effects of AGE accumulation on ART outcomes in study I and in study II we improved ART outcomes in very severe ART patients by decreasing postprandial glycemic levels and AGEs with sitagliptin, a new antihyperglycemic agent. Materials and Methods: Study I: Toxic AGE (TAGE), pentosidine (Pent), and carboxymethyl lysine (CML) in blood and follicular fluid (FF) were measured in 157 ART-patients. We analyzed associations of AGE with ART outcomes. Study II: Sitagliptin, 50 mg/day, was administered for 2 months before ART in 41 very severe ART repeaters with 7.6±0.7 (SEM) attempts of previous failed ARTs and 41.0±0.5 years of age. 75g-oGTT was normal and borderline in 39 and 2 patients, respectively. All patients had failed with metformin. TAGE measurements and oGTT were done before and one month after sitagliptin in 36 patients. Results: Study I: TAGE, Pent, and CML in FF, and TAGE in serum showed significant negative correlations with follicular and embryonic develoment and fertilization. Among 12 factors, only age, Pent in FF, and TAGE in serum were correlated with ongoing pregnancy significantly. Women with serum TAGE above 7.24 U/mL showed decreased oocyte numbers and ongoing pregnancy rates, even with younger age or lower day-3 FSH. Study II: Sitagliptin significantly decreased plasma glucose levels at 30, 60, and 120 minutes after 75 g glucose in oGTT, but not fasting levels. Serum TAGE was decreased, unchanged or increased in 17, 6 or 13 patients, respectively (groups D, U or I). The number of day-2-superior-embryos was increased by sitagliptin in 47 and 50% of patients in groups D and U, respectively, being significantly higher than 8% in group I. Consequently, 3 ongoing pregnancies and 2 abortions were achieved in 41 ART with sitagliptin (rates of clinical and ongoing pregnancy: 12% and 7.3%, respectively), while 8 abortions alone in their 244 previous ART attempts without sitagliptin (3.2% and 0%, respectively). Conclusions: Serum TAGE and FF Pent accumulations correlated highly with poor follicular and embryonic developments and with unlikelihood of ongoing pregnancy. Serum TAGE predicts poor ART outcomes independently of age and day-3 FSH. Decreasing postprandial glycemic levels and TAGE by sitagliptin is possibly a novel therapy for ovarian dysfunction.

O-027 A novel long protocol of GnRH agonist and hMG regimen: a dramatical increase in pregnancy rate by induction of diminished but significant mid-cycle LH surge.

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Objective: In the long protocol of GnRH agonist and hMG regimen, desensitization of pituitary prevents premature LH surge and thus it has been believed that mid-cycle LH surge cannot be induced. Serendipitously, however, we have found a novel protocol to induce diminished but significant LH surge, increasing dramatically the rates of clinical and ongoing pregnancies. Designs: a prospective randomized study. Materials and Methods: Hundred forty-two initial IVF/ICSI cycles in 140 women with age younger than 41 years and day-3-FSH less than 15 IU/L were assigned prospectively and at random to receive the conventional long protocol (control group) or a novel protocol (surge group). In both groups, buserelin acetate was administered nasally at 3 times of 300 μg daily from mid-luteal phase in the preceding cycle until 15:00 pm on the day of hCG administration. In the surge group, 300 μg buserelin acetate was additionally administered twice one hour before and just before administration of 10,000 IU hCG at 21:00 to 24:00 pm, while hCG alone with no additional buserelin acetate was administered in the control group. In 25 patients, concentrations of LH, FSH, PRL, TSH, GH, hCG, E2 and P4 in serum were measured one hour before, just before and one hour after hCG administration. Results: The rates of clinical and ongoing pregnancies per stimulated cycle were significantly ($P<0.01$ and $P<0.05$, respectively) higher in surge group (41% and 34% in 71 cycles) than control (18% and 15% in 71 cycles). In surge group 86% and 14% of 14 hormone-measured women showed increase and plateau, respectively, in serum LH levels after administration of buserelin and hCG, while all of 11 hormone-measured women in control group showed decrease in LH after hCG (incidence of LH surge: 86% vs. 0%, $P<0.0001$). The profiles of GH and P4 changes around hCG administration also significantly differed between surge and control groups. Conclusions: Under pituitary desensitization by nasal administration of GnRH agonist, diminished but significant LH surge can be induced by administration of GnRH agonist at increased dosage within one hour before hCG. This novel protocol significantly improved embryonic development and ongoing pregnancy rate, suggesting critical role of mid-cycle surge of LH and other hormones in oocyte maturation.

O-028 GnRHa: a promising alternative of HCG to trigger ovum maturation in minimal stimulation cycle

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The objective of this study was to assess the effect of recombinant HCG and GnRH agonist to trigger ovum maturation in minimal stimulation cycle. This comparative study, at the First People's Hospital of Yunnan Province, was performed on 189 infertile IVF embryo transfer candidates. Inclusion criteria were normal basal hormonal profile, <lt38 years old of age, cause of infertility were male factor, tuble abnormalities. A total of 189 infertile patients undergoing IVF/intracytoplasmic sperm injection (ICSI) treatmen with Clomiphene Citrate (CC)/human menopausal gonadotrophin (HMG) were prospectively studied. All the cycles were gouped according to two different ovum maturation trigger agents: recombinant human chorionic gonadotrophin (rec-HCG) or GnRHa. Oocyte retrieval was performed 32-34 h after reHCG or GnRHa injection subcutaneously. One to three embryos were transferred on day 3. The principal outcome wes to evaluste clinical pregnancy and embryo implantation rates. The two groups were similar in terms of patients' age, day 2 FSH and LH, number of retrived oocyte, oocyte retrival rate, fertilization rate, cleavage rate and number of transferred embryo. In contrast, the pregnancy rate (13.0 versus 25.6%) was better in the GnRHa group, but there was no signicant difference between the two groups. The implantation rate was significantly higher in the GnRHa group (25.42 versus 10.45%, p<lt0.05) compared with rec- HCG group. In conclusion, GnRHa seemed to be a promising and better alternative of HCG to trigger alternative of HCG to trigger ovum maturation in minimal stimulation IVF cycle.

O-029 The observation of effect of two different COH protocols on the IVF-ET outcome of aged patients

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To explore the influence of two different COH protocols on IVF-ET outcome, and the better COH protocols for aged infertility. Two hundred infertility patients who were 35 -45 years old received in vitro fertilization and embryo transfer (IVF-ET) from May 2010 to May 2011 were randomly divided into two groups, the patients in group A accepted the GnRHa+ Gn short protocol while the patients in group B accepted the GnRH ant+ Gn protocol. Duration of stimulation, amps of gonadotropin, estradiol level on hCG day, number of oocytes retrieved, fertilization rate, total embryos obtained, high quality embryo obtained, embryos transferred, embryos frozen,implantation rate per transfer,clinical pregnancy rate per transfe, miscarriage rate between A, B groups were compared. The level of serum estrogen (E2) on the HCG injecting day and the number of oocytes retrieved of group B were less than that of group A. The duration of stimulation, dosage of Gn of group B were higher than that of group A, but there was no statistical significance. There were no significant difference in the fertility rate, the high quality embryos rate, abortion rates between the two groups.The clinical pregnancy rate in group B was higher than those in group A (35% vs 21%), there was statistical significance between two groups ($P<0.05$). Although the estradiol level on hCG day and the number of oocytes retrieved are lower in the GnRH-ant protocol, which does not affect the implantation rate and clinical pregnancy for aged infertility, the GnRHant protocol is excelled which can have better IVF-ET outcome.

O-030 EFFICACY OF SEQUENTIAL TREATMENT PROTOCOL WITH HIGHLY PURIFIED URINARY FSH AND RECOMBINANT FSH FOR CONTROLLED OVARIAN STIMULATION

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OBJECTIVE: To evaluate impact of sequential stimulation protocol with different FSH isoforms on oocyte quality and IVF/ICSI outcomes. DESIGN: A prospective randomized, open-label, controlled study. MATERIAL AND METHODS: A total of 204 IVF/ICSI cycles were included in this study between June 2010 and January 2011. The inclusion criteria were (1) age between 25-34 years old; (2) BMI between 18-25 kg/m^2; (3) First IVF/ICSI; (4) normal ovulatory cycles; (5) both ovaries present and normal uterus; (6) infertility attributable to tubal, male, or idiopathic factors. The participants underwent a luteal phase gonadotropin releasing hormone agonist down-regulation protocol. After pituitary down-regulation was confirmed, the patients were randomized into three groups according to computer-generated random numbers: group A (n=67) received recombinant FSH, (r-FSH Gonal-F, Serono, contains more less-acidic FSH isoform) only until hCG administration; group B (n=72) received highly purified urinary FSH (HP-FSH Fostimon IBSA, contains more acidic FSH isoform) only until hCG administration; group C (n=65) sequentially treated HP-FSH for first 5 days and followed r-FSH until hCG administration. In three groups, FSH starting doses 150 IU/day for first 5 days and then adjusted according to ovarian response. IVF/ICSI outcomes among three groups were compared. RESULTS: There was no significant difference for duration and cause of infertility, age of patients and type of IVF/ICSI procedure, doses of total gonandotropin and days of stimulation as well as number of embryo transfer. CONCLUSION: ovarian stimulation seems to be affected by different FSH isoforms. The sequential protocol improves oocyte maturity and embryo cleavage, and increases pregnancy compared with stimulation with acidic HP-FSH alone.

O-031 Application of elective single embryo transfer (e-SET)

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Introduction: Elective single embryo transfer (e-SET) is now considered to be one optional way to solve multiple gestations. The objective of this report is to discuss strategies of SET. Material & Methods: From Jul.2009 to Dec.2010, We performed 1380 IVF cycles with infertility cause of tubal factor, including 4 groups: 1-SET of 4-8 cells (168 fresh and 46 frozen cycles), 2-single frozen blastocyst transfer (SBT,98 cycles), 3-multi-embryo transfer of 4-8 cells(794 fresh and 164 frozen cycles), 4-multi-blastocyst transfer (110 cycles). The relationship between multiple factors (age of patients, COH protocol, embryo quality, et al.) and clinical pregnancy rates (PRs) as well as multiple gestation rates were statistically analyzed. Results: 1.PRs achieved 24.3% in group 1, which was significantly lower than in group 2 (42.8%), group 3 (42.9%), and group 4(53.6%). PRs were obviously lower in group 1 (24.9% of fresh, 23.7% of frozen) compared with group 3 (43.6% of fresh, 39.6% of frozen). 2.Age: PRs had no notable differences between younger patients(<35) and elder ones(>35) in group 1 (26.4% to 22.1%), group 2 (43.6% to 41.5%). Minimal difference appeared in group 3 (45.2% to 39.1%). But in group 4, PRs of younger ones were higher than that of older ones (58.2% to 40.7%). 3. COH protocols: In group 2, PRs of low stimulation reached 56.8%. In group 4, PRs were significantly higher in low stimulation group (58.4%) than in others. 4. Embryo quality: In group 2, PRs of transferring high-quality blastocysts (52.7%) were significantly higher than transferring low-quality ones (39.4%). PRs of transferring 2 high-quality ones (59.4%) were slightly higher than transferring 1 high-quality plus 1 low-quality blastocysts(48.4%), as well as transferring 2 low-quality ones (47.2%). But in group 1 and 3 the influences were not so obvious. 5. Multiple gestation rates: Multiple gestation rates were obviously lower in SET group (0%) than in multiple transfer group.Conclusions: Though PRs of multi-embryo transfer are higher than that of SET (both fresh and frozen), frozen SBT can obtain equal PRs as well as frozen multi-blastocyst transfer while patients are over 35 years old. Besides, PRs of high-quality frozen SBT is twice than that of low-quality frozen SBT. Based on the data, frozen SBT, especially high-quality frozen SBT, has been demonstrated to be a valuable choice for elder patients to reduce multiple gestations.

O-032 A Novel function of Human Pinopodes by Expressing L-Selectin Ligand during Window of Implantation

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Background: Apical surfaces of human endometrial epithelium and endothelium are key elements for the initiation of molecular interactions to capture the blastocyst or leukocyte, respectively. The L-selectin adhesion system has been strongly proposed to play an important role in the initial steps of trophoblast adhesion and promotion of integrin-dependent processes, ultimately culminating in the establishment of the embryo-maternal interface. On the basis of these facts, we hypothesized a novel role for uterodomes as the first embryo-fetal contact sites to contain the highest subcellular expression of L-selectin ligand suggesting its role in early adhesion as predicted. Thus, the objective of this study was therefore to determine the subcelluar pattern of distribution of the L-selectin ligand (MECA-79) in human endometrial apical membrane region during the window of implantation.Materials and Methods: Endometrial biopsies of 5 fertile females ranging in age between 21 and 36 years in the midluteal phase, that is, days 20-24 of a regular menstrual cycle, were studied using several approaches, including scanning electron microscopy (SEM), immunostaining for light microscopy and transmission electron microscopy (TEM), and immunoblotting as well as statistical analysis of the area-related numerical densities of immunoreactive MECA-79-bound nanogolds to detect the expression pattern and the subcellular distribution pattern of L-selectin ligand (MECA-79) in human endometrium during the window of implantation.Results: The endometrial biopsies were scored according the dating criteria of Noyes et al. by an experienced histologist. The SEM images of the midluteal phase specimens revealed that fully developed uterodomes were abundant in our samples. DAB immunostaining and immunoflouresent staining as well as immunoblotting revealed that MECA-79 was expressed in the midluteal phase specimens. The results of immunogold TEM illustrated the expression of MECA-79 in human uterodomes in the midluteal phase and a higher area-relate numerical density in uterodomes compared to that of the uterodome-free areas. Conclusions: This is the first demonstration of the molecular localization of MECA-79 in the human uterodomes which may indicate a novel role for uterodomes to be capable of shear-stress-dependent tethering-type adhesion in the initial phases of human embryo implantation.

O-033 Obstetric outcomes of ART pregnancies in women over 35 years old

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[Objective] In late years, assisted reproductive technology (ART) has progressed remarkably, and pregnancies by ART are increasing. On the other hand, detailed analysis of the obstetric outcomes of ART pregnancies is still insufficient. For appropriate perinatal management, we studied obstetric complications in women over 35 years old who conceived by ART. [Methods] The study enrolled 418 women over 35 years old conceived by ART. As a control group, 3,517 women conceived spontaneously and delivered at our hospital from January 2005 to December 2010 were used. The obstetrical complications were compared with this control. [Results] The ratio of ART pregnancies in women over 35 years old showed increasing trend of approximately 12%. The twin-birth rate decreased sharply, but it was still approximately 5 times higher than in spontaneous pregnancies. The mean maternal age and the number of primiparous pregnancy were higher in ART pregnancy (39.1±2.8 years old, 82.6%) than in spontaneous pregnancy (37.4±2.2 years old, 37.7%). The incidence of premature birth (before 34 weeks of gestation), PIH, and placenta accreta were also significantly higher (8.6% vs. 2.6%, 8.9% vs. 4.2%, 2.6% vs. 0.9%, respectively). If limited for the singleton pregnancies, incidence of placenta previa was also significantly higher (2.5% vs. 1.2%) in addition to premature birth, PIH and placenta accreta (6.2% vs. 2.4%, 6.7% vs. 4.2%, 3.1% vs. 0.9%, respectively). The incidence of the placenta accrete was significantly higher in women over 40 years old than that in women 35-39 years old among ART pregnancies regardless of singleton or multiple gestation (5% versus 0.8%). Uterine disease was a risk factor of the placenta accreta (OR 2.7, IC1.2-6.05) other than the ART (OR 2.44, IC 1.15-5.14). [Conclusion] Incidence of premature birth before 34 weeks, PIH and the placental disorder are significantly higher in the ART pregnancies than that in the spontaneous pregnancies over 35 years old. Furthermore, incidence of the placenta accreta was particularly high in the ART pregnancies over 40 years old thus careful management is required.

O-034 The age of 35 years is the critical age for successful TESE-ICSI in nonobstructive azoospermic patients with normal karyotype and nonmosaic Klinefelter syndrome.

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Introduction: It was reported that the sperm retrieval rates of MD-TESE (microdissection testicular sperm extraction) declined with age in nonmosaic Klinefelter syndrome (KFS) patients. However, effect of aging of KFS on fertility rate and pregnancy outcomes is unknown. We retrospectively reviewed our experience of men with nonobstructive azoospermia (NOA) treated with TESE-ICSI over 5-year period. We analyzed fertilization and pregnancy rates in patients with nonmosaic KFS comparing those in NOA patients with normal karyotype treated in the same period of time. Material & methods: Between 2004 and 2009 a total of 164 patients with NOA (118 with normal karyotype; 46 with KFS) underwent successful MD-TESE by a single surgeon. All sperm retrieved from testes were cryopreserved until ICSI. All patients were followed at least one year after the last embryo transfer. Patients backgrounds, fertilization rate, and pregnancy outcome were compared. The Mann-Whitney U test was used to compare variables between two groups. Comparison of fertilization rate, pregnancy rate and abortion rate between two groups (NOA patients with normal karyotype and nonmosaic KFS; 35 years old and more than 35 years old) were done by χ^2 test. Results: Serum concentrations of FSH were significantly higher in nonmosaic KFS than in NOA patients with normal karyotype. However, serum concentrations of FSH were significantly lower in those younger than 35 years of age than in those older than 35 years of age in both groups. Serum concentrations of testosterone were significantly lower in nonmosaic KFS than in NOA patients with normal karyotype. However, serum concentrations of testosterone were higher in those younger than 35 years of age than in those older than 35 years of age in both groups. Fertilization rates and clinical pregnancy rates were significantly higher in nonmosaic KFS than in NOA patients with normal karyotype. However, fertilization rates and clinical pregnancy rates decreased significantly over 35 years of age in both groups. Conclusions: Sperm from testes of nonmosaic KFS had better fertilizing ability than those from NOA patients with normal karyotype. However, sperm fertilizing ability decreased after the age of 35 years of old in both groups.

O-035 Comparing study of effectiveness of nifedipine and magnesium sulfate for acute tocolysis of preterm labor and threatened preterm labor

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Introduction: Preterm labor with 10% prevalence is one of the most important complications in newborns with prematurity and makes problems emotionally and economically especially in families with long history of infertility. Preterm labor is described with dilatation more than 1cm, effacement more than 85% and threatened preterm labor is defined as occurrence of contractions without dilatation fewer than 37 th weeks of pregnancy. Therefore all gynecologists try to find the best method to control preterm labor. All medications used in preterm labor have some complications. Because of that, we aimed to use nifedipine with most beneficence and least complications and cost. Materials and Methods: This clinical trial study was performed on 220 patients with preterm- labor and threatened preterm labor during 2009-2010 years in kosar Hospital of Urmia University of Medical Sciences. Patients divided in to two groups after description treatment to them and getting informed consent. Within each group 55 patients were treated with Nifedipine orally and 55 patients were treated with magnesium sulfate intravenously. Data were collected based on patients response to medications and analyzed with Spss software. Results: In this study the effectiveness of nifedipine in preterm labor group was more than Mg-sulfate but not statistically significant and in threatened preterm labor group the effectiveness was higher significantly (p=0.001). Also the mean time from treatment to delivery was significantly more in nifedipine group. (PV=0.000) Nifedipine group had less maternal complications and better fetal outcomes significantly. (PV=0.000) Conclusion: These findings suggest using nifedipine would be considerable for prevention of preterm labor and threatened preterm labor.

O-036 The Change of Serum Anti-Mullerian Hormone (AMH) Level by Chemotherapy and Operation in Premenopausal Women with Gynecological Cancer

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Objectives: Women who underwent chemotherapy (chemo) sometimes resulted in premature ovarian failure (POF). Recently, the serum AMH level has been shown to be a marker of ovarian reserve. The objectives of this study are (1) to observe the change of serum AMH level as a marker of ovarian reserve by chemo and operation (ope) for patients with gynecological cancer, (2) to evaluate the pathological finding of the ovary after chemo in the AMH measured cases. Subjects: 5 premenopausal women, who underwent chemo with or without ope against gynecological cancer in our department, were studied. Mean age of them was 28.4 (range 13-45) years, and they were two cases of ovarian germ cell tumor (GT), one case of epithelial ovarian cancer (OC), one case of corpus uteri cancer (CUC) and one origin-unknown case of cancer pleuritis and peritonitis (CPP). The performed chemo regimens were BLM+VP-16+CDDP for GT, DTX+CDDP for CUC, TC (PTX+CBDCA) for OC, three regimens; TC, CPA+ADM+CDDP and PLD for CPP. Design and Methods: Informed consents were obtained from patients in this study. Serum AMH, LH, FSH, and E2 level were measured before, during, and after chemo and ope. AMH level was measured ourselves using AMH immunoassay kit (Immunotech).Results: (1) Serum AMH level of all cases were gradually decreasing according as chemo and it could not be detected after the second or third course. (2) In two GT cases, who were in her teens and twenties, serum AMH level re-increased in a few months after the final course of chemo. Afterward, their AMH value reached to the low plateau level as compared with the same aged women. They have regular menstrual cycles, and normal serum gonadtropin-E2 levels. However, their ovarian reserves are presumed to be impaired and the almost equivalent level with thirties' and forties' level in terms of AMH level. (3) The pathological study revealed that (a) ovarian follicles were degenerated, decreased their number, and impaired their growth, (b) the small blood vessels and fibrosis in the cortex were proliferated. Conclusion: (1) Even if they have normal menstrual cycle, and normal gonadtropin-E2 levels, the ovarian reserves are impaired by chemo and ope. It is seemed to be able to predict it by measuring the serum AMH. (2) The patient who shows hypergonadtropic-hypogonadism is not always POF during and right after chemo. We should evaluate their ovarian reserve after some periods in terms of ovarian follicle development.

O-037 Alternative treatment options for patients with ovarian insufficiency basedon Natural Cycle IVF

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Introduction: In general, patients with low ovarian reserve are difficult to manage and have a relatively poor prognosis. Using traditional stimulation protocols like the long GnRH agonist protocol or the GnRH antagonist protocol in these patients often results in low ovarian response and poor pregnancy rates. In natural menstrual cycles without ovarian stimulation, every month the female body naturally selects the best possible oocyte for ovulation. Nevertheless, hormonal changes due to ovarian insufficiency can influence and change the individual menstrual cycle pattern of each patient over the time, which may result in difficulties to conceive naturally. We observed the menstrual cycle pattern of patients with low ovarian reserve in order to distinguish different stages of ovarian insufficiency. Once we were able to describe the different stages, we developed new treatment approaches for each patient group based on Natural Cycle IVF. Material & Methods: In 2010, the menstrual cycle pattern of 10 patients with AMH <1.0 nmol/L were observed. Patients were 22 to 42 years old. Blood samples were drawn to determine FSH, LH, E2 and transvaginal ultrasound scans were performed on different days of the cycle. Depending on the cycle pattern of each patient, we offered individualized treatment approaches based on Natural Cycle IVF using either Ibuprofen or Clomifen to control ovulation, GnRH agonists to induce ovulation and either Ethinyl-Estradiol or combined oral contraceptive pills to regulate the cycle. Fresh embryo transfer was performed on day 2 or embryos were frozen and transferred later in artificial cycles. Patients were informed about off label use of the medication and informed consents were signed. Results: Based on our observation, we could describe four different stages of ovarian insufficiency. We were able to perform oocyte retrievals and embryo transfers in all 10 patients. A total of 33 natural cycles were initiated. Premature ovulation occurred in 3 cycles (9.0%) and no retrieval was attempted. Among the attempted 30 oocyte retrievals, 21 (70.0%) were successful. Out of those 21 oocytes 11 (36.6% per retrieval) were mature and 10 (33.3% per retrieval) were immature. ICSI resulted in 8 fertilizations (72.7% per mature oocyte). Out of 8 transfers, 3 (37.5%) resulted in biochemical pregnancy. Two patients delivered (25.0%), one patient had a miscarriage at 8 weeks of pregnancy. Conclusion: Our experience shows that ovulation can successfully be controlled by the use of NAIDs or clomiphene citrate and does not necessarily require GnRH analogues for pituitary suppression. This knowledge opens new space for development of alternative protocols respecting the patients' own physiology with no need for heavy stimulation. Patients with ovarian insufficiency may benefit from this approach, which can be offered before referring them to egg donation.

O-038 CORRELATION OF BODY MASS INDEX WITH OUTCOME OF IN VITRO FERTILIZATION IN A DEVELOPING COUNTRY

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Introduction: To correlate ovarian response to stimulation and IVF outcome according to the body mass index (BMI). Materials and Methods: We retrospectively reviewed all patients who underwent IVF cycle in our institution from January 2008 to October 2010. Three hundred and twenty eight patients underwent 342 in vitro fertilization cycles or ICSI and were divided into four subgroups according to BMI; underweight BMI; 18.5kg/m2; normal weight BMI-18.5-24.9; overweight BMI-25.0-29.9 and obese more than 30.0 kg/m2. Results: Three hundred and forty two in vitro fertilization cycles or ICSI from our IVF database were studied. According to classification by BMI, 8.8% of women were underweight (BMI; 18.5 kg/m2), 43.3% were normal weight (BMI 18.5-24.9) 41.2% were overweight (BMI 25.0-29.9) and 6.1% were obese (BMI more than 30.0 kg/m2). The total days of stimulation was almost similar, approximately 10-11 days but total dose of gonadotropin required, increased as BMI increased (p value 0.04). We found that with increasing BMI negative co-relation was seen with clinical pregnancy rate (p value=0.40) with pregnancy rate decreasing from 31.2% in normal weight woman to 21.9% in obese women. In the present study no difference was seen in the number of oocyte retrieved but a decreased fertilization (75.8% in normal weight to 66.1% in obese) and cleavage rate (72% to 61%) was seen with decreased number of cryo-preserved embryos (4.32 to 2.4) with increasing BMI. This study shows that poorer oocyte quality is seen with increasing BMI which results in reduced clinical pregnancy rate. In our study no deleterious effect of low BMI was seen on IVF outcome and clinical pregnancy rate. Conclusion Female obesity impairs IVF outcome potentially by impairing oocyte quality but does not affect ovarian response to stimulation.

O-039 Bilateral ovarian endometriomas removal does not affect the IVF outcome

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Introduction: Endometriosis represents nowadays probably the most frequent cause of female infertility or subfertility. At our institution endometriosis is diagnosed in more than 40 % of infertile patients. Treatment of choice is by all means laparoscopic surgery which leads to a more than 60 % pregnancy rate, regardless the stage of the disease. For the rest of the patients in vitro fertilization (IVF) is the most appropriate treatment of choice. The impact of ovarian endometriomas on IVF outcome remains still controversial and is a matter of debate. Whereas some studies have documented that ovarian endometriosis is associated with a reduced ovarian reserve and consequently lower pregnancy rates, others failed to prove this association. The present study was designed to elucidate if bilateral ovarian endometriomas removal whether or not affects IVF outcome. Materials and methods: In 34 patients (group A) underwent IVF program the only cause of infertility was bilateral ovarian endometriomas which had been previously surgically removed. The control group (group B) represents 168 patients who underwent IVF procedure due to tubal factor. The inclusion criteria for the study were age less than 37 years, regular ovulatory cycles, normal basal gonadotrophin level and normal spermiogram. Among others, following parameters were analyzed and compared between the both groups: fertilization rate, pregnancy rate per cycle and per embryo transfer (ET), take home baby rate (THBR) per cycle and per ET. Results: In none of the compared parameters statistically significance was reached. In the group "A" pregnancy rate per cycle and per ET was 44.1% and 55.6 % compared to the group "B" where was 38.7 % and 42.5 % respectively. THBR per cycle and per ET in the group "A" was 38.2 % and 48.1 %, whereas in the control group was 33.3 % and 36.6 % respectively. Conclusions: Severe ovarian damage may occur in ovaries operated for ovarian endometriomas but the present study showed that bilateral endometriomas removal does not affect IVF outcome in our patients. Pregnancy rate and THBR were even higher, though not statistically significant, than in patients with tubal factor of infertility. We believe that appropriate surgical technique is of paramount importance to preserve functional ovarian tissue in patients with endometriomas leading to favourable IVF outcome if needed.

O-040 The new stripping technique for low protrusion rate uterine myoma in TCR

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[Objective] In present study, we studied the effectiveness of the new stripping technique in TCR (transcervical resection) for low protrusion rate uterine myoma. [Design] Four cases had low protrusion rate (less than 40%) of uterine submucosal myoma. Before TCR, cervical dilation was done with more than 5 pieces of laminalia. Our new stripping technique of myoma is shown as follows. At first, transverse incision was made on the top of myoma nodule. The tip of a loop electrode was inserted to the space between myoma nodule and the muscle layer of uterus without electrocagulation, and myoma nodule was detached from normal layer of uterine muscle. Until the protrusion rate reach more than 70% and diameter of myoma reach to less than 2cm, resection and detachment are continued. [Results] In four case, TCR was successful without complication. [Conclusion]The new stripping technique in TCR has contributed to success TCR for low protrusion rate uterine myoma.

O-041 Evaluation of short-term and long-term outcome of tubal conservation in the treatment of tubal ectopic pregnancy

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OBJECTIVES: To evaluate short-term and long-term outcomes of tubal conservation in the treatment of tubal ectopic pregnancy such as laparoscopic salpingostomy (LS) and methotrexate (MTX) treatment. We assessed in terms of consequent tubal preservation and tubal patency for the short-term outcome, and pregnancy rate and ectopic pregnancy rate for the long-term outcome. METHODS: This study include 131 cases who underwent LS (LS group) and 21 who were treated with systemic administration of MTX (MTX group) among 216 ectopic pregnancy cases treated in the past 8 years. Serum human chorionic gonadotrophin (hCG) concentration and Chlamydia trachomatis IgG and IgA antibodies were measured before the treatment, and serum hCG concentration was measured until it turned to negative after the treatment. Four months after the treatment tubal patency was assessed by using hysterosalpingography or hydrotubation (113 of LS group and 13 of MTX group). We evaluated the long-term outcome of the cases (57 of LS group and 9 of MTX group) desiring future pregnancy and were able to follow over a year. RESULTS: The short-term outcome of LS group / MTX group was as follows; tubal preservation rate: 93.1% (122/131) / 95.2% (20/21), tubal patency rate: 96.5% (109/113) / 84.6% (11/13). There was no significant difference between the groups, and these rates were not affected by serum hCG concentration before the treatment, C. trachomatis antibodies or fetal cardiac activity. Also, no difference was detected in the long-term outcome of LS group /MTX group; intrauterine pregnancy rate: 26.3% (15/57) / 33.3% (3/9), ectopic pregnancy rate: 8.8% (5/57) / 0% (0/9). However, it was distinctive of five recurrent ectopic pregnancy cases that all of them were positive for C. trachomatis antibodies and were detected peritubal adhesion at the first surgery and that four of them were ipsilateral tubal ectopic pregnancy.

O-042 2000IU hCG in high responders does not affect the outcomes of IVF

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To evaluate the effect of 2000IU and 5000IU hCG in high responders during IVF-ET. One hundred and eighty-two IVF cycles were analyzed from high responders based on oocyte quantities, embryo transferred times and peak E_2 levels. On the day of hCG administration, if E_2 levels were ≥3000 but <5000pg/ml, patients received 5000IU (group B). For E_2 levels≥ 5000 pg/ml, patients received 2000IU (group A and C). If patients failed to get pregnant after they transferred embryos≥3 times, we cryopreservated all their embryos after oocyte retrieval (group C). Mean ages were 30.18 ±3.19, 30.33±3.29 and 29±2.63 for groups A, B and C. Peak E_2 levels in group A or C was higher than group B (6788±2975, 5597±2240vs. 4630±815 pg/ml), as well as the mean number of oocytes retrieved (21.53±7.4, 22.89±8.71vs. 18.34±4.84). Proportion of mature oocytes (90.56%, 89.45%vs. 87.65%), fertilization rates (71.97%, 74.4%vs. 74.07%), high qualityrates (66.16%, 61.87%vs. 58.23%), chemical PR (44.83% vs. 44.83%) and clinical PR were similar. No incidence of moderate or severe OHSS was found in 2000 IU hCG, but two moderate OHSS patients were found in 5000IU hCG group. Taken together, 2000IU hCG is safe and efficient to induce adequate oocyte maturation, fertilization while eliminating the risk of OHSS in high responder patients.

POSTER PRESENTATIONS

P-001 The influence of crowding on Leydig cell, weight gain, testosterone and cortisole levels in mice

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Aims: Human typically encounter in the modern, developed world with stressors that originates largely from social and interpersonal interactions. Epidemiological data suggest that stress is related to the development of metabolic disease and infertility. In the present study the effects of crowding on Leydig cell, cortisole and androgen levels were investigated. Methods: 60 male mice were divided into 6 groups. Control groups (2 cages, 5 mice/cage), groups 1 as mild stress groups (2 cages, 10 mice/cage) and groups 2 as severe stress (2 cages 15 mice/cage). Out of six groups, three groups kept for one month and three next groups kept for two months. The mice were anaesthetized with an intra peritoneal injection of ketamine and xylazine after one and two months. The testosterone and cortisol levels in plasma were assessed by radioimmunoassay. Then, the samples of testes were removed and processed for light and electron microscopy. The data has been compared using statistical methods (SPSS, ANOVA, $P<0.05$). Results: The results showed that the differences in weight gain and plasma testosterone levels in animals exposed to stress for one month were not significant as compared with control group ($P<0.01$); Whereas the differences in the number of leydig cells in groups 3 were significantly increased as compared with control group ($P<0.01$). The differences in other study groups weren't significant. The plasma cortisol levels in group 2 were significantly increased as compared with control group ($P<0.02$) and the plasma cortisol levels in group 3 were also significantly decreased as compared with control group ($P<0.006$); whereas the differences in other groups weren't significant. The results showed that the differences in the numbers of leydig cells, weight gain, plasma testosterone and cortisol levels, in animals exposed to stress for two months weren't significant. Transmission electron microscopy revealed decreasing the numbers of secretory granules in the Leydig cells in mice of group 2 and 3 as compared with control group. Conclusion: These results showed that crowding stress can affect on number of leydig cells and plasma cortisol levels on male mice.

P-002 Identification of basic reprogramming factors associated with a pluripotent potential of *in vitro* cultured spermatic stem cells in domestic animals.

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Spermatogonial stem cells (SSCs) that are present in the mouse testis have the ability to give rise to pluripotent ES-like cells under in vitro conditions. However, properties of the potential have poorly understood in domestic animal species. Transcription factors *POU5F1*, *SOX2*, *c-MYC*, *KLF4* and *NANOG* have been known to be essential for maintaining pluripotency of stem cells and generation of iPS cells. In the present study, we have identified these reprogramming factors in the testis of domestic animals. Spermatogonial stem cells/gonocytes in both pig and bull testis expressed UCHL-1 and VASA, which are known as germ cell specific markers in both species. Interestingly, germ cells that are express UCHL-1 and VASA simultaneously expressed POU5F1 and NANOG protein, and the expressions of *SOX2*, *c-MYC* and *KLF4* genes were also observed. Under in vitro cultivation of stem/progenitor spermatogonia, NANOG and POU5F1 protein were specifically expressed and ES cells-like colonies were formed together with the expressions of germ-cell specific markers. In addition, spermatic stem cells differentiated into various cell lineages including three germ layers. These results suggest that unipotent germ stem cells in domestic animals have a reprogramming potential into pluripotent stem cells in in vitro culture condition.

P-003 Effect of diet contain sesame seed on the rat testis

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Introduction: The development of a new dietary adjunct with a novel natural antioxidant impact on the male fertility in general has been increasingly expressed in recent time. However, the purpose was to evaluate the effects of sesame seed on adult male rats testis using unbiased. Methods and Materials: Histopathology, biochemical and hormonal studies. Thirty adult male rats were divided into two groups of 15 rats each. The regimen group received diet containing 30% sesame seed, while the control group received diet along eleven weeks. Five microns of uniformly random transverse sections of processed testicular tissues were equally analyzed. Testis was analyzed using Graph pad t test software and $P<0.0001$ was considered extremely statistically significant.Results: The results showed that no significant body weight rats, weight and volume testis and percentage volume seminiferous tubules vessels. However, the mean cells number of epithelial and percentage volume of epithelial, lumen and interstitial of this tubules were extremely significant ($P<0.0001$) respectively, in the experimental group compared to control. Conclusion: Sesame seed intake may improved testicular parameters, fertility and sperm production in the males.

P-004 Analysis of Y chromosome microdeletions in Kashanian infertile males

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Introduction: Studies have shown that approximately 10-15% of couples have infertility problem and a male factor can be accounted for half of these cases. Deletion in three non-overlapping regions on long arm of Y chromosome (AZFa, AZFb and AZFc) is caused has defective spermatogenesis and ultimately infertility in men. According to recent studies males who have Y chromosome deletions and followed by ICSI may transmit their deletions to a son offspring. So before ICSI all such men should undergo screening of these deletions for preventing transmitting to their son. Methods & Material: Screening of Y chromosome micro deletions was done in 50 infertile men referring to kashan infertility center for ART. Genomic DNA was extracted from peripheral blood lymphocytes by standard ethanol chloroform techniques. PCR performed on each samples for detection of micro deletions, using 8 STS markers based on EAA/EMQN guideline and 12 STS markers which used in Iran and neighbor countries. Results: In this study, none of patient has these deletions. Conclusions: This study failed to find any deletion in the patients who came for ICSI to this centre, so it appears that other factors are important in the infertility of this population.

P-005 The usefulness of testicular sperm extraction with the Trucut biopsy needle

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Introduction: Currently, testicular sperm extraction (TESE) can be performed in many ways to obtain testicular spermatozoa from azoospermic patients. TESE is mostly performed by invasive surgical procedures under total or lumbar anesthesia, which are traumatic for patients and also very technically demanding. We performed TESE under intravenous anesthesia by using the Trucut biopsy needle. This technique is less technically demanding and less invasive than open surgical procedures. Further, this method is advantageous because TESE can be repeated, unlike open surgical procedures. The purpose of this study was to assess the usefulness of TESE performed using the Trucut biopsy needle. Methods: In this study, the subjects were 105 infertile men who underwent TESE that was performed using the Trucut biopsy needle. The relationship of serum follicle stimulating hormone (FSH) levels with the sucess rate of TESE was analyzed. In TESE, the presence or absence of testicular spermatozoa was examined at first. Subsequently, the motility of the testicular spermatozoa was investigated. Small amount of testicular tissue was collected for histopathological examination. Results: Testicular spermatozoa were retrieved from a total of 64 (61%) patients of which 57 (88%) had an FSH level <16 mIU/ml. Among patients in whom TESE was successful, motile sperms were identified in 44 (69%) of which 43 (98%) had FSH levels <16 mIU/ml. In 11 (23%) patients with a histopathological diagnosis of germ cell aplasia, testicular spermatozoa were retrieved. Conclusion: TESE performed using the Trucut biopsy needle is as effective as open surgical procedures in patients with FSH levels <16 mIU/ml. However, in patients with FSH level >16 mIU/ml, the proportion of patients who had immotile spermatozoa increased, even if testicular spermatozoa were able to retrieved. Therefore, for patients with FSH levels >16 mIU/ml, other procedures of TESE are preferred because the possibility of obtaining motile testicular spermatozoa is higher.

P-006 An evaluation of the confounding effect of sperm abnormalities on pregnancy and implantation rates and miscarriage rates following in vitro fertilization-embryo transfer using sibling oocytes

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Introduction: The possibility exists that sperm with certain abnormal parameters may be able to fertilize oocytes with in vitro fertilization embryo transfer (IVF-ET) and produce normal looking embryos that are inferior and less likely to result in a normal pregnancy. The best way to test this hypothesis would be to compare the pregnancy outcome following the fertilization of oocytes from the same pool of oocytes shared by two males, one with and one without normal semen parameters. Materials and Methods: Donor oocyte recipient pairs sharing the same pool of donated oocytes were identified where one male partner had a subnormal semen specimen whereas the other male partners semen specimen was considered normal. To be considered an abnormal specimen the motile density had to be $<10x10^6$/mL, or motility <30% irrespective of sperm concentration or normal morphology using strict criteria <4%, or the presence of >80% antisperm antibodies or a hypo-osmotic swelling test of <50%. Clinical (gestational sac at 8 weeks) and live delivered pregnancy rates and implantation and spontaneous abortion rates were compared with these two groups following embryo transfer. Only 1 cycle per patient was used. Many but not all recipients with husbands with subnormal semen specimens had the oocytes fertilized by intracytoplasmic sperm injection (ICSI) whereas the majority of the recipients without male factor had conventional oocyte insemination. Results: There were 138 paired cycles compared. There was no significant difference in fertilization rates for those with male factor (785/1088, 72.2%) vs. no male factor (817/1184, 69.0%). There was no difference in clinical pregnancy rate/transfer 52.2% (72/138) vs. 48.6% (67/138) or live delivered pregnancy rate/transfer 42.0% (58/138) vs. 43.5% (60/138) or implantation rate 29.6% (114/385) vs. 27.8% (109/392) (chi-square analysis). The miscarriage rate was twice as high with male factor (19.4%, 14/72) vs. 10.4% (7/67) (p=0.16, Fishers exact test). Conclusions: The presence of male factor in the era of ICSI does not seem to have a negative impact on IVF outcome as evidenced by comparing outcome of sibling oocytes of which half were fertilized by males with and half without subnormal semen parameters. Though the miscarriage rate was twice as high with male factor the slightly higher clinical pregnancy rate per transfer resulted in no difference in the live delivered pregnancy rate per cycle.

P-007 Testicular sperm retrieval using a new multiple-holes puncture needle

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Testicular fine needle aspiration (TFNA) was initially used for diagnosis purposes and it is now sometimes used to recover sperm from the testicles for ICSI. This study aimed to compare the reliability of testicular sperm extraction by a new multiple-hole needle aspiration (MHNA) with TFNA in infertile males with azoospermia. This retrospective cohort study involved 68 patients with azoospermia. Detailed clinical and laboratory examinations were performed and two semen analyses were obtained from each patient. A 20-ml 14 gauge 13-mm needle with multiple-hole was used for MHNA, and a 20-ml 21 gauge 13-mm needle was used for TFNA. The 68 patients underwent testicular sperm retrieval were divided into two groups according to their sperm retrieval methods (MHNA group n=30; TFNA group n=38). The motile sperm retrieval rate in MHNA group was 70%, significantly higher than that in TFNA group of 39.5%. Five ICSI cycles were performed in MHNA group, and four cases get pregnant. Testicular MHNA in infertile males is a simple, reliable and minimally invasive technology for testicular sperm retrieval. It is more effective than TFNA in testicular sperm extraction.

P-008 The impact of body mass index on sperm recovery and serum reproductive hormone levels in an infertility setting-An analysis of 445 azoospermic cases -

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Introduction: The relationship between metabolic syndrome and health disorders or the decline in male sexual functions is a major topic of discussion; moreover, reports describing a correlation between obesity and sperm count have appeared in the literature. A retrospective study regarding the impact of body mass index on reproductive hormones and potential sperm recovery was conducted involving infertility cases treated at this clinic on an outpatient basis over the past decade. Materials and Methods: The subject population consisted of 795 infertility outpatients treated in this clinic between April 2000 and March 2010; 350 and 445 cases of subfertility, e.g., varicocele, and azoospermia (obstructive azoospermia= OA, 187 cases; non- obstructive azoospermia= NOA, 258), respectively, were diagnosed. Additionally, 30 fertile controls were included. Levels of reproductive hormones, triglyceride, HDL-cholesterol and uric acid and BMI were examined. Results: Total and free testosterone levels displayed significant elevation in NOA sperm recoverable groups. Correlations in fat metabolism, uric acid or BMI were not detected among azoospermia, subfertility and fertile control subjects. Conclusion: No relationship was apparent between sperm recovery potential and body mass index. This retrospective study assessed patients at a single facility; thus, future large-scale population-based longitudinal studies are necessary.

P-009 The frequency of males with sperm with low hypoosmotic swelling test scores (which prevents morphologically normal embryos from implanting) in couples having in vitro fertilization-embryo transfer

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Introduction: The hypo-osmotic swelling (HOS) test defect is an interesting semen abnormality. If <50% of the sperm when placed in a hypo-osmolar solution fail to show tail swelling, a defect in the functional integrity of the sperm membrane is detected. This abnormality does not inhibit fertilization but dramatically prevents the embryos from implanting because of transfer of a toxic protein present from sperm to the zona pellucida which then become incorporated in the embryo membrane causing a functional impairment of the embryo membrane, which impairs the implantation process. Despite the fact that the knowledge that this defect markedly inhibits embryo implantation has been known for over 25 years, and the defect is greatly obviated by intracytoplasmic sperm injection (ICSI), this simple inexpensive test is rarely performed by most in vitro fertilization (IVF) centers. The objective of this study was to determine how common is this abnormality in an IVF population in males with normal semen parameters where conventional oocyte insemination rather than ICSI would generally be performed without the knowledge of a low HOS score.Materials and methods: Over a 10 year period couples undergoing IVF-embryo transfer (ET) were retrospectively identified where the standard semen parameters were deemed normal (motile density >$8x10^6$/mL, morphology using strict criteria >4%, and antisperm antibodies by direct immunobead <20%. The frequency of low HOS test scores was then determined.Results: A low HOS score was found in 250 of 1993 males evaluated a frequency of 12.5% in this population of males with otherwise normal semen parameters. Conclusions: Because ICSI adds extra embryologist time, increases the expense of an already expensive procedure, and may decrease the chance of successful pregnancy and possibly increase the risk of mono-zygotic twins it is generally not performed routinely when males have normal semen parameters. Thus IVF centers not performing this simple inexpensive test may be choosing the wrong oocyte insemination technique and subjecting the couple to the risks and expense of IVF with little likelihood of success when ICSI would have worked extremely well. Hopefully, this study will encourage other IVF centers to add this simple inexpensive test to their routine semen analysis to help prevent some couples from undergoing expensive IVF-ET without much chance of pregnancy because of the wrong oocyte insemination technique.

P-010 Effects of several culture conditions on the primordial germ cell proliferation

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Objective: The aim of this study was to compare the in vitro effects of different concentrations of retinoic acid (RA), and co-culture with STO cells on the ESCs-derived primordial germ cells (PGCs) proliferation. Materials and Methods: CCE mouse ESCs were cultured for 1 day in order to embryoid body (EB) formation and then cultured for 4 days in the presence of 5 ng/ml BMP4 for PGC induction. In order to PGC enrichment, ESCs derived germ cells cultured for 7 days in the presence of different doses (0-5 micromolar) of RA both in the simple and STO co-culture systems. Expression of Mvh, PGC specific marker, was evaluated using quantitative PCR. Data analyses were done with ANOVA and Tukey posttest. Results: The results of PCR showed higher significant expression of Mvh in 3 micromolar RA concentrations on the top of the STO feeder layer. Conclusion: Quantitative RT-PCR was used to estimate the level of germ cell gene expression. The results confirmed that the addition of 3 micromolar RA concentrations on the top of the STO feeder layer has a positive role in PGC enrichment.

P-011 Early development of *in vitro* aged eggs after intracytoplasmic sperm injection

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Introduction: Recently, rescue approaches for fertilization failure eggs (unfertilized eggs) using intracytoplasmic sperm injection (ICSI) have been addressed (rescue ICSI). We believe that target eggs used for rescue ICSI have already aged by the time of reinsemination and are therefore essentially different from fresh unfertilized eggs. This study examined the development of embryos derived from the *in vitro* aged eggs after ICSI. Material and Methods: BDF1 mice were used for this experiment. Superovulated eggs were cultured for 6~24 h *in vitro* for aging. Some of these aged eggs were stained by immunofluorescence, and the spindles and chromosome alignments were observed. For the other eggs, we performed ICSI, and fertilized eggs were cultured for 96 h *in vitro*. To evaluate the quality of ICSI embryos, we performed chromosome analysis at the 8-cell stage. Additionally, we analyzed the ratio of inner cell mass cells and trophectoderm cells in the blastomere by evaluating Oct4 and Cdx2 positive cells at the blastocyst stage. Results: In 6-, 12-, 18-, and 24-h aged group, respectively, 85.9%, 57.1%, 26.9%, and 6.4% of eggs had morphologically normal spindle and chromosome alignment. The rates of these eggs in 12-h or more aged group were significantly lower than that in the fresh group (91.0%). The fertilization rates after ICSI were 90.2% and 74.5% in 6- and 12-h aged group and 88.7% in the fresh group, and the developmental (fertilized eggs to blastocysts) rates of these eggs were 78.4%, 25.0%, and 80.2%, respectively. The developmental rate was significantly lower for the 12-h aged group than for the other groups. At the 8-cell stage, the numerical aberration of chromosome was detected in 65.5% of all observed metaphase plates in the 12-h aged group, which was significantly higher than that in the fresh group (29.3%). The number of Oct4 and Cdx2 positive cells in the blastocyst was 4.3 and 2.8, respectively, in the 12-h aged group, and 5.5 and 2.1, respectively, in the fresh group. The number of Oct4 and Cdx2 positive cells in 12-h aged group was significantly lower than that in the fresh group; however, there was no difference in the Oct4/Cdx2 ratio. Conclusions: *In vitro* aging caused the abnormality of cleavage apparatus in eggs and this abnormality lead to numerical aberration of chromosome and reduction of blastomere in embryos. This study suggested that the rescue ICSI should be performed for unfertilized eggs until 6h after the first insemination.

P-012 One step closer to the development of a rapid bioassay to determine if adequate luteal phase progesterone supplementation is provided during in vitro fertilization-embryo transfer cycles

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Introduction: One of the functions of progesterone is to interact with a progesterone receptor that develops on gamma delta T-cells when exposed to an allogeneic stimulus. This 34 kDa protein suppresses natural killer cell function by stabilizing perforin granules and causes a shift from thymic (T) helper (H) 1 cells to TH2 cells, thus shifting the emphasis from the cellular to the humoral immune system. This protein is called the progesterone induced blocking factor (PIBF). To date this protein has only been measured by an immunocytochemistry technique which is not a practical assay for rapid turn-around for results. The PIBF has recently been synthesized by recombinant DNA technology thus opening the possibility of the development of an enzyme linked immunosorbent assay (ELISA). However for an ELISA to be developed it is first necessary to determine if PIBF is a soluable protein. Materials and methods: Serum samples were blindly assessed for the presence of PIBF using a novel sandwich ELISA we developed with full length (amino acid 1 to 757) recombinant human PIBF, rabbit polyclonal antibody to amino acid 1-300, and affinity purified goat polyclonal antibody to the internal region of PIBF conjugated to horseradish peroxidase. Color was developed using tetramethylbenzidine substrate stopped by 2N H2SO4 and read at 450 lambda. Bovine serum albumin served as a negative control. Results: Soluble PIBF was detected in several samples taken from pregnant women, with highest levels in pregnant patients at over 10 microgram/ml concentration. There were significant inter-individual differences. Conclusions: PIBF, does indeed also exist in a soluble form and can be detected in serum. With further refinement of our ELISA we aim to improve sensitivity and specificity, allowing for the simple measurement of PIBF on a rapid, high throughput basis, making it a more practical test to determine with a bioassay if the amount of progesterone supplementation is sufficient or not. Hopefully once a sensitive and specific PIBF ELISA is developed levels can be determined during the luteal phase that define successful vs. non-successful embryo transfers.

P-013 Effect of ovarian induction on the ultrastructure of corpus luteum during luteal phase at implantation period

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Introduction: During ovarian induction for IVF cycle, a large number of follicles develop also some ultrastructural changes occurred in corpus luteum that affect on pregnancy rate. The aim of this study was to evaluate ultrastructural changes of mouse corpus luteum after ovarian induction using hMG and hCG during luteal phase at implantation period. Material and Methods: 20 females NMRI mice 6-8 weeks old were divided into two groups: Control group; the mice were rendered pseudopregnant and hyperstimulated group; the mice were rendered pseudopregnant after the ovarian induction. The samples were obtained from the ovary in each group at he same time during luteal phase at implantation period. Ultrastructural changes were assessed using electron microscopy study. Results: Our results showed that some identifiable changes were seen in structure and ultrastructure corpus luteum in hyperstimulated group. These changes included enhancement of the apoptosis, vacuole formation and intercellular space, whereas the angiogenesis was decreased. The findings indicated decrease in organelle condensation in cytoplasm of hyperstimulated group such as mitochondria and polyribosome, furthermore, choromatin condensation of nuclei were observed in some cell. Conclusions: Briefly, the ovarian induction using hMG & hMG result in some ultrustuctural changes on the corpus luteum at implantation period which could be affected on the pregnancy rate.

P-014 Autophagy in the ovarian granulosa cells

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Object: Autophagy is the intracellular clearance system which breaks proteins in the cell to make a vesicle named autophagosome. It is well known that the autophagy abundantly appears in the starvation status and the early stage of mammalian embryos just after fertilization. The cumulus cells are differentiated from the mural granulosa cells during follicle development especially just before ovulation. The cumulus cells are associated with the transportation of nutrition to an oocyte and the control of the meiosis of an oocyte. The association of autophagy in the mural and cumulus granulosa cells with the maturation and quality of an oocyte has not been reported. In this study, we examined the relation between the expression of autophagy and the oocyte quality using LC3 which is one of the markers for autophagosome. Method: The mural and cumulus granulosa cells was obtained from the patients for assisted reproductive technology treatment. The oocyte quality was divided by the morphology of cumulus oocyte complex (COC); mature, immature and dysmature. Autophagy was detected by the immunofluorescence staining of the cumulus cells of the COC and the mural granulosa cells. Apoptosis in the granulosa cells was also detected by the nuclear fragmentation stained with Hoechst 33342. The stain positive cells rate was calculated in the both staining methods. Results: The rates of autophagy in cumulus and mural granulose cells were 45.2%, 33.3%, 46.8% and 6.38% ($p<0.01$) (mature, immature, dysmature and mural granulosa cells respectively). The rate of apoptosis in cumulus and mural granulose cells were 1.01%, 0.92%, 3.44% and 1.11% ($p<0.01$) (mature, immature, dysmature and mural granulosa cells respectively). Conclusions: In autophagy, cumulus cells groups were higher than that of mural granulose cells. There was no difference among the cumulus COC groups. Meanwhile the dysmature group indicated the highest incidence in apoptosis. Thus autophagy is not related with the quality of COC.

P-015 The promoter-1031(T/C) polymorphism in tumor necrosis factor-α associated with polycystic ovary syndrome

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A tumor necrosis factor-α (TNF-α) is a multifunctional proinflammation cytokine that has been considered as one of pathogenesis for various immune-related diseases. The promoter-1031(T/C) polymorphism in a TNF-α gene was reported that it plays an part in reproduction-related diseases. And polycystic ovary syndrome (PCOS) is a common gynecological disease of women in reproductive age women. Here, we performed a comparative study of -1031(T/C) polymorphism of TNF-α gene with PCOS in a Korean population. The -1031(T/C) polymorphism of TNF-α gene was analyzed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) in a total of 217 PCOS patients and 144 matched female controls of healthy women. The frequency of T/T, T/C, and C/C genotypes of -1031(T/C) polymorphism in TNF-α gene showed different proportion (p-value = 0.0003, odd ratio (OR) = 2.53). In addition, the frequencies of co-dominant (p-value = 0.0003, OR = 2.81), and dominant alleles (p-value = 0.0001, OR = 2.81) showed association between PCOS and control group. This is the first study in PCOS with -1031(T/C) polymorphism in TNF-α gene. And we concluded that -1031(T/C) polymorphism in TNF-α gene is associated with PCOS in a Korean population. Therefore, we expect that it may be considered as a clinical biomarker to diagnose for PCOS, and is helpful in understanding the etiology for the pathogenesis of PCOS.

P-016 Association between INS-VNTR polymorphism and polycystic ovary syndrome in a Korean population

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Polycystic ovary syndrome (PCOS) is a common disorder of the women in reproductive ages. But its etiology is not fully understood yet. Variability in the number of tandem repeats of the insulin gene (INS-VNTR) is known to associate with PCOS, and it is associated with an increased risk of diabetes mellitus and other cardiovascular diseases. The aim of our study was to analyze an association between the -23/Hph I polymorphism of INS-VNTR and PCOS in a Korean population. A total of 218 PCOS patients and 141 controls were analyzed by restriction fragment length polymorphism (RFLP) method for the INS-VNTR polymorphism. Statistical analysis of genotyping results were performed using HapAnalyzer. X2 test and logistic regression were utilized to analyze the association between two groups. A p-value under 0.05 was considered statistically significant. The frequencies of A/A and A/T genotypes indicated a similar change between PCOS patients and controls. In addition, no association was found between PCOS and control subjects (p-value = 0.0544, odd ratio (OR) = 1.69). Our present data demonstrated that INS-VNTR polymorphism is not associated with PCOS in Korean women. Thus, it is suggested that INS-VNTR polymorphism is not a key factor in the etiology and the pathogenesis of PCOS in a Korean population.

P-017 Application of non-elective blastocyst culture

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This article summarized 387 non-elective blastocyst culture and cultivation of blastocyst transfer for patients and pregnancy from February to September, 2010. We analyzed and summarized the cultivation of blastocyst transfer and pregnancy with the 387 patients. Method: We extended the cleavage stage of embryos to the blastocyst stage in vitro to observe blastocyst formation rate and quality, and analyzed the relationship between embryo quality of the cleavage stage and blastocyst formation and the impact of blastocyst formation to clinical pregnancy. The results showed that a total of 3513 embryos of 387 patients had been cultured blastocysts and 1489 blastocyst had been got. The blastocyst formation rate was 42.35%. I-II stage embryo blastocyst formation rate was significantly higher than that of grade III-IVembryos ($P<0.01$); Clinical pregnancy rate of blastocysts was significantly statistically significant at 5,6,7 days after transplantation ($P<0.01$). The incidence of ectopic pregnancy of blastocyst frozen transplantation was significantly lower than that of cleavage stage embryo transfer ($P<0.01$). Conclusion: 1. The rate of high quality embryonic blastocysts formation was significantly higher than low quality embryos. 2. The formation of the pregnancy rate was higher at 5 days after blastocysts transplantation, which is low at 6,7 days. 3. Compared with embryo transfer on day 2, blastocyst transfer was more consistent with the natural phenomenon. Blastocyst transfer could improve the embryo implantation rate in IVF-ET and the clinical pregnancy rate and reduce the number of embryo transfer.

P-018 Oocyte activation with Ca ionophore A23187 and roscovitine for ovulated mouse oocytes to produce haploid parthenogenones

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ICSI is a essential for male factor infertility. However, total fertilization failure was observed in 2-3% of ICSI cycles and disorder of oocyte activation is assumed to be a main cause of this unfavorable phenomenon. We previously reported a new oocyte activation technique using a combination of Ca ionophore A23187 and puromycin. Puromycin, a one of protein synthesis inhibitor, showed a positive effect for oocyte activation during mouse and human oocyte, however, there is a concern of safty to use puromysin for early developmental stage of oocyte and embryo. In this study, we evaluated the efficacy of a new combination of Ca ionophore A23187 and roscovitine, which is potentselective reversible inhibitor for MPF for the oocyte activation. Retrived mice oocytes were treated with a combination of A23187 and roscovitine (roscovitine group) or using a combination of A23187 and puromycin (puromycin group) or with A23187 (control group). Activated oocytes with 1PN2PB were observed in 88.3% in roscovitine group, 94.4% in puromycin group and 2.2% in control group. ($P<0.01$: control vs roscovitine or puromycin). The DNA content of activated oocytes in the roscovitine group was virtually a half of that of MII oocytes (47.8±10.7%). Most of activated oocytes in roscovitine group showed haploid chromosome number (96.7%). These results indicate the efficacy and the safty of oocyte activation using a combination of A23187 and roscovitine.

P-019 The Availability of Recombinant FSH (recFSH) in Minimum Ovarian Stimulation by the Deference of the Anti-Müllerian Hormone (AMH) Level

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BACK GROUND: Anti-Müllerian hormone (AMH) is increasingly used to quantify ovarian reserve, but it has not yet realized its full clinical potential in assisted reproduction technology. We usually use minimum ovarian stimulation by only clomifene citrate (CC), in cases, by CC with recFSH. We investigated the availability of recFSH in minimum ovarian stimulation by various AMH level patients. MATERIALS AND METHODS: We investigated 238 IVF cycles (mean patient age of 36.3) from August 2010 to March 2011. Patients were induced minimum ovarian stimulation by only CC or CC with recFSH. We cultured embryo and cryopreserved at expanding or full expanded blastocyst. Serum AMH concentrations were divided into seven groups (<5 (a) , 5≤ <10 (b) , 10≤ <15 (c) , 15≤ <20 (d) , 20≤ <25 (e) , 25≤ <30 (f) , 30≤ (g) pmol/L). We investigated that we obtained the number of eggs by an IVF cycle and the rate of blastocyst cryopreservation (BC). RESULT: We obtained more number of eggs with recFSH than only CC over 5 pmol/L AMH level. (only CC a: 1.2, b: 1.4, c: 1.2, d: 1.6, e: 1.9 f: 2.0, g: 1.9 eggs/IVF cycle, with recFSH a: 1.1, b: 2.6, c: 4.1, d: 4.7 e, 4.1 f: 4.0, g: 8.3 eggs/IVF cycle.) The rate of BC higher with recFSH than only CC less 10 pmol/L AMH level. The highest rate in 25≤<30 pmol/L AMH level in both group. (only CC a: 13.3, b: 21.4, c: 50.0, d: 40.0, e: 33.3 f: 100, g: 33.3 %, with recFSH a: 37.5, b: 42.9, c: 34.4, d: 38.5 e, 50.0 f: 87.5, g: 35.6 %.) CONCLUSION: This study demonstrated that minimum ovarian stimulation by CC with recFSH effective to improvement the quality of embryo for low AMH level patients.

P-020 Results of 1027 office-based diagnostic hysteroscopies before IVF and evaluation the pregnancy and take home baby percentages between patients with normal and abnormal uterine findings.

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Objective: To determine the findings of office hysteroscopy in an in vitro fertilization (IVF) population, also evaluate the pregnancy and take home baby percentages between patients with normal and abnormal uterine findings. Moreover, we compare the cost-effectivines of office hysteroscopy among other procedures. Methods: Between May 2007 and May 2008, hysteroscopic findings in 1027 patients admitted to our IVF Unit were analyzed retrospectively. Patients were divided into two groups, as normal and abnormal uterine findings. We examined their record for IVF outcome. The pregnancy and take-home baby percentages were determined between the groups. Pearsons chi-square test was used for categorical variable. $p<0.05$ were considered statistically significant. Results: All hysteroscopic parameters were considered normal 792 cases (77.1%); in 235 cases (22.9%) various pathological conditions were found. Endometrial pathology were found more frequently in older age women (p<,000). We found statistical differences for pregnancy percentages between groups with no pathology and with pathology (p<,003). Also there was a statistical differences for take-home baby percentages between these groups. The cost of all office hysteroscopy was analyzed and compared with the other procedures. Conclusions: There was a trend toward better pregnancy and take-home baby percentages in women with normal hysteroscopic findings. Because of this routine uterine cavity evaluation should be perfomed in all patients. But for first line examination, non invasive and cheap procedures should be recommended.

P-021 Serum levels of macrophage inhibitory cytokine 1 (MIC 1) as a predictor of miscarriage in early pregnancy of women treated for repeated reproductive failures

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Imbalances in the intrauterine cytokine milieu around the time of implantation may play a causative role in early pregnancy failure. Cytokines appear to be an important component of a communication network at the feto-maternal interface. Experimental evidence suggests that MIC-1 has immunomodulatory activity and acts to maintain pregnancy by suppressing the production of proinflammatory cytokines. Studies of serum MIC-1 levels in several cohorts of patients have revealed potential clinical use in the monitoring of pregnancy. The aim of our study was to evaluate the prognostic value of serum MIC-1 levels in pregnant women treated for repeated reproductive failures. In the study, 59 women treated for repeated reproductive failures (15 women with RSA and 44 women with RIF) were included at positive blood pregnancy test. Serum samples were collected from the women between 4-16 weeks of pregnancy and MIC-1 concentrations were measured using a sensitive enzyme-linked immunosorbent assay (BioVendo, CzR). Serum levels of MIC-1 in 5th week of gestation were significantly higher (p=0.033) in the group of women pregnant by ART procedure in comparison with the group of spontaneously pregnant women. No difference in MIC 1 levels was observed between the groups in later gestational weeks. Eight women (13.56%), 2 from the RSA group and 6 from RIF group, miscarried in 9-10 week of gestation and their MIC 1 concentrations were significantly lower (p=0.045) than those with ongoing pregnancies. Low MIC-1 serum levels were detected before miscarriage in the pregnant women treated for repeated reproductive failures, suggesting possible predictive and causative role of this cytokine and that the application of therapeutic agents that would lead to up-regulation of MIC-1 prevent miscarriage.

P-022 A drop in serum estradiol the day after human chorionic gonadotropin (hCG) shot does not adversely affect pregnancy rates per embryo transfer in women with very decreased oocyte reserve

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Introduction: The majority of hyperstimulated women having in vitro fertilization-embryo transfer (IVF-ET) show a rise in serum estradiol (E2) the day after hCG. However, women with markedly diminished oocyte reserve with usually no more than 1-3 follicles may follow the course more akin to normal ovulatory cycles when the serum E2 drops after the luteinizing hormone (LH) surge as progesterone (P) rises. The objective of the present study was to determine if such a drop in this special group of patients has any negative impact on the pregnancy rate (PR) or implantation rate following ET. Materials and methods: Over a 10 year period women undergoing IVF-ET with a day 3 serum follicle stimulating hormone (FSH) >15 mIU/mL were identified. Only the first cycle of IVF-ET was evaluated for serum E2 and P and E2/P ratio on the day of and the day after hCG injection. They were divided into 2 groups: those with an increase vs. those women with a decrease in serum E2 the day after hCG injection. P level and E2/P ratio were also compared not only between these 2 groups but in those who conceived vs. those who did not. Comparisons were made with chi-square analysis. Results: There were 403 IVF-ET retrievals selected: 325 with E2 rising (grp 1) and 78 with E2 dropping (grp 2). Thus 19.3% showed a drop in E2. The clinical and live delivered PRs/transfer were similar in grp 1 and grp 2, 31.1% (61/196) and 28.1% vs. 38.6% (17/44) and 22.7% (p=NS, chi-square). Implantation rates were also similar, 22.1% (70/317) vs. 28.0% (21/75). There was no difference in the level of E2 on day of hCG, 490pg/mL grp 1 vs. 478 pg/mL grp 2. Comparing E2/P ratio in pregnant vs. non-pregnant women grp 1 vs. grp 2 provided no new insights. For grp 1, 49 released the oocyte (15.0%) before the retrieval vs. 14 (17.9%) in grp 2 and no eggs were retrieved in 39 (12.0%) grp 1 vs. 13 (16.7%) grp 2 women (p=NS). Conclusions: A decrease in serum E2 the day after hCG shot in women with diminished oocyte reserve has no adverse consequences for IVF-ET.

P-023 Analysis of the limitations for the numbers of attempts of infertility treatment using ART.

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Purpose: The successful treatments using ART, including IVF-ET, ICSI-ET and frozen-thawed ET, have offered satisfactory results for a number of infertile couples. However, there still exist refractory infertile couples even they are treated by modern ART. The aim of the present study is to analyze the limitations for the numbers of attempts by ART, the final stage of infertility treatment, especially in such patients. Method: Between Jan. 2006 and Dec. 2009, 157 infertile couples were treated by ART only in our institute and the final results of their treatments were obtained. Patients who were not yet completed their treatments or those who had experienced the ART treatments in other clinics were excluded. In all, 247 fresh embryo transfers and 163 frozen-thawed embryo transfers were included. As for blastocyst transfers, the results in 115 treatment cycles from 45 couples were analyzed. Results: The pregnancy rates (PR) per cycle and that per patient were 37.2% and 58.6%, respectively. The multiple PR was 5.4%, including only twin pregnancies. In all, the cumulative PR reached plateau (98.9%, 95.7%) when the number of oocyte picking-up and that of embryo transfer carried out were 3 cycles and 4 cycles, respectively. The approximate limitation of the total numbers of embryos for achieving pregnancy was designated as 7. As for blastocyt transfers, the cumulative PR reached plateau (90.3%) when the number of embryos transferred was 3. The approximate limitation of the total numbers of blastocysts for achieving pregnancy was designated as 6. Conclusions: These findings should be informed for the decision making before the patients start to be treated by ART.

P-024 The predictive value of AMH for ovary reserve and response during IVF-ET

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Introduction: There is an urgent need for a reliable and early marker for the detection of a declining number of follicles, and prediction of spontaneous pregnancy potential and assisted reproduction technology outcomes. Recent studies shown that Anti-Mullerian hormone (AMH) maybe such a promising and reliable marker. We therefore carried out a prospective trial to study the predictive value of AMH for ovary reserve and response during IVF-ET. Material & Methods: (1) The level of AMH protein secretion in serum was examined by ELISA in 228 cases of infertile woman, the level of AMH protein in follicular fluid and the AMH mRNA relative concentration in granulosa cells were examined by ELISA and RT-real-time-PCR in 71 cases who received IVF-ET treatment. Results: (1) A positive correlation was found between serum AMH and AFC ($p<0.01$) in 228 cases of infertile women, and it correlated negatively with Age, Age at Menarche and Years Since Menarche ($p<0.01$, $p<0.01$, $p<0.01$), serum AMH level and AFC correlated each other and declined significantly with age. (2) There is no significant difference between the bAMH with the AMH protein level on day 5 after Gn injection and on oocyte retrieval day ($p>0.05$) in 71 cases of IVF-ET treatment patients. (3) A positive correlation was found between serum AMH with FF AMH and relative concentration of AMH mRNA in GC ($p<0.01$,$p<0.01$) in 71 cases of IVF-ET treatment patients, there are significant differences comparing serum, FF AMH level and GC AMH mRNA relative concentration with the ovary response ($p<0.05$). (4) A positive relationship was found between bAMH and the number of oocytes retrieved ($p<0.01$), and there are positive correlations between the AMH mRNA level with the number of fertilized oocytes and the number of cleavage ($p<0.01$,$p<0.01$) (5). The basic AMH level in 12 OHSS patients is significantly higher than the 12 ovary poor response cases ($p<0.01$). Conclusions: There is obvious relationship between serum AMH level with ovary reserve and response. The serum AMH protein level is of more clinical value to predict the ovary reserve and response, and may be applied in clinic as a molecule marker.

P-025 Whether Day 3 FSH/LH ratios is a good predictor of IVF prognosis in GnRH antagonist protocol?

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Introduction: As most of us in this field know, Day 3 FSH is a simple and easily-available ovarian reserve marker and that, its value is limited when FSH is significantly elevated. We also know that Day 3 LH also has limited value in ovarian reserve marker. Some previous studies have shown FSH/LH ratios could be used as ovarian reserve marker. The problem was that these studies were limited by small sample sizes and almost all studies were performed in GnRH agonist protocol and not in GnRH antagonist protocol. Objective: The purpose of this study was to examine whether day 3 FSH/LH ratios could predict IVF outcome in GnRH antagonist cycles. Design: A retrospective cohort study. Materials and methods: We retrospectively reviewed 228 patients undergoing IVF using the GnRH antagonist protocol from January 2004 to December 2008. Inclusion criteria included age < 40, day 3 FSH < 10 IU/L. Day 3 FSH/LH level of included patients were checked within 3 months of IVF start. The patients were divided into 3 groups. The groups were based upon Day 3 serum FSH/LH ratios. Group I consisted of 117 patients with serum FSH/LH < 2, Group II consisted of 64 patients with 2< FSH/LH <3 and Group III consisted of 47 patients with FSH/LH > 3. We compared characteristics and cycle outcomes of patients. Results: The characteristics of patients by age, parity and cause of infertility were similar among the 3 groups. The number of oocytes (Group I: 14.5, Group II: 12.5, Group III: 9.46) and the number of 2PN (Group I: 10.5, Group II: 8.7, Group III: 6.18) were significantly decreased when day 3 FSH/LH ratios increased. Ongoing pregnancy rates significantly decreased as FSH/LH ratio increased. [Group I: 47.8%(56/117), Group II: 35.9%(23/64), Group III: 19.1%(9/47), P<0.01] Conclusions: We observed that increased FSH/LH ratios were associated with poor IVF outcomes in GnRH antagonist cycles. Moreover, as FSH/LH ratios increased, the number of oocytes, the number of 2PN and ongoing pregnancy rates decreased. Therefore, we suggest that Day 3 FSH/LH ratios can be used as simple and easily-available ovarian reserve marker and a good predictor of IVF prognosis.

P-026 Luteal blood flow in patients undergoing GnRH agonist long protocol

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Background: Blood flow in the corpus luteum (CL) is closely related to luteal function. It is unclear how lutealblood flow is regulated. Standardized ovarian-stimulation protocol with a gonadotropin-releasing hormone agonist(GnRHa long protocol) causes luteal phase defect because it drastically suppresses serum LH levels. Examiningluteal blood flow in the patient undergoing GnRHa long protocol may be useful to know whether luteal bloodflow is regulated by LH. Methods: Twenty-four infertile women undergoing GnRHa long protocol were divided into 3 groups dependenton luteal supports; 9 women were given ethinylestradiol plus norgestrel (Planovar) orally throughout the lutealphase (control group); 8 women were given HCG 2,000 IU on days 2 and 4 day after ovulation induction inaddition to Planovar (HCG group); 7 women were given vitamin E (600 mg/day) orally throughout the luteal phasein addition to Planovar (vitamin E group). Blood flow impedance was measured in each CL during the mid-lutealphase by transvaginal color-pulsed-Doppler-ultrasonography and was expressed as a CL-resistance index (CL-RI). Results: Serum LH levels were remarkably suppressed in all the groups. CL-RI in the control group was more thanthe cutoff value (0.51), and only 2 out of 9 women had CL-RI values < 0.51. Treatments with HCG or vitamin Esignificantly improved the CL-RI to less than 0.51. Seven of the 8 women in the HCG group and all of the womenin the vitamin E group had CL-RI < 0.51. Conclusion: Patients undergoing GnRHa long protocol had high luteal blood flow impedance with very lowserum LH levels. HCG administration improved luteal blood flow impedance. This suggests that luteal blood flow isregulated by LH.

P-027 Comparison of the effect of GnRHa long and short protocol in older women during IVF/ICSI

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Objective: This retrospective study aimed to compare the effect of GnRHa long and short protocol in women aged 35 and above after IVF/ICSI attempts. Methods: A total of 401 fresh IVF/ICSI cycles were followed in my study. In women aged 35-39 years group, 233 cycles were included in the long protocol and 105 cycles in the short protocol. In another age group, women aged 40 years and above, 14 and 49 cycles were included in the long and short protocol, respectively. Results: In women aged 35-39 years group, there were significant differences in the days of Gn using (9.75±2.03 vs 8.26±1.76, P<0.05) and the amount of Gn (31.57±9.38 vs 26.48±7.64, P<0.05) in GnRHa long and short protocol respectively. The EMT on the dayHCG (10.00±2.26mm, P<0.05) in long protocol were thicker than that in short one (9.20±1.90mm, P<0.05). The number of oocytes retrieved (7.46±4.77 vs 5.2±3.96), cleavage (4.65±4.04 vs 3.56±3.07), excellent embryos (3.36±2.12 vs 2.31±1.99) and the rate of excellent embryos (69.07% vs 60.19%) in the long and short protocol were significant difference (P<0.05), as well as the implantation rate (21.5% vs 13.0%, P<0.05) and the pregnancy rate (39.7% vs 26.8%, P<0.05). In another age group,the days of Gn using (10.60±1.34 vs 7.74±1.82) and the amount of Gn (37.50±7.49 vs 26.25±7.59) in long and short protocol respectively were significantly different (P<0.05). There were significant difference in the number of oocytes retrieved (6.91±6.75 vs 3.80±3.14, P<0.05), excellent embryos (2.43±1.16 vs 1.47±1.30, P<0.05) and the rate of excellent embryos (68.56% vs 53.62%, P<0.05). But there were no significant difference in implantation rate and pregnancy rate. Conclusion: women aged 35 years and above who received long protocol needed more ampoules of Gn and longer period of treatment. More oocytes, a greater rate of high quality embryos and a higher clinical pregnancy rate can be obtained in GnRHa long protorol.

P-028 Small amount of testosterone administration improves ovarian response to ovulation induction in poor responders

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Objective: It is well known that there are some patients who cannot respond properly by the exogenous administration of gonadotropin preparations. It is also reported that plasma estrogen can modify the activities of gonadotropin receptors. We have already reported that exogenous administration of estrogen is effective to improve the response of gonadotropin administration in these patients, however there are still some patients who cannot be treated by this method. Present study was performed to elucidate whether exogenous administration of estrogen with a small amount of testosterone was effective to improve the response of gonadotropin administration in the patients of severe poor responders. Method: Six patients who had resistance to gonadotropin therapy, and could not succeed by the administration of the exogenous estrogen therapy were subjected (35.4+/-1.9 years old). All of them had experiences of previous treatment of hMG (up to 600/day), and could not respond to the treatment. Then, exogenous estrogen (conjugated equine estrogen: 2.5 mg/day) and testosterone cream (trans-dermal, 0.6mg/day) was administered for 2 weeks with Gn-RH agonist. After that, hMG preparation of 150IU/day was administered to the subjects with estrogen, testosterone and Gn-RH agonist in gradual increasing method up to 600/day, and the follicular growths were observed by the transvaginal ultrasonic scanner. Then, 10000iu of hCG was injected when the matured follicles (diameter over 18mm) were observed, and the proper luteal support (progesterone vaginal tablets, 60 mg/day) was also done. Results: The ovulations were observed in four of six patients, and one could conceive with this method. Conclusion: The exogenous testosterone administration with estrogen and Gn-RH agonist is effective to the ovulation induction in the patients of severe poor responders. It was suggested that the exogenous testosterone and estrogen might have some effect to improve the sensitivity of the gonadotropin receptors in the ovaries.

P-029 rFSH combined with uhMG had significantly increased pregnancy compared with rFSH alone undergoing ovarian stimulation following a long protocol in IVF

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Objective: The purpose of this study was to compare the pregnancy rates resulting from ovarian stimulation with rFSH combined with uhMG or rFSH alone in women undergoing IVF cycles. Material & methods: The study included 144 cycles (134 patients) between January 2007 and December 2007 in IVF cycles, and controlled ovarian hyperstimulation were performed with GnRH agonist long protocol. The patients were classified in two groups: Group A- rFSH alone for controlled ovarian hyperstimulation following a GnRH agonist long protocol (69 cycles, n=63). Group B- rFSH combined with urinary hMG for controlled ovarian hyperstimulation following a GnRH agonist long protocol (75 cycles, n=71). We compared patients age, the number of retrieved oocytes, transferred embryos, the percentage of metaphase II oocytes and clinical pregnancy between the two groups. Results: The age of the patients, the percentage of metaphase II oocytes, the number of transferred embryos and abortion rates were comparable in two group. The number of retrieved oocytes (17.6 ± 8.7, 12.3 ± 6.3; P<0.001) and embryos (13.0 ± 7.1, 8.5 ± 4.9; P<0.001) were significantly higher for group A than for group B. However, pregnancy outcomes including clinical pregnancy rates (55.1%, 64.0% P<0.01), ongoing pregnancy rates (44.9%, 52.0% P< 0.01) and implantation rates (19.6%, 20.7% P<0.01) were significantly higher for group B than for group A. Conclusions: Our data indicates that the use of rFSH combined with urinary hMG was significantly higher clinical pregnancy rate than rFSH alone undergoing ovarian stimulation following an agonist long protocol. Comparing our analysis of the two groups, the use of rFSH combined with urinary hMG had significantly increased pregnancy, despite a lower number of retrieved oocytes and fertilized oocytes. The hypothesis was that embryo quality and endometrial receptivity were significantly improved after using rFSH combined with urinary hMG, compared with rFSH alone, probably due to the presence of LH. Therefore, LH activity may influence treatment response and outcome in IVF cycles. Further studies were that data from a larger population are needed to precisely estimate any difference between uhMG and rFSH.

P-030 A comparison of pregnancy outcome in women with normal oocyte reserve according to the use of high or low dose follicle stimulating hormone (FSH) stimulation

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Introduction: The main advantage of high dosage FSH controlled ovarian hyperstimulation (COH) is a greater embryo yield allowing better odds of having top quality embryos and more cryopreserved embryos for future transfers. The main advantage of low FSH stimulation is reduced expense and less risk of ovarian hyperstimulation syndrome (OHSS). The objective was to determine if more embryos from which to choose provides higher pregnancy rates (PRs) per transfer or could the need to cryopreserve for OHSS risk reduce the chance of pregnancy on first transfer (fresh or frozen)? Finally, would the chance of having more embryos with high stim result in a greater chance of having an extra frozen embryo transfer resulting in a higher PR per oocyte harvest&quot.Materials and methods: A retrospective review was done on 1st oocyte retrievals over a 10 year period on women with normal ovarian reserve. The data were stratified according to 2 age groups (35 and 36-39) and according to whether high dose FSH COH (187.5 IU) or low dose FSH (>187.5 IU) was used. Comparisons were made according to the first fresh ET, the PR per 1st transfer including a frozen ET and per oocyte harvest. Results: Fresh transfers only clinical and live delivered PR, abortion rate, and implantation rate for women 35: high dose FSH 50.2% (210/418), 41.9%, 16.7% (35/210) and 32.1% (322/1004); low FSH 51.0% (99/194), 44.8%, 12.1% (12/99) and 34.6% (149/431). First transfer (fresh or frozen) 49.2% (245/498), 41.2%, 16.3% (40/245) and 31.0% vs. 49.6% (118/238), 43.7%, 11.9% (14/118) and 33.0% (178/539). PR per harvest 64.4%, 53.0%, and 17.7% vs. 57.0%, 49.4%, and 13.2%. For ages 36-39 the comparative values for fresh ET were for high FSH stimulation 35.4% (92/260), 26.9%, 23.9% (22/99) and 19.7% (137/694) vs. 28.7% (27/94), 25.5%, 11.1% (3/27) and 20.1%. First transfers 33.9% (99/292), 26.4%, 27.3% (27/99), and 18.6% (146/787) vs. 32.7% (34/104), 28.8%, 11.8% (4/34) and 22.2% (51/230). PR per harvest 41.8% (135/323 retrievals), 32.5%, and 22.2% (30/135) vs. 30.5% (40/131 retrievals), 26.7% and 12.5%). Conclusions: The main advantage of high stimulation over low stimulation from a PR standpoint is that women aged 36-39 have a 25% higher pregnancy rate per harvest. Otherwise, the outcome between the two methods were similar. The couple must weigh reduced risk and cost with low stim vs. one additional benefit for high stim, i.e., possibly more embryos for future transfers.

P-031 The effects of laser assisted hatching on pregnancy rates

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Background: For infertile women aged over 35 years, failure of the ZP (zona pellucida) to rupture is believed to be associated with a decreased implantation rate in In Vitro Fertilization (IVF) or Intra Cytoplasmic Sperm Injection (ICSI). Objective: In this research Laser assisted hatching was offered to patients with advanced maternal age to evaluate a possible benefit. Materials and methods: Nine hundred thirty two cycles of IVF/ICSI in females were analyzed. Women included in this study were allocated in 4 groups. In group I and II, embryos were cultured and transferred with and without LAH in women aged under 35, where as embryos of group III and IV were examined with and without LAH in women aged over 35. Laser manipulations were performed using a suturn Tm3 system using 2-3 pulses of 0.8 millisecond with 400 voltage duration. The size of the hole made in the zona was measured to be 5-10 µm, depending on the zona thickness of each individual embryo.Results: The performance of LAH significantly increased clinical pregnancy rates in all patients. The chemical pregnancy (50.99% and 31.61% respectively), clinical (50% and 30.69% respectively) and multiple pregnancies (22.27%and 5.94% respectively) significantly differ between group I and II. In the patients with advanced female age over 35 the performance of LAH significantly increased chemical (30.12%) and clinical pregnancy (27.71%) rates compared to whom that without LAH (18.96%) and (16.37%) respectively. Conclusion: Our data demonstrate in the patients who were less than 35 years old, multiple pregnancy rates were significantly increased compared other groups who aged over 35 years old. In addition benefit of LAH in improving pregnancy ratesafter IVF or ICSI in women of advanced age (over 35) was shown.

P-032 Correlation between semen intracellular parameters and fertilization rates following ICSI

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Introduction: Nowadays, using functional tests alongside with the conventional semen analysis could be effective in foreseeing the treatment results. This study was set out to investigate how mitochondrial membrane potential, DNA fragmentation index and apoptosis in ejaculated spermatozoa correlate with each other and also laboratory outcomes after intracytoplasmic sperm injection (ICSI). Materials and methods: We examined ejaculated spermatozoa from 120 patients undergoing ICSI treatment. Sperm DNA fragmentation Index was assessed by sperm chromatin dispersion test (SCA). Mitochondrial membrane potential (MMP) and incidence of apoptosis were also evaluated by flow cytometry. Result: There were significant positive correlation between apoptosis, MMP and DNA fragmentation index but these parameters did not show any significant correlation with laboratory ICSI outcomes. Conclusion: Despite the existence of a relationship between intracellular factors, in this study no relationship between these parameters with fertilization rate was observed. This result could be caused by choosing high quality sperms according the classical factors by an embryologist in ICSI process and more research might be helpful.

P-033 Whole ooplasmic transfer by direct injection method using Piezo driven system for prevention of mutated mitochondrial DNA transmission

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Mitochondrial diseases are caused by pathogenic mutation in mitochondrial DNA (mtDNA) and treatment option for patients having mitochondrial disease are severe. Mitochondrial DNA is transmitted maternally and in Assisted Reproductive Technology (ART), it has been proposed that ooplasmic transfer which is the technique to exchange mutated mtDNA to normal mtDNA by replacing oocyte cytoplasm using nuclear transfer techniques is the potential treatment to prevent mtDNA transmission. Ooplasmic transfer has been applied by using existing method, i.e. cell membrane fusion method in animals and human. However, these reconstructed oocytes are caused mtDNA heteroplasmy as donor karyoplast (donor nuclei with cytoplasm) including donor mtDNA is fused with recipient cytoplasm which has mtDNA from recipient result in mixture of two different mtDNA in the oocyte. Therefore, it is necessary to invent new techniques to avoid mtDNA heteroplasmy. Here, we developed innovative technique of ooplasmic transfer in germinal vesicle (GV) oocytes which we inject GV (donor nuclei only, with no cytoplasm) directly into oocyte using piezo drive system termed as Germinal Vesicle Injection (GVI). Bovine GV oocytes were used as their oocyte size is similar to human GV oocytes. Donor GV was taken from oocytes after disrupting oocyte membrane, removed cytoplasm attached around GV membrane and then directly injected to recipient enucleated GV oocytes using Piezo drive system (Primetech Co. Ltd., Japan). Inner diameter (ID) of injection pipette should be 30 μm adjusted outer diameter of bovine GV which has been thought that 30 μm ID of injection pipette is invasive for the oocytes. But we improved the survival rate of oocytes after injection up to 95% (58/61) by using 1 μm of thickness of pipette with minimum intensity of piezo pulses. In vitro maturation and in vitro developmental rate to the blastocyst stage after fertilization of GVI oocytes were 81% (47/58) and 22% (8/47), respectively and there were no significant differences between non treated GV oocytes (81%, 70/86 and 25%, 11/44). These results indicate that our new GVI technique is effective and non invasive method for bovine oocytes. We hypothesize that our GVI technique will be able to avoid mtDNA heteroplasmy if all mitochondria attached tightly around donor GV are completely removed or inactivated. More detailed experiment should be performed before clinical trial. But we anticipate that our GVI tecnique is the potential treatment to prevent mutated mtDNA transmission.

P-034 Elevation of the Risk of Monozygotic Twinning by Expanded Blastocyst Transfer

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[Objectives] During *in vitro* fertilization and culture, monozygotic twinning (MZT) cannot be completely avoided even when fertilization is performed by single embryo transfer, and the rate of MZT after *in vitro* fertilization is higher than that after natural fertilization. MZT is considered to occur through the splitting of the inner cell mass during hatching via the narrow opening of the zona pellucida. We recently encountered 6 cases where MZT resulted from single blastocyst transfer, which was performed after manipulation to ensure complete hatching via the zona pellucida. We report these cases with discussions about the methods of fertilization and the background for embryonic development. [Subjects] Among all cycles of embryo transfer performed in our clinic between January 2006 and April 2010, 494 cycles involved single blastocyst transfer (95.5% of the cases), and pregnancy resulted from 196 cycles (pregnancy rate, 39.7%). The subjects who underwent these 196 cycles were the subjects of this study. During embryo transfer in each case, complete hatching via the zona pellucida was ensured by a physical method. [Results] MZT occurred in 3.1% (6/196) of pregnancies. The MZT rate was 4.8% (4/84) with fresh embryo transfer and 1.8% (2/112) with vitrified-warmed embryo transfer. The MZT rate was analyzed in relation to the fertilization method: the rate was 3.9% (4/103) with conventional *in vitro* fertilization (C-IVF)-derived blastocyst transfer and 2.2% (2/93) with intracytoplasmic sperm injection (ICSI)-derived blastocyst transfer. The rate of MZT after fresh embryo transfer was 6.7% (3/45; C-IVF-derived embryo) vs. 2.6% (1/39; ICSI-derived embryo). The MZT rate after vitrified-warmed embryo transfer was 1.7% (1/58; C-IVF-derived embryo) vs. 1.9% (1/54; ICSI-derived embryo). Further, the MZT rate was analyzed in relation to the embryo grade: the rate was 3.3% (6/184) after transfer of high-grade embryo (at least 3BB) and 0% (0/12) after transfer of low-grade embryo. Of the high-grade embryos whose transfer resulted in MZT, the growth stage at the time of transfer was blastocyst in 0% embryos, expanded blastocyst in 6.9% (4/58) embryos, and hatching/hatched blastocyst in 2.2% (2/92) embryos.

P-035 Evaluation of sperm chromatin integrity following IMSI or HBA for pre-ICSI sperm selection

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Objective: For almost two decades ICSI has been successfully used to obtain high fertilization and pregnancy rates in couples with severe male infertility. In recent years however new sperm selection methods such as intracytoplasmic morphologically selected sperm injection (IMSI) and hyalorunan-binding-assay (HBA) has been developed to identify the most competent gametes before ICSI procedure. In this prospective study we evaluated the integrity of sperm chromatin following selection with the above methods. Materials and methods: After obtaining informed consent ejaculated sperm samples were collected from infertile patients requiring ICSI treatment. Samples were initially prepared using discontinuous gradient centrifugation re-suspended and divided into four groups. IMSI group (A): normal morphology sperm containing no vacuoles in the nuclear area was selected by observation under x1000 magnification inverted microscope. HBA group (B): in Petri dish a 2µl droplet of hyalorunan (HA) containing medium (Sperm Slow, MediCult, Denmark) was connected to another droplet containing the suspended sperm. After incubation the HA-bounded sperm cells were retrieved from the junction zone of the two droplets. ICSI control group (C): sperm with normal morphology and excellent motility was selected by observation under x 200 magnification conventional ICSI equipment. Unselected control group (D): sperm was randomly selected from the initially prepared suspensions. Sperm chromatin integrity as the main outcome measure was evaluated by sperm chromatin dispersion test using a commercially available kit (Halosperm, Halotech, Spain). Pair wise comparisons were made between the experimental and control groups using chi-square test to check for statistical significance. Results: The sperm chromatin fragmentation rates were 4.5% (2/48), 2.6% (4/151), 10.9% (7/71) and 19.9% (48/289) for groups A, B, C and D, respectively. Differences were statistically significant in favor of HBA group compared to group C ($p=0.03$) and D ($p<0.0001$). Compared to the two control groups the IMSI group showed a non significant trend towards lower sperm chromatin fragmentation values. Conclusions: Our preliminary data suggests that both HBA and IMSI are effective methods for non-invasive selection of sperm cells with high sperm chromatin integrity. Further studies are needed to evaluate the impact of these new sperm selection techniques on assisted reproduction treatment outcome.

P-036 Differential effects of urinary and recombinant chorionic gonadotropin on oxidative stress responses in decidualizing human endometrial stromal cell.

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(Introduction) Human chorionic gonadotropin (hCG) is one of the earliest signals secreted by the implanting embryo. In addition to its well-known luteotropic function in early pregnancy, hCG also acts directly on decidualizing endometrium. Recently, we demonstrated that recombinant hCG (rhCG) prevented apoptosis in decidualizing human endometrial stromal cells (HESCs) exposed to oxidative stress. Two hCG preparations are widely used clinically: rhCG, produced by recombinant DNA technology, and urinay hCG (uhCG), extracted from urine of post-menopausal women. However, an analysis of the direct effects of rhCG and uhCG on the decidual phenotype of HESCs has not yet been done. In this study, we investigated the effects of uhCG and rhCG on the morphological and functional profiles of decidualizing HESCs. (Material & Methods) HESCs from hysterectomy specimens were isolated and incubated with 8-bromo-cyclic adenosine monophosphate and medroxyprogesterone acetate in the presence or absence of recombinant hCG (rhCG) or urinary hCG (uhCG). Hydrogen peroxide was used as the source of reactive oxygen species (ROS). The amounts of PRL in culture media were examined. Western blot was performed for protein analysis. The level of apoptosis was analyzed by cell detection ELISA Kit. (Results) Neither rhCG nor uhCG alter the morphological appearance of the decidual HESC cultures, although rhCG attenuated prolactin expression, a major decidual marker protein. rhCG represented protective effect on decidualizing HESCs to oxidative cell death through enhanced expression of SOD2, a cardinal enzyme in the cellular defense against oxidative damage. rhCG signaling also selectively limits activation of the apoptotic machinery in decidualizing HESCs by enhanced Bcl-2 expression whereas uhCG induces the expression of Fas ligand. (Conclusion) Our results suggest that rhCG might be a preferable agent to protect the maternal decidua against oxidative damage in pregnancy, especially at the time of implantation and beyond.

P-037 Heparin prevents programmed cell death induced by oxidative stress in human decidualized endometrial stromal cells

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(Introduction) Heparin is clinically administered for the prevention of thromboembolic disorders during pregnancy, especially for antiphospholipid antibody syndrome. A recent clinical trial demonstrated a beneficial effect of heparin administration in the luteal phase on implantation rate as well as the live birth rate in women with repeated implantation failure. In addition, heparin exerted direct effects on human endometrial stromal cells (HESCs), independently of its anticoagulant activity. However, the precise effects of heparin on decidualization process ramains uncertain. Recently, we demonstrated that human endometrial stromal cells (HESCs) become extraordinarily resistant to oxidative stress-indued apoptosis upon decidualization. Therefore, we hypothesized the possible direct action of heparin on HESCs decidualization, that might induce the expression of genes participating in resistance to oxidative stress, and that would result in improved implantation. (Methods and Materials) Primary HESCs cultures were established, propagated, and confluent cultures remained were decidualized with 8-br-cAMP (0.5 mM) and MPA (10-6M), and with or without various concentration heparin or 4 days. Hydrogen peroxide (H2O2) treatment was used as a source of ROS. The amounts of PRL in culture media were examined. Western blot was performed for protein analysis. The level of apoptosis was analyzed by cell detection ELISA Kit. (Results) Treatment with 8-Br-cAMP plus MPA pronounced stimulated PRL secretion which is known as a decidualizing marker. Addition of heparin at various concentrations to decidualized HESCs caused a dose-dependent incrase of PRL production. Decidualized HESCs treated with heparin dose-dependently decreased the cell death rate induced by oxidative stress. Although heparin treatment on its own did not elevate the expression of FOXO1 on non-dedidualized HESCs, heparin dose-dependently augmented MPA/cAmp-induced FOXO1 and Mn-SOD expression. Furthermore, heparin elicits phosphorylation PI3K-signal transduction pathway in primary decidualized HESCs. (Conclusion) These results demonstrate that heparin-treated decidualized HESCs acquired resistance to oxidative stress by induced expression of FOXO1 and Mn-SOD, suggesting that heparin may improve the uterine environment for successful implantation.

P-038 The role of 5'AMP-activated protein kinase (AMPK) in human endometrial stromal cells

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Objective: 5'AMP-activated protein kinase (AMPK) is a fuel sensing enzyme that responds to decreases in cellular energy state as reflected by an increase in the AMP/ATP ratio. Our objective was to clarify the role of AMPK in cultured human endometrial stromal cells (ESC). Materials and Methods: Human ESC were cultured, and the effect of IL-1, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) on the production of interleukin (IL)-8 and monocyte chemoattractant protein (MCP)-1 was examined. The supernatants were collected, and IL-8 and MCP-1 were measured by ELISA. The activation of AMPK was detected by Western blot analysis using anti-phosphorylated AMPK antibody. This study was approved by the institutional review board. Results: IL-1 increased the production of IL-8 and MCP-1 by ESC in a dose-dependent manner. On the other hand, IL-1-stimulated IL-8 and MCP-1 production was significantly decreased following treatment with AICAR [IL-8: IL-1β (1 nM); 73.0±15.9 ng/mL, IL-1β and AICAR (1 mM); 46.7±6.0 ng/mL ($p<0.01$); MCP-1: IL-1β (1 nM); 8,136.3±1,394.6 pg/mL, IL-1β and AICAR (1 mM); 4,685.1±432.8 pg/mL ($p<0.01$)]. The phosphorylations of AMPK were determined by treatment with AICAR. Conclusions: The inhibitory effect on the production of IL-1-stimulated IL-8 and MCP-1 via a mechanism involving AMPK were observed. The results suggest that AMPK may play an important role of anti-inflammatory effect resulting to reduction of IL-8 and MCP-1 production. It is suggested that AMPK may contribute to the modulation of endometrial local factor in implantation processes.

P-039 Pinopode Formation upon Progesterone, hCG, and Clomiphene Citrate treatment

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Background: Induction of ovulation is one of the primary steps in assisted reproduction while considered as one of the main reasons for endometrial inadequacy in luteal phase. Hormonal treatment with progesterone or human chorionic gonadotropin (hCG) appeared to improve pregnancy rates following hormone therapy for ovarian hyperstimulation and became a common practice. However, little is known about endometrium status upon treatment with these hormones. Thus, this study was designed to investigate the effects of some routinely used hormones, on the pinopode formation. Materials and Methods: A total of seventy five fertile and infertile females ranging in age between 21 and 36 years participated in our studies. Study participants underwent ovarian hyperstimulation with a long protocol method of a GnRH agonist or clomiphene citrate (CC) or received no hormonal treatment considered as control group. Participants in GnRH agonist- or CC-stimulated groups were randomized into designed experimental groups receiving progesterone, estradiol valerate or hCG which are routinely used to improve endometrial status. Results: Statistical analysis indicated that the area-related numerical density of pinopodes in biopsies taken from patients who received progesterone treatment as a luteal phase support following GnRH agonist-stimulated cycles were significantly higher than those who received hCG treatment and those who received no treatment as well as those in natural control group. Our findings also indicated that pinopode density in patients who received CC plus estradiol valerate was significantly higher than those who received CC plus progesterone or CC alone. Pinopode density in patients who received CC plus progesterone or CC were significantly decreased compared to that of the natural control group. Conclusions: Our data demonstrated that progesterone following GnRH agonist-stimulated cycles increased pinopode density while has no significant effect when combined with CC. Instead, estradiol valerate increased pinopode density when induction of ovulation was with CC. Moreover, it was shown that pinopode formation was reduced in stimulated cycles with CC while data from induction of ovulation using GnRH agonist protocol did not show this effect.

P-040 IS THERE ANY CORRELATION OF UTERUS POSITION AND DEEPTH EMBRYO TRANSFER ON PREGNANCY RATE IN IVF

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Introduction: To evaluate is there any correlation of uterus position and deepth embryo transfer on pregnancy rate in IVF. Material and methods: Cohort observational analytic study with retrospective data obtained from medical record of patients participated in IVF programme at Harapan Kita Maternity Hospital Jakarta from January 2008 to December 2008. Results: There were 50 patient, pregnancy 19 (38%) and no pregnancy 31 (62%). Position of uterus: anteflexcy 29 patient (pregnancy 34.5%) and retroflexcy 21 patient (pregnancy 42.9%). Mean of deepth embryo transfer on pregnancy success was 7.1 cm (SD 0.59), and unsuccess pregnancy is 6.98 cm (SD 0.63). Mean of the serum level estradiol was 1,450.98 ng/dL for pregnancy success, and 796.27 ng/dL for unsuccess pregnancy. There were no significant correlation between uterus position and deepth embryo transfer with pregnancy rate (p=0.547); (p=0.506). There was significant correlation between serum level estradiol and pregnancy rate (p=0.04). Conclusion: there were no significant correlation between uterus position and deepth embryo transfer with pregnancy rate. Furthermore, there was significant correlation between serum estradiol levels with pregnancy rate.

P-041 Influence of antisperm antibodies (ASA) on the outcome of infertility treatment

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Introduction: There has been suggested that antisperm antibodies (ASA) can impair the fertilizing capacity of human spermatozoa, acting negatively on sperm motility and cervical mucus penetration, and at the level of in vitro gamete interaction. The literature demonstrates that the various previously used treatments for immunological infertility, i.e. medical therapy, intrauterine insemination with husband's spermatozoa (AIH) and in vitro fertilization and embryo transfer (IVF-ET), usually had poor success. The purpose of this study was to investigate the influence of ASA on the outcome of infertility treatment.Materials and Methods: We treated 28 couples whose female partners were ASA positive and 13 couples whose male partners were ASA positive during 2007-2011. Clinical results of ASA positive couples were compared.Results: First, we compared the results of IVF-ET (not including ICSI) between ASA positive females (5 cycles) and tubal factor (118cycles). Both female groups were restricted under 35 years old in order to remove the influence of oocyte aging. Clinical pregnancy rate (gestational sac+) per embryo transfer of ASA positive females was 100%, and that of tubal factor was 37.0 % (n.s.). Fertilization rate of ASA positive female was 61.3%, and that of tubal factor was 74.7 % (n.s.). Abortion rate of ASA positive female was 0%, and that of tubal factor was 25.9 % (n.s.). Next, we compared the results of IVE-ET (including ICSI) between ASA positive females (27 cycles) and ASA positive males (8cycles) in all ages. Clinical pregnancy rate (gestational sac+) per embryo transfer of ASA positive females was 33.3%, and that of ASA positive males was 33.3 % (n.s.). Fertilization rate of ASA positive females was 71.2%, and that of ASA positive males was 68.8 % (n.s.). Finally, we compared the results of AIH between ASA positive females (39 cycles) and ASA positive males(19cycles) and ASA negative couples (14602 cycles) in all ages. Clinical pregnancy rate (gestational sac+) of ASA positive females was 2.6%, and that of ASA positive males was 5.3%, whereas that of ASA negative couples was 7.3%, which was not significantly different. Conclusions: Our results suggested that conventional IVF-ET was effective to ASA positive females, and pregnancy rate of IVF-ET was not different between ASA positive females and ASA positive males. AIH was also effective to both ASA positive females and ASA positive males.

P-042 The effect of ovarian stimulation on expression level of E-cadherin, Leukemia inhibitory factor, Progesterone receptor and αvβ3 Integrin genes in mouse blastocysts

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Introduction: The appropriate interaction between blastocyst and endometrium is essential for a successful implantation. There are many factors involved in cross talk between embryo and endometrium including hormone receptors, cytokines, adherence molecules and et ct. there are studies which show lack of these genes can be resulted in implantation failures. In this study the expression level of some of these genes in superovulated mouse blastocysts was compared with their expression in blastocysts from natural cycle. Materials and Methods: About 20 Female NMRI mice were injected with 7.5IU pregnant mare serum gonadotropin and the uteruses were flushed 3.5 days after given human Chorionic Gonadotropin and about 120 blastocysts were obtained from. Also 60 female non stimulated mice mated with males and natural cycle blastocysts were flushed out from the uteruses 3.5 days after mating. Expression level of E-cadherin, Leukemia inhibitory factor, Progesterone receptor and αvβ3 Integrin genes was examined in 120 blastocysts obtained from hormone treated mice and in 120 natural cycle blastocysts by Quantitative real time PCR method. Student t-test was used for data analyzing. Results: Results revealed that the expression level of all studied genes were significantly lower in the hormone treated group than the natural cycle blastocysts ($p<0.05$). However the highest decreasing rate was seen in β3 gene and the lowest change was seen in αv gene. Conclusion: Although ovarian stimulation in ART cycles is utilized to obtain more oocytes, it may has some disadvantages by down regulation of some genes involved in implantation process in blastocysts.

P-043 Usefulness of elective single blastocyst transfer in the first treatment cycle for infertile women aged less than 35.

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Purpose: The modern trend of transferring less number of embryos is based on the idea that the satisfactory pregnancy rate (PR) could be maintained and the multiple pregnancies should be avoided. For such purposes, elective single blastocyst transfer (eSBT) might be a useful treatment. However, it is still unclear that eSBT is effective for both of younger women with relatively higher potential of conception and elder women with relatively lower potential of conception. The aim of the present study is to know the indication of eSBT in the first treatment cycle for infertile women. Method: Between Aug. 2006 and Dec. 2010, 124 infertile women were treated by the first single embryo transfer following IVF or ICSI using fresh ejaculated sperm in our institute. Results: The overall clinical PR was 33.0% (41/124). There were no multiple pregnancies. The PRs were 42.2% (27/64) in women aged less than 35 and 23.3% (14/60) in women aged 35 and more. There was a significant difference between them (P=0.02). As for eSBT, the overall PR was 31.0% (17/55). The PRs were 46.2% (12/26) in the former and 17.2% (5/29) in the latter. There was also a significant difference between them (P=0.02). The PR (33.3%) by day2/3 embryo transfer was better than that (16.7%) by day 4/5 embryo transfer in the elder women (P=0.13). Conclusions: These findings suggest that eSBT seems to be useful in the first treatment cycle for infertile women aged less than 35. However, the PR by eSBT in the first treatment cycle for women aged more than 35 was disappointing. For such women aged 35 and more, day 2/3 single embryo transfer might be useful at least in the first treatment cycle. Alternatively, transferring of two embryos could be allowed for such women. Further study is required why some embryos derived from elder women are not suitable for longer culture in vitro.

P-044 A Summary of The clinical outcome of minimal stimulation IVF/ ICSI

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In order to reach the goal of reducing the treatment cost, avoiding the risk of OHSS and multiple pregnancy, gaining full-term single live birth. Natural cycle, minimal stimulation IVF and vertrification frozen, single blastocyst transfer have been applied in Reproductive Medical Centre of the First Peoples Hospital of Yunnan Province since 2009. This summary prospectively studied 28 natrual IVF cycles, 1065 minimal stimulation cycles, 435 fresh embryo transfer cycles and 182 frozen embryo transfer cycles from March 2009 to September 2010, blastocyst formation rate was 46%, accumulative pregnancy rate was analyzed. The average age of patients in natural cycle was 30.8&plusmn 3.3 years. In 28 single blastocyst transfer cycles, the pregnancy test positive rate was 52.6% (15/28), the clinical pregnancy rate was 42.9% (12/28). The average age of patients in minimal stimulation cycle was 33.5 ± 3.8 years. Fresh embryo transfer was performed in 435 cycles. The pregnancy test was positive in 184 cycles (42.3%, 184/435), the clinical pregnancy was achieved in 178 cycles (40.9%, 178/435). In frozen embryo transfer cycles, pregnancy test positive rate and clinical pregnancy rate was 48.4% (88/182) and 44.5% (81/182) respectively.

P-045 Ovarian Response and Reserve Evaluation in consecutive IVF/ICSI-ET cycles

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Objective: To evaluate if IVF/ICSI-ET effects ovarian response and reserve in consecutive cycles and to put forward it's clinical outcome. Methods: It has been retrospectively investigated 329 patients who had more than 3 IVF/ICSI-ET cycles between 2000-2008 among 5000 patients applied to our centre. Patients age, basal hormone level, leading follicule count on day 6-7, gonadothropine dose applied, oocyte and embryo numbers gained and pregnancy outcome have been noted. Patients divided in to 4 age groups as less than 30 (129) group 1,between 30-34 (78) group 2, between 35-40 (129) group 3 and more than 40 (6) group 4. Results: Although basal hormone level and number of oocytes and embryos gained isn't different, it has been found a significant rise in gonadothropine dose to get same result in each group. However leading follicule number decreased in consecutive cycles for group 1 and 2, there wasn't a significant difference for group 3 and 4. After third cycle pregnancy and live birth rates were increased. Conclusion: Although the rise in required gonadothropine dose to get the same result in consecutive cycles is pointing a decrease in ovarian response; it doesn't show an ovarian reserve diminish. But leading follicle number decline of group 1 and 2 in repeating cycles is indicating ovarian reserve downward for ART patients especially younger than 35 years. It's thought-provoking that why ovarian reserve seems to be reduced in younger patients and not in older ones for repeating cycles. This clinical outcome could be due to naturally decreased ovarian capacity by age.

P-046 Our 11-year experience with sperm cryopreservation for patients with malignant disease

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Introduction; In recent years, damage to reproductive function has been a frequent and well-documented side effect associated with the treatment of malignant disease. Because the gonadotrophic effect of a drug depends on its type, dose and the number of treatment cycles, it is impossible to predict which patients will have normal spermatogenesis and which will remain azoospermic after treatment. Sperm banking before starting treatment for a malignant disease is currently considered the most effective method for preserving fertility. The aim of this study was to describe the state of cryopreservation for each year of the study period, the profiles of patients with malignant disease who wished to cryopreserve sperm, the conditions of the stored cryosperm, and the usage conditions of the stored sperm in our hospital. We also analyzed various issues related to our 11 years of experience with banking sperm. Methods & patients; The number of sperm cryopreservations each year between 1999 and 2009 was determined. Then, the characteristics of forty-one patients with malignancy who desired to bank sperm were recorded; these characteristics included the patients'age, marital status, type of malignant disease, sperm condition at the time of cryopreservation and usage condition of the cryopreserved sperm. Results; The sperm of 88 % (36/41) of patients was successfully collected and cryopreserved, yielding a total of 45 semen samples. The number of new cryopreservations per year is currently stable. Most of the patients were young, single and childless. Half of the patients were diagnosed with testicular tumors. Nearly half of the cryopreserved sperm had counts >40 million/ml and a motility >50%. With respect to the usage condition of the cryopreserved sperm samples, 36 % (16/45) of the samples are still being stored, 38 % (17/45) correspond to the samples of patients missing in the follow-up and 27 % (12/45) had been used for ART or had been disposed of. The number of accumulated samples has gradually increased. Conclusion; Important factors for long-term sperm banking are the follow-up with patients after cryopreservation and the continuity of sperm banking facilities. Thus, a close connection between the sperm banking facility and the hospital at which the patients are being treated and followed patients with malignant disease is important. Furthermore, it is preferable that sperm banking facilities should be public large-scale clinics rather than private clinics.

P-047 A Successful pregnancy and live birth after intracytoplasmic sperm injection with cryopreserved limited numbers of spermatozoa stored in the practical container

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Introduction: Conventional freezing procedures are not suitable for a few spermatozoa. Some authors have attempted to use various types of containers such as zona pellucida for the cryopreservation of small numbers of spermatozoa. Unfortunately, these containers are the only currently available options and they cannot be used universally. In our previous study (Endo et al, J Mamm Ova Res 2011), we have established a novel simple freezing technique for a single spermatozoon using Cryotop (Kitazato Biopharma, Japan) and Cell Sleeper (Nipro, Japan), which are available commercially and used universally. The aim of this study was to evaluate the efficiency of performing intracytoplasmic sperm injection (ICSI) using sperm stored in these containers. Materials and methods: Spermatozoa were frozen in two different containers, Cryotop or Cell Sleeper. Cryotop consists of a fine polypropylene strip attached to a plastic handle and is equipped with a cover straw, and Cell Sleeper is a vial type of cell-freezing container, which equipped with an inner tray and is sealed with a screw cap. Individual sperm were transferred to the containers using the ICSI pipettes equipped with a micromanipulator. Immediately they were frozen in accordance with our method and stored in the cryotanks. In the day of oocyte retrieval, the frozen sperm were thawed and retrieved. After following ICSI, a part of the oocytes were activated by 10 IU ml/ml of calcium ionophore A23187 (Sigma, USA) for 15 min. Fertilized embryos were cultured until day 5, and single embryo transfers were performed in all cycles. Results: A total of 192 spermatozoa obtained from six patients were frozen in 25 containers (7.7 cells per container). Two couples were underwent ICSI program and stored sperm (19 cells) were thawed. Sixteen sperm were successfully retrieved and 3 of them were lost. Sperm were injected individually into matured 12 oocytes and unused 4 retrieved-sperm cells were re-cryopreserved. Fertilization was observed in 8 (67%), and all zygotes were cleaved. After embryo transfer, one woman was resulted in singleton pregnancy and concluded with full term delivery of healthy boy (2632g). Conclusions: Our clinical data shows a rare case of a successful delivery after transfer of a blastocyst derived from ICSI using the limited numbers of sperm stored in the practical containers. Having a method of reliable sperm storage for severe male factor patients may reduce multiple testicular surgical operations.

P-048 An evaluation of a modified slow cool cryopreservation technique and the efficacy of a graduated estrogen regimen vs. natural cycles on pregnancy rates following frozen embryo transfer

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Introduction: Many in vitro fertilization-embryo transfer (IVF-ET) centers do not seem to have high pregnancy rates following the transfer of embryos that have been cryopreserved using traditional slow cool methods. The present study evaluated the efficacy of a simplified slow cool method that avoids what we believe is the weak point of the usual method which in the planar freezer and instead uses an alcohol bath with slow cool controlled rate freezer (Biocool) with a one-step removal of the cryoprotectant. Furthermore the technique would compare the efficacy of natural vs. graduated estradiol (E2) regimen on subsequent pregnancy rates (PRs).Materials and methods: The women were placed on the oral E2 regimen. In general natural cycles were recommended if there was a contraindication for E2, e.g., history of breast mass or cancer, thrombosis, migraine headaches or if the E2 regimen was started but was stopped because of side effects. Embryos were either all cryopreserved at the 2 pronuclear phase in cases of risk of ovarian hyperstimulation syndrome or for inadequate endometrial thickness. Otherwise the normal policy was to allow twice the number of embryos to cleave to day 3 as intended to transfer and transfer the best half and freeze the rest. The embryos not allowed to cleave to day 3 were frozen at the 2 pronuclear stage. Results: Only 125 women were assigned to natural cycles vs. 1812 to the graduated E2 regimen. The clinical PR per transfer was 33.6% with natural cycles vs. 42.5% with the E2 regimen (p=0.06, chi-square analysis). The live delivered PRs were 27.2% vs. 34.8% (p=0.10, chi-square analysis). The respective implantation rates were 18.7% vs. 21.6% (p=0.25, chi-square analysis).Conclusion: The simplified slow cool cryopreservation protocol using a Biocool alcohol controlled rate freezer in lieu of the planar freezer with a one-step removal of the cryoprotectant resulted in a very adequate pregnancy rate with frozen-thawed ET. The policy is to use up all embryos and thus these data included transfers that were sometimes predominantly using embryos that were de-selected or even twice frozen twice thawed. There was a trend for higher clinical pregnancy rates using the graduated E2 protocol. One must weigh the much increased use of the E2 hormone replacement not merely during the frozen ET cycle but during the first trimester against the possible slightly lower pregnancy rates using a natural cycle.

P-049 The effect of vitrification on Histone Modification of regulatory regions of H19, Igf2 and Mest imprinted genes in mouse embryo

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Introduction: The effect of embryo vitrification and warming on epigenetic status of preimplantation embryo has to be evaluated. Histone modification, as an epigenetic modification system, can control gene expression. Imprinted genes H19, Igf2 and Mest are involved in regulating proper embryo development and imprinting disorders of these genes can be associated with some syndromes. This study was investigated gene expression level and modification of three histone markers in transcriptional regulatory regions of these genes in vitrified-warmed mouse embryos. Materials and Methods: Fresh blastocysts from NMRI mice were considered as control group. Also Vitrified-warmed and non vitrified 8-cell embryos cultured into blastocyst stage were mentioned as experimental groups (I) and (II) respectively. Expression level of H19, Igf2 and Mest and also some histone modifications in regulatory regions of these genes were studied in the three groups. To gene expression evaluation, Real time PCR was carried out on about 105 embryos in each group. On the other hand, acetylation of histone3 at lysine9 and methylation of histone3 at lysine4 as gene activators and methylation of histone3 at lysine9 as a gene suppressor were evaluated by ChIP assay method on 120 blastocysts in each group. REST test and ANOVA were used to data analysis. Results: According to our study the expression level of all genes in group II was decreased significantly in comparison with control group ($P<0.01$). In group III only H19 and Igf2 had significant decreasing in comparison with fresh blastocysts ($P<0.01$). Significant increasing in di-methylated histone3 at lysine9 was seen in the regulatory regions of all genes in vitrified group ($P<0.01$). Also tri-methylated histone3 at lysine4 and acetylated histone3 at lysine9 were decrease in some regulatory regions but not all of them ($P<0.01$) in comparison with control group. Additionally, di-methylated histone3 at lysine9 in regulatory regions of Igf2 and H19 but not Mest was significantly increased in group III. Conclusion: These results indicate that vitrification and culture condition can down regulate and modify histone pattern of regulatory sequences of H19 and Igf2 in blastocysts. Total histone pattern in each group was coordinated with gene expression changes. Among different modifications, di-methylation of histone3 at lysine9 has the most coordination with gene expression changes in different groups.

P-050 Withdrawn

P-051 Which is more effective for pregnancy freezing embryos: in day-2, day-3 stage or blastocyst?

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Introduction: In IVFand ICSI, the pregnancy result of frozen embryo transfer has improved compared with the fresh embryo transfer in recent years. Moreover, it can be expected that the risk of ovarian hyperstimulation can be reduced when all embryos are frozen. When all embryos freeze, the optimal developmental stage for cryopreseving embryos remains controversial. Materials and Methods: All embryos freezing in our clinic are either frozen does at day-2,3 and then remainder is frozen by blastocyst. Thawed embryo transfer the first time replaces early day-2,3 embryo or in some cases by blastocyst. When the first trial is not successful, the left embryo is transferred by blastocyst. The appropriate transfer protocols at the natural period or the hormone replacement is selected. It was determined whether day-2,3 stage or blastcyst was more useful by this protocol. Results: Thawed embryo was transferred in 41 cases and in 79 cycles and the pregnancy was a success in 24 cases and in 25 cycles (58.5% per cases/ 31.6 % per cycle) in total. Ten out of 35 cases that transferred the first time day-2,3 early embryo reached pregnancy (28.6%). One out of six cases that transferred the blastocyst the first time reached pregnancy (16.7%). The second transfer attempt reached pregnancy in one out of 25 non-pregnancy cases in the early stage embryo. 24 cases were transferred the second time by blastocyst (11 cases early embryo to grew blastocyst among these) and 11 cases (five cases in the growth blastocyst) reached pregnancy (45.8%). Two cases reached pregnancy in five non-pregnancy cases in the second transfer by the early embryo in blastocyst at the initial transfer (40%). The E2/P ratio in the case that succeeded in a pregnancy by the second time compared with the first time pregnant cases, there was no significant difference. Six cases ended in miscarriage within 25 cycles of the pregnancy. (24.0%) Conclusions: Early stage day-2,3 and blastocyst are acceptable when all embryos freezing are executed at the frozen time of the embryo. In the second transfer, it proved better that thawed early stage embryo were used in the transfer after growing to a blastocyst.

P-052 Highest liquid nitrogen quality for vitrification process : Micro bacteriological filtration of LN2

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Objective: To evaluate any risks of contamination of microorganisms in liquid nitrogen using Ceralin Filter Equipment. Design: Analisys on-site (Octapharma plant) and in-situ (laboratory) of microorganism in LN2 for validation of the filter Ceralin equipment. Materials and Methods: Ceralin Filter Equipment is ceramic membrane made of multiple layers, formed into a multi-channel element.pore size of the ceramic membrane is 0.1 µm ; thus the Ceralin can be considered as a sterilizing filter in accordance to FDA Guidelines on Aseptic Processing 1987: *"A sterilizing filter produces a sterile effluent when challenged with the microorganisms Pseudomonas diminuta at a given concentration (107 UFC/cm^2 of filtering surface)"* The on-site validation procedure consists of carrying out sampling and analysis campaigns upstream/downstream from the filter. This analisys of samples was made using impaction on agar-agar : bioimpactor MistralTM. with a flow 100 to 300L/min and a volume of sample of 200L. Results: A significant reduction of microorganisms has been achieved by the Ceralin filter on-site. Guidelines for filter validation (integrity tests) are not described nor performed in cryogenic conditions. Thus, correlations between integrity test, bacterial challenge and particle challenge in standard conditions and in liquid nitrogen have been investigated. These studies lead to the strict specifications for Ceralin (only membranes whose bubble point value is higher 2.2 bar). Validation of the filter efficiency in-situ has been done and can be done by customer when wanted. Conclusions: Ceralin equipment offers a solution for a safer vitrification process. It guarantees no contamination from liquid nitrogen and go onwards potential regulation imperatives related to this process.

P-053 Comparing developmental potential and ultra structure of human blastocysts after re-vitrification

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Introduction: Vitrification has shown to be a suitable and effective method of cryopreservation, but little is known about re-vitrification of human embryos. The purpose of this study was to compare the developmental potential and ultra structure of human blastocysts after re-vitrification and determining the best stage of human for re-vitrification. Materials and Methods: 95 cleavage embryos were obtained from the patients who didn't want to have embryo freezing because of different reasons. After getting informed constant, the embryos were divided into two groups. In the first group, 42 embryos (4-8 cell stage embryos) were vitrified and warmed using the Cryotop technique. After evaluation the survival rate, the alive embryos were vitrified and warmed again with the same method. After thawing, the survival rate was assessed and the alive embryos were cultured for 3-4 days and in the end of cultivation, the blastocyst rate was determined. In the second group, 53 embryos (4-8 cell stage embryos) were vitrified and warmed with the above mentioned method. After warming and evaluation their survival rate, the alive embryos were cultured for 3-4 days and then the blastocyst rate was evaluated. The obtained blastocysts were re-vitrified and warmed and the survival rate was assessed. Ultra structure of the obtained blastocysts in both group after re-vitrification were studied, too. Results: There was not significant difference between survival rates of two groups after vitrification-warming (92% vs. 89% respectively for the first and second groups). 20 out of 47 embryos (43%) of the second group reached to blastocyst stage after thawing. The survival rate in the first group after re-vitrificatin was 89.7%, meanwhile it was 30% in the second group and there was significant difference ($P<0.05$). The blastocyst rate after re-vitrificatin in the first group was 25.7%. Ultra structural study revealed disorganization of cytoskeleton of the blastocysts after re-vitrification in the both groups. The regularity of mitochondria was also disappeared and some vacuoles were seen in the cytoplasm of re-vitrified blastocysts. Conclusion: The results showed that re-vitrification of cleavage embryos has less harmful effects comparing to re-vitrification of blastocysts. The reason for delay of blastocyst formation could be ultrastructural changes following re-vitrification.

P-054 Examination of blastocyst transfer after pronucleate embryos were slowly freeze-thawed

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Objective: We examined the usefulness of blastocyst transfer after pronucleate embryos (2PN) were slowly freeze-thawed. Subjects: One hundred and four cycles in 98 patients who received slowly freeze-thawed 2PN were involved in this study from January through December 2010. Freezing was applied to the following cases: patients with multiple conception failures, Clomifene citrate in poor-responders, for the prevention of OHSS, when ET falls on a holiday, there are surplus embryos available, etc. Methods: When freezing, a blastocyst was exposed to 20% serum PBI for five minutes, to 1.5 M PROH for 10 minutes, and to 1.5 M PROH+0.1 M sucrose for 10 minutes, then placed in an alcohol bath at 10degree celsius in a programmable freezer. When thawing, straws were left to stand at room temperature for 40 seconds after being taken out of liquid nitrogen, then dipped in water at 23degree celsius for 10 seconds, and transferred into a thawing solution. The thawing solution comprised 1.0 M PROH+0.1 M sucrose decreasing by 0.25 M / 5 minutes and the specimen was cultured for five days after being dipped in 0.1 M sucrose and PBI for 5 minutes, respectively. The embryos transplanted it in a HRC. Results: The 2PN survival rate was 96.7% (540/558), the cleavaged rate was 99.0% (535/540), the rate of blastocyst formation for D5 was 31.2% (169/540), and blastocyst transfer became possible for 74% (77/104) of frozen embryos in the thawing cycle. The pregnancy rate after blastocyst transfer was 55% (39/77), the pregnancy rate in the thawing cycle was 39% (41/104), and the abortion rate in the thawing cycle was 26% (11/41). The pregnancy rates after blastocyst transfer and in the thawing cycle classified by age were 83% (5/6) and 83% (5/6) in the 20s, 60% (15/25) and 46% (15/32) between 30 and 34 years old, 56% (14/56) and 48% (16/33) between 35 and 39 years old, 50% (5/10) and 29% (5/17) between 40 and 42 years old, and 9%(1/11) and 6%(1/16) for over 43 years or older, respectively. The pregnancy rates after blastocyst transfer and in the thawing cycle classified by the applied conditions were 66% (4/6) and 36% (4/11) for multiple conception failures, 16% (2/12) and 15% (3/19) for Clomifene citrate, 66% (20/30) and 58% (20/34) for the prevention of OHSS, 62% (5/8) and 55% (5/9) for ET days falling on a holiday, 62% (5/8) and 60% (6/10) for freezing of surplus embryos, and 30% (4/13) and 22%(4/21) for others.Conclusion: In this examination, it is thought to be effective in a case 40 years or younger.

P-055 5/-(N-Ethylcarboxamido) adenosine (NECA) improves angiogenesis in transplanted human ovarian tissue: potential for fertility preservation

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Introduction: In the past 3 decades by enormous progress in cancer therapy the possibility of successful recovery from this disease has enhanced. However, many of cancer patients become infertile after treatment and this can be an important concern for people at reproductive age. There are limited ways to restore fertility of women post-treatment such as cryopreservation of embryo, oocyte and ovarian cortex. One of the promising methods to restore fertility ability of these patients is ovarian tissue banking (OTB). OTB is based on the isolation of fragments of cortex of ovary before cancer therapy, freezing and transplanting them after cancer treatment.There are many drawbacks such as apoptosis and considerable reduction of follicular density in the transplanted ovary. Many studies have shown that these problems mainly result from ischemic damages and not from freezing/thawing process of the tissue. One solution to reduce ischemic damage is enhancing angiogenesis after transplantation of ovarian cortex tissue. Based on the angiogenesis capacity of adenosine, the aim of this study was to evaluate the angiogenesis potency of NECA, and adenosine agonist, in triggering angiogenesis of transplanted human ovarian tissue. Material and Methods: Human ovarian tissue fragments were transplanted to nude mice. Four hours post transplantation (PT), mice were treated with NECA, while control group received only vehicle. The transplanted fragments from each group were recovered on days 2, 7 and 30 and studied for expression of Angiopoietin1, Angiopoietin2, VEGF-121 and VEGF-189 at both gene and protein levels (by real time PCR and western blot respectively) and vascular density. RESULT(S): On the 2nd day PT, the level of Ang1 gene expression was significantly lower than that in controls, while the opposite result was obtained for VEGF-189. These results were also confirmed at the protein level. The differences were, however, not statistically significant for Ang2 and VEGF-121. The density of vessels in NECA group elevated significantly at day 7 PT compared to pre-treatment state.CONCLUSION(S): Since the process of angiogenesis is more critical in the first 48 hours PT, our results suggest a beneficial role for NECA in this process.

P-056 Assessing risks of birth defects in ART babies

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The incidence of congenital malformations in infants born after reproductive technology has been much discussed in the literature with controversial conclusions. Slightly more than 4% of babies born via assisted reproductive technology (ART) may have major birth defects. One of the explanation may be due to the higher rate of multiples pregnancies which may stands for a higher rate of abnormalities. In this paper we try to overview the literature figures and, also, we present a case of twin pregnancy, obtained by IVF, in which one of the fetuses was affected by duodenal atresia. The pathology is due to failure of recanalization of this intestinal segment. Its frequency varies between 1/2500 and1/10000 live birth and it has high risk of an associated chromosomal anomaly. The diagnosis was based upon follow up ultrasound scans (recognition of the classic double bubble sign, associated with polyhydramnios) and the prognosis was good after the surgical cure, despite the premature birth. As a fact, we conclude that councelling about possible risks associated with fertility treatment (including multiple pregnancy and fetal malformations) is an important part of the fertility treatment process and, therefore, obstetricians and pediatricians, as well as certified infertility counselors, need to become sources of an accurate information.

P-057 Two cases of ovarian abscess after oocyte retrieval

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Ultrasound-guided transvaginal oocyte retrieval has been known as he standard ovum pick up (OPU) method at in vitro fertilization and embryo-transfer (IVF-ET). Pelvic abscess formation is one of the complications induced by OPU. We report 2 cases of ovarian abscess which occurred after OPU. Case 1 was a 29 years old nurse. We punctured and aspirated fluid from an endometrioma followed by OPU. Eleventh day after OPU, she developed fever and bilateral ovarian swelling with an abscess-like mass observed by transvaginal ultrasonography. She was hospitalized and underwent intravenous antibiotic treatment. Because of the continuing high inflammatory reaction, we punctured and drainaged the ovarian abscess. MRSA was detected in the content fluid. By changing antibiotic which targeted MRSA, her inflammatory reaction improved immediately. Case 2 was a 43 years old housewife. She developed fever and abdominal pain at second day after OPU. Her symptoms and the laboratory findings improved immediate after the start of antibiotic treatment. MRSA should be considered as an etiologic agent in cases of pelvic inflammation after OPU.

P-058 An experience of an infertility treatment for a woman with benign metastasizing leiomyoma

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Benign metastasizing leiomyoma (BML) is a rare disease in which solitary leiomyoma-like nodules are present in a distant location. As BML is found primarily in women of reproductive age, the origin of BML is presumed to be the uterus. Approximately 150 case reports of BML have been published. We report an experience of an infertility treatment in a woman with BML. A 40-year-old nulliparous woman was referred to our University hospital for surgical operation of the abdominal wall tumor and the uterine myoma. Past history includes an abdominal myomectomy for the uterine myomas, which were diagnosed as cellular leiomyoma in the pathological examination, 4 years prior to referral. On the first visit, the patient has a growing abdominal tumor of 6 cm diameter and a uterine tumor of 6 cm diameter. After through workup, she underwent an abdominal tumor resection and an abdominal myomectomy in our hospital. The pathological examination was reported as cellular leiomyoma of the uterus, metastasizing to the abdominal wall. As she may have the risk of tumor spreading or metastasizing, furthermore having the right tubal occlusion, we decided to perform in vitro fertilization and embryo transfer for an infertility therapy. Under the long luteal protocol, 19 oocytes were retrieved, followed by moderate ovarian hyperstimulation syndrome. The patient achieved a single pregnancy by a thawed embryo transfer in natural cycle, resulting in delivering a healthy infant by cesarean section, in which any developing tumors were not observed. Before starting an infertility therapy in a woman with BML, making a diagnosis of BML precisely is essential. Meticulous sampling of the pathology specimen should be undertaken to exclude leiomyosarcoma, which unlike BML, has an aggressive course.

P-059 A Transgenic Mouse Model of Breast Cancer Using tissue specific MMTV Promoter

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Background: Piwil2, a member of AGO/PIWI family of proteins, has been reported to be expressed in precancerous stem cells (pCSCs), tumor cell lines and various types of human cancers. In human breast cancer, previous findings have been determined Piwil2 and its effector signaling pathways as key factors in the proliferation and survival of breast cancer stem cells. To study the roles of Piwil2 in breast cancer, a transgenic mouse model has been developed containing mouse mammary tumor virus (MMTV)-piwil2cDNA construct. Methods: Suitable vector containing pMMTV-piwil2CDNA was constructed and linearzed by two desirable restriction enzymes. Then, the MMTV-piwil2cDNA construct was injected into the pronuclei of 0.5-day NMRI embryos. Results: Five transgenic founder male mice were produced as determined by southern blot and PCR analyses. Four founder animals were able to transmit the transgene to their offspring. Conclusion: Tumorigenesis effects of Piwil2 on the breast tissue in the F1 generation are being investigated.

P-060 Effect of genistein administration on intratesticular testosterone level and spermatogenesis in rats treated with busulfan

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Objective: To investigate the effect of genistein (isoflavon) administration on spermatogenesis recovery of the rats treated with busulfan (chemotherapy drug).Materials and Methods: 185 male rats were assigned to Control, Busulfan, Busulfan/GnRH agonist (-a), Busulfan/Genistein 50 mg, and Busulfan/Genistein 100 mg treatment groups. A single intraperitoneal injection of busulfan (25 mg/kg) was done to induce a chemical damage to testis. Three weeks after the busulfan injection, an additional injection of GnRH-a (0.625 mg) or oral administration of genistein (50 mg or 100 mg) for 4 weeks was done. Thirteen weeks after the busulfan injection, serum and intratesticular testosterone (ITT) levels, weights of testes, and histological characteristics of the testes were investigated. Results: Testosterone level in serum (2.70 ng/ml) was significantly reduced after busulfan injection (1.65 ng). Additional treatments of GnRH-a (1.11 ng) and genistein (50 mg: 1.52 ng, 100 mg: 1.71 ng) did not reverse the reduced testosterone level by busulfan. However, ITT level (Control, 67.7 ng / g-testis) was significantly increased after busulfan injection (365.1 ng), and the increased ITT level was reversed by additional GnRH-a (159.6 ng) and genistein 50mg (170.0 ng), 100mg (155.0 ng) treatments. Weight of testis (Control, 3.49 g) was significantly decreased by busulfan injection (1.29 g), but the reduced weight of testis was partially recovered by GnRH-a (1.77 g) or genistein (50 mg: 1.85 g, 100 mg: 1.70 g) treatment. Histological examination revealed that busulfan induced a severe damage to spermatogenesis, but the damaged spermatogenesis was partially recovered by both additional GnRH-a and genistein treatments. Conclusions: Genistein enhanced the recovery of the chemically impaired spermatogenesis through the suppression of ITT level. Therefore, we suggest that genistein is a safe substitute for hormones to recover the impaired spermatogenesis of human cancer patients treated with chemotherapeutic drug, with less risk of side effects.

P-061 The role of Doppler in predicting endometrial thickness, pattern and blood flow in infertile women undergoing IVF cycle in a tertiary care institute

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Introduction: Endometrial receptivity is crucial to implantation of an embryo. 2D power Doppler was used to evaluate the association between endometrial thickness, its pattern and blood flow and pregnancy outcome in women undergoing in-vitro fertilization. Material & Methods: A total of 140 infertile women attending the infertility clinic at All India Institute of Medical Sciences, New Delhi between January 2009 to December 2010 was included in this prospective study. The study was approved by the Institutes Ethics Committee. The cause of infertility in these patients was tubal factor, male factor, and unexplained infertility. All patients were stimulated using the standard long protocol. The endometrial thickness, pattern and blood flow was recorded on the day of HCG administration. Results: The mean age was 34.5 years and mean duration of infertility was 7.6 years. 69% of patients had primary infertility and 31% had secondary infertility. The mean endometrial thickness was 8.4mm with endometrial blood flow in zone 1 in 38 patients, zone 2 in 44 and zone 3 in 58. 28.4% women with an endometrial thickness between 6.4 to 12mm achieved a successful pregnancy. Also a higher pregnancy rate was observed when the blood flow was in zone 3(49.2%) compared to zone 1 (16.7%). Conclusions: The endometrial thickness and vascularity have a predictive value in implantation of an embryo in women undergoing assisted reproductive techniques. However, the study sample was small to make a definite statement.

P-062 Reduced blastcyst formation rate in women in advanced reproductive age with high Anti-Mullerian hormone level

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BACKGROUND: Anti-Mullerian hormone (AMH) is widely thought to be a useful marker of ovarian reserve. It is also known that serum AMH level declines with aging. However, there are some exceptional cases with still higher serum AMH levels in advanced reproductive age. We focused on this group and studied the results of blastcyst culture for IVF or ICSI. METHOD: We studied 35 cycles of IVF-ET or ICSI undergone at Suzuki memorial hospital from September 2009 to February 2011. Prior to ovarian stimulation, serum AMH concentrations were measured. These cycles are divided in three groups. Group A: high AMH level (>20 pmol/ml) - advanced age (>=36) group (n=6), Group B: low to normal AMH level (<20 pmol/ml) - advanced age (>=36) group (n=14), Group C: younger age (<36) Group (n=15). Number of oocyte yield, fertilization rate, blastcyst forming rate were compared. RESULT: Mean oocyte yield in group A was 10.5, which is significantly higher than that in group B (4.6) and C (5.9). No significant differences in fertilization rates were observed among three groups; Group A (56%), Group B (51%), Group C (54%). Blastcyst forming rates were as follows; Group A (14%), Group B (39%), GroupC (54%). Significant reduced blastcyst forming rate in group A comparing with group C were noted (p=0.026). CONCLUSION: Although relative large number of oocyte yield may be expected, women in advanced age with higher serum AMH levels were characterized by significant reduced blast cyst forming rate.

P-063 Effects of salpingectomy on ovarian response in controlled ovarian hyperstimulation for in vitro fertilization: a reappraisal

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Objective: To evaluate the effects of salpingectomy on ovarian response in controlled ovarian hyperstimulation (COH). Setting: University-based tertiary medical center. Study design: Retrospective study Patients: 36 women who underwent controlled ovarian stimulation cycles for in-vitro fertilization (IVF) before and after salpingectomy Interventions: Laparoscopic salpingectomyMain outcome measures: The number of dominant follicles and oocytes aspirated before and after salpingectomy. We also evaluated maximal estradiol levels, duration of stimulation and average daily dose of gonadotrophins. Results: The overall number of dominant follicles and the number of oocytes aspirated before and after salpingectomy were comparable (7.2 ±3.8 vs. 7.3 ±3.7 and 10.2 ± 6.6 vs. 10.3 ±7.4 respectively). Maximal E2 levels and the daily doses of gonadotrophins before (1899 ± 185 pg/ml and 217.8 ±10.0 IU) and after surgery (1997 ±231 pg/ml and 239 ±16.3IU) were also similar. There was no significant different in the number of dominant follicles before and after surgery on the operated side (3.8 ± 2.2 vs. 3.7 ±2.0). Regression analysis to assess the effect of unilateral versus bilateral salpingectomy showed no effect on the main outcome measured. Conclusions: Unilateral or bilateral salpingectomy does not influence ovarian response in controlled ovarian hyperstimulation.

P-064 Semen analysis and evaluation of ICSI outcome in patients infected with hepatitis B virus

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Introduction: Hepatitis B infection represents the most important virus to manage in assisted reproduction techniques (ART) Tunisian candidates, screening of this sexually transmitted virus is recommended. The success of ART in couple infected with hepatitis B virus (HBV) was not clearly established. Several inflammatory and biological disturbances exist in hepatitis B and interference with the biological steps of ART was possible. The aim of our study was to verify a possible correlation between infection with hepatitis B and sperm abnormalities and the biological assessment of IVF. Materiel and Methods: This is a case-control study of 56 couples infected with hepatitis B virus (cases) followed by consultations of the Unit of Reproductive Medicine of Sousse and candidates for in vitro fertilization (IVF). HBV markers were tested for both partners in case of at least one positive for hepatitis B virus surface antigen (HbsAg). Their recruitment was done between February 2004 and February 2010. These markers included hepatitis B surface antibody (anti-HBs) and Hbe antigen and antibody (HbeAg and anti-Hbe). Serologies were assessed by commercially available assays.Results: The HbsAg rate was significantly higher in men 61% than in women 39%, p= 0.04. No serology HBeAg type was positive. Among the 59 couples infected with hepatitis B s only 3.4% of couples were jointly infected. The most frequent sperm abnormalities in patients with HBsAg was the asthenozoospermia, 62.5% vs 20% in the control group, P=0.001. The oligospermia was significantly higher in the control group, p=0.005. Tubal obstruction was the most common abnormality among infected women 95% vs 50% in the control group, P=0.008. The sperm bacteriology was significantly higher positive in cases with a prevalence of 28.8% against 9.2% in control group, p= 0.01. The pregnancy rate among cases and controls was equal respectively 12.1% and 16.7% with an insignificant difference. Conclusion: The asthenozoospermia was the most common abnormality in infected man with hepatitis B virus. But in infected women, the tubal obstruction was the most frequent anomaly and was associated with Chlamydia trachomatis.

P-065 Effect of Chemotherapy on Spermatogenesis in Testicular Cancer Patients

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[Purpose] We evaluated fertility status after treatment for Testicular cancer (TC) by conducting a large, retrospective study. [Materials and methods] A retrospective study was conducted of 103 patients who was diagnosed unilateral gonadal germ cell tumor between 1983 and 2010 in our facility, and free of relapse. Semen analysis and serum hormonal level (lutenizing hormone: LH, follicle-stimulating hormone: FSH, testosterone: TST) were obtained for each patient, as well as the fertility follow-up. Seventy-six patents were treated with chemotherapy after orchidectomy. [Results] Thirty-seven patients were performed routine semen analysis before chemotherapy. The mean sperm motility was below (40.1±28.3%), while the mean sperm concentration was above the normal (27.0±33.0*10^6/mL). In non-azoospermic patients (n=32), fifteen were asthenozoospermic. After chemotherapy, seminal parameters were recovered to the normal value within 30±31 months in twenty-two patients. Patients treated with 5 and more cycles of chemotherapy were hard to recover the normal sperm condition. With regard to the hormone level, FSH was kept high in the patients who didn't recover the semen data, while TST has been kept within the normal range. Of 21 patients who had attempted to achieve pregnancy with their partners, nine patients had succeeded and 7 patients had children (60±39 months after treatment). In the patients who succeeded to have children, sperm concentration and motility just before pregnancy were 53±36*10^6/ml and 61±26%, respectively. [Conclusions] About half of the TC patients showed poor sperm quality before orchidectomy. Our data demonstrated that the patients who recovered spermatogenesis after chemotherapy showed improved sperm concentration above 20*10^6/ml within 2 years and recovered fertility. Patients treated with high dose chemotherapy or 5 and more cycles of PVB (cisplatin, vinblastine, and bleomycin) or BEP (bleomycin, etoposide, and cisplatin) showed poor recovery of spermatogenesis, but their TST values were kept within normal level.

P-066 Reproductive outcomes following laparoscopic myomectomy in patients with infertility

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Objectives: To evaluate the outcomes of gasless laparoscopic myomectomy in infertile patients. Methods: We conducted a retrospective clinical analysis of 170 patients who underwent laparoscopic myomectomy for infertility. Gasless laparoscopic myomectomy was performed by lifting two K-wires inserted into the lower abdomen subcutaneously. The patients were followed up for more than one year. We evaluated clinical findings, operative findings and obstetrical outcomes. Results: One hundred ten of the 170 patients (64.7%) conceived after myomectomy. Sixty-six (60%) received no treatment for infertility after the operation. Sixty patients (36.3%) remained infertile, despite most (68%, 41/60) receiving ART (assisted reproductive technology). Average age and body weight were significantly lower in the pregnant than in the nonpregnant group (33.7 vs. 36.7 years; 53.9 vs 56.5 kg.). The mean duration of infertility before laparoscopic myomectomy was shorter in the pregnant than in the nonpregnant group (3.4 vs 5.9 years). The size, location, number and weight of myomas did not differ between the two groups. The drop on hemoglobin concentration, operative time and concomitant surgeries did not influence fertility outcomes. The rate of umexplained infertility factors was higher in the pregnant than in the nonpregnant group (58/110, 19/60; p=0.008). Conclusions: Gasless laparoscopic myomectomy is useful, effective and safe for infertile patients. This operative procedure is an important therapy for infertile patients with myomas regardless of their location, number or size.

P-067 The role of CINC/gro in the rat ovulatory process

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CINC/gro is a CXC-family chemokine similar to interleukin-8 in rats. CINC/gro is one of the factors that regulate the ovulation process. However, the mechanism that regulates atresia of post-ovulation in ovaries is not clearly defined. Therefore, we aimed to determine whether antibody-blocking of CINC/gro might change the number of ovulated oocytes and modulate neutrophil infiltration. We investigated the effect of antibody on the level of inflammatory cytokines production, and on atresia of the follicles. Apoptosis was measured the TUNEL method and by analysis of the mRNA expressions of Bcl-2 and Bax. Anti-CINC/gro antibody treatment decreased the number of ovulated oocytes. The mRNA levels of COX-2 and IL-1 beta were decreased by antibody treatment, whereas that of TNF alpha was increased. TUNEL analysis indicated a large number of apoptotic cells in the antibody group compared to control as well as a significant increase in the Bax/Bcl-2 ratio 24 h after hCG administration. These findings suggest that ovulation is accelerated by neutrophil infiltration into the theca layer. CINC/gro appears to synergize with IL-1 beta for ovulation. In contrast the data suggest that expression of CINC/gro suppresses TNF alpha expression and that expression of CINC/gro therefore prevents follicles from undergoing atresia and apoptosis.

P-068 A Commercially Available Dual-Buffered IVF Handling Medium Containing HEPES and MOPS Maintains Stable pH and Supports Human Sperm Survival, Normal Fertilization Following ICSI and Embryo Development

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Introduction: Maintenance of stable pH is important to reduce stress in the culture environment and optimize embryo development. Buffers, like HEPES and MOPS, are used to stabilized pH of media used outside the incubator. Current commercially available media utilize a single buffer. However, use of a single buffer limits the ability to adjust optimal buffering capacity. For example, the pKa, or optimal buffering of MOPS at 20^{o}C is 7.20, while HEPES has a pKa of 7.55. At 37^{o}C HEPES has a pKa of 7.31, while MOPS is 6.95. Furthermore, potential toxicity concerns exist with elevated concentrations of buffers used in mono-buffered media. Therefore, media utilizing a single buffer may not be ideal. Use of a dual buffered media containing MOPS and HEPES may permit benefits of both buffers and provide a more appropriate buffering over a range of temperatures, while permitting use of lower buffer concentration.
Materials and Methods: A dual-buffered medium containing HEPES and MOPS (pH Secure^{TM},Irvine Scientific), was tested against control HEPES buffered media. pH of media was measured and compared. Standard QC assays including a 24h HSSA and a 96h 1-cell MEA were used to assess media efficacy. Finally, human oocytes were split between each media and held for ICSI and resulting fertilization compared. Data were analyzed using Fishers exact test.
Results: The pH of pH Secure^{TM} and control HEPES media were 7.27 and 7.33, and change in pH following 1 month were not significantly different (0.08 vs. 0.07), respectively. pH Secure^{TM} maintained sperm motility at rates equivalent to control media after 24h, resulting in a 14.8% and a 20.2% decrease in motility, respectively (n=5). Following 2h exposure to pH Secure^{TM} or control media, no differences in blastocyst formation (80% vs. 76.5%) or hatching (45% vs. 28) were observed following 96h of culture (n=2). Finally, no differences in rates of normal fertilization following ICSI were apparent in either pH Secure^{TM} (78.6%) or control HEPES media (74.2%) (n=47 patients, 594 oocytes).
Conclusion: Use of a dual-buffered medium containing HEPES and MOPS maintains a stable pH environment, supports human sperm survival, normal fertilization following ICSI and mouse embryo development at rates comparable to traditional HEPES buffered medium. Combining two buffers to regulate media pH offers the potential advantages of reducing individual buffer concentration, as well as the ability to optimize buffering capacity.

P-069 Development of Mouse and Human Embryos in a Low Humidity Incubator

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Objective: Recently, a dry culture system has been designed - without a water tray - which could diminish the risk of any contamination. At ASRM2010 we have already reported that water evaporation and osmolarity changes could be efficiently controlled in a dry culture system. The aim of the present study was to compare the efficacy of dry and humid culture systems by evaluating blastocyst formation rates of mouse and human embryos. Materials and Methods: Two separate incubators were used to compare embryo development. For the dry and humid incubator system an EZ-Culture System and a conventional AP30 incubator were used respectively (ASTEC, Japan). 2,225 2-cell mouse embryos were cultured with either the dry or humid systems. Moreover after obtaining informed consent 32 4-8 cell human embryos were also randomly assigned to culture with one of the above systems. All media droplets were covered with mineral oil and the media were exchanged every 48 hours. Blastocyst development rates were evaluated after 3 days. Results: For mouse embryos 93.1% and 91.9% developed to blastocyst stage with the dry and the humidfied incubator system, respectively. Similarly the blastocyst formation rates of human embryos used in the experiment were not significantly different 62.5% and 56.3% with the dry and humid incubator, respectively. Conclusions: Our results suggest that the development of both mouse and human embryos in a dry culture system were effectively identical to those of embryos cultured under standard humidified culture conditions. Although results are still preliminary regarding human application in our opinion, it might not be necessary to use a humidified atmosphere for culturing mouse or human embryos. The dry culture system used in the present study is efficient, hygienic and requires only little maintenance. Hence this new dry incubation system could reduce the risk of a possible contamination and contribute to lowering maintenance costs of an in-vitro fertilization program.

P-070 Contribution of oocyte and embryo cleavage quality in obtaining blastocysts

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Introduction: Oocyte growth and quality are dependent on the normal growth and differentiation of the oocyte harboring follicule. Oocyte quality is assessed by the absolute number of mature oocytes and number of embryos used. The evaluation of Oocyte quality correlated with the rate of fertilization and embryo quality after ICSI. The aim of this study was to assessing oocyte and cleavage embryonic quality according to the quality of blastocysts. Material and Methods: This retrospective study included forty nine infertile couples, who undergo ICSI with transfer of blastocyst at the unit of Reproductive Medicine of the University hospital Farhat Hached, Sousse, Tunisia. Embryo transfer performed during 2009-2010. The average age of women and men were equal respectively 32 ± 3.5 and 37.69 ± 4.92 years with duration of infertility equal 6.6 ± 3.68. Sperm and oocytes preparation techniques for intracytoplasmic sperm injection and evaluation of embryo quality were interpreted according to WHO criteria. Results: The mean number of aspirated oocytes was 9.3 ± 3.69. The majority of oocytes were good qualities (65.3). Mean fertilization rate was 76.23 ± 18.1. The average percentage of abnormal sperm forms was proportional to the average rate of blastocyst tops ($p = 0.03$). The average number of oocytes obtained was proportional to the average number of blastocysts. The average number of cleavage embryos obtained (5.59 ± 2.38) was inversely correlated with the average number of blastocysts (1.51 ± 1.06). The oocyte maturation and fertilization rate were inversely proportional to the rate of blastocysts. The mean pregnancy rate was 44.9 %. Conclusions: A high percentage of good qualities oocytes associated with high rates of fertilization were favorable for obtaining blastocysts and good pregnancy rate. The average percentage of abnormal sperm forms was proportional to the average rate of blastocyst tops ($p = 0.03$). But oocyte maturation and fertilization rate were inversely correlated to the blastocysts rate.

P-071 Involvement of first cytokinesis on gene expression at blastocyst stage

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Although we showed that cytokinesis at first cell cycle affect post-transfer viability, the mechanism has been unclear. The aim of present study is to investigate effect of duration and cleavage pattern at first cytokinesis on gene expression at blastocyst stage that related to implantation and pregnancy reorganization. Bovine *in vitro* fertilized (IVF) embryos were cultured in well of the well culture dishes contained 125 µl CR1aa medium supplemented with 5% calf serum for 168 h post insemination (hpi). Development of the embryos was monitored using time-lapse cinematography and embryos developed to blastocyst stage were individually examined gene expression (*AKR1B1*, *CDX2*, *IFNτ*, *IGF2R* and *PLAC8*) using real-time PCR. As result in multiple regression analysis, duration at first cell cycle had a correlation with *IFNτ* ($P = 0.002$) and *IGF2R* expressions ($P = 0.005$), and *IGF2R* expression in embryos cleaved after 27.25 hpi was lower than *in vivo* derived embryos ($P < 0.05$). *IFNτ* expression was also affected a number of blastomere ($P = 0.014$), and the expression level in embryos undergoing direct cleavage from one-cell stage to three-four blastomeres was lower than those characterized by two blastomeres and *in vivo* derived embryos ($P < 0.05$). Furthermore, *AKR1B1* expression in embryos with fragmentation was higher than embryos without fragmentation and derived *in vivo* ($P < 0.05$). Higher pregnancy rate observed for animals in which transferred blastocysts had undergone first cytokinesis within 27 hpi and indentified by the presence of two blastomeres without fragmentation, than among those with abnormal cytokinesis. These results indicate that duration and cleavage pattern at first cytokinesis may involve to gene expression at blastocysts stage, which are related to implantation and pregnancy reorganization, resulting in impact of post-transfer viability. This work was supported by the Research and Developmental Program for New Bio-Industry Initiatives.

P-072 Zona-free oocyte formed a one pronucleus zygote following piezo intracytoplasmic sperm injection can subsequently develop to the blastocyst in micro-well culture system. (Case Report)

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Introduction: Before performing intracytoplasmic sperm injection (ICSI), cumulus-oocyte complexes are treated by hyaluronidase to remove their cumulus cells. Occasionally, the ooplasms can escape from their zona pellucida during this procedure. The mature and morphologically normal zona-free oocytes can be fertilized with ICSI and then cultured to the blastocyst stage (J. Ding et al. Human Reproduction, 1999). ,
Material & Methods: A 33-year-old primary unexplained sterility woman underwent an ICSI cycle in our clinic. The clomiphene citrate/HMG method was used in her ovary stimulation. Briefly, from day 3 of her menstruation, 100 mg clomiphene (Shionogi, Japan) was given every day for 7 days. From the day 7 of her menstruation, 150 IU HMG (Fujipharma, Japan) was injected per 2 days for twice. On the day 9, 0.25 mg antagonist (MSD, Japan) and 10000 IU hCG (ASKA, Japan) were injected. Finally, one oocyte-cumulus complex was retrieved on day 11. After 4 hours of culture in HTF medium (Irvine, CA) with 10% Serum Substitute Supplement (SSS) (Irvine Scientific, CA) in 6% CO2 in air, the cumulus cells were removed by gently peppitting in 40 IU hyaluronidase in order to performing ICSI. Unfortunately, the matured ooplasm was loosing its zona pellucida. The zona-free oocyte was injected with one spermatozoon from her husband by using piezo-ICSI and cultured in HTF medium with 10% SSS in 6% CO2 6% O2 and 90% N2. After 16 hours of culture, the fertilized oocyte developed a one pronucleus (1PN) zygote. The 1PN zygote was cultured in 50µl SICM medium with 10% SSS (COOK, Australia) in a micro-well drilled by melting the 35 mm dish bottom with a heated steel rod. On the third day of culture, SICM medium was carefully removed and 50µl SIBM medium (COOK, Australia) supplemented with 10% SSS was added to continuously culture. On the fifth day, no blastocyst was formed but a good expanding blastocyst was formed on the eighth day. ,
Conclusions: The results suggest that zona-free oocyte can be fertilized by piezo ICSI, and the fertilized zygote can develop to a good blastocyst using micro-well culture system. Combine with the previous result that 1PN-derived blastocyst could result in pregnancy and birth in human assisted reproductive technology (Lyn Gras et al., Human Reproduction, 1999), the blastocyst derived from zona-free 1PN zygote may be used for clinical treatment.

P-073 A CUMULATIVE EMBRYO SCORING SYSTEM USED TO PREDICT PREGNANCY OUTCOME

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Objective: To investigate the efficiency of our Cumulative Embryo Score (CES) system for selecting the optimal embryos to transfer Design: A prospective study Materials and Methods: This study was performed on 436 IVF-ET cycles from January 2010 to February 2011 in which one(64 cycles), two(206 cycles) or three embryos(166 cycles) were transferred. The quality of embryos was assessed using our CES system. Early-cleavage embryos received two points, but non early-cleavage embryos did not receive any point. In addition, we also gave the embryos a point from 5 to 1 according to the grades of embryos (G1 to G5) on day 2 and 3, respectively. Therefore, each individual embryo could receive the point from 2 to 12. The CES of individual embryo was used to evaluate the developmental potential of the embryos. The IVF-ET cycles were categorized into nine groups according to the CES and the number of embryos transferred: CES group A1(score 9-12, 1ET), A2(score 19-24, 2ET), and A3(score 26-36, 3ET), CES group B1(score 5-8, 1ET), B2(score 13-18, 2ET), and B3(score 15-25, 3ET), CES group C1(score 1-4, 1 ET), C2(score 1-12, 2ET), and C3(score 1-14, 3ET), respectively. Results: When one embryo was transferred, the pregnancy rates of A1, B1 and C1 groups were 50.0%, 19.3% and 9.5%. When two embryos were transferred, the rates of A2, B2 and C2 groups were 67.3%, 52.8% and 45.0%. When three embryos were transferred, the rate of the A3 group was 72.0%, those of the B3 and C3 groups were 50.0% and 33.3%, respectively. The mean CES/embryo in the pregnant group was 7.78 that were significantly higher than that in the non pregnant group 6.23. When one embryo transferred, the mean CES/embryo in the pregnant group was 7.93, but the CES in the non pregnant group was 5.54($P<0.05$). When two embryos transferred, the mean CES/embryo in the pregnant group was 7.85, but the CES in the non pregnant group was 7.00. When there embryos transferred, the mean CES/embryo in the pregnant group was 6.93, but the CES in the non pregnant group was 6.72.Conclusion: In the present study, a close correlation between the pregnancy rates and the CES was observed. The cut-off values of the CES/embryo according to the number of embryos transferred in the pregnant groups may be a good indicator to select the embryo and to decide the number of embryos for transfer.

P-074 Cytoplasmic halo as an additional indicator to predict the quality of the results of IVF and ICSI embryos

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Introduction: The important thing that determines the success of IVF is selection the best viable embryos transfered.There are some assessment such as Z scoring system,fragmentation and number of blastomere.Sometimes, we look the clear area on the cortex of zygote at the day of fertilization check, called cytoplasmic halo. It is due to the movement of cytoplasmic organelles specially mitochondria to the center, closer to the pronucleus. Several previous studies mentioned, the formation of cytoplasmic halo at zygote stage is the indication of good quality embryos (Gada,et al,2007;Shimura,et al,2006). But these studies have not compared between ICSI and IVF. So, in this study, we compared the number of embryos that presence halo and the quality of embryos resulted between ICSI and IVF. Material & Methods: The study was conducted on embryos of 55 Aster Clinic patient in January 2010 to May 2011(34 ICSI and 21 IVF). At zygote stage, along with fertilization check, the presence of the halo was recorded. On the day of ET, embryo quality was assessed (excellent, good, moderate, poor) based on number of blastomeres, fragmentation, blastomere size and suitability. Result: We found that both fertilization program (IVF and ICSI), percentage of excellent and good embryos (23,15% and 48,05%) was significantly higher on embryos halo(+) than embryos halo(-)(19,67% and 8,95%). While the percentage of moderate and poor embryos on embryos halo (+)(19,67% and 8,95%) lower than embryos halo (-) (30,27% and 17,04%). The number of zygote halo (+) in ICSI (54,56%) is significantly lower than IVF (75,38%). While the percentage of zygote halo (-) in ICSI (46,02%) is significantly higher than in IVF (24,71%). This is also supported by the lower quality of embryos performed ICSI than IVF, either on embryos that have a halo or not. In ICSI, the percentage of excellent, good, and moderate embryos was lower than IVF embryos, while the percentage of poor quality embryos was higher. The lower embryo quality in ICSI embryos probably caused by more treatment to the oocytes prior to ICSI performed. Its damage caused the migration of mitochondria in ooplasma disturbed so the halo was not formed and the lack of ATP around pronucleus which produces the low quality embryos (Fujimoto,et al,2011). Conclution: Cytoplasmic halo can be an additional indicator for predicting the embryo quality fertilized by ICSI and IVF. Percentage of embryos with halo (+) was higher in embryos fertilized by IVF compared to ICSI.

P-075 Withdrawn

P-076 Effects of L/D stimuli and the circadian clock on reproductive physiology in female mice

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Background: The effects of light/dark (L/D) and the circadian clock on physiology should be separately examined because L/D stimuli influence not only physiology but also the circadian clock. Results: We uncoupled the two factors by comparing the reproductive physiology including estrous cycle progression and ovulation profile of wild-type mice and that of mice that had completely lost the circadian clock, *Per1/Per2* double-knockout (*Per1/Per2*) mice and *Per2/Cry1* double-knockout (*Per2/Cry1*) mice, under L/D and constant darkness (D/D). Under L/D, no abnormality was observed in estrous cycle progression, but perturbation of timely ovulation and accompanying decline of reproductive ability were observed in *Per1/Per2* and *Per2/Cry1* mice. Although pituitary-derived LH surge, which is prerequisite for ovulation, occurred at almost the same timing in wild-type mice and *Per1/Per2* mice, expression pattern of *Cox2*, the gene responsible for timely ovulation, was perturbed in the ovary of *Per1/Per2* mice. Since overexpression of CLOCK and BMAL1 and REV-ERB ALPHA, componets of the circadian clock, suppressed the expression of *Cox2* in cultured ovarian granulosa cells, respectively, *Cox2* expression is linked to the circadian clock. Taken together, the data suggest that the perturbation of timely ovulation in *Per1/Per2* mice was likely due to the lack of the ovarian circadian clock. Under D/D, although no abnormality was observed in estrous cycle progression in wild-type mice, *Per1/Per2* and *Per2/Cry1* mice showed drastic elongation of estrous stage, which caused elongation of the whole estrous cycle. Both wild-type mice and *Per1/Per2* mice showed abnormal ovulation profiles as observed in *Per1/Per2* mice kept under L/D. Conclusions: Our results indicate that cooperation of L/D stimuli and the circadian clock optimizes female reproductive performance.

P-077 Numbers of CGG repeats on the FMR1 gene in Japanese patients with premature ovarian insufficiency

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Objective: To define the number of CGG repeats on FMR1 in Japanese patients with premature ovarian insufficiency (POI) and normal controls and to identify correlations between number of CGG repeats and age at the onset of amenorrhea in these patients. Design: Retrospective, controlled cohort study.Setting: Outpatient department of academic tertiary center. Patients: One hundred and twenty-eight consecutive Japanese patients with sporadic, non-syndromic POI and 98 controls with normal menstruation.Interventions: DNA was obtained from plasma of each subject. Main Outcome measures: Whether the distribution of numbers of CGG repeats differs between patients with POI and controls and whether the number of these repeats correlates with age at the onset of amenorrhea in such patients.Results: We identified ≥40 repeats in all alleles except for eight in seven patients with POI with intermediate numbers of repeats and two in the premutation range in two patients. The prevalence of >40 repeats was significantly greater in the patients. Age at the onset of amenorrhea negatively correlated with ≥36 CGG repeats in patients with POI. Conclusions: Over 36 CGG repeats in the FMR1 might intensify the etiology of POI at least up to the premutation range.

P-078 Genetic analysis of spermatozoa in four infertile patients with macrocephalic sperm head syndrome

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Objective: Total teratospermia with predominantly macrocephalic (large headed) spermatozoa is a rare abnormality. The purpose of this study was to evaluate the chromosomal content of spermatozoa in cases of macrocephalic sperm head syndrome (SM) and to describe the association between various phenotypes, sperm chromosomal abnormalities, and the mutation of the aurora kinase C gene (AURKC). Materiels and methods: The patients were four infertile patients with macrocephalic sperm head syndrome (91%, 82%, 100% and 60%respectively). Morphological aspects for MS were detailed after shorr staining. The chromosomal content of the spermatozoa was analyzed by fluorescent in situ hybridization (FISH) using X, Y, 18 centromeric probes. AURKC sequence analysis was carried out for all patients. Result(s): Two morphological aspects for MS were highlighted: MS with irregular head and multiple flagella, MS with regular head, all the nonselected spermatozoa were abnormal, diploid, or polyploid. Sequence analysis showed that the 3patients with total syndrome were homozygous for the AURKC c.144delC deletion. Conclusion(s): In conclusion, we confirmed that the absence of AURKC c.144delC is not a sufficient condition to indicate ICSI in cases of SM. In fact, the very low proportion of normal haploid spermatozoa contraindicated ICSI for our four patients. We suggest performance of these analysis before including (or not), in an ICSI program, patients with macrocephalic sperm head syndrome.

P-079 Gene Expression Profiling of Karyotypically Normal, Trisomy 12 and XXY Human Embryonic Stem Cells

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Human embryonic stem cells (hESCs) are well known to have the ability to proliferate unlimitedly and to differentiate into all cell types. Recently, a number of reports have been published in which chromosomal abnormalities has been observed in hESCs. We analyzed gene expression profiles of normal cell lines (SNUhES3: S3 and SNUhES4: S4), two variants of SNUhES3 (trisomy 12: S3v12 and XXY: S3vX), and one variant of SNUhES4 (trisomy 12: S4v12) using the Illumina HumanRef-6 BeadChip including 48,095 probe sets to find epigenetic difference between normal cells and chromosome variants. Gene expression patterns were showed that 55 genes were up-regulated and 28 genes were down-regulated in S3v12 to compare with S3 and 98 up-regulated genes and 75 down-regulated genes were identified in S3vX. Also, 87 genes were up-regulated and 61 genes were down-regulated in S4v12 to compare with S4. In particularly, three genes, PUS7L (synthetase), INHBE (growth factor) and PKIB (kinase inhibitor), were up-regulated and two genes, ERG1 (KRAB box transcription factor) and GALNT9 (glycosyltransferase) were down-regulated in S3v12, S4v12 and S3vX compared to normal hESC lines. Gene expression profiling in variant hESC lines with trisomy 12 or XXY chromosome as well as normal hESC lines may provide insights to the development of research field for cell differentiation and clinical approach such as cell replacement therapy. This research was supported by a grant (SC-1150) from Stem Cell Research Center of the 21st Century Frontier Research Program funded by the Ministry of Education, Science and Technology, Republic of Korea.

P-080 Effect of dibutyryl cAMP during in vitro maturation on the developmental competence of bovine oocytes after ICSI

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Introduction: Bovine intracytoplasmic sperm injection (ICSI) is useful technique for production of calves from limited supply of gametes. In bovine ICSI, in vitro-matured oocytes are used, but it is well known that the developmental competence of in vitro-matured oocytes is lower than that of in vivo-matured oocytes. The content of cAMP of follicular oocytes is increased during follicular development, and differ from individual immatured oocytes. The objective of this study was to examine effect of treating bovine oocytes with dibutyryl cAMP (dbcAMP) during in vitro maturation (IVM) on nuclear maturation and embryonic development after ICSI. Material& Methods: As a basic medium, TCM199 supplemented with 1mg/ml recombinant human albumin, 1 IU/ml recombinant human FSH and 50 ng/ml EGF was used. In the first experiment, bovine cumulus-oocyte complexes (COCs) were cultured in the basic medium supplemented with 0, 10, 100 and 1000 μM dbcAMP for 21 h. We examined the effect of the concentration of dbcAMP during IVM on nuclear maturation and the blastocyst development after ICSI. In the second experiment, we examined the effect of treating COCs with 10 μM dbcAMP on sperm aster formation in bovine IVM oocytes at 5 h after ICSI. In the third experiment, using time-lapse microscopy, we observed the effect of treating COCs with 10 μM dbcAMP during IVM on the speed of embryo development after ICSI. Results: In the first experiment, treating COCs with 0 to 1000 μM dbcAMP during IVM did not affect the percentage of MII oocytes (69 to 79 %). Blastocyst rate in the group of 10 μM dbcAMP (56%) was significantly higher than that in the others (0 μM: 24%, 100 μM: 43% and 1000 μM: 31%). In the second experiment, the percentage of sperm aster formation in bovine IVM oocytes treated with 10 μM dbcAMP was significantly higher than that in the untreated IVM oocytes (82 % vs. 52%). In the third experiment, timing of cleavage and blastocyst development after ICSI in the group of 10 μM dbcAMP was significantly faster than that in the untreated group (cleavage, 22.2±0.5 h vs. 25.6±0.8 h, blastocyst, 162.1±2.2 h vs.170.3±3.2). Conclusions: We demonstrate that treating bovine COCs with 10 μM dbcAMP together with FSH and EGF during IVM does not affect nuclear maturation at 21 h, but stimulates the sperm aster formation after ICSI, accelerates the speed of embryo development, and improves the blastocyst rate.

P-081 Androstenedione induces abnormalities in morphology and function of developing oocytes, which impairs oocyte meiotic competence

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Previous studies have suggested that intraovarian excess androgen, including androstenedione, is a main cause of polycystic ovary syndrome. However, the mechanism leading to polycystic ovary by androgen is currently unclear. To obtain further insight into the effects of androgen on ovarian folliculogenesis and oogenesis, early secondary follicles were isolated from the mouse ovaries and were cultured individually in vitro with or without androstenedione (10^{-11}-10^{-5} M) for 12 days. In this single follicle culture system, early secondary follicles could develop to preovulatory stage. Androstenedione treatment reduced survival rates of follicles and promoted the formation of follicles with abnormal morphology, such as the lack of cumulus and mural granulosa cells and misshapen oocyte. Significantly higher estradiol secretion was observed in androstenedione-exposed follicles. In addition, androstenedione treatment prevented the alteration in chromatin configuration and influenced expression of growth differentiation factor 9 in the oocytes. When follicles cultured with androstenedione for 12 days were treated with human chorionic gonadotropin and epidermal growth factor, the first polar body exclusion, chromosome alignment on metaphase plate, and spindle assembly were inhibited in the oocytes. These results demonstrate that excess androgen and perhaps its metabolite, estrogen, induce abnormalities in morphology and function of developing oocytes, which impairs oocyte meiotic competence.

P-082 Preimplantation genetic diagnosis for spinal muscular atrophy using mini-sequencing and genetic linkage analyses at the blastocyst stage

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Background: Approximately 94% of spinal muscular atrophy (SMA) cases are caused by common homozygous absence of the SMN1 gene. Although preimplantation genetic diagnosis (PGD) is an alternative option for couples at risk of having a child with single gene disorder, blastomere biopsy has been employed in most in vitro fertilization procedures. Methods: In the present study, we validated and applied protocol clinically for PGD through the use of blastocyst biopsy, whole genome amplification, mini-sequencing genotype coupling with genetic linkage of SMN gene involving three informative microsatellite markers, and thawed embryo transfer. Results: We report data to identify the SMN1 gene deletion on eighteen clinical embryos obtained from one participating couple, where both partners are heterozygous SMA carriers with 1-SMN1/3-SMN2 genotypes. Approximately 78% (14/18) of blastocysts were successfully amplified in a single PGD cycle. Among these embryos, ten (72%, 10/14) were diagnosed as unaffected, two (14%, 2/14) as affected, and two embryos (14%, 2/14) had no conclusive diagnosis due to allele drop-out (ADO). Two unaffected embryos were thawed and transferred in the next cycle resulting in a singleton pregnancy, and the birth of a healthy girl who carries the 1-SMN1/3-SMN2 genotype. Conclusions: The strategy of PGD using blastocyst biopsy and thawed embryo transfer increased the reliability of the results and permitted more time for the execution of molecular diagnosis. The improved protocol for PGD could be adopted for other monogenic diseases in the future.

P-083 Preimplantation Genetic Diagnosis (PGD) for translocation carrier with mild stimulation and single vitrified blastocyst transfer : Case Report

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Introduction: In population of chromosomal translocation carriers, hyperstimulation is still applied for PGD cycle to increase the number of retrieved oocytes, because it is considered that they have high risk of meiotic non-disjunction. Then, how many oocytes does carrier couple need to conceive a healthy baby? This is the new approach for PGD with mild stimulation which is developed from our experience in two decades. Material and methods: Twelve couples consisting of subjects with a mean age of 35.2 years who are carriers of chromosomal translocations.The patients underwent mild ovarian stimulation, which was carried out by administration of clomiphene citrate in combination with a minimum amount of urinary HMG (75IU X less than 4 times) or rec. FSH (75IU X less than 3 times). Administration of clomiphene citrate was initiated from day 3 of the menstrual cycle at 50mg/day and was continued until the day before administration of the GnRH agonist as the maturation trigger (GnRHa nasal spray, 300μg) and 32-35 h before oocyte retrieval as described by Kato. IVF was performed for all oocytes, and blastomere biopsy was performed on the embryos reaching at least the 6-cell stage on day 3. Culture of all the biopsied embryos was continued, and embryos developing to the blastocyst stage were vitrified. FISH analysis was performed on all biopsied blastomeres using 3 to 4 appropriate FISH probes specific for translocated segments. Cryopreserved blastocysts which showed normal/balanced were thawed and intrauterine transfer of single blastocysts was performed. Results: Eighty-nine Cumulus Oocyte Complex (COC) were collected in 35 OR cycles from 12 patients, average number of COC per OR cycle were 2.5(89/35). Ninety one % (77/85) were fertilized, 28% (20/72) were transferable at the 8-cell stage and 13 of the blastocysts which showed alternate were thawed and intrauterine transfer was performed by single blastocyst transfer for 8 patients. A positive heart beat was obtained in 7 patients, pregnancy rate per ET was 54% (7/13). Finally, the delivery rate was 46% per ET (6/13) and 17% per OR (6/35), and one case is ongoing at 20 weeks. There were 6 deliveries of 6 healthy babies, consisting of 2 males and 4 females, with no major malformations at birth. Conclusions: ESHRE PGD consortium's data (2009) with 417 patients showed that the average numbers of COC to obtain a fetus were 64.1(No. of COCs/No. of FHB) which was five times of our data (12.7: 89/7). There haven't been any reports concerning to assess the appropriate number of oocyte to obtain a pregnancy for carrier couple, however, our results and subsequent natural pregnancy outcomes which was reported by Sugiura-Ogasawara may indicate that it is enough around 10 oocytes for a delivery. And this conclusion strongly suggests that to decrease the number of oocytes requires not only accurate diagnosis skill but also improved IVF-ET technique in facilities.

P-084 Noninvasive morphological examination for germinal vesicle oocytes predict their maturity process of completion

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Objective: Especially, derived from small size follicle, germinal vesicle (GV) oocyte sometimes can be obtained in human IVF. Those human GV oocytes have been researched as in vitro maturation (IVM) for a long time. However, IVM studies conducted with GV stage oocytes have not produced promising results with regard to oocyte maturity and blastocyst development. We noninvasively examined GV stage oocyte by means of morphology with microscope and analyzed whether or not these morphologic parameters can predict their maturity and quality. Method: This study was designed 197 IVF cycles from May 2010 to March in 2011. All GV oocytes were obtained from small size follicles (＜ 8mm diameter) for natural cycle IVF and cultured in vitro in P1 medium (Irvine Scientific, USA). Cumulus cells were gently removed by pipetting with hyaluronidase, then cumulus-free GV oocytes were morphologically examined by contrast-phase microscope (Olympus Optical Co., Japan) at ×400 magnification. We measured two times in a different orientation and calculated the average diameter of whole cytoplasm, GV and chromatin configuration using by Octax Eyeware software (OCTAX Microscience GmbH, Germany). Cumulus-free GV oocytes were cultured in P1 culture medium at 37℃ and 5% CO_2, 5% O_2 and 90% N_2. All oocytes were confirmed the presence of GV or first polar body and classified as immature (GV, MI) or mature (MII) after 24 hours in vitro culture. Results: A total of 198 GV oocytes were obtained from 127 patients. Patient age varied from 24 to 48 years old (38.9 ± 4.0 years old). The GV oocytes were retrospectively examined after removing cumulus cells. Of 148 GV oocytes were cultured for 24 hours, it was found that there were 44 GV, 25 MI and 79 MII. (53.4% matured) Mean diameter of oocyte cytoplasm of GV, MI and MII were 114.75μm, 116.2μm and 116.44μm,respectively. The mean diameter of GV were 32.67μm, 32.37μm and 32.73μm, respectively. The mean diameter of chromatin configuration were 8.54μm, 8.63μm and 9.06μm, respectively. In addition, 1.4% of GV oocyte contained two chromatin configurations (2/148), these two oocytes remained in GV after 24 hours culture. Student-T test was used for statistical analysis. Conclusion: Matured oocytes contained significantly larger size chromatin configuration than immature oocytes **(P<0.05).** Different size of chromatin configuration may be related to GVBD and oocyte maturity. The oocyte which had two chromatin configurations did not mature, therefore it appears that the morphology of chromatin configuration is relating mechanism of oocyte maturation. Furthermore, specific morphologic parameters may provide information of oocyte maturation in IVM..

P-085 hCG is critical for the uterine decidual response in mice

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Introduction: The process of implantation, necessary for all viviparous birth, consists of tightly regulated events, including apposition of blastocyst, attachment to the uterine lumen, and differentiation of the uterine stroma. In rodents and primates the uterine stroma undergoes a process called decidualization. Decidualization, the process by which the uterine endometrial stroma proliferates and differentiates into large epithelioid decidual cells, is clitical to the establishment of fetal-material communication and the progression of implantation.On the other hand, it was said that fertilization rate of hCG injected mice was lower than normal mice. So, this our study investigate into the relation between decidualize after hCG injection in mice and their fertility. Material & methods: Mice were maintained in the designated animal care facility at Meiji University according to the institutional guidelines for the care and animals. The artificial decidual response has been previously described (Finn, C.A., *et al.*, 1972, Biol. Reprod. 7:82-86). Briefly, mated female ICR mice (8-10 weeks old) by castrated male after PMSG and/or hCG treatment (1time and/or 3times) were injected 25µl sesame oil into uterine horn at 3.5d.p.c. to induce decidualization. The contralateral horn was not traumatized and served as a control. These mice were sacrificed and extracted both uterine horns at 7.5d.p.c. The ratio of wet weight in uterine horn after sesame oil injection to the other no-stimulated one was calculated. At the time of dissection, uterine tissues and ovaries were fixed with 4% paraformaldehyde (PFA). To perform histological analysis and count number of oocyte, these samples were then dehydrated by ethanol washes, embedded with paraffin, sectioned longitudinally and stained with hematoxylin and eosin. Finally we perfomed embryo transfer to hCG injected mice to check the fecundability. Results: Whereas uterine horn of control group was able to decidualize, uterine horns of hCG injection all groups were not able to perform decidualization significantly (untreated group: 22.58±3.32g versus hCG injection group: PMS+ hCG 1.10±0.03g, h 2.44±1.0g, 3(PMS+ hCG) 2.16±0.56g, 3hCG 3.21±0.77g; $p>0.05$). In pathological analysis, the cells of uterine endometrium in hCG injected group was not hypertrophied and polygon. Pregnacy rate of hCG injected group (50%) was half of control group (100%) after embryo transfer and the number of pups in hCG group (56.2%) was significantly fewer than control group (23.3%). The body weight of these pups were lighter than control group (hCG group: 1.2±0.39g versus control: 1.55±0.25g) significantly. Conclusions: Our study showed that hCG is critical for the uterine decidual response and infecundity in mice. It was essential to make clear the mechanism between decidual hypoplasia with hCG and infertility in the further investigation.

P-086 Cytogenetic study in early spontaneous abortion after IVF and ICSI

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Objective: To investigate the incidence of chromosomal abnormalities in early pregnancy loss after IVF and ICSI. Methods: From January 2004 to December 2007, a total of 166 cases of miscarriage villi samples after IVF-ET were collected for cytogenetic analysis. The average age of cases was 38.9. All IVF cycles were performed by natural cycle or mini IVF in our center. Cytogenetic analysis was performed at special biochemical analysis center (SRL, Japan). Results: Cytogenetic results were obtained for 158 (ICSI 63, IVF 95) samples and 117 samples (74.1%) showed chromosomal abnormalities. Autosomal trisomies of chromosomes 4, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21 and 22 were detected in 80 samples (68.4%). In 80 samples, 22 samples were trisomy 22(27.5%), 17 samples showed trisomy 15 (18.8%) and 10 samples were trisomy 21 (12.5%). Double trisomy was detected in 13 samples. In 117 samples, 8 samples showed sex chromosome monosomy and all samples showed 45,X. Triploidy was detected in 1 sample and tetraploidy was detected in 3 sample. All of polyploidy abnormality were performed by conventional insemination. Structual rearrangements were detected in 8 samples (6.8%). Normal karyotype samples accounted for 41 (25.9%). ICSI samples more showed aneuploidy than conventional insemination patients; 82.5% vs. 68.4% ($P<0.05$). There was no difference of average age between two groups. Comparison between conventional insemination and ICSI did not show significant difference in the type of chromosome abnormalities. Conclusion: Chromosome abnormalities are identified in 74.1% of all spontaneous abortions. In this study, the average age of IVF patients is 39.5. Due to this background, incidence of abnormal chromosome of miscarriage villi showed higher than previous reports. In autosomal trisomy samples, incidence of trisomy 22 was particularly higher than other chromosome trisomies. Furthermore, the frequency of trisomy 15 was higher than previous reports. These results indicate that the proportion of chromosomal abnormalities may be different between normal pregnancy and IVF or ICSI. We also found that ICSI samples were more likely to have aneuploidy than IVF samples.

P-087

Day 7 β-hCG level after frozen-thawed blastocyst transfer can be a predictor for pregnancy outcomes?

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Introductions: There are only few large-scale studies about outcomes after single embryo transfer. In this time, we analyzed the results after frozen-thawed single blastocyst transfer to find out the correlation between serumβ-hCG levels supplied from single implanted embryo and pregnancy outcomes. Materials and methods: We investigated total of 7,523 cycles of frozen-thawed single blastocyst transfer performed from Jan. 2004 to Dec. 2008. The age of patients was from 25 to 44 years. All cases were confirmed both serum β-hCG levels on day 7 after blastocyst transfer and the results about pregnancy outcomes. And also were excluded all multiple pregnancies and ectopic pregnancies. All patients retrieved oocyte under the natural cycle or clomiphene based minimal stimulation protocol. All obtained blastocyst vitrified using Cryotop® (Kitazato, Japan). Frozen-thawed single blastocyst transfer performed on day 5 after spontaneous ovulation or on cycle day 18 in hormone replacement cycles. Serum β-hCG levels checked out 7 days after transferred embryo. Patients were divided into eight groups depending on the β-hCG levels. Pregnancy rates and live birth rates in each β-hCG level were analyzed in 3 age groups (a. 25-34 year, b. 35-39 year, c. 40-44 year). Results: Patients with day 7 β-hCG levels $\geq$30mIU/mL showed comparatively high pregnancy rates and live birth rates regardless age group. Conclusions: Our study suggested that day 7 β-hCG level after single frozen blastocyst transfer may be used for a predictor for pregnancy outcomes.

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