The Third World Congress on Mild Approaches in Assisted Reproduction

— Embracing Mild IVF and IVM —

Date: July 30 and 31, 2010
Venue: Pacifico Yokohama, JAPAN

PROGRAM & ABSTRACTS
Dear Friends and Colleagues

It is our great pleasure and honor to welcome you to the Third World Congress on Mild Approaches in Assisted Reproduction to be held on July 30 and 31, 2010, in Yokohama, Japan.

The Congress will focus on the objectives of ISMAAR to raise scientific and public awareness about mild, safe, more physiological and affordable approaches in assisted reproduction as its key topics. The Faculty will dedicate their time and efforts in promoting practical aspects of Mild IVF and IVM.

In addition, global concerns about preservation of fertility for cancer female patients, protection of human fertility, safety, regulation, accessibility and affordability of ART will dominate the debate and agenda at the Congress.

Yokohama is an international port city right next to Tokyo, and it is, therefore, within easy reach from both Tokyo Station and Narita International Airport. The city has developed as the base for foreign trade in Japan since 1859 and that it is an ideal place for exchanging information and ideas with colleagues from all over the world.

We look forward very much to welcoming you to Yokohama in 2010, and to discussing our accomplishments and plans for the future of ART.

Sincerely yours,

Osamu Kato
Congress president
Dear Friends and Colleagues

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We look forward to welcoming you to Yokohama in 2010, and to discussing our accomplishments and plans for the future of ART.

Geeta Nargund

President ISMAAR
How to get to PACIFICO Yokohama

By Air
- Haneda Airport
  - Airport Limousine Bus: 30min
  - Keikyu Express: 24min
- Narita Airport
  - Airport Limousine Bus: 90min
  - JR Narita Express: 90min

By Train
- Shibuya Station
  - Tokyo Toyo Line: Express (Direct link to Minato Mirai Line) 75min
  - JR Tokaido Line: 30min
- Shin Yokohama Station
  - JR Yokohama Line: 3min
  - Yokohama Subway: 8min

By Car
- Metropolitan Expressway
  - Toward Tokyo Station, Yokohama Phase 1: 8min
  - Toward Tokyo Station, Yokohama Phase 2: 6min

Parking
- Minato Mirai Public Parking
  - Capacity: 1,180 cars
  - Open hours: 24hrs
  - Fee: 200¥/30min (10% off 6:00-9:00)
- Riski Park Parking
  - Capacity: 100 cars
  - Open hours: 8:00-21:00
  - Fee: 250¥/30min
- Bus/Large vehicle Parking
  - Capacity: 40
  - Open hours: 24hrs
  - Fee: 500¥/30min (10% off 7:00-9:00)

Please visit our website www.pacifico.co.jp for more information.
Note: The 28th Annual Meeting of Japan Society of Fertilization and Implantation (JSFI) to be held on July 28-29 at the same venue.
1. **Instructions for ORAL Presentation**

Clarity of slides is vital for a successful scientific presentation.

1) All the speakers are requested to keep to the time previously allotted for each. Green and Orange lamps will tell you how much time is remaining in your presentation. A green lamp will indicate when one minute remains, while an orange will light up when no time remains.

2) All presentations will be done on PC.

3) Please bring your own PC and a backup of your data. The only PC media that will be available are CD-R, DVD-R, or USB.

4) PCs with Windows XP and PowerPoint 2003 are to be used. PCs with Macintosh OS X and PowerPoint 2004 are to be used. Windows Vista/Macintosh users: Please bring your own computer for your presentation.

5) Animation will be available. However, sound effects are not available. If your presentation data is in PC media, please make sure that the data is compatible with Media Players for Windows or Quick Time for Macintosh. Standard PC fonts (for Windows or Macintosh) should be used.

6) If you are using your own PC, please make sure to bring an AC adaptor (standard 2-pin type). For projector output purposes, a VGA cable will be provided. Please confirm whether your PC is equipped with an RGB jack (mini D-sub 15 pin type) as standard. If you use a different type of RGB jack to connect to an external monitor, please bring it with you. XGA (1024x768) is the suitable monitor size. For purpose data projection, please adjust your screen setting to XGA. Please cancel your screen saver and power saving settings in advance, especially if your data includes video image and sound.

7) Please bring your PC or PC data to the PC Preview Desk in front of the presentation room at least 30 minutes prior to your presentation to register and submit it to test the connection and view your file.

8) We will issue a receipt after checking your data. Please exchange the receipt for your PC at the Operator's Desk, beside the stage.

9) Please use the mouse and keyboard on the podium for your presentation. You are required to handle your data yourself, using the mouse and keyboard connected to the PC.

10) The copied data for your presentation will be deleted by the secretariat after the meeting.
2. Instructions for POSTER Presentation

1) Presenters are requested to follow the schedule below when mounting your poster on the assigned board and removing your poster materials from the board.

2) Set-up : July 30 (Fri) 08:30-10:00 (tentative)
   Dismantling : July 31 (Sat) from 16:00 (tentative)

3) Posters : 90cm wide x 180cm high

Your poster presentation number will be posted on your assigned board and the poster can be attached to the board.

Push pins for mounting your poster materials will be provided on site.

• All posters not removed will be discarded.
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
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<td>08:00</td>
<td>Registration</td>
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<tr>
<td>09:00-09:35</td>
<td>Opening Remarks</td>
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<tr>
<td>09:00-09:35</td>
<td>Plenary Lecture 1: <em>Mild IVF</em></td>
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<tr>
<td></td>
<td>Chair: Geeta Nargund</td>
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<td>Chair: Osamu Kato</td>
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<td>Speaker: Bart Fauser</td>
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<tr>
<td>09:35-10:35</td>
<td>Session 1: <em>Low-cost IVF for developing countries</em></td>
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<td>Chair: Ian Cooke</td>
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<td>Speaker1: Willem Ombelet</td>
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<td>Speaker2: Ian Cooke</td>
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<td>Speaker 3: Sudarsan Ghosh Dastidar</td>
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<td>Co-Sponsored by EIKEN CHEMICAL CO., LTD.</td>
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<td>TOSOH CORPORATION</td>
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<tr>
<td>10:35-10:55</td>
<td>Coffee Break</td>
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<tr>
<td>10:55-11:55</td>
<td>Session 2: <em>Natural/modified natural cycle IVF using GnRHa</em></td>
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<td>Chair: Osamu Kato</td>
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<td>Chair: Sherman J. Silber</td>
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<td>Speaker1: Antonis Makrigiannakis</td>
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<td>Speaker2: Sherman J. Silber</td>
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<td>Co-Sponsored by Mochida Pharmaceutical Co., LTD.</td>
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<td>11:55-12:30</td>
<td>Poster Viewing</td>
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<tr>
<td>12:30-13:30</td>
<td>Sponsored Luncheon Symposium I: <em>Mild ovarian stimulation with clomiphene citrate</em></td>
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<td>Chair: Makio Shozu</td>
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<td>Speaker1: Reishi Misumi</td>
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<td>Speaker2: Shokichi Teramoto</td>
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<td>Co-Sponsored by Schering-Plough K.K.</td>
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<tr>
<td>13:30-13:50</td>
<td>Coffee Break</td>
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<tr>
<td>13:50-14:50</td>
<td>Session 3: <em>Non invasive assessment of oocytes and embryos: Only one embryo to transfer</em></td>
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<td>Chair: Shehua Shen</td>
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<td>Chair: Tadashi Okimura</td>
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<td>Speaker1: Juana Crespo</td>
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<td>Speaker2: Denny Sakkas</td>
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<td>Speaker3: Kazuo Yarnagata</td>
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<td>14:50-15:10</td>
<td>Coffee Break</td>
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<tr>
<td>15:00-16:10</td>
<td>Afternoon Session 1: <em>Poor Responder</em></td>
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<td>Chair: Timur Gürgan</td>
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<td>Speaker1: Milton Leong</td>
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<td>Speaker2: Luis Arturo Ruvalcaba Castellon</td>
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<td>Speaker3: Mesut Oktener</td>
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<tr>
<td>15:30-15:50</td>
<td>Session 4: <em>In vitro maturation of oocytes</em></td>
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<td>Chair: Yoshiharu Morimoto</td>
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<td>Chair: Milton Leong</td>
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<td>Speaker1: Svend Lindenberg</td>
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<td>Speaker2: Atsuku Fukuda</td>
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<td>Speaker3: Jin-Ho Lim</td>
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<td>16:10-16:35</td>
<td>Afternoon Session 2:</td>
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<td>Chair: Milton Leong</td>
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<td>Speaker: Timur Gürgan</td>
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<td>16:35-17:55</td>
<td>Free Communications:</td>
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<td>Chair: Luis Arturo Ruvalcaba Castellon</td>
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<tr>
<td>18:00-20:00</td>
<td>Welcome Reception Gala Dinner</td>
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Note: The 28th Annual Meeting of Japan Society of Fertilization and Implantation (JSFI) on July 28-29 at the same venue.
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Chair</th>
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<th>Sponsor</th>
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<tbody>
<tr>
<td>09:00-09:35</td>
<td>Plenary Lecture 1: &quot;Mild IVF&quot;</td>
<td>Chair: Geeta Nargund, London, UK</td>
<td>Chair: Osamu Kato, Tokyo, Japan</td>
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<tr>
<td>09:00</td>
<td>Speaker</td>
<td>&quot;Mild ovarian stimulation for IVF&quot;</td>
<td>Bart Fauser, Utrecht, The Netherlands</td>
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<tr>
<td>09:35-10:35</td>
<td>Session 1: &quot;Low cost IVF for developing countries&quot;</td>
<td>Chair: Willem Ombelet, Genk, Belgium</td>
<td>Chair: Ian Cooke, Sheffield, UK</td>
<td>Co-Sponsored by EIKEN CHEMICAL CO., LTD. TOSOH CORPORATION</td>
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<tr>
<td>09:35</td>
<td>Speaker 1</td>
<td>&quot;Infertility care in poor-resource countries: The Arusha project&quot;</td>
<td>Willem Ombelet, Genk, Belgium</td>
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<tr>
<td>09:55</td>
<td>Speaker 2</td>
<td>&quot;Low-cost IVF for developing countries&quot;</td>
<td>Ian Cooke, Sheffield, UK</td>
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<td>10:15</td>
<td>Speaker 3</td>
<td>&quot;Low Cost Controlled Ovarian Stimulation in ART - Our Experience&quot;</td>
<td>Sudarsan Ghosh Dastidar, Kolkata, India</td>
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<td>10:35-10:55</td>
<td>Coffee Break</td>
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<tr>
<td>10:55</td>
<td>Speaker 1</td>
<td>&quot;Impact of 19,467 IVF cases/year based on natural cycle IVF&quot;</td>
<td>Yuji Takehara, Tokyo, Japan</td>
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<td>11:15</td>
<td>Speaker 2</td>
<td>&quot;REPEATED IMPLANTATION FAILURE: IMMUNOLOGICAL ASPECTS AND EVIDENCE BASED TREATMENT MODALITIES&quot;</td>
<td>Antonis Makrigiannakis, Herakleion, Greece</td>
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<td>11:35</td>
<td>Speaker 3</td>
<td>&quot;Natural/modified natural cycle IVF&quot;</td>
<td>Sherman J. Silber, Missouri, USA</td>
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</table>
| 13:50-14:50 | **Chair:** Shehua Shen, California, USA  
              **Chair:** Tadashi Okimura, Japan                                                   |
| 13:50       | Speaker 1: "Non invasive assessment of oocytes and embryos: improving embryo selection to transfer"  
              Juana Crespo, Valencia, Spain                                                   |
| 14:10       | Speaker 2: "Assessment of embryo viability before transfer: Genomics, Proteomics and Metabolomics"  
              Denny Sakkas, Connecticut, USA                                                  |
| 14:30       | Speaker 3: "Assessment of Embryo Quality by Live-cell Imaging"  
              Kazuo Yamagata, Hyogo, Japan                                                   |
| 14:50-15:10 | **Coffee Break**                                                                        |
| 15:10-16:10 | **Afternoon Session 1: "Poor Responder"**                                               |
| 15:10       | **Chair:** Timur Gürgan, Ankara, Turkey                                                 |
| 15:10       | Speaker 1: "Nurturing the ovaries: Treatment of patients with high FSH with delayed stimulation using low dose gonadotrophins"  
              Milton Leong, Hong Kong                                                       |
| 15:30       | Speaker 2: "Poor Responders Updated"                                                   
              Luis Arturo Ruvalcaba Castellon, Jalisco, Mexico                               |
| 15:50       | Speaker 3: "Efficacy of IVF-CloLomiphene Citrate /Letrozole+Antagonist Protocol in Severe Poor Responders"  
              Mesut Oktem, Ankara, Turkey                                                     |
| 16:10-16:35 | **Afternoon Session 2: "Highly Glycosylated FSH (HG-FSH): Available Clinical Results and Investigational Issues"** |
| 16:10       | **Chair:** Milton Leong, Hong Kong                                                     |
| 16:10       | Speaker: Timur Gürgan, Ankara, Turkey                                                  |
| 16:35-17:55 | **Free Communications**                                                                 |
| 16:35       | **Chair:** Luis Arturo Ruvalcaba Castellon, Jalisco, Mexico                            |
| 16:35       | Speaker 1: "Consequences of cryopreservation protocols of metaphase II oocyte on translation processes"  
              Sandrine CHAMAYOU, Sant'Agata Li Battiati, Italy                                |
| 16:55       | Speaker 2: "An individualized stimulation algorithm reduces oocyte numbers and removes hyper-stimulation syndrome without affecting pregnancy rates."  
              John Yovich, Perth, Australia                                                   |
| 17:15       | Speaker 3: "Are Mild ART-derived blastocysts more favorable than conventional COH-derived ones?"  
              Yasushi Takai, Saitama, Japan                                                  |
| 17:35       | Speaker 4: "Vitrified-warmed blastocyst gives significantly superior clinical result compared to fresh blastocyst transfer in COH cycles --Time to transfer less and vitrify more"  
              Guoqing Tong, Nanjing, PR China                                               |
<p>| 18:00-20:00 | <strong>Gala Dinner</strong>                                                                         |
|             | at The Pan Pacific Yokohama Bay Hotel Tokyo                                           |</p>
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<tr>
<td>09:00-09:35</td>
<td>Plenary Lecture2 : &quot;Current status of mild IVF in Japan&quot;</td>
<td>Chair: Geeta Nargund, London, UK</td>
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<td>Chair: Willem Ombelet, Genk, Belgium</td>
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<td>09:00</td>
<td>Speaker</td>
<td>Osamu Kato, Tokyo, Japan</td>
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<td>Chair: Milton Leong, Hong Kong, China</td>
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<td>Co-Sponsored by Merck Serono Co., Ltd.</td>
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<tr>
<td>09:35</td>
<td>Speaker 1 &quot;In Vitro Maturation of Human Oocytes&quot;</td>
<td>Svend Lindenberg, Copenhagen, Denmark</td>
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<td>09:55</td>
<td>Speaker 2 &quot;Optimal IVM-IVF for routine clinical use*</td>
<td>Aisaku Fukuda, Osaka, Japan</td>
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<tr>
<td>10:15</td>
<td>Speaker 3 &quot;Natural Cycle IVF combined with IVM&quot;</td>
<td>Jin-Ho Lim, Seoul, South Korea</td>
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<td>10:35-10:55</td>
<td>Coffee Break</td>
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<td>Chair: Geeta Nargund, London, UK</td>
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<td>10:55</td>
<td>Speaker 1 Stuart Campbell, London, UK</td>
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<td>11:15</td>
<td>Speaker 2 &quot;One-Stop Fertility Assessment –The role of advanced ultrasound technology&quot;</td>
<td>Geeta Nargund, London, UK</td>
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<tr>
<td>11:35</td>
<td>Speaker 3 &quot;Use of a catheter Designed for Vaginal Ultrasound Guided Embryo Transfer&quot;</td>
<td>Marwan Shaykh, Florida, USA</td>
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<tr>
<td>11:55-12:30</td>
<td>Poster Viewing</td>
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<tr>
<td>12:30-13:30</td>
<td>Sponsored Luncheon Symposium II : &quot;Fertility as social priority&quot;</td>
<td>Chair: Timur Gürgan, Ankara, Turkey</td>
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<td>Chair: William Ledger, Sheffield, UK</td>
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<td>12:30</td>
<td>Speaker 1 &quot;European attitudes to fertility treatment and reimbursement. Current position and future prospects&quot;</td>
<td>William Ledger, Sheffield, UK</td>
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<td>12:50</td>
<td>Speaker 2 &quot;Epidemiology and Prevention of Infertility in Society with Fewer Children&quot;</td>
<td>Harumi Kubo, Tokyo, Japan</td>
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<td>13:30-13:50</td>
<td>Coffee Break</td>
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### Session 6: "Vitrification for oocytes and embryos"

**Chair:** Masashige Kuwayama, Tokyo, Japan  
**Chair:** Stanley P. Leibo, Louisiana, USA  
**Co-Sponsored by:** KITAZATO BioPharma. Co., Ltd.

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<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Topic</th>
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</table>
| 13:50 | Speaker 1 | "Vitrification for oocytes and embryos"  
Stanley P. Leibo, Louisiana, USA |
| 14:10 | Speaker 2 | Joni Stehlik, New York, USA  
"Oocytes vitrification for milder IVF in Europe"  
Monica Antinori, Rome, Italy |
| 14:30 | Speaker 3 | "Blastocyst Vitrification Towards Single Embryo Transfer in the United States"  
Terry Schlenker, Colorado, USA |
| 14:50 | Speaker 4 | "Oocytes vitrification for milder IVF in Europe"  
Monica Antinori, Rome, Italy |

**Coffee Break**

### Session 7: "Fertility preservation for cancer patients"

**Chair:** Bunpei Ishizuka, Kanagawa, Japan  
**Chair:** Yu Ohyama, Chiba, Japan  
**Co-Sponsored by:** Future Mother Co., Ltd.

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<thead>
<tr>
<th>Time</th>
<th>Speaker 1</th>
<th>Topic</th>
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</table>
| 15:30 | Speaker 1 | "Fertility Preservation Techniques and Strategies before and after Cancer Treatment"  
Timur Gürgan, Ankara, Turkey |
| 15:50 | Speaker 2 | "The current approach to oocytes vitrification for cancer patients in Japan"  
Takafumi Utsunomiya, Oita, Japan |
| 16:10 | Speaker 3 | "Fertility Preservation for Cancer Patients"  
Sherman J. Silber, Missouri, USA |
| 16:30 | Speaker 4 | "Ovarian tissue vitrification for cancer patients"  
Noriko Kagawa, Tokyo, Japan |

**Closing Remarks**
### Agenda of the Work Shop in Mild Approaches in Assisted Reproduction

**Date; August 1st, 2010**

Pacifico Convention Plaza Yokohama  
1-1-1, Minato Mirai, Nishi-ku, Yokohama, Japan

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<th>Time</th>
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<td>9:00-9:05</td>
<td>Opening Remark</td>
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<td>9:05-9:50</td>
<td>Natural / Mild stimulation IVF protocol</td>
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<td>Yuji Takehara (Kato Ladies Clinic)</td>
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<td>10:05-10:50</td>
<td>Clinical results of Mild stimulation using clomiphene citrate</td>
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<td>Sherman Silber (Infertile center St. Louis)</td>
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<td>11:05-11:50</td>
<td>A novel procedure for severe cases of adenomyosis-surgical treatment by the triple-flap method for reconstruction of the uterine wall Osada procedure for massive adenomosis</td>
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<td>Hisao Osada (Kato Ladies Clinic)</td>
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<tr>
<td>11:50-13:00</td>
<td>Lunch</td>
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<td>13:00-13:45</td>
<td>The efficacy and safety of managing ectopic pregnancies with transvaginal ultrasound-guided local injections of absolute ethanol.</td>
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<td>Hirotsune Kajima (Minato Mirai Yume Clinic)</td>
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<td>14:00-14:45</td>
<td>The Development and Usage for Assisted Reproduction Medical tools.</td>
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<td>Shokichi Teramoto (Shinbashi Yume Clinic)</td>
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<td>14:45-14:50</td>
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Poster session

P-001 Relations between the timing of transfer, expansion size and implantation rates in frozen thawed single blastocyst transfer


Kato Ladies Clinic

P-002 Rescue ICSI of oocytes that failed to appear the fertilization corn and cytoplasmic flare 6 hours post-insemination in conventional IVF

Tomohisa Wada, M. Kanihata, A. Kuwahata, M. Ochi, C. Kani, T. Horiuchi

1 Ochi Yume Clinic Nagoya
2 Prefectural University of Hiroshima

P-003 PSYCHOLOGICAL DISTRESS MEASUREMENTS AMONG INFERTILE WOMEN UNDERGOING IN-VITRO-FERTILIZATION

Suneeta Mittal, Latit Kumar, Samana G, Anupama Bahadur

All India Institute of Medical Sciences

P-004 DDB inhibit development of experimental endometriosis and induce apoptosis of endometrial stroma

Ali Farid Ali, Laila Farid, Mostafa Fouad, Ahmed Abd el shafy

1 Heliopolis research center
2 Ain shams university

P-005 Comparison of Galectin-9 and Galectin-3 Expression and Localization in the Human Uterodome

Mehri Azadbakht, Maryam Kabir-Salmani, Mitsutoshi Iwashita

1 Department of Biology, Razi University, Kermanshah, Iran
2 Department of Genetic Medicine, National Institute for Genetic Engineering, Tehran Iran
3 Department of Obstetrics and Gynecology, Kyorin University School of Medicine, Tokyo, Japan

P-006 Effect of maturation in vitro on spindle morphology in human oocytes

Mette Munk, Novella-Maestre, Svend Lindenberg, Steen Smidt Jensen

1 Copenhagen Fertility Center
2 Instituto Valenciano de Infertilitat (IVI), University of Valencia
3 Section for Reproductive Biology and ART, Copenhagen Fertility Center

P-007 Effect of Progesterone in cultures of follicles derived from polycystic ovaries (PCO) mouse model

Mehri Azadbakht, Ali Bazdar, Ali Amini

Department of Biology, Razi University, Kermanshah, Iran

P-008 Experience of mild stimulation IVF for patients with repeated IVF failures (Cases series)

Julia Vosnesenskaya, Yakovenko S.A.

IVF Clinic “Altra Vita”

P-009 Evaluation of the intrauterine insemination and controlled ovarian hiperstimulation cycle outcomes in our clinic

Begüm Aydogan, Orkun Cetin, Sezai Sahmay, Berna Aslan

1 Istanbul University Cerrahpasa Medical School Obstetrics and Gynecology Department
2 Cerrahpasa Medical School, Istanbul, TURKEY

P-010 The long-term difference in ovarium effects of recombinant and urinary FSH in stimulation protocols with agonists

Ales Sobek Jr, Hladikova Blazena, Tkadlec Emil, Sobek Ales

1 Fertimed
2 Palacky university, Olomouc
P-011 Recombinant FSH and urinary FSHs are all effective in ovarian stimulation in IUI cycles; a prospective study.
MESUT OKTEM 1, AHMET ERDEM 2, MEHMET ERDEM 2, NURAY BOZKURT 2, EDA DEMIR 2, ESRA NAS 5, ONUR KARABACAK 1
1 GAZİ UNIVERSITY SCHOOL OF MEDICINE, DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, DIVISION OF REPRODUCTIVE MEDICINE AND INFERTILITY
2 GAZİ UNIVERSITY SCHOOL OF MEDICINE, DEPARTMENT OF OBSTETRICS AND ANKARAGYNECOLOGY, DIVISION OF REPRODUCTIVE MEDICINE AND INFERTILITY

P-012 Supplementation of clomiphene citrate cycles with Cimicifuga racemosa or ethinyl oestradiol--a randomized trial.
Ahmed Y. Shahin, Omar M. Shaaban, Alaa M. Ismail
Assiut University, Women’s Health Center, Department of Obstetrics and Gynecology

P-013 Demographic characteristics and clinical profile of poor responders in IVF / ICSI – A comparative study
Nabaneeta Padhy, Asmita mahla, Sathya Balasubramanyam, Divyashree, Thangam Varma
INSTITUTE OF REPRODUCTIVE MEDICINE, MADRAS MEDICAL MISSION

P-014 Post-translational modifications of IGFBP1 at the embryo-maternal interface
Maryam Kabir-Salmani 1, Mitsutoshi Iwashita 2, Keiji Sakai 2, Hiromi Shibuya 2
1 National Institute of Genetic Engineering and Biotechnology
2 Kyorin University School of Medicine

P-015 Patient friendly approach to oocyte pick-up procedure.
Mette Munk 1, Svend Lindenberg 2, Suzan Lenz 2, Steen Smidt Jensen 2, Claus Christoffersen 2
1 Copenhagen Fertility Center
2 Section for Reproductive Biology and ART, Copenhagen Fertility Center

P-016 The midluteal decline in serum estradiol levels is deleterious to embryonic implantation during in vitro fertilization and embryo transfer
Yang Jianzhi, Liping Zhu, Xiaoming Teng, Fan Yang, Jianming Yu, Yu Wang
Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine

P-017 Assessment of endometrial and ovarian characteristics using three dimensional power Doppler ultrasound to predict response in frozen embryo transfer cycles
Tamara Žáčková 1, Iykka Y Järvelä 2, Juha S Tapanainen 2, Jaroslav Feyereisl 1
1 Institute for the Care of Mother and Child (UPMD), Department of IVF, Charles University
2 Department of Obstetrics and Gynecology, Oulu University Hospital

P-018 Ultrasound Guided Direct Injection Of GnRHa In The Treatment Of Lieomyoma
Ali Farid. Ali 1, Laila Farid 2, Mostafa Fouad 2, Ahmed Abd el shafy 2
1 Heliopolis research center
2 Ain shams university

P-019 Comparison of embryo transfer guided by vaginal versus abdominal ultrasound: a pilot study
Daniel Bodri, Zamora Maria-José, Coll Oriol
Clínica EUGIN

P-020 Ultrasonographically guided transvaginal hydrolaparoscopy (UTHL) with single use instruments – a safe way in the examination of tuboovarian complex.
Ales Sobek Jr. 1, Blazena Hladikova 2, Koutna Olga 2, Sobek Ales sr 2
1 Fertimed infertility centre, general hospital Prostejov
2 Fertimed
P-021 Endometrial sonographic characters predicting pregnancy following recurrent clomiphene induction in unexplained infertility.

Ahmed Y. Shahin
Assiut University, Women’s Health Center, Department of Obstetrics and Gynecology

P-022 Effect of artificial oocyte activation using Ionomycin on ICSI outcome

Shahnaz Razavi1, Shirin Reisi1, Mohamad Hosein Nasr Esfahani 2
1 Department of Anatomy, Isfahan Medical University
2 Royan Institute, (Isfahan campus), ACECR

P-023 Zeta sperm selection: A suitable method for recovery of sperm with low DNA fragmentation and protamine deficiency

Shahnaz Razavi, Shirin Reisi
Department of Anatomy, Isfahan Medical University

P-024 Effects of antisperm antibodies on fertilization, cleavage and pregnancy rate in infertile couples undergoing In-vitro Fertilization at a selected centre in Sri Lanka

Varuni Tennakoon1, Surangi G. Yasawardene 2, Deepal S. Weerasekera, James W. Catt 3
1 Prathana, Centre for IVF
2 Department of Anatomy, Faculty of Medical Sciences, University of Sri Jayawardenepura
3 Optimal IVF

P-025 Influence of immunoglobulin isotype and sperm surface location of antisperm antibodies on fertilization, cleavage and pregnancy rate in human - A Sri Lankan study

Varuni Tennakoon1, Surangi G. Yasawardene 2, Deepal S. Weerasekera, James W. Catt 3
1 Prathana, Centre for IVF
2 Department of Anatomy, Faculty of Medical Sciences, University of Sri Jayawardenepura
3 Optimal IVF

P-026 TREATMENT OF CERVICAL INSUFFICIENCY ABORTION BY AUTOLOGOUS HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS, MODERN TREND

1 Heliopolis research center
2 Ain shams university
3 Kaf EL-Sheikh University

P-027 Yeast Beta Glucan inhibit development of experimental endometriosis and induces apoptosis of endometrial stroma

Ali Farid. Ali 1, Laila Farid 2, Ahmed Abd el shafy 2
1 Heliopolis research center
2 Ain shams university

P-028 Comparison of α 6 integrin expression in mouse embryonic stem cell derived germ cells differentiated in STO co-culture system

Zohreh Makoolati 1, Mansoureh Movahedin 2, Mehdi Forouzandeh-Moghadam 2
1 Tarbiat Modares University
2 Tarbiat Modares University, Anatomical sciences department

P-029 Case Report: Efficacy of Mild stimulation and single vitrified blastocyst transfer of Preimplantation Genetic Diagnosis (PGD) –Why do we need so many oocytes?-

Kato Ladies Clinic
P-030 New strategy of ovulation induction for poor responders

Kohzo Aisaka¹, H. Hiraike¹, Y. Ikezuki¹, S. Obata¹, O. Hiraike², H. Mori³
1 Hamada Hospital, Tokyo
2 University of Tokyo
3 Teikyo University

P-031 Successful outcome in high survival rate and pregnancy rate after Single Embryo Transfer (SET) using cryotop vitrification method. – A clinical analysis for three years.

Tsuyoshi Okubo¹, Teruaki Hayashi¹, Tomohiro Sueyoshi¹, Junichiro Fukuda¹, Masahige Kuwayama², Keiichi Kato², Osamu Kato²
1 Shimbashi yume clinic
2 Kato ladies’ clinic

P-032 Effects of new protocol for estrogen and progesterone replacement with FSH/HMG simulation on patients with premature ovarian failure: a report of a case in whom fertility was successfully induced.

Yodo Sugishita¹, Mari Watanabe², Nobuhito Yoshioka², Juichiro Saito², Midori Tamura², Bunpei Ishizuka²
1 Department of Obstetrics and Gynecology, St. Marianna University School of Medicine
2 St. Marianna University School of Medicine

P-033 Heterotopic cesarean scar pregnancy: Case report

Wai Leng Wong, Lee SL, Ho TH, Tan HK
Singapore General Hospital

P-034 Heterotopic pregnancy following assisted reproduction and embryo transfer

Lay Kieng NG, Lee SL, Hemashree R, Yu S L
Singapore General Hospital

P-035 The chromosomal constitution of embryos developing from tri-pronuclear zygotes during assisted reproductive technology

Saori Maruyama¹, Naoki Aoyama², Yasuhiro Mchikura², Hiroshi Ishikawa¹, Hrokazu Usi¹, Osamu Kato², Makio Shozu¹
1 Chiba University
2 Kato Ladies’ Clinic
3 Towako Ladies’ Clinic

P-036 Utility of natural cycle ART

Masanori Ochi, A. Kuwahata, T. Wada, M. Kamihata
Ochi Yume Clinic Nagoya

P-037 The Effectiveness of Clomiphene Citrate in Suppressing the LH Surge in the Minimal Stimulation IVF Protocol

Satoshi Kawachiya, Kato K, Osada H, Takehara Y, Kato O
Kato Ladies Clinic
Mild ovarian stimulation for IVF

Bart CJM Fauser
University Medical Center, Utrecht, The Netherlands

The history of IVF has been characterized by profound ovarian stimulation protocols, in an attempt to optimise pregnancy rates per cycle. These approaches, aiming at generating many oocytes, were meant to counterbalance inherent shortcomings in in vitro oocyte fertilisation, embryo culture, as well as embryo selection for transfer and transfer. Another reason often put forward to justify profound stimulation is the cryostorage of surplus embryos, providing additional pregnancy chances in subsequent unstimulated cycles. Over the years ovarian stimulation protocols have become extremely complex and time consuming, associated with much patient discomfort and considerable complication rates. Moreover, costs of applied medication may outweigh the cost of IVF treatment itself.

Mild IVF may involve mild ovarian stimulation, mild transfer policies (i.e. single embryo transfer in selected patients), or both. Both strategies result in a reduction in the pregnancy rate per cycle. Only when cryopreserved embryo transfer cycles are included in success rates, overall pregnancy rates become comparable in IVF units with good laboratory performance. The aim of milder forms of ovarian stimulation is to render stimulation less complex, less time consuming and less costly, while improving patient acceptability by reducing side effects and chances for complications. Reported pregnancy rates per started cycle also reduced following mild stimulation. However, mild stimulation improves the cost effectiveness of IVF (hopefully resulting in augmented access to IVF) and may also reduce drop-out rates. Therefore, cumulative pregnancy rates of a given treatment strategy are likely to be similar as shown by some recent studies. Certainly, the recent introduction of GnRH antagonist helped to reduce overall drug consumption and considerably reduced the duration of stimulation. Therefore, more IVF cycles can be performed in a given period of time when viewed from a per treatment rather then a per cycle paradigm.

Further improvement of embryo selection and cryostorage is warranted for the wider acceptance of mild ovarian stimulation and single embryo transfer. Moreover, more individualized approaches may reduce both hyporesponse (and cycle cancellation) and hyperresponse and therefore further improve safety and efficacy of treatment. Hence, the focus of further development in ovarian stimulation should shift from mild stimulation towards mild ovarian response.
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 Epididymal 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.
Infertility care in poor-resource countries: The Arusha project

Willem Ombelet  
Department of Obstetrics and Gynecology, Genk Institute for Fertility Technology  
(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.
Low cost IVF for developing countries

Ian Cooke
Director of Education, IFFS; CEO Low Cost IVF Foundation

“Low cost IVF for developing countries” has become a mantra for reducing costs of a treatment cycle in the hope that a cheaper regime will be applicable to low resource environments. Such regimes are characterised by grossly reducing drug, equipment and laboratory consumable costs. However staff costs, even although lower than seen in developed world clinics, are not included; these will need to cover medical, nursing and administrative staff. Counselling staff, recruited slowly and often unwillingly in better resourced environments, are also required. The availability of adequate, dedicated space may depend on whether a unit is to be created in the public or the private sector. The commitment to develop a unit in the public sector requires political will and allocation of resources for which there are other, seemingly more pressing, demands. Calculated costs need ultimately to be validated as cost-effectiveness in a local venue, but, as elsewhere, initial results are likely to be poorer than those subsequently obtained.

Funds are required to establish a unit and to maintain it but, after a modest time, such units will need to be self-sustaining. Although external funds may be available, it is proving difficult to generate these. Further, to initiate a clinic, staff need to be trained. This would best be undertaken in an adjacent country where training costs may be better contained and where there is an appreciation of the conditions likely to be experienced on the trainees’ return.

There is a need to recognise the various levels of health care, particularly if the WHO objective of primary care is not the fundamental unit. Referral systems need to be established, so that basic investigation, and treatment such as IUI, can be carried out at earlier stages. In addition, it is rational to be able to care for the woman, pregnant by IVF, in an adequate maternal and child health care system.

The ultimate charge for a treatment cycle may not be as low as the 100€ advocated by Habbema (2008), to be included in a national health service. It will depend on the income of the population quintiles and how far down the quintiles there is political will to reach. It will also be dependent on the results and there will need to be a recognition of what can be achieved in a simple system. Cumulative live birth rates are attractive following successive treatments, but costs mount and lift IVF beyond common grasp. They will also be influenced later by adoption of cryopreservation and sperm preparation in HIV patients.

Access to IVF will remain limited where there is a system of out-of-pocket expenses, the greater this contribution, the greater the risk of catastrophic expenditure for a family. Although there is ethical agreement that IVF should be available in low resource economies, establishing the first such unit is demanding. Developing more peripheral units to provide a national network, the first unit acting as a training centre, will be a much greater challenge.
Low Cost Controlled Ovarian Stimulation in ART - Our Experience

Sudarsan Ghosh Dastidar
GD Institute for Fertility Research

Introduction;
Cost of Controlled Ovarian Hyperstimulation (COH) using standard mid luteal GnRH agonist protocol (GnRH-A) and FSH is often beyond the reach of relatively poor resource patients. Furthermore, the incidence of ovarian hyperstimulation syndrome (OHSS) is reported to be higher in long GnRH-A protocol. It has also been suggested that treatment related stress over a prolong duration in GnRH-A protocol is the most important reason for patient to dropout from IVF program. Recently, mild ovarian stimulation has been advocated by many workers with the aim to develop cost effective, patient friendly and safer protocols where risks of treatment are minimized. Thus we undertook a study to compare the result of a minimal ovarian stimulation with CC-FSH/hMG and GnRH Antagonist (Study Group A) protocol with those of standard long GnRH-A protocol used in IVF (Control Group B). This study was conducted with the purpose to design a stimulation protocol which is cost effective, less stressful and of shorter duration.

Material and methods;
A prospective clinical trial was designed. Study group consisted of 54 patients undergoing IVF using minimal stimulation protocol using Clomiphene Citrate (CC) 100 mg per day from Day-2 to Day-6 of cycle and HP-FSH/hMG (150 IU) on D4, D6 and daily from D7. Serial TVS started from D7 along with serum E2 level estimation. GnRH Antagonist (Cetrorelix 0.25 mg sc) administrated daily when lead follicle measures 13-14 mm in diameter and continued till day of hCG. Women in the control group (matched for age and cause of infertility, n=62) underwent standard long mid luteal GnRH agonist (GnRH - A) - gonadotropin stimulation during the same study period.

Results;
The number of oocytes retrieved was significantly lower in the CC-FSH/hMG group compared to the long GnRH – A protocol. However, the clinical pregnancy rate per transfer was not significantly different between the protocols (A, B : 31.3%, 36.6%). Average cost of COH in CC- FSH/hMG antagonist group was nearly 40-50% less compared with that in standard GnRH-A protocol. Furthermore, there was zero incidence of moderate to severe ovarian hyperstimulation in study group.

Conclusions;
CC-gonadotropin with GnRH antagonist appears to be a reasonably efficient alternative approach to offer safe, low cost IVF to patients of poor economic status. Significantly fewer oocytes in CC group is most likely to translate into non-availability of excess embryos for cryo-preservation and thus a lower cumulative pregnancy rate with fresh and frozen – thawed embryos transfer. However, this might not be a significant drawback since added costs of cryo-preservation and subsequent thaw cycles itself is a financial deterrent for many patients in developing countries, especially where insurance coverage is not offered.
Impact of 19,467 IVF cases/year based on natural-cycle IVF

Yuji Takehara
Kato Ladies Clinic

Although in the initial period, IVF was based on natural cycles, today’s IVF standard is based on controlled ovarian hyperstimulation (COH). COH is a convenient method for doctors, technicians and nurses, but not so good for the patients, because of side effects such as OHSS, the high cost of the injection medicines, and the burden of daily outpatient visits. We have encountered loss of consciousness in a patient whose blood data and echo findings did not show critical OHSS. Fortunately, this patient recovered completely and subsequently became pregnant and delivered normally. Since then, we have become suspicious about COH, as fertility-clinic patients are basically healthy and should under no circumstances be exposed to life-threatening side effects. That prompted us to start minimal-stimulation IVF and later revert to completely natural-cycle IVF. Here, we present our current IVF protocols and our results for the year 2009.

Our IVF protocols include 1: completely natural cycles, 2: clomiphene citrate (CC)-only cycles, 3: CC plus low-dose hMG/rFSH injection cycles, 4: CC plus nasal hMG cycles, 5: Femara cycles, and 6: daily hMG/rFSH injection cycles in patients suffering from pituitary dysfunction. Last one is almost identical to so-called stimulation cycle, but the percentage is quite low and very rare in our clinic.

GnRH agonists are applied to induce intrinsic LH surge to cause final oocyte maturation, hCG injection is contraindicated in order to avoid oocyte damage and promote production of residual follicles in the subsequent menstruation cycle. Aspiration of follicles is done without any anesthesia using fine 21 G needles. In all cases, embryo transfer (ET) is performed as a single embryo transfer by a transvaginal echo-guided procedure. Day-2 embryo transfer, namely, cleavage embryos, is employed when no tubal factors exist, and blastocyst transfer is selected in cases with tubal problems and/or repeated day-2 ET failures. In cryopreserved blastocyst transfer, laser-assisted hatching is applied to slow the growth of embryos in which it takes more than 120 hours from insemination for the blastocyst to form. All blastocyst transfers are based on completely natural ovulation cycles as far as the menstruation cycles are regular. Hormone-replacement ET is adopted for patients whose cycles are anovulatory or irregular.

The total number of needle aspirations in 2009 was 19,467 at the Kato Ladies Clinic alone. The patients ranged in age from 21 years old to 54 years old, with an average age of 39.4±4.7 years old. The hCG-positive rates (β-hCG>20.0) subsequent to embryo (d2) or blastocyst (d5) transfer without cryopreservation in groups 1 to 6 in which the aforementioned protocols were applied, were 43.9, 28.2, 33.2, 19.8, 60.5 and 26.7%, respectively. The clinical pregnancy rates (FHB+ % per transfer) in groups 1 to 6 were 35.1, 19.9, 24.3, 13.7, 47.4 and 13.3%, respectively.

In conclusion, completely natural cycles yielded the best pregnancy rates when the retrieved oocytes became ET-possible embryos. These data encourage us to promote natural-cycle IVF and we have no valid reason to rely on stimulation protocols.
The immunological relationship between mother and conceptus still remains a mystery, although the recent advances in molecular biology have lighten a lot of the parameters that participate in feto-maternal cross-talk during implantation. The atypical expression of major histocompatibility complex (MHC), the specific role of some hormones and cytokines, as well as the modified function of cellular constituents of the feto-maternal interface, represent substantive parameters of fetomaternal immunotolerance during implantation. However the implantation process is currently considered the most important limiting factor for the establishment of a viable pregnancy and the fertility physician is often called upon to perform the unpleasant task of counselling an infertile couple after repeated implantation failure (RIF). Aetiology is often not clear and treatment options are indistinct. Some of these include hysteroscopic treatment, myomectomy, preimplantation genetic diagnosis for aneuploidy screening (PGS), assisted hatching, blastocyst transfer, zygote intra-Fallopian transfer (ZIFT), salpingectomy of hydrosalpinges and immunological treatment. Since some of these remedies have not been proven to be effective (the evidence behind some of these is robust), assisted reproduction programmes should resist offering treatment options that are not evidence based at least until well designed randomized studies show the value of what are today considered as empirical treatments.
Gentle ovarian stimulation protocols have several advantages over conventional IVF protocols, including less medication and few injections, producing less eggs, but eggs of higher quality. “Mini-IVF” is safe, patient friendly, and is physiologically more natural. It may be more cost effective if results are comparable to conventional protocols. Vitrification of embryos allows transfer of thawed embryos in subsequent cycle when the endometrium is more receptive. 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 single embryo transfers for gentle stimulation protocols and vitrification in preference to standard conventional IVF stimulation protocols. Now a randomized control trial is required.
Non invasive assessment of oocytes and embryos: improving embryo selection to transfer

Juana Crespo
INSTITUTO VALENCIANO DE INFERTILIDAD

The method used worldwide to select the embryos to be transferred is the static morphological criteria based in punctual observations during embryo development. The implantation rates have been minimally improved in the last decade and now, IVF labs tend more and more to the single embryo transfer to avoid multiple pregnancies. Therefore, there is a need to develop non-invasive methods applicable to the entire IVF population to improve oocyte and embryo selection, increasing the implantation rates. Nevertheless, a perfect morphologically embryo does not always exclude chromosomal abnormalities. The aims of our lines of research are in one hand to develop a new non-invasive metabolomic approach to identify normal embryos from those with aneuploidies using the spent culture media before embryo transfer. These metabolomic profiles will be obtained with an UPLC (ultra performance liquid chromatography) coupled to a Mass spectrometry instrument. In the other hand we purpose the oxygen consumption measurements from oocytes and embryos together with the assessment of the kinetics of embryo division to be applied routinely in the clinical embryology laboratory in order to assess embryo quality by using an automated instrument with programmable measurement cycles for unattended operation. This would allow data to be collected on respiration rates of single oocytes and embryos during development as well as taking measurements of embryonic development by analysing time-lapse images in real time to quantify the timing of cell division.

Once these parameters will be determined as appropriated we will be able to:
1. Introduce a novel quantitative method measure oocyte quality (actually this determination is based in subjective morphological features).
2. Substitute the standard procedures to select embryos for transfer following exclusively morphological criteria by those combining embryo media metabolomic profile with morphology, division kinetic and respirometry.

By undergoing this project we will be able to improve implantation rates decreasing embryo manipulation (strictly necessary for the standard procedures) which considerably alters in vitro culture conditions.
Assessment of embryo viability before transfer: Genomics, Proteomics and Metabolomics

Denny Sakkas
Yale University School of Medicine, Department of Obstetrics & Gynecology

Utilization of assisted reproductive technologies (ART) continues to increase annually worldwide. The high success rates enjoyed through IVF are attained in many cases only through the simultaneous transfer of multiple embryos. The risks related to multiple gestations include preterm delivery with all of the known clinical sequelae. Decreasing the prevalence of multiple gestations while maintaining or improving overall pregnancy rates remains the most significant contemporary goal of infertility research.

The sentinel issue that prevents physicians from limiting the number of embryos transferred is the inability to accurately estimate or profile the reproductive potential of individual embryos within a cohort of embryos using existing diagnostic techniques (i.e., morphological evaluation). If embryologists and physicians could accurately and consistently identify viable embryos, IVF practitioners would be able to reduce multiple gestations while ensuring good pregnancy outcomes. Ideally this technology must fit certain parameters in that it must be capable of predicting embryo viability through a rapid, invasive or non-invasive sampling, and reproducible platform so as to not encumber current IVF clinical practice.

Gene expression analysis and proteomic profiling, and more recently, analytical examination of the embryonic metabolome are showing great promise in fulfilling the necessary criteria for performing embryo profiling. Metabolomic profiling of embryo culture media using optical and non-optical spectroscopies is providing a greater insight into the identification of embryos with increasing reproductive potential. In the near future technologies using genomic, transcriptomic, proteomic and/or metabolomic information will be routine practice in the clinical IVF laboratory allowing for a more accurate assessment of which embryo will lead to a normal live birth.
A significant number of human pregnancies end up in early losses following assisted reproductive technologies (ARTs). Recently, it is apparent that most problems arise from chromosomal abnormalities in the embryos. Although the mechanisms for the onset of these abnormalities are still under discussion, nondisjunction and chromosomal breakage during chromosome segregation process at very early stages are likely causes. Then, to monitor this process without affecting embryo viability, we tried to establish a ‘minimum damage’ live-cell imaging technology of the preimplantation embryo. By means of this technique, we assessed the chromosome integrity in ART-generated embryos and the linkage between chromosome abnormalities found in early cleavage stage and their impact on the developmental potentials was evaluated.

A mixture of mRNAs encoding enhanced green fluorescent protein (EGFP) coupled with α-tubulin and monomeric red fluorescent protein 1 (mRFP1) fused with histone H2B was used as fluorescent probes. Anaphase II/telophase II stage oocytes were injected with mRNA, transferred to the imaging system and long-term imaged until blastocyst stage. By optimizing the devices and conditions for imaging, the procedure itself was not detrimental to full-term development even after a prolonged imaging process. When ICSI embryos were monitored for their chromosome integrity, we found that some embryos with normal morphology as seen by conventional light microscopy had abnormal chromosomal segregation (ACS) at the first mitotic division. Pieces of chromosomes were misaligned during the first metaphase and formed micronuclear-like structures at the interphase of the 2-cell stage. The incidence of ACS in ICSI embryos (range 10.4-46.7%) was significantly higher than in IVF embryos (1.7%). Similar ACS was also found in ROSI-generated mouse embryos, but even more frequently. Giemsa staining and Immunostaining revealed that these fragments were derived from double-strand DNA breaks in the sperm genome. About half of the embryos with ACS developed into normal-looking morulae or blastocysts and implanted, but almost all of them aborted spontaneously before embryonic day 7.5. These data suggested that the ACS during first mitosis appears to be a major cause of early pregnancy losses in ART-generated mouse embryos. In other words, our live-cell imaging technique allowed the identification of defective embryos among normal-looking 2-cell mouse embryos. Thus, the overall pregnancy rate could be improved by excluding such embryos before performing embryo transfer. Moreover, the ICSI procedure increases the risk of chromosomal breakage, so we might need to reevaluate the safety of ICSI in human ART.
In Vitro Maturation of Human Oocytes

Svend Lindenberg  
Copenhagen Fertility Center

Human in vitro maturation techniques have now developed into a clinical useful tool in the treatment of specific well characterized groups of infertile women.

The IVM procedure is specifically useful in the following situations:

1) In programs aiming at cryopreservation of human oocytes for preserving fertility

2) In situations where OHSS is eminent and early follicular puncture is indicated

3) In FSH/hCG primed IVM treatment where rescue IVM is used in connection with support of the few MII oocytes retrieved.

Using the above mentioned approaches IVM associated techniques seems to add a valuable new entity to the human ART technology.

The results from these treatments approaches 30% baby take home rates and this treatment modality is also becoming a very important new technology in clinical management of high risk patient in our clinics.
2

Optimal IVM-IVF for routine clinical use

Aisaku Fukuda

IVF Osaka Clinic

Although IVM-IVF is a relatively new option for assisted reproductive technologies (ART) promising significant benefits (e.g., prevention of OHSS, lower medication cost and less stress for the patient), IVM-IVF success rates are still less than conventional ART. The first successful IVM-IVF in the world was achieved in 1994 and in Japan by our group in 2000. From October 1999 through December 2009, we have performed 777 cycles of IVM-IVF (566 through fresh transfer and 211 cycles via frozen-thawed transfer) and have achieved 102 successful pregnancies (22.2%). When we divide the latter nine years into three terms (term 1: 2001-2003, term 2: 2004-2006 and term 3: 2007-2009) and compare them, the average pregnancy rates of each term in total (fresh and frozen cycles) and fresh cycles only increased incrementally, 14.9%, 23.3%, 33.9% and 17.1%, 21.7%, 35.4%, respectively. The pregnancy rate of term 3 was significantly higher compared either to term 1 (P<0.01) or term 2 (P<0.06). Multiple strategies such as frozen cycles, low dose FSH, HCG pretreatment, Metformin administration and LH surge by GnRH agonist, alone or in combination, have been attempted in the meantime. We focused on Metformin in this study, because Metformin was routinely used in term 3. Fresh cycle IVM-IVF was performed on 152 PCO patients in term 3, either with Metformin pretreatment of 1500mg/day for at least four weeks (Group A; n=61) or without Metformin (Group B; n=91). Follicular monitoring began from cycle day 7, and if needed, 150 units/day of FSH were administered for 3 days on average until follicles reached 10 mm in diameter. HCG of 10,000 i.u. 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. The pregnancy rate and number of oocytes retrieved in group A (51.2% and 12.2) were significantly higher (P=0.01) than in group B (30.9% and 9.1). However, there were no significant differences in the rates of maturation (53.0% vs. 50.4%), fertilization (82.8% vs. 87.4%), and good quality embryos (37.3% vs. 28.2%). Furthermore, the doses of FSH (285 i.u. vs. 259 i.u.) and the levels of E2 (244 pg/ml vs. 229 pg/ml) and T (0.6ng/ml vs. 0.51 ng/ml) were not significantly different. The study suggests that Metformin pretreatment of PCO patients improves clinical outcome of IVM-IVF comparable to conventional ART by increasing the number of oocytes retrieved. Metformin did not change the levels of T and E2, and neither did the dose of FSH required and the rates of maturation, fertilization and the number of good quality embryos. IVM-IVF might be applied as a first choice of ART for PCO patients with Metformin pretreatment because of its advantages for the patient, both medical and financial.
Natural cycle IVF combined with IVM

Jin-Ho Lim
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.
Stuart Campbell
St George’s Hospital Medical School, Academic Department of Obstetrics and Gynaecology
One-Stop Fertility Assessment – The role of advanced ultrasound technology

Geeta Nargund
The Centre for Reproduction & The Advanced Technology

One-Stop Fertility Assessment
The role ultrasound scan is pivotal in the assessment of fertility. A routine gynaecological scan does not provide the information required to assess the fertility potential. Therefore the term “Fertility scan” is coined to describe a comprehensive assessment of uterus, endometrium, ovaries including blood flow measurement and rule out pelvic pathology. This can be combined with the assessment of fallopian tubes (HycoSy).

Fertility scan
- **Uterus:**
  - position, mobility
  - dimensions
  - congenital anomaly (3D)
  - fibroids / adenomyosis
  - uterine cavity investigation by hydrosonography
  - uterine blood flow; uterine artery PI & PSV
- **Endometrium:**
  - thickness
  - morphology
  - colour or power Doppler assessment
- **Ovary:**
  - morphology: normal, polycystic or multicystic
  - position and mobility
  - volume / antral follicle count
  - stromal blood flow (PSV)
  - identification of cysts, endometrioma, dermoid etc
  - Doppler assessment of cyst
  - dominant follicle; mean diameter, Doppler
  - perifollicular blood flow
  - presence of hydrosalpinx
- **Pouch of Douglas:**
  - free fluid
  - masses
The technique of embryo transfer is a very important determinant of the success of In Vitro Fertilization. We have recently developed a Transfer catheter to be used specifically for vaginal ultrasound guided embryo transfer.

The COOK Echotip Soft-Pass Transvaginal Probe Embryo Transfer Catheter (TVP Catheter), is a soft catheter with an echogenic tip, an outer sheath, a lock to fix the inner catheter, preventing it from moving, and a stopper on the outer sheath, which will determine its travel within the endometrial cavity. The design of the catheter is to allow a Transvaginal probe to be used for embryo transfer, enhancing visualization and eliminating the need for a speculum during transfer. The catheter is designed with a cervical stop that is tapered on the back-end to allow for easy removal from the vaginal cavity. The cervical stop can be adjusted and placed to a desired location, which is determined by an initial Transvaginal probe scan of the cervical canal and endometrial cavity. The guide catheter which is 17 centimeters in length has a bulb tip on it to help negotiate through a difficult cervix. On the back-end of the guide catheter, there is a securing adapter (Tuohy-Borst locking mechanism), which can be tightened to assure that the transfer catheter does not advance or withdraw during insertion. The guide catheter is also made of a material that has memory, allowing a curve to be preset for placement.

The transfer catheter is made using a soft material with an echogenic tip, it also has a stiffening cannula on the back portion to allow for more pushability.

The materials that make up the Echotip Soft-Pass Transvaginal Probe Embryo Transfer Catheter (TVP catheter) have been through biocompatibility testing and approved for use.

Results:

<table>
<thead>
<tr>
<th>Age</th>
<th>Pregnancy Test</th>
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<tr>
<td>&lt;35</td>
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<td>35-37</td>
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<tr>
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<tr>
<td>40-42</td>
<td>0%</td>
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<tr>
<td>&gt;42</td>
<td>20%</td>
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The COOK TVP embryo transfer catheter for use with vaginal ultrasound, was found to have several advantages which include:

1. Better visualization than abdominal ultrasound.
2. Inner catheter is exposed only in the uterus, hence decreasing the chance for contamination.
3. No need for an ultrasound technician.
4. No need for stylet except in very rare difficult cases.
5. No need for full bladder.
6. Avoids touching the fundus.
7. Not cumbersome. (No need for speculum)
8. The catheter made the physician more comfortable and sure of his transfer.
9. The catheter was used successfully in patients who are obese, with history of c-section scar, severe retroversion, severe antversion or uterine anomalies.

Conclusion:
The use of the TVP catheter with vaginal ultrasound guidance, gives the physician comfort and confidence in his or her transfer, and it is not cumbersome to use.
Vitrification for oocytes and embryos

Stanley P Leibo
Department of Biological Sciences, University of New Orleans Audubon Center for Research of Endangered Species

Twenty-five years ago, Rall and Fahy described the ice-free cryopreservation of mouse embryos by a procedure that they referred to as vitrification. Since then, this procedure has been successfully applied to the embryos of at least 11 mammalian species, success meaning the birth of live offspring. The procedure has been especially efficient for the cryopreservation of human oocytes and embryos. Because the procedure of vitrification has been so widely adopted by the international clinical community, it is difficult to document precise figures. Dozens of experimental reports have described the births of children derived from oocytes or embryos that had been cryopreserved by vitrification. Nevertheless, it can be reasonably estimated that many, many hundreds, probably thousands of children have now been born from vitrified oocytes or embryos. Parenthetically, it should also be noted that spermatozoa have also been successfully vitrified.

In the first report by Rall and Fahy and almost universally endorsed by most investigators in the field, the keys to successful cryopreservation by vitrification have been considered to be the use of highly concentrated solutions of cryoprotectants and high cooling rates. Considerable attention has been devoted to designing devices and embryo holders that yield increasingly high cooling rates. Stated simply, “if fast cooling is good, faster cooling must be better.” There have also been efforts to construct embryo containers that will prevent viral or microbial contamination during the cooling process itself or during storage in liquid nitrogen.

Given the success that has been achieved with vitrification of reproductive cells, it is hard to argue that the method has flaws or limitations. Nevertheless, there are data that suggest that even rather low concentrations of cryoprotectants can also be used successfully to vitrify oocytes and embryos. Furthermore, re-examination of some of the older data as well as new observations indicate that the rate at which cryopreserved specimens are warmed is probably more important than cooling rate to yield high survival. Since cryopreservation of human oocytes and embryos has become an essential aspect of assisted reproduction in humans (and in animals), efforts to refine and optimize these methods will certainly continue.
Joni Stehlik
New Hope Fertility Center
The ability to cryopreserve human oocytes confers significant advantages in clinical assisted reproduction. The procedure could benefit especially those couples and countries where embryo cryopreservation can’t be applied because of religious concerns and legal restrictions. Moreover, oocyte cryopreservation can be applied to preserve fertility of female cancer survivors and patients at risk of spontaneous premature ovarian failure, to delay childbearing and to quarantine oocytes in oocyte donor program.

Since for a long time the slow cooling methodology, originally validated in the 1990’s, has been perceived inefficient, adoption of oocyte cryopreservation remained limited and the majority of ART clinics couldn’t offer it to patients as a trustable procedure to support the infertility management.

Thanks to the introduction of an ultra rapid cooling technique, called vitrification, that produces a glass-like solidification of cells, completely avoiding intra-cellular ice crystallization during the cooling and warming process, a new perspective has been offered regarding the management of ART attempts.

According to the current literature, regardless the kind of protocol and cryocarrier applied, human oocytes vitrification can provide worldwide excellent results in terms of survival, embryo cleavage, pregnancy and healthy babies born.

For these reasons, vitrification can be considered a valid tool applicable even to milders, cases where the reproductive strategy provides a small number of oocytes to be injected.

Due to the limitations imposed by its legislation in 2004 and thanks to the following introduction of vitrification, Italy can be considered the best example in Europe of an effective application of a “friendly ovarian stimulation” that provides oocytes with a such considerable quality that once cryopreserved with an efficient protocol can offer to patients reliable results.
Introduction;
Trophectoderm (TE) biopsy can be performed on either day 5 or day 6 post oocyte retrieval relative to blastocyst development. Accordingly, cryopreservation becomes an essential clinical component as limited time is available for genetic analysis prior to the timing for a fresh transfer. The aim of this study was to evaluate the efficacy of vitrification following TE biopsy of human blastocysts for comprehensive chromosomal screening (CCS) that could contribute to the practical application of single embryo transfer in the United States.

Material and methods;
A total of 184 patients (mean 37.6yrs) were recruited for TE biopsy followed by vitrification with a subsequent frozen blastocyst transfer (FBT). A hole in the zona pellucida was created on D3 of culture. At the blastocyst stage, TE cells protruding out of the breach were biopsied for genetic analysis. Comprehensive chromosome screening was performed for all 23 pairs of human chromosomes. Biopsied blastocysts were vitrified utilizing the cryotop device with a DMSO/ethylene glycol protocol. Only euploid (correct number of chromosomes) blastocysts were selected for transfer in a subsequent FBT.

Results;
The survival rate following vitrification and warming of biopsied blastocysts was 97.38% (335 out of 344). A total of 56 women (30.4% of patients) received a transfer of a single chromosomally euploid blastocyst in an FBT cycle. Clinical pregnancy with positive fetal heart tones (FHT) was detected in 34 of the 56 patients (60.7%) with 35 positive FHT implantations (62.5%) from 56 blastocysts transferred. Only 3 women experienced an early pregnancy loss within the first trimester (8.6%). During the same time period, 272 patients underwent an FBT with non-biopsied conventionally frozen blastocysts. Survival rate after thawing was 83% (681/820) (P<0.0001), with a 57% (156/272) clinical pregnancy rate (FHT) (P<0.001) and 35% (211/600) implantation rate (FHT) (P < 0.0001).

Conclusions;
High survival, pregnancy and implantation rates were observed following blastocyst biopsy, CCS and vitrification. Vitrification is a successful means for cryopreservation of human biopsied blastocysts and allows for time interval required for genetic analysis of D5 and D6 human blastocysts in an IVF cycle. In addition, these results indicate the clinical potential for successful single embryo transfer.
There are many male and female patients of young age diagnosed with some form of invasive cancer. With current treatment regimens, including aggressive chemotherapy, radiotherapy, bone marrow transplantation, and surgery, the cure rate for some malignancies now is very high. These treatments, however, can lead to gonadal failure and permanent infertility. Fertility preservation is a significant concern for such men and women faced with cancer treatment. Today, 1 in 700 young adults is a cancer survivor and it is estimated that 1 in every 250 adults will be a childhood cancer survivor in 2010. In United States, more than 20,000 children and young people of reproductive age are exposed to chemotherapy and/or radiotherapy every year. Also, delaying childbearing for social or financial reasons leads to more women suffering from fertility threats due to early-stage cancers discovered.

Advances gradually obtained in cancer management performed using surgery, radiotherapy and chemotherapy have resulted in significantly improved cure rates especially in young patients with certain malignancies. Thus, the patients may hope relatively higher cure rates associated with long term survival. Nevertheless, currently used cancer therapies are often detrimental to fertility. The ovaries are highly susceptible to such therapies and may be damaged significantly after chemotherapy and/or radiotherapy. Therefore, the patient may suffer from premature menopause and infertility which may impact her quality of life and self-esteem significantly.

The most common adverse effect of cancer therapies is premature menopause. Even if the patient experiences normal menstrual cycles after cancer therapy, premature menopause will be more likely due to the reduced follicular reserve. Several alternatives have been attempted in effort to preserve fertility in young women undergoing cancer treatment. Although the ovarian tissue cryopreservation has recently been the focus of intense investigation, cryopreservation of embryos and mature oocytes has several advantages over ovarian tissue preservation. Also there are some strategies for minimizing female gonadal toxicity caused by cancer therapy including use of radiation shields, transposition of the ovaries out of irradiation field, suppression of ovaries by administration of gonadotropin releasing hormone agonists during adjuvant chemotherapy. In addition, fertility-saving surgical approaches are used in selected women with gynecologic cancers instead of more radical surgical procedures. Similarly, fertility preservation options such as conservative surgical approaches including partial orchiectomy with or without cryopreservation in testicular cancer patients and at least sperm cryopreservation in other male cancer patients should be offered before initiating therapy. Use of embryonic stem cells as a source for gametes also emerges as a hope in male and female cancer survivors.
The current approach to oocytes vitrification for cancer patients in Japan

Takafumi Utsunomiya
St. Luke Clinic

OBJECTIVE:
Aggressive chemotherapy and radiotherapy have greatly enhanced the life expectancy of young cancer patients, but these treatments cause massive destruction of the ovarian reserve resulting in infertility or sterility. However, oocyte cryopreservation can preserve their fertility of these patients after cancer treatment. We applied a minimal ovarian stimulation protocol using clomiphene citrate (CC) to retrieve mature oocytes, and the cryotop vitrification method to cryopreserve them in Japan.

MATERIALS AND METHODS:
Seventy two unmarried hematopoietic defect patients with informed consent who underwent the CC cycle from January, 2007 to March, 2010. Fifty mg CC was administered from cycle day 3 and 75 IU recombinant FSH was administered every other day from day 8 until the leading follicle developed to 18 mm in diameter. Administration of CC was then stopped, and 300µg GnRH-agonist (buserelin) was given as a maturation trigger. Oocytes were retrieved from 30 to 36 hrs following the administration of the GnRH-agonist using a 22 gauge needle with local anesthesia (Teramoto, 2007). The retrieved oocytes were denuded before vitrification. The cryotop method (Kuwayama 2005) was used to vitrify the oocytes. The oocytes were equilibrated in 7.5% ethylene glycol and 7.5% DMSO in modified medium 199 (M-199) for 15min before being transferred into the vitrification solution (VS) for 30 sec. Oocytes were then transferred into onto the cryotop with minimum volume, and immediately submerged into liquid nitrogen.

RESULTS:
Oocyte retrieval and cryopreservation was successful in 89% (64/72) of the patients. No patients had any adverse side effects using the minimal ovarian stimulation protocol and aspiration with a 22 gauge needle under local anesthesia. The mean age of the patients was 27.2 (±5.1, S.D.). The mean numbers of oocyte retrieval cycles per patient was 1.7, and the mean number of retrieved oocytes per patient was 7.1, and per cycle was 4.2. The mean number of morphologically normal cryopreserved oocytes per patient was 5.6, and per cycle was 3.4. The type of cancers included acute and chronic leukemia, malignant lymphoma, aplastic anemia, and myelodysplastic syndrome.

CONCLUSION:
Our data showed that the minimal ovarian stimulation protocol using CC for oocyte retrieval with a 22 gauge needle and with local anesthesia was a safe, simple, effective method of preserving fertility for unmarried cancer patients.
A successful case of human ovarian tissue transplantation between monozygotic (MZ) twin sisters discordant for premature ovarian failure (POF) was first reported in 2005 (1). Menstrual cycles resumed after four months, and spontaneous pregnancy occurred after the second ovulation, leading to the birth of a healthy child. Subsequently, a consecutive series of seven more successful cases was reported for a total of eight, all demonstrating ovulatory cycles with normalised serum FSH levels (2,3). Spare ovarian cortical tissue from the donor ovary was cryopreserved for future grafting as a backup in case the first transplant became depleted of follicles and ceased to function. In a ninth case a different technique was used, microvascular transplantation of a whole ovary, and this too led to a prompt return of normal cycles, pregnancy by natural conception and the delivery of a healthy child (4). Thus far, 12 pregnancies and eight healthy babies have resulted from these cases, none of whom required immunosuppression.

Despite this apparent success, there has been concern whether ovarian tissue grafts, either fresh or cryopreserved, have only transient function (5,6,7). So far, there have been a few successful cases reported of thawed autotransplanted ovarian tissue in former cancer patients (8,9,10,11,12,13), but information about graft longevity is sparse, and successes were only sporadic case reports. The present report represents a long-term followup of the duration of function of fresh and frozen human ovarian grafts in a large series so as to estimate the degree of follicle loss from ischemia, and from cryopreservation.

One solution to ischemic loss, microsurgical transplantation of an intact, whole ovary is technically much more difficult and risky than cortical grafting (14,15,16,4). It would, however, be preferable and simpler if a cortical grafting technique could minimize loss. A long-term follow-up of our series of MZ twins offered an unusual opportunity to study the duration of function of fresh ovarian cortical grafts to evaluate oocyte loss from the transplant itself without the confusion created by cryopreservation, and to try to improve results with cryopreservation by vitrification (17). We now have evidence of long-term ovarian function in the current twin series suggesting that a substantial reserve of follicles survives in fresh cortical grafts despite being subjected to lengthy ischaemia compared to vascular transplantation. We have also found that slow freezing causes significant loss of oocyte viability compared to the vitrification technique. Ovarian transplantation in humans is a robust procedure, even after cryopreservation, and vitrification might prove to be more effective than slow freezing.
Ovarian tissue vitrification for cancer patients

Noriko Kagawa
Kato Ladies’ Clinic

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 of the urgent patients who does 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. Based on the high efficiency ultra rapid vitrification, the Cryotop method.

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.
Mild ovarian stimulation with clomiphene citrate

Shokichi Teramoto
Shinbashi Yume Clinic

Clomiphene citrate is a very common and popular drug. It is often used for the treatment of infertility from the early stage. Users can immediately feel the effect of the drug, but its negative effect can also be experienced, that is, ovulation does not occur immediately. Luteinization without ovulation (i.e., luteinized unruptured follicle) often occurs as well. Nevertheless, many physicians performing infertility treatment do not pay particular attention to the reason behind this problem and they try to overcome such problem by using human chorionic gonadotropin (HCG). Delayed ovulation is actually a noteworthy phenomenon. The author speculated that delayed ovulation is attributable to the competitive inhibitory effect of enclomiphene, an isomer of clomiphene citrate, on estrogen and reported in 2007 an ovarian stimulation method taking advantage of the inhibitory effect of clomiphene citrate on luteinizing hormone (LH) surge. In this report, a standard treatment regimen is described in which 150 IU of a follicle stimulating hormone (FSH) preparation is administered every other day from day 8, as an effective method of replenishing exogenous FSH. However, it was found that this procedure is an immature replenishing method that could lead to overdosage. This presentation first presents an introduction to clomiphene cycle and then describes the method for replenishing a minimal necessary amount of exogenous FSH based on the endogenous FSH level, which I established after 2007. In the latter part of the presentation, I will propose the most efficient ovarian stimulation method.
European attitudes to fertility treatment and reimbursement. Current position and future prospects

William Ledger
University of Sheffield

Despite the avowed desire of the European Union to achieve harmonisation across its member states, great diversity exists between different member countries in their level of provision of, and reimbursement for ART. Some countries number amongst the most generous in the World in their support of treatment for infertile couples whilst others lag far behind. There is a clear correlation between the level of State support and uptake of ART, as evidenced most recently by the abrupt decline in number of IVF cycles performed in Germany after reduction in coverage of assisted reproduction by statutory health insurances in 2004.

Failure of the State to adequately support its infertile couples carries several adverse consequences. Not only does this mitigate against a policy of single embryo transfer, incurring short and long term costs of multiple pregnancy and resultant handicap to the State, but failure to support also prevents the birth of much-wanted children who will in due course more than reimburse the costs of their creation over their lifetime of contribution to State finances through taxation.

Mild approaches to assisted reproduction flourish in countries where patients do not have to pay large sums from their own resources. Acceptance of a policy of multiple frozen embryo transfers, repeated mild stimulation or natural unstimulated IVF cycles is highest in countries with generous State support and policies to reduce multiple pregnancies as a result of ART work best where the State can mandate single embryo transfer through contracts for reimbursement with clinics.

There are no signs that the disparity across Europe is likely to correct in the near future. The politics of recession are not friendly to subsidy for treatment of non-life threatening health problems and the prospects for European ART are not rosy. However the continuing fall in total fertility rate seen in most Western countries may persuade some national Governments to provide support for ART in order to help correct population decline.
Since there has been no improvement in the current downward trend in Japan’s birth rate, the growing population of potential patients with infertility is a significant social problem. Underlying the factors of infertility are psychological factors related to the current stress of modern-day society, sexually transmitted infections, increased smoking rates among young females, weight abnormalities such as obesity and underweight resulting from diet, an age-related decrease in reproductive function resulting from late marriage and late childbearing (social infertility), and increasing numbers of patients with polycystic ovary syndrome, endometriosis, or uterine myoma. Eighty to 90% of these factors contributing to infertility are derived from personal lifestyle and are considered to be preventable.

It is proposed that to prevent infertility, males and females of reproductive age undergo regular checkups of reproductive function once a year. It is expected that this kind of effort may lead to improvement of the downward trend in the birthrate of the society with fewer children.
Nurturing the ovaries: Treatment of patients with high FSH with delayed stimulation using low dose gonadotrophins

Milton Leong
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 previous history, or by low antral follicle count, or by raised FSH, low AMH, or a combination of some of 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 these 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 also plays a part in this humiliating decision.

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, then sometime they must have a developing oocyte cumulous complex with a follicular shell producing estrogen. They are not responsive to even high doses of FSH in the beginning because their follicles are not ready to respond, the receptors are not ready. But, given the right timing, we should be able to take over from their own FSH and continue follicular maturation which will wilt when their own FSH wanes. Because, for these patients, even if respond to FSH stimulation, their functioning follicles will stop growing, or prematurely luteinised if left to themselves.

In 2007 we started a protocol of delayed stimulation, where patients with raised FSH (>15U/L) on day 2-3, and typically AFC would be 0-2. We do US every 3-5 days, depending on findings, until at least one follicle reaches a diameter of over 9mm, 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 about 10%.

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.
Low response in ovarian stimulation is a problem in fertility patients; it can be a sign of ovarian aging.

Low response can be defined as a patient that with few antral follicles on day 2 of the cycle.

The main features of low response are: reduce of ovaries volume and antral follicles, background of low response stimulations, basal FSH of 10 to 15 UI/ml. Estradiol lower than 500 pg/ml on the administration of the hCG day.

Administrate growth hormone (GH) to potentiate the effect of exogenous gonadotropins and to modulate the action of FSH on granulosa cells by up-regulating the local and synthesis of insulin-like growth factor-I. The IFG-I amplifies the effect of gonadotropin action at the level of both the granulosa and theca cell.

Advantage of the flare up schemes is: avoid the excessive ovaries suppression, achieve an initial stimulus-receptor GnRH and allowing endogenous secretion reinforces the effect of gonadotropins exogenous.

The GH co-treatment was started in the late luteal phase simultaneously with the GnRH agonist when the flare-up effect took place to increase the cohort of follicles recruitable in the approaching treatment cycle.

As higher concentrations of estradiol in pre-ovulatory follicular fluid predict a higher chance of pregnancy.

The growth hormone (GH) showed the most consistent relationship with different parameters of embryo quality and higher concentrations of GH in follicular fluid were associated with rapid, good cleaving embryo morphology and a high embryo implantation potential.

Springer Science concludes that poor responder women undergoing repeated assisted reproduction treatment and co-stimulated with GH achieve more oocytes, higher fertilization rate if growth hormone started in the luteal phase of previous cycle, as compared with women of the same status treated with GnRH-a long protocol.

Two lines of circumstantial evidence support use of exogenous DHEA to augment ovarian stimulation in women aged 35–40 who are poor responders. controlled studies demonstrate marked augmentation of serum IGF-I concentrations with oral administration of physiological DHEA (Morales et al., 1994; Diamond et al., 1996; Casson et al., 1998) On this basis, we postulated that in patients <41 years old, with previously demonstrated poor response and normal FSH concentrations, administration of oral DHEA combining with gonadotrophin stimulation would result in enhanced ovarian response.

FMR1 permutation is known like as a cause of POF (up to 20% of female carriers may develop POF) with a relative risk of 10.

It was found a correlation between the number of repeats CGG and the amount of the FSH and AMH levels.

FMR1 molecular analysis and the FSH, AMH and inhibins A and B hormone studies could help to predict the POF in low responders.
Introduction;
Poor response to ovarian stimulation in in vitro fertilization (IVF) treatment still remains to be a challenge in IVF practice. Alterations in the stimulation protocol, such as changing the gonadotropin or gonadotropin-releasing hormone (GnRH) agonist dose, use of microdose flare protocols, clomiphene citrate (CC) or letrozole (L) protocols, or use of a GnRH antagonist have varying degrees of success in improving outcomes. The objective of this study was to confirm the effectiveness of the clomiphene citrate or letrozole+ GnRH antagonist protocol in very poor responders.

Material and methods;
We evaluated the cycles of poor responder patients who underwent IVF between 2006 and 2009. We evaluated 32 very poor responders who had failed previous IVF cycles with microdose flare protocol or GnRH antagonist protocol and were then treated with the CC/L+GnRH antagonist protocols as follows: Clomiphene citrate 100 mg/day or letrozole 2.5 mg/day was initiated on day 2 and continued for 5 days. On day 4, administration of human menopausal gonadotropins (hMG) 300–450 IU/d were commenced. When the leading follicle exceeded 13 mm in diameter, 0.25 mg of GnRH antagonist (cetrorelix acetate) was started daily until the day of human chorionic gonadotropin (hCG) administration. hCG was given when 2 leading follicles were ≥17 mm. Vaginal oocyte retrieval was performed 35–36 hours after hCG, and embryo transfer was performed on day 2 or 3. Luteal support was given by daily vaginal progesterone suppositories. Clinical pregnancy was defined as the presence of an intrauterine gestational sac on transvaginal ultrasound.

Results;
32 patients underwent 42 IVF cycles using the CC/L+GnRH antagonist protocol. Mean age of patients was 37.02 ± 5.9 years, mean day-3 serum FSH was 13.9 ± 4.6 mIU/ml, total dose of hMG used was 2118.1 ± 1411.5 IU, follicles ≥17 mm on hCG day was 0.9 ± 0.8, E2 and progesterone levels on the day of hCG administration were 362.1 ± 281 pg/mL and 0.7 ± 0.9 ng/mL, respectively. Cancellation rate of cycles was 78.5%. Number of oocyte-cumulus complexes and metaphase II oocytes were 2.8 ± 1.4 and 2.2 ± 1.5 respectively. Fertilization rate was 70.7% and number of embryos transferred was 1.8 ± 0.9. Pregnancy rate (β-hCG positivity) per cycle attempt was 7.1 % (3/42), clinical pregnancy rate per cycle attempt was 4.7% (2/42), clinical pregnancy/ET was 22.2% (2/9), live birth rate per cycle attempt was 2.3% (1/42), and live birth /ET was 11.1% (1/9). All clinical pregnancies were occurred in CC group.

Conclusions;
Poor responders who had previously failed to respond to microdose flare protocol or GnRH antagonist protocol may benefit from CC/L+GnRH antagonist protocols despite of high cancellation rate. CC/L+GnRH antagonist protocols may provide an alternative option for severe poor responders.
Highly Glycosylated FSH (HG-FSH): Available Clinical Results and Investigational Issues

Timur Gürgan
Hacettepe University, Faculty of Medicine, Dept of Ob&Gyn, Reproductive Endocrinology and IVF Unit, Ankara

FSH is a family of molecules carrying different amounts of sugars (glycosylation). Due to their content in sialic acid, more glycosylated molecules exert a more acidic activity. Acidity is used in analytical tests. Natural FSH is a wide mix of the different molecules. Acidic isoforms (human derived) have longer half life, higher biopotency with lower in vitro estradiol production effect. They are highly selective for the follicular threshold and cause slow follicular growth in comparison with the least acidic isoforms. FSH varies over time according to well defined patterns. In the follicular phase there is predominance of the very acidic forms in the recruitment phase can be responsible for better selection of good quality follicles and may maintain longer (physiological) pre-antral phase. Throughout the reproductive life there is progressive increase of more biopotent, acidic forms with advancing age. This may be due to compensative to the general ovarian resistance, more precisely, reaction to the pathologic shortening of the early follicular phase.

Industrial FSH products are very different in their glycoforms composition. There are source-related differences. Less sugars in the products derived from engineered rodent cells. More sugars in the products sourced from menopausal women. There are also significant differences within the same source type. The process engineering strategy may make the difference. Human derived FSH products contain highly glycosylated FSH and there is also differences in between different drugs even if they are simply called recombinant FSH or Human derived ones.

Highly glycosylated FSH (hFSH) produces different pattern of follicle growth. This effect may cause:
- Less follicles recruited
- Slower growth rate in pre-antral phase
- Fast development once entering the antral phase
- Minor loss of follicles during maturation
- Reduced number of small follicles at time of hCG (<OHSS)

And possibility has an impact on the improved outcomes:
- Better quality of oocytes/embryos
- Higher implantation rates
- Improved outcome from frozen embryos

Pivotal studies showed that in good prognosis patients with a fixed starting dose of gonadotrophin, there is no difference between hFSH and rFSH with respect to:
- Mean number of oocytes retrieved
- Clinical pregnancy rate
- Live birth rate
- Safety and tolerability

But on the theoretical basis stimulation with a acidic FSH may result in a more selective recruitment and in a slower (physiological) antral phase.
- It is well established that a proper pre-antral phase is mandatory for good quality oocytes
  - DNA imprinting
  - Genetic synthesis
  - Proteic synthesis
- Another important questions which should be asked is: Does the pattern of activation of the oocyte genoma really vary according to the (FSH) stimulation regimen? and Are cumulus cells as well sensitive to alternative stimulation types?

We have just finished a study on the “Effect of FSH stimulation Regimens on The Pattern of mRNA expression in human Cumulus Cells”. And the our results showed that:
- FSH isoforms are likely to play an important physiological role
The available FSH drug products differ each other in terms of isoform content. Clinical data available so far showed interesting outcomes from the sequential use of differently glycosylated FSHs. More molecular investigations or the metabolomics of different fluids and culture medias need to be done to elucidate better the effect of different FSH glycoforms and Mix in the complex dialogue between the oocyte and cumulus cells during the follicular and luteal phases. Different CHO (controlled hyperstimulation) treatments induce different responses in CC biochemical pathways. Our data on cumulus transcriptome profiling showed that the group of genes involved in the biological function and molecular process can be expressed differently on relation with the used FSH glycoforms. More studies need to be done (mainly proteomics) to elucidate the continued dialogue between the oocytes and its somatic environment to reach its final maturation and competency at ovulation.
Consequences of cryopreservation protocols of metaphase II oocyte on translation processes

Sandrine CHAMAYOU1, Bonaventura G2, Guglielmino A1, Alecci L1, Tibullo D3, Diraimondo F3, Barcellona M.L.2

1 Unità di Medicina della Riproduzione - Fondazione HERA

Introduction;
The main protocols applied for oocyte cryopreservation are slow freezing/rapid thawing (SF/RT) and vitrification. The oocyte contributes to the future embryo with the genetic haploid maternal heritage and the energetic and metabolic reserves necessary for the future development of the embryo. One of these fundamental molecules is mRNA. In the literature, there are no data about mRNA conservation of proteins that act at different cellular level according to the different cryopreservation protocols. The aim of our study was to evaluate the modification of mRNA amount of three groups of proteins involved in 1) DNA structural organization (NAP1L1, TOP1, H1F0H1), 2) mitochondrial energetic pathways (ATP5GJ, SDHC) and 3) cell cycle regulation and processes (CLTA, MAPK6, CKS2) according to the different freezing protocols and compared with the mRNA amount in the fresh metaphase II oocytes.

Material and methods;
The oocytes used in this study were surplus oocytes donated by the patient after written consent. The fresh oocytes and the SF/RT oocytes were cultured and prepared as previously described (Chamayou et al, 2006). A pool of oocytes were vitrified on cryotop support according to the protocol described by Kuwayama et al (2005). Each pool contained 15 oocytes. Total RNA of each group was isolated using PicoPure RNA isolation kit (Arcturus, Sunnyvale, CA) and treated by DNAse I, RNAse-free. Extracted RNA was used as template for cDNA synthesis. For RT-PCR analysis of mRNA expression, 1.0 µg of total RNA (in 20 µL reaction volume) was reverse-transcribed using Reverse Transcriptase enzyme added of an aliquot of oligo-dT primers in a standard reaction. Each reaction was evaluated by 2% agarose gel electrophoresis and ethidium bromide–stained gels digitally photographed. Semi-quantitative evaluation by densitometric analysis was performed by Scion Image software (USA).

Results;
There is an overall decrease of mRNA extracted from cryopreserved oocytes compared with fresh oocytes at all level in the cell. The amount of mRNA of genes involved in DNA structural maintenance were respectively 22,2% after SF/RT and 74,2% after vitrification if compared to mRNA amount in fresh oocytes. The respective results were 30,6% and 61,5% for mRNA of proteins involved in energetic pathways; and 25,9% and 51,9% for mRNA of proteins acting in cell cycle and processes.

Conclusions;
We previously observed that the cleavage rate and the cellular quality of embryos produced from cryopreserved oocytes after SF/RT was significantly inferior if compared to the results obtained from sibling fresh oocytes (Chamayou et al. 2006). The results of the present study confirm cellular damages generated by SF/RT protocol. The decreasing of mRNA after vitrification was less pronounced than mRNA extracted from fresh oocytes but less than SF/RT protocol. Both freezing protocols compromise translation processes in the oocyte but vitrification appears to be more conservative.
An individualised stimulation algorithm reduces oocyte numbers and removes hyper-stimulation syndrome without affecting pregnancy rates.

**John Yovich, James D Stanger**
PIVET Medical Centre

**Introduction:**
The risks associated with excessive follicle recruitment have encouraged a reduction in hormone stimulation regimens for IVF. The problem with reducing FSH dosage is the variation in response of individuals to a standard dose. In this presentation, we report the initial outcome of creating an individualised dosage regimen (PIVET Algorithm) for each patient where the aim of stimulation was to recover 8 oocytes per collection.

**Material and methods:**
Creation of the PIVET Algorithm of FSH dosage is based upon the patients Age, Body Mass Index, Anti-Mullerian Hormone, Day-5 Antral Follicle Count and adjusted for Day-2 FSH level and smoking history. This was all undertaken in a preliminary Assessment Cycle in which the patient was reviewed on Day 21 to calculate the FSH stimulation dosage for the forthcoming IVF treatment cycle. Where possible the dosage was not altered during follicle development tracked over 7-12 days utilising ultrasound and serum hormone levels (E2, P4 and LH). Most patients had an Antagonist regimen but the dose was independent of the stimulation protocol and included some cases of Down-Regulation and Flare stimulation. The results were compared to the previous 7 years of activity measuring the number of oocytes and embryos per oocyte collection and comparing clinical pregnancy rates per fresh transfer.

**Results:**
Compared to 2002-2008 data, individualised stimulation in latter 2009 significantly reduced the number of oocytes/collection from 10.2 to 7.5 over all patient age groups and from 11.5 to 8.9 in the “at risk” group of women under 35 years of age. This had the effect of reducing the number of embryos from 6.3 to 4.4 (total population) and 7.4 to 5.3 for the younger group. The clinical pregnancy rate per fresh transfer was unchanged from 30% to 32% (total population) and 42% to 44% for the younger group. There have been no incidences of admissions for ovarian hyper-stimulation since commencing use of the PIVET Algorithm compared to annualised rates of 2.6% in previous years.

**Conclusions:**
Using key patient parameters that reflect ovarian reserve to apply an individualised FSH hormone dosage for follicle recruitment, the PIVET Algorithm significantly reduces the number of oocytes recovered and embryos generated per collection whilst removing the risk of ovarian hyper-stimulation syndrome, but without reducing the pregnancy rate from fresh embryo transfers.
Are Mild ART-derived blastocysts more favorable than conventional COH-derived ones?

Yasushi Takai¹, Ken Ohara², Eri Kamiya², Shigetaka Matsunaga³, Munetaka Ito³, Masahiro Saitoh², Naoki Hayashi⁴, Osamu Ishihara⁵, Hiroyuki Seki²

1 Department of OB/GYN, Saitama Medical Center/Saitama Medical University
2 OB/GYN, Saitama Medical Center
3 OB/GYN, Sekishindo Hospital
4 Muse Ladies Clinic
5 OB/GYN, Saitama Medical University

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).

Material 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 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 difference 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).

Conclusions;
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.
Introduction;
In general, it is believed that blastocyst transfer significantly increase pregnancy rate in human ART practice. By reducing number of embryo transferred, blastocyst transfer decreases multiple pregnancy related complications. With sequential culture medium marketed and physical oxygen culture condition (low O2 concentration) benefiting blastocyt formation known, blastocyst culture and transfer are widely undertaken in the international ART practice. Early rescue ICSI for low fertilization (less than half oocytes fertilized) practiced in Chinese ART society in order to generate more viable embryos for replacement also increases the probability of blastocyst formation, which I believe is a significant innovation in human IVF.

Material and methods;
In an university-affiliated woman and child health hospital IVF program, Patients underwent IVF/ICSI treatment with standard GnRHa long protocol. Clinical pregnancy rate and implantation rate were compared among three groups, such as Day3 cleavage embryo tranfer, Day3 and Day5 sequential transfer and Day5/6 blastocyst transfer only. Surplus Day5/6 blastocyst were vitrified with cryotop ( , Japan) tools for subsequent transfer in natural ovulatory cycle or HRT cycle if pregnancy was failed to be established in fresh transfer cycles. Clinical pregnancy rate and implantation rate were analyzed and compared particularly between fresh transfer cycles and vitrification-warmed transfer cycles

Results;
We ask an immediate question: shall we perform blastocyst transfer for all the patient? We have retrospectively analyzed our data on blastocyst transfer performed in last two years. It is found that sequential transfer (one or two cleavage embryos transferred on day3 followed with one blastocyst transferred on day5) did not increase pregnancy rate and clinical pregnancy rate in all age groups, in comparison with day3 cleave transfer (two or three cleavage embryos transferred only on day3). No significance was seen in day5 blastocyst transfer compared with either day3 cleavage embryo transfer or sequential transfer. Comparable clinical pregnancy rate at 40% was seen in patients below 35-years-old in the three groups.

However, it is surprisingly found that vitrified-warmed blastocyst transfer resulted in much better results – higher clinical pregnancy rate (59.8%) and higher implantation rate (40%) compared with fresh blastocyst transfer in controlled stimulation cycles (see table2). Vitrified-warmed blastocyst is generally considered less superior as they are the left-over embryos after blastocyst with better morphology were transferred in COH cycles. Therefore, the striking result indicated that stimulated endometrium in COH cycles is hostile for blastocyst implantation as good blastocyst resulted in lower implantation rate in fresh transfer cycles. Natural ovulation cycle or hormonal replacement cycle gives more receptive endometrium for blastocyst implantation. Therefore, it is recommended that more blastocyst vitrification should be done with patients with less optimal endometrium in COH cycles. The vitrified blastocyst transferred in the subsequent natural ovulation cycle or hormonal replacement cycles gives better clinical result.

Conclusions;
In a general population, blastocyst transfer does not significantly increase clinical pregnancy rate. When patients have suboptimal endometrium, such as "C" type (even "B" type) echo under ultrasound scan or elevated progestin level, fresh blastocyst transfer should be avoided. Subsequent vitrified-warmed blastocyst transferred in non-COH cycle with optimized endometrium dramatically increases clinical result. More attention should be paid to endometrium not so-called embryo quality based on morphology. We will present single fresh blastocyst transfer and single vitrified-warmed blastocyst data in the conference.
Introduction;
Single blastocyst transfer is applied clinically to prevent multiple pregnancies. In recent years, high survival rate has been obtained with established vitrification methods, therefore, frozen thawed single blastocyst transfer is performed. The aim of this study is to provide reliable data for frozen thawed single blastocyst transfer results compared by the timing of transfer, expansion and implantation rates. Retrospective data analysis of over 3,500 frozen thawed single blastocyst transfer cycles.

Material and methods;
Data from women who had given their consent for frozen thawed single blastocyst transfer from June 2007 to January 2008 was retrospectively assessed. The oocytes retrieved by mild stimulation were inseminated by c-IVF or ICSI. Normally fertilized embryos with two pronuclei were cultured until blastocyst stage and vitrified at 140µm or more of the diameter of expanding blastocyst cell line using cryotop methods. Frozen thawed single blastocyst transfers were performed in natural cycle using Gn-RH agonist for induction of ovulation. Implantation rates were assessed using x2 distribution after frozen thawed single blastocyst transfer at the timing of 98-99, 110-111, 122-123, 134-135, and 146-147 hrs after ovulation, and also assessed their implantation rate after the frozen thawed single blastocyst transfer with the size of expanding blastocyst cell line at 140, 150, 160, 170, 180, 190, and 200µm on day5.

Results;
A total of 3,588 cycles of frozen thawed single blastocyst transfer were performed from June 2007 to January 2008 (mean age 37.6±4.1 years old). Implantation rates after frozen thawed single blastocyst transfer at the timing of 98-99, 110-111, 122-123, 134-135, and 146-147hrs after ovulation were 55.2, 57.5, 61.4, 58.3, and 47.8% respectively. Higher implantation rates were obtained at the timing of 122-123hrs after ovulation. Implantation rate after the frozen thawed single blastocyst transfer with the size of expanding blastocyst cell line at 140, 150, 160, 170, 180, 190, and 200µm on day5 (122-123hrs after ovulation) were 40.0, 47.1, 45.9, 50.7, 61.1, 63.2, and 64.8%. Implantation rates were increase as their size expands.

Conclusions;
Higher implantation rates are obtained after frozen thawed single blastocyst transfer when the blastocyst is vitrified at the later stage of expanded blastocyst and transferred at the promising timing.
Rescue ICSI of oocytes that failed to appear the fertilization corn and cytoplasmic flare 6 hours post-insemination in conventional IVF

Tomohisa Wada¹, M. Kamihata¹, A. Kuwahata¹, M. Ochi¹, C. Kani¹, ²; T. Horiuchi²

1 Ochi Yume Clinic Nagoya
2 Prefectural University of Hiroshima

Introduction;
We have performed rescue ICSI of oocytes that failed to extrude the second polar body 6 hours post-insemination in conventional IVF since 2004. In rescue ICSI, the criteria for choosing unfertilized oocytes are very important. Recently, using time-lapse cinematography, the appearance of a fertilization corn in surface of oocytes, and cytoplasmic flare in the ooplasm during fertilization were observed about 2.2 hours after extrusion of second polar body (Mio, JMOR 23, 27-35, 2006). Therefore, the objective of this study was to investigate whether the fertilization corn and cytoplasmic flare were added as new criteria for judging fertilized oocytes in addition to the extrusion of second polar body for Rescue ICSI.

Material and methods;
From April 2004 to December 2008, we studied rescue ICSI of oocytes that failed to extrude the second polar body, and did not find fertilization corn and cytoplasmic flare as the additional criteria, individually for 612 cycles and 1619 cycles induced by clomiphene-HMG-GnRHa administration. Conventional IVF was performed 3 hours after ovum pick up, and the final sperm concentration was adjusted to 1*10⁵/ml. In Rescue ICSI, the spindle was fixed at the 12 o’clock position if it could be visualized using ICSI Guard (OCTAX) and the first polar body was fixed at the 12 o’clock position if the spindle could not be visualized. The oocytes were examined for fertilization 10-13 hours after rescue ICSI, and two pronuclei (2PN) were judged as normal fertilization. Cleavage of the oocytes and blastocyst development were assessed on day 2 and day 5. All blastocysts were vitrified and stored for a single blastocyst transfer in the next cycle.

Results;
The rescue ICSI in the failure of 2PB extrusion (1PB group) or the no appearance of fertilization corn and cytoplasmic flare (no flare group) performed 18.6% (114/612) or 15.2% (246/1619) of total cycle, respectively. Normal fertilization rate of rescue ICSI in the no flare group (84.8%, 408/481) was similar to that in the 1PB group (80.9%, 165/204), but 3PN rate in the no flare group (3.3%, 14/481) was significantly (P <0.05) lower than that in the 1PB group (7.8%, 16/204). The percentage of good cleaved embryos at day 2 was no significantly difference between the no flare group (17.9%, 73/408) and the 1 PB group (17.0%, 28/165), but the percentage of good blastocysts at day 5 in the no flare group (27.0%, 110/408) was significantly (P<0.01) higher than that in the 1PB group (16.4%, 27/165). Pregnancy rates of a single blastocyst transfer were no significantly difference between both groups (20.5%, 17/83 and 21.4%, 6/28).

Conclusions;
These results clearly confirmed that rescue ICSI using fertilization prediction based on fertilization corn and cytoplasmic flare, in addition to the extrusion of second polar body, was very useful for preventing the incidence of polyspermy, and producing good blastocysts at day 5.
PSYCHOLOGICAL DISTRESS MEASUREMENTS AMONG INFERTILE WOMEN UNDERGOING IN-VITRO-FERTILIZATION

Suneeta Mittal, Latit Kumar, Samana G, Anupama Bahadur
All India Institute of Medical Sciences

Introduction;
Infertility has been characterized as creating a form of chronic stress that can cause to a variety of psychological difficulties. Women undergoing IVF treatment often experience anxiety, depression and uncertainties about the treatment outcome. Stigma and discrimination are the common family and societal reactions faced by the infertile women. The overall aim of the study was to understand and assess the psychological distress in terms of self esteem, guilt, sexuality and depression among women undergoing In-vitro-fertilization (IVF) cycle.

Material and methods;
The study has been conducted at Assisted Reproduction Technology (ART) facility, Department of Obstetrics & Gynaecology, AIIMS, New Delhi. The sample for the study consists of fifty eight consecutive infertile women undergoing IVF cycle during June 2008 - January 2009. Infertility Questionnaire (IFQ) and Beck Depression Inventory (BDI-II) measures were used in the study. Patients were asked to voluntarily fill up questionnaire before IVF cycle.

Results;
One of the most significant finding of the study is that about one fifth of the conceived women had lesser level of distress on IFQ measure when compared with the non-conceived group. On an overall basis, 22% of the affected women population on IFQ score was found to be at mild to moderate level of distress. 55% of the women were found to be adversely affected on their sexual satisfaction count. It was further found that 6% of women faced severe level of distress on this variable. Another significant finding is that half of the conceived women had lower depression level as compared to non-conceived women on BDI measure. The BDI-II scores indicated that 31% of the women subjects were found to be facing moderate level of depression, while another 25% of women faced mild level of depression. Moreover, by and large the two instruments though separately used in this study gives almost identical result trends.

Conclusions;
Younger the age or higher the traditional gender role orientation, greater is the increased motivation for the child. The study suggests that a comprehensive psychological evaluation is prerequisite for choosing interventions. Special focus needs to be given on enhancing the sexual health/concerns of the infertile women. Mental health of the infertile women undergoing the treatment for a better sustenance requires monitoring on a continuous basis.
P-004

**DDB inhibit development of experimental endometriosis and induce apoptosis of endometrial stroma**

*Ali Farid. Ali¹, Laila Farid², Mostafa Fouad², Ahmed Abd el shafy²*

1. Heliopolis research center
2. Ain shams university

**Introduction:**
A new hepatoprotectant and it has antioxidant, antiproliferative, antiinflammatory and hepatoprotectant against chronic viral hepatitis.
Pathophysiology of endometriosis involve ectopic attachment and proliferation of endometrial; tissue, exudative streee and inflammation.
This study evaluate the effect of DDB on the mouse model endometriosis and on culture of human endometrial cells

**Material and methods:**
Human proliferative phase endometrial biopsy was established as an organ culture or used for human endometrial stromal cells isolation.
To establish endometriosis in ovariectomized mice, endometrial tissue were maintained in 1 mm estradiol for 24h and subsequently injected intraperitoneal.
Dose of DDB injected in the study group (n=12) was 5mg/kg body weight, placebo in control group (n=10). The animal is then sacrificed and endometrial implants are measured and stained.
TUNEL for apoptosis were measured.
Studies for human endometrial stroma cells evaluate the effect of DDB on apoptosis by detection of Casapses 3-7.

**Results:**
Mice treated with DDB had significant lower number and volume of endometrial implanmts than controls.
DDP induced concentration dependent way of apoptosis.

**Conclusions:**
DDB significantly reduced number and size of endometrial implants in a mouse model of endometriosis as well as induce human endometrial stromal cells apoptosis this will open a new era in the treatment of endometriosis.
Comparison of Galectin-9 and Galectin-3 Expression and Localization in the Human Uterodome

Mehri Azadbakht¹, Maryam Kabir-Salmani², Mitsutoshi Iwashita³

1 Department of Biology, Razi University, Kermanshah, Iran
2 Department of Genetic Medicine, National Institute for Genetic Engineering, Tehran Iran
3 Department of Obstetrics and Gynecology, Kyorin University School of Medicine, Tokyo, Japan

Introduction;
Galectins are a family of proteins that bind to galactose-containing ligands. Members of this family have been implicated in a variety of functions including: cell growth, cell adhesion, apoptosis, and inflammation, all of which are important for endometrial function. It is appeared that the galectins are regulatory molecules which perform functions depending on their extracellular location and their specific cell surface receptors. Preliminary evidence suggests that galectins, through binding to the extracellular domains of one or both subunits of an integrin, may positively or negatively modulate integrin activation, and affect binding with extracellular ligands. Galectin-3 seems to be an endogenous cross-linker of the CD98 antigen, leading to the activation of integrin mediated adhesion. Galectin-9 is one of the very few epithelial markers that are strictly regulated during the menstrual cycle, with a significantly increased expression during the secretory phase. Galectin-9 is a very intriguing to follow up with structural and functional studies in the human endometrium. The aim of this study was to comparison of Galectin-9 and Galectin-3 expression and localization in the human uterodome, especially during the frame of the implantation window.

Material and methods;
Endometrial biopsies in the proliferative, early, and mid-secretory phases from women with regular menstrual cycle were studied using immunostaining for light and transmission electron microscopies (TEM) and statistical analysis of the area-related numerical densities of galectin-9-bound and galectin-3-bound nanogold.

Results;
Images of immunostaining for light microscopy demonstrated a strong expression of galectin-9 at the luminal and glandular endometrial epithelium in the mid-secretory phase compared to the galectin-3. Photomicrographs of immunogold staining for TEM illustrated the localization of galectin-9 and galectin-3 in the uterodomes. Statistical and morphometric analysis showed a significantly higher area-related numerical density of galectin-bound nano-golds in the uterodomes compared to that of the uterodome-free areas of the luminal epithelium (p<0.001). There was a significantly increase in the densities of galectin-9-bound nano-golds compared to galectin-3-bound nano-golds(p<0.001).

Conclusions;
High expression of galectin-9 in the bulbous ultrastructure of the human endometrial epithelium, called uterodomes during the frame of implantation window compared to galectin-3 suggests that galectin-9 can be considered as a marker of endometrial receptivity and should play an important role during the initial events of human embryo implantation. The expression of galectin-9 in the surface epithelium, combined with the functional aspects of galectin-9 make it an intriguing factor for cell-cell interaction in the human endometrium, as well as during human implantation.
P-006

Effect of maturation in vitro on spindle morphology in human oocytes.

Mette Munk¹, Novella-Maestre², Svend Lindenberg³, Steen Smidt Jensen³

¹ Copenhagen Fertility Center
² Instituto Valenciano de Infertilidad (IVI), University of Valencia
³ Section for Reproductive Biology and ART, Copenhagen Fertility Center

Introduction;
Incomplete cytoplasmic maturation of in vitro matured (IVM) oocytes has been known to cause microtubule and filament alterations, which may result in abnormal pronuclear formation and later failed embryogenic development. In the present study we examined the influences of in vitro maturation of human ova to the MFII stage in a standardized in vitro maturation medium compared to in vivo matured MFII stage ova prior to the ICSI procedure.

Material and methods;
A total of 44 patients admitted for IVM and 44 patients admitted for IVF/ICSI were analyzed using a polscope for evaluating the presence and placement of the spindle in relation to the polar bodies in all MFII oocytes produced after egg collection prior to ICSI or after 28 h in vitro maturation following the IVM protocol.

Results;
We found the same mean number of oocyte retrieved (IVM 4.9 oocyte versus ICSI 5.9 oocyte), the mean number of MFII oocytes after collection of IVF/ICSI oocytes were 5.1 versus 2.1 for the IVM oocytes after 28 h maturation.

Conclusions;
These findings indicate that in vitro matured human oocytes either mature from MF I to MFII normally having a normal spindle appearance after 28 hours in vitro maturation or deteriorate completely. This indicates that IVM matured oocytes if they reach the MFII are normal, and the obvious lower implantation rate in IVM might be due to other factors such as impaired endometrial development during the IVM procedure.
Effect of Progesterone in cultures of follicles derived from polycystic ovaries (PCO) mouse model

Mehri Azadbakht, Ali Bazdar, Ali Amini
Department of Biology, Razi University, Kermanshah, Iran

Introduction;
Polycystic ovary syndrome (PCOS) is a heterogenous disease characterized by hyperandrogenaemia, hirsutism, oligo- or amenorrhoea and anovulation. The variable phenotypic expression of reproductive and metabolic abnormalities in PCOS patients leads to differences in oocyte developmental competence, defined as the ability of the oocyte to complete meiosis and undergo fertilization, embryogenesis and term development. A common feature of PCOS is the accumulation of small subcortical follicles and increased ovarian stromal volume, causing a characteristic ultrasound image and a basis for the most commonly used designation for the syndrome. In PCOS, hyperandrogenism also affects ovarian steroidogenesis, and decrease intra-ovarian concentration of progesterone. The aim of this study was to examine the effect of progesterone on in vitro culture and maturation of ovarian follicles derived from polycystic ovaries mouse model.

Material and methods;
PCO was induced with daily administration of testosterone enantate 1 mg/100 g body weight for 1 to 4 weeks. Control mice were injected only with vehicle. Mouse ovaries were underwent histological examination and were categorized into PCO and control groups. Follicles were mechanically isolated, each follicle cultured individually in microdrops 20 µl of MEM-α culture medium supplemented with 5% FBS, 100 mIU/ml rFSH (Gonal-f), 10 ng/ml rEGF, with 3 mg/ml progesterone (treatment I) and without progesterone (treatment II) under mineral oil for 12 days. On day 12, in vitro maturation was induced by using 7.5 IU/ml HCG. Growth and survival rates of the follicles were assessed during the culture period and the in vitro maturation of the oocytes was studied.

Results;
Our findings show that, four week testosterone enantate treatment significantly increased the primordial follicles, and significantly decreased the preantral and antral follicles compared to one week testosterone enantate treatment (p>0.05). The survival rates of follicles in the PCO group, treatments I and II was lower than control group treatments I and II (p<0.05). After 24h oocytes were assessed for percentage of in vitro maturation. The percentage of GVBD oocytes in PCO group, treatments I and II (20.6 and 18.7) decreased compared to control group, treatments I and II (34.7 and 32.2), respectively; (p<0.05). The percentage of MII oocytes in PCO group, treatments I and II (49.6 and 22.6) increased compared to control group, treatments I and II (49.6 and 22.6) increased compared to control group, treatments I and II (19.4 and 12.2), respectively; (p<0.05). The percentage of degenerated oocytes decreased in PCO and control groups, treatment II (20.6 and 32.2), respectively; (p<0.05). There was no significant differences in PCO and control groups, treatment I (40.3 and 42.6), respectively; (p<0.05).

Conclusions;
Progesterone improves oocytes in vitro maturation rate in cultures of follicles derived from polycystic ovaries (PCO) mouse model.
Experience of mild stimulation IVF for patients with repeated IVF failures (Cases series)

Julia Vosnesenskaya, Yakovenko S.A.
IVF Clinic "Altra Vita"

Introduction;
Repeated IVF failures related to such problems as age of patients, low ovarian reserve, poor response, unsatisfactory quality of oocytes and embryos, low blastocyst rate remain to be a serious problem for infertility treatment. In regular clinical practice doctors have to recommend IVF treatment with donor eggs which is not acceptable for many couples.

Material and methods;
This category of patients was offered to undergo minimal stimulation program with use of clostylbegit and with embryo transfer in next menstrual cycle (Kato-Teramoto protocol). From October 2009 to May 2009 fifty three stimulations with participation of fifty one patient on criteria were age-over 39 y.o., FSH over 9, antral follicle count less than 6, AMH less than 1, inadequate response in previous attempts, blastocyst rate 0% in usual protocols with antagonists, more that two ineffective IVF attempts in anamnesis. Average age of the patients was 38 years (27-48), average FSH level - 13,6 (1,7-39), average antral follicles count on the moment of the beginning of the stimulation - 6,8 (2-22), average number of IVF attempts in anamnesis - 1,8 (0-7).

Results;
120 mature oocytes were received in 53 protocols, on the average 2,3 oocytes per stimulation are retrieved. Seventy seven embryos were vitrified, 1,4 per stimulation on average. Six of the frozen embryos were blastocysts, 51 were frozen on day three and 20 were frozen on day two. In 33% of the cases cryoconsrvation did not take place because of not receiving oocytes or absence of embryos for vitrification.

Conclusions;
For the moment twenty eight transfers were performed in cryo cycles with use of HRT. In twelve cycles we awaiting for HCG result, in eleven cycles pregnancy did not occur, five clinical pregnancies were received. In all cases we received single fetations pregnancies, no extra uterine pregnancies. Evaluating preliminary data on mild stimulation effectiveness in chosen category of patients we can talk about an extra opportunity of getting pregnancy using patients own oocytes.
P-009

Evaluation of the intrauterine insemination and controlled ovarian hyperstimulation cycle outcomes in our clinic

Begüm Aydogan1,2, Orkun Cetin1, Sezai Sahmay2, Berna Aslan2

1 Istanbul University Cerrahpasa Medical School Obstetrics and Gynecology Department
2 Cerrahpasa Medical School, Istanbul, TURKEY

Introduction;
The evaluation of the intrauterine insemination (IUI) and controlled ovarian hyperstimulation (COH) outcomes of the patients who admitted to Istanbul University Cerrahpasa Medical School Reproductive Endocrinology Department during January 2007 - April 2009.

Material and methods;
The study was designed as retrospectively and performed during January 2007 and April 2009 at Istanbul University Cerrahpasa Medical School Reproductive Endocrinology Department. 1321 cycles of infertile patients were analyzed retrospectively. After the standard infertility investigation, a controlled ovarian hyperstimulation with clomiphen citrate (CC) was used for the first cycle of patients that have an ovulation dysfunction, oligoasthenozoospermia, unexplained infertility, polycystic ovary syndrome (PCOS) and endometriosis. The ovarian response for the first cycle and cancelled cycles were taken into consideration and increased dose CC or gonadotropin treatment were preferred for the second cycle treatment. The cycles were followed by transvaginal ultrasonography. Human chroic gonadotropin (HCG) was administered whenever at least one follicle has reached to 18mm during ultrasound examination. After 36 hours following HCG administration, the intrauterine insemination was performed by sperms that were prepared by Swim-up and Percoll gradient technics.

Results;
The COH cycle number and the IUI cycle number were found, 1321 and 1091 respectively. The pregnancy rate per cycle was % 4 for the controlled ovarian hyperstimulation and it was % 4.8 for intrauterine insemination. In 284 cycle, CC 50gr was used and the pregnancy rate per cycle was found to be %3.1. In 518 cycle, CC 100gr was used and the pregnancy rate per cycle was calculated as %3.8. 277 cycles were performed by using human menopausal gonadotrophine (HMG) and the pregnancy rate per cycle was calculated as % 5. In 149 cycle recombinant (rec) follicle stimulating hormone (FSH) were used and the pregnancy rate per cycle was found to be % 6.7. The differences between HMG and rec FSH group was not statistically significant.

Conclusions;
When the results of our clinic data are taken into account, the pregnancy rates per cycle were lower according to %10-20 rate of the literature. COH and IUI are both effective treatment modalities for the patients who possess appropriate criteria for indications.
The long-term difference in ovarium effects of recombinant and urinary FSH in stimulation protocols with agonists

**Ales Sobek Jr.¹, Hladikova Blazena¹, Tkadlec Emil², Sobek Ales¹**

1 Fertimed
2 Palacky university, Olomouc

**Introduction:**
Although the diverging effects of recombinant FSH (rFSH) and urinary-derived FSH gonadotrophins (uFSH) have been previously recognized, analyses of long-term data are rare. The aim of the study was to compare the effect of rFSH and uFSH in long and short analog protocols in a group of infertile clinic patients over a period of 14 years.

**Material and methods:**
In a retrospective analysis, 3632 patients were stimulated for IVF/ICSI in a short (SU – short urinary, SR – short recombinant) and long (LU – long urinary, LR – long recombinant) analog protocols during the period 1996–2008. Only the first IVF/ICSI cycle from each patient was included. Patients treated with combination of uFSH and rFSH and low responders, who were solely treated with antagonists, were excluded from this study. In each patient, we recorded basal FSH, the dose of gonadotropins used for stimulation, dose of FSH needed for retrieval of one oocyte (IU), estradiol (E2) levels 2 days prior to the egg collection (pmol/l), the number of retrieved oocytes and clinical pregnancies. Time (years), age of the patient on the day of egg collection and the type of stimulation protocol (nominal variables with four levels: LU, LR, SU, SR) were included in the model as fixed effects. We analysed the data by fitting generalized additive models (GAM), using Akaike information criterion for selecting the best model structure.

**Results:**
In groups LU (876 patients), LR (543), SU (1144) and SR (195), mean ages were 29.0, 29.59, 28.92 and 30.52 years, respectively. Mean basal FSH in these four groups were 6.51, 6.55, 7.20 and 7.28 nmol/l, respectively. In patients stimulated with uFSH (LU, SU), there was 23.9% higher consumption of FSH (IU) needed for stimulation (LU: 2443, SU: 2342 vs. LR: 1823, SR: 1820; p< 0.001). The dose of FSH needed for recovery of 1 oocyte (IU/oocyte) was 30.2% higher in uFSH (LU: 253, SU: 265) than rFSH group (LR: 169, SR: 186; p < 0.001). The E2 level (pmol/l) prior to egg collection was 15% lower (p< 0.001) in uFSH group (LU: 2348, SU: 2655) than rFSH group (LR:2657, SR:2878). The number of retrieved oocytes was lower in uFSH (LU: 10.60, SU: 10.08) than rFSH (LR:11.74, SR: 10.89; p < 0.01). In a period of 14 years, we also detected an overall increase in mean basal FSH (p< 0.01), decrease in mean E2 (p< 0.05) and number of retrieved oocytes (p< 0.001), and increase in total dose of FSH (p< 0.05) and the dose needed for recovery of 1 oocyte (p< 0.01) independently of the stimulation protocol and age. Stimulation with long protocols resulted in more pregnancies than short protocols (40%/ET vs. 31.1%/ET; p< 0.001). There was no significant difference between uFSH and rFSH in terms of pregnancy outcome.

**Conclusions:**
In a group stimulated with rFSH, we detected lower consumption of FSH and lower dose of recombinant FSH needed for the recovery of 1 oocyte, higher E2 level before oocyte collection and higher number of collected oocytes. Stimulation with long protocols resulted in more pregnancies than in short protocols. There was no significant difference between uFSH and rFSH in terms of pregnancy outcome. The decrease in ovarian function described by basal FSH and E2 levels and by the number of retrieved oocytes was observed over the period of 14 years.
Recombinant FSH and urinary FSHs are all effective in ovarian stimulation in IUI cycles; a prospective study.

MESUT OKTEM¹, AHMET ERDEM², MEHMET ERDEM², NURAY BOZKURT², EDA DEMIR², ESRA NAS², ONUR KARABAÇAK¹
1 GAZİ UNIVERSITY SCHOOL OF MEDICINE, DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, DIVISION OF REPRODUCTIVE MEDICINE AND INFERTILITY
2 GAZİ UNIVERSITY SCHOOL OF MEDICINE, DEPARTMENT OF OBSTETRICS AND ANKARAGYNECOLOGY, DIVISION OF REPRODUCTIVE MEDICINE AND INFERTILITY

Introduction;
To compare the clinical results of recombinant FSH and urinary FSHs in ovarian stimulation for IUI cycles.

Material and methods;
211 IUI cycles were prospectively randomized into three groups for unexplained infertility cases: 70 in recombinant FSH (Gonal-F, Group I), 72 in purified urinary FSH (Merional, Group II), and 69 in highly purified urinary FSH (Fostimon, Group III). The main outcome was pregnancy rate (β-hCG positivity). Secondary outcomes were number of follicles ≥17 mm on hCG day, days of stimulation, total dose of gonadotropins used, miscarriage and multiple pregnancy rates.

Results;
The pregnancy rate was higher in recombinant FSH group. (21.4% (15/70) in recombinant FSH group, 20.8% (15/72) in purified urinary FSH group, and 15.8% (11/69) in highly purified urinary FSH group). Despite a trend for higher pregnancy rate in recombinant FSH group, the difference did not reach statistical significance (p=0.586). Mean number of follicles ≥17 mm on hCG day was significantly higher in recombinant FSH group compared with purified FSH group (1.6 ± 1.2 vs. 1.2 ± 0.6, p=0.01). Mean FSH dose composed per cycle was significantly higher in purified urinary FSH group compared with recombinant FSH group (993.1 ± 674.3 vs. 809.4 ± 455.8 IU p=0.02). There were no significant difference in terms of days of stimulation, miscarriage and multiple pregnancy rates (p>0.05).

Conclusions;
Recombinant FSH and urinary FSHs were all effective in ovarian stimulation in IUI cycles.
Supplementation of clomiphene citrate cycles with Cimicifuga racemosa or ethinyl oestradiol--a randomized trial.

Ahmed Y. Shahin, Omar M. Shaaban, Alaa M. Ismail
Assiut University, Women’s Health Center, Department of Obstetrics and Gynecology

Introduction;
The anti-oestrogenic activity of clomiphene citrate (CC) on the cervical mucous and endometrium may be the reason for the relatively low pregnancy rates in CC induction cycles. Various follicular-phase supplements have been tried to improve cycle outcome in these patients.

Material and methods;
This study compared follicular-phase supplementation with either phytoestrogen (PE) or ethinyl oestradiol (EE) in CC induction cycles for the treatment of unexplained infertility. A total of 134 patients were randomly allocated to each treatment group (67 each).

Results;
The PE group needed significantly fewer days for adequate follicular maturation, had a thicker endometrium and higher oestradiol concentration at the time of human chorionic gonadotrophin injection (all P < 0.001). The PE group had higher luteal-phase serum progesterone compared with the EE group. No significant difference was found regarding clinical pregnancy rates (14.0% versus 21.1%, respectively).

Conclusions;
In conclusion, the cycle characteristics in unexplained infertility women treated with clomiphene citrate induction and timed intercourse improved after follicular-phase supplementation with PE compared with EE supplementation. Further studies are needed to confirm the mechanism beyond these effects.
Demographic characteristics and clinical profile of poor responders in IVF / ICSI – A comparative study

Nabaneeta Padhy, Asmita Mahla, Sathya Balasubramanyam, Divyashree, Thangam Varma
INSITUTE OF REPRODUCTIVE MEDICINE, MADRAS MEDICAL MISSION

Introduction;
Ovarian response varies considerably among individuals and depends on various factors. Poor response in IVF yields lesser oocytes and is associated with poorer pregnancy perspective. Cycle cancellation due to poor response is frustrating for both clinician and the patient. Studies have shown that women conceiving after poor ovarian response have more pregnancy complications like PIH & preeclampsia than women with normal ovarian response. In addition, poor ovarian response could be a predictor of early menopause. This paper studies various demographic and clinical profile of poor responders and tries to look at the known and unknown factors which could contribute to poor ovarian response in IVF.

Material and methods;
Data were collected retrospectively from 104 poor responders who had less than 4 oocytes at retrieval and compared with 324 good responders for factors like age, BMI, type of subfertility, duration of subfertility, environmental factors like stress at work, smoking, pelvic surgery, chronic medical disorder, indication of IVF, basal FSH, mean age of menopause in their mothers etc.

Results;
Among the poor responders 60.57% were above 35 years of age compared to 36.41% in control group, which is statistically significant. Mean age of menopause in mother was found to be 4 years earlier in poor responder group. Male factor and unexplained infertility were significantly (p=.0007) higher in good responders (p=.0032). Significant proportion (31.73%) of women in study group had undergone some pelvic surgery (p=0.0186).

Conclusions;
Apart from age and endometriosis and prior pelvic surgery also could be used as predictors for poor ovarian response. Heredity also plays a major role in determining ovarian response.
Introduction;
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 post-translational 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.

Material 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.

Results;
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. 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.

Conclusions;
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.
Patient friendly approach to oocyte pick-up procedure.

Mette Munk¹, Svend Lindenberg², Suzan Lenz², Steen Smidt Jensen², Claus Christoffersen²
¹ Copenhagen Fertility Center
² Section for Reproductive Biology and ART, Copenhagen Fertility Center

Introduction;
Since we introduced ultrasound guided oocyte retrieval for IVF local analgesic using a Para cervical block and sedation has been the main procedure for oocyte retrieval. We have further improved the method by testing a local analgesic in the vaginal vault in the position for the puncture, in which the local analgesic were placed ultrasonically guided just prior to the puncture.

Material and methods;
In a prospective observational study where 1 doctor continued the Para cervical block method for analgesic and sedation with Rapifen, was compared to 3 other doctors using the ultrasound guided placement of the local analgesic and Rapifen sedation. A VAS score for pain perception during the procedure and after were recorded in 100 patients (20 in the Para cervical blockage group and 80 in the ultrasound guided group).
A total of 1287 oocyte retrievals were observed from 01.01.09 to 01.01.10, no drop out. 167 oocyte collections were done by the Para cervical blockage and 1120 using the ultrasound guided procedure.

Results;
The live pregnancy rate per aspiration (week 12 of gestation) was found to be 24% (40/167) in the Para cervical blockage group and 23% (253/1120) in the ultrasound guided group. No differences in number of oocytes, fertilization rates or cleavage rate nor implantation rates were seen between the groups. However a significant lower volume of sedatives were used in the ultrasound guided group as well as lower VAS score for pain during and after the treatment.

Conclusions;
Local analgesic applied under ultrasound guidance in the place for puncture of the vaginal vault during oocyte pick up in human ART is a simple and safe procedure, providing further comfort to the patient compared to traditional Para cervical blockage.
The midluteal decline in serum estradiol levels is deleterious to embryonic implantation during in vitro fertilization and embryo transfer

Yang Jianzhi, Liping Zhu, Xiaoming Teng, Fan Yang, Jianming Yu, Yu Wang
Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine

Introduction;
Embryo implantation remains the most inefficient process in the complex series of events that culminate in the establishment of an In Vitro Fertilization-Embryo Transfer (IVF-ET) pregnancy, two critical components that dictate the success of this rate-limiting step are embryo quality and endometrial receptivity. Improvement of embryo culture techniques has greatly advanced the clinical efficacy of IVF-ET. However, the reasons why only a relative minority of morphologically normal embryos transferred in IVF-ET cycles successfully implant are not fully understood. Some investigators have shown that supraphysiologic E2 levels have a detrimental influence on endometrial receptivity and IVF outcome. However, others did not find high E2 levels to be detrimental to IVF outcome. Hence, the impact of serum E2 concentrations on the outcome of IVF-ET is still under debate.

Our objective is to determine whether the peak E2 level and its midluteal decline during controlled ovarian hyperstimulation (COH) in IVF-ET and Intracytoplasmic sperm injection (ICSI) cycles impairs embryo implantation.

Material and methods;
One hundred sixty-two patients undergo IVF/ICSI protocol for retrospective matched analysis and eighty-three patients undergo for prospective analysis in University-affiliated IVF and infertility unit between 2007 and 2009; one hundred sixty-two patients were retrospective divided into three matched groups: group A, in which patients are nonconception in fresh cycle and conception in Freeze Embryo Transfer (FET) cycle, group B, in which patients are conception in fresh cycle, group C, in which patients are nonconception in fresh cycle and FET cycle; Serum levels of E2 concentrations were measured on the day of human chorionic gonadotropin (hCG) administration in these three groups. Additional studies were performed on eighty-three patients in two groups according to whether the cycle resulted in clinical pregnancy (group M) or not (Group N), and E2 also were measured on the day of hCG administration and the 7 day after oocyte retrieval.

The cycle characteristics measured included the numbers of oocytes retrieved, fertilization, cleavage, embryo transfer(ET) and frozen embryo; in group A,B,C, the patient and treatment cycle parameters had to be comparable when the data was compared according to the age, infertility duration, infertility cause, procedure used for fertilization (IVF or ICSI), COH protocol(long or short). To reduce the bias inherent in including multiple cycles per patient, only first cycles were included in the trial.

Results;
Serum E2 levels on the hCG day were no significant differences among group A, B, C (2988.438±217.127 vs 2716.633±188.193vs2992.636±232.172.), and There were no statistical differences between group M and group N when measuring the E2 on hCG day (3531.333±312.988 vs 4101.575±409.489) and the E2 on midluteal phase(1318.200±300.803 vs 1542.387±289.936). But the decline of E2 between hCG day and midluteal day is significantly higher in group N than in group M (2128.188±267.575 vs 3191.667±499.842). There were no statistically significant differences in number of oocytes retrieved and number of embryos transferred among group A, B, C. The group A had a higher number of fertilization rate, cleavage embryo, frozen embryo compared with the group C.

Conclusions;
Our analysis refutes the negative role of superphysiologic levels of E2 on the day of hCG administration or on the midluteal phase on the embryo implantation, but its dramatic decline at the midluteal phase is deleterious to embryonic implantation during in vitro fertilization and embryo transfer, this results will instruct us how to assign the embryo between fresh cycle and FET cycle, it will help us to improve the pregnancy rate of IVF/ICSI.
Assessment of endometrial and ovarian characteristics using three
dimensional power Doppler ultrasound to predict response in frozen
embryo transfer cycles

Tamara Žáčková¹, Ikka Y Järvelä², Juha S Tapanainen², Jaroslav Feyereisl¹

¹ Institute for the Care of Mother and Child (UPMD), Department of IVF, Charles University
² Department of Obstetrics and Gynecology, Oulu University Hospital

Introduction;
The ability to identify a receptive uterus prospectively by a noninvasive method would have an invaluable clinical impact
on treatment efficiency and success rates. The aim of this prospective study was to evaluate whether any of the
endometrial or ovarian parameters measured using 3D power Doppler ultrasound would predict the outcome in frozen
embryo transfer (FET) cycles.

Material and methods;
Thirty women with no known gynecological pathology (e.g. endometriosis, fibroids, any operation to gynecological organs)
undergoing FET were recruited. The FET was carried out in the natural menstrual cycle 3-4 days after the first positive LH
test result. Blood samples for hormonal analysis were collected, and three-dimensional (3D) ultrasonographic examination
(Voluson Expert 730, Kretz Zipf, Austria) was performed on the day of the FET and repeated with analysis of the total hCG
one week later, at the time of expected implantation. The outcome measures were endometrial pattern, thickness,
volume, vascularization index (VI), subendometrial volume and VI, dominant and non-dominant ovarian volume and VI.
The subendometrial region was considered to be 5 mm beneath the border between endometrium and myometrium. At
the second visit, the power Doppler mode was not used to examine the uterus.

Results;
The demographic, clinical, and embryological characteristics were similar between the pregnant (15/30) and nonpregnant
groups (15/30). There were no differences between the groups in endometrial/subendometrial thickness, volume, or
vascularization index (VI). The endometrial triple-line pattern was more often present in the pregnant group on the day of
the FET (93.3% vs. 40.0%, 95% CI 25.5-81.2%). No differences in the ovaries were observed on the day of the FET. At
the second visit, the triple-line pattern was still more often present in those patients who had conceived (91.7% vs.
42.9%, 95% CI 18.5-79.1%), and their corpus luteum was more active as judged by the rise in 17-hydroxyprogesterone
and estradiol levels. No differences were observed in the dominant ovarian vasculature.

Conclusions;
According to our results, measurement of power Doppler indices using 3D ultrasound on the day of the FET does not
provide any additional information concerning the outcome of the cycle. The existence of the triple-line pattern on the day
of the FET seems to be a prognostic sign of a prosperous outcome after FET. The dominant ovary in the pregnant group
seems to be already activated one week after the FET.
P-018

Ultrasound Guided Direct Injection Of GnRHa In The Treatment Of Lieomyoma

Ali Farid. Ali¹, Laila Farid², Mostafa Fouad², Ahmed Abd el shafy²

1 Heliopolis research center
2 Ain shams university

Introduction;
Is to test this hypothesis that a second form of GnRH and corresponding receptor exists in the fibroid and that GnRH agonists interact directly with GnRH receptors present in fibroids, and produce a new modality of treatment of fibroid avoiding hypoestrogenic state and bone loss by ultrasound guided injection of GnRH in the fibroid.

Material and methods;
10 women had uterine myoma, diagnosis of myoma is based on clinical examination and 3D ultrasound, Minimal invasive outpatient using sedation, GnRHa is given directly into the center of the myoma by 3D transvaginal ultrasound guided injection.

Results;
Statistically significant decrease of uterine volume 414.2± 44.3 before treatment vs. 201±33.1 after treatment, Statistically significant decrease of myoma volume 169.8± 13.7 before treatment vs. 18.7 ±2.3 after treatment, Statistically significant increase in Hb concentration 7.3 ± 1.6 vs. 13.2 ±2.1 vs. after treatment.

Conclusions;
Ultrasound Guided Injection Of GnRHa In The Treatment Of Lieomyoma is a new modality and new delivery system, reducing the cost of treatment, no effect on bone metabolism, no hypoestrogenic symptoms.
Comparison of embryo transfer guided by vaginal versus abdominal ultrasound: a pilot study

Daniel Bodri, Zamora Maria-José, Coll Oriol
Clinica EUGIN

Introduction;
The embryo transfer procedure is the final, crucial step during the course of an in-vitro fertilization cycle. Abdominal ultrasound guided embryo transfer was shown to increase chances of live birth compared with the "clinical touch" method. However only a few studies have evaluated the use of vaginal ultrasound for the same purpose.

Material and methods;
In the context of a pilot study embryo transfer guided by transvaginal ultrasound was prospectively evaluated in 35 recipients of donor oocytes during October-November 2009. Mostly two fresh embryos (mainly at cleavage-stage) were transferred to the uterine cavity under vaginal ultrasound guidance using the Kitazato embryo transfer catheter. Patients of the study group were compared with 68 recipients who underwent embryo transfer during the same two-month period using abdominal ultrasound guidance and Edwards-Wallace echogenic catheter. Patients in the vaginal ultrasound group voided their urinary bladder before the procedure. Conversely in the abdominal ultrasound they were asked to have a full bladder to facilitate echographic visualization. Embryo transfer was performed by a single operator (D.B.) in close collaboration with the embryologist (two-stage technique). Main outcome measures were the clinical pregnancy per embryo transfer and embryonic implantation rate.

Results;
No differences were observed between recipient age (40.6±5.1 versus 40±5.2 years, p=0.61), the number of transferred embryos (1.9±0.3 versus 1.9±0.4, p=0.89) and endometrial thickness (10.3±2.7 versus 9.9±2 mm, p=0.44). Clinical pregnancy rate (57.1% 20/35 versus 42.6% 29/68, p=0.41) and implantation rate (35.4% 23/65 versus 28.3% 36/127, p=0.47) were not significantly different between the vaginal versus abdominal ultrasound group. Twinning rates were comparable (15% 3/20 vs 24.1% 7/29, p=0.52). No extrauterine pregnancy occurred in any of the groups.

Conclusions;
The preliminary findings of our pilot study suggest that embryo transfer guided by vaginal ultrasound is a feasible method which gives comparable results compared to embryo transfer guided by abdominal ultrasound. This new method potentially could lead to the optimization of embryo transfer procedure especially in cases where the echographic visualization is suboptimal with abdominal ultrasound (obesity, retroverted uterus, large fibroids). A randomized clinical trial is warranted to compare pregnancy rates and patient commodity with guidance with the two different ultrasound techniques.
Introduction;
The access to the peritoneal cavity during laparoscopy can result in serious complications such as vessel or bowel perforation. UTHL is a new outpatient procedure which combines transvaginal hydrolaparoscopy (THL) and ultrasound (US) imaging in pelvic examination. It provides ultrasonographically controlled access to the abdominal cavity through the thin posterior vaginal wall, which markedly reduces risk of complications. It is used as a diagnostic tool in visualization of the tuboovarian complex in infertility clinic patients and it is in this sense an alternative to diagnostic laparoscopy (LSK). The description of this new method in terms of the safe, ultrasound guided access to the peritoneal cavity was the aim of this study.

Material and methods;
In UTHL the pouch of Douglas was filled with saline solution via the Fallopian tubes during hysteroscopy, which was the first part of the examination. Puncture of the pouch of Douglas as well as the insertion of the trocar with optics was then done under ultrasound control. The tubo-ovarian structures and the posterior pelvis were visible optically and on ultrasound at the same time (video) Single-use instruments (picture) were used in UTHL. Chromopertubation with methylene-blue was done in all cases. The success rate of the pouch of Douglas puncture, the complication rate, the ability to examin both tubes, length of postoperative stay, length of the sick leave were evaluated.

Results;
We evaluated 1105 UTHL procedures performed between 2001-2009. Complete evaluation of the tubo-ovarian structures was achieved in 96.2%. In 0.9 % patients only unilateral visualization was possible. The procedure was stopped in 2.9% after hysteroscopy was performed, since there was no fluid present in pouch of Douglas after hysteroscopy. We detected no injury of big vessels and one bowel perforation (0.09%) in a patient with adhesions in pouch of Douglas and endometriosis, which was solved immediately in a general hospital. Mild adhesions were detected in 16% of the women, 5.5% had grade I endometriosis. There was unilateral tubal occlusion in 5.4%, and bilateral in 4.2%. In 14.3% elective operative laparoscopy for tubal obstruction and/or adhesions was recommended. The average postoperative stay was 2 hours and average sick leave was 3.2 days .

Conclusions;
Ultrasonographically guided transvaginal hydrolaparoscopy is a modification of standard hydrolaparoscopy offering an instant ultrasound control during the whole procedure. The method is used for direct visualization and evaluation of tuboovarian complex in infertile clinic patients, but it could be theoretically used also in other surgical branches. The method is very well tolerated by the patients and has much shorter postoperative stay in comparison with conventional laparoscopy. The application of disposable instruments reduces markedly risk of infectious complications. The procedure has proved to be a valuable alternative to standard diagnostic laparoscopy in examination of tuboovarian complex and pelvic status in infertile clinic patients. The use of ultrasound enhances rapidly the safety of access to the pouch of Douglas and minimizes complications.
Endometrial sonographic characters predicting pregnancy following recurrent clomiphene induction in unexplained infertility.

Ahmed Y. Shahin
Assiut University, Women's Health Center, Department of Obstetrics and Gynecology

Introduction;
Patients with unexplained infertility managed repeatedly with clomiphene citrate need parameters to predict pregnancy to save them further unsuccessful trials and shorten their treatment to pregnancy interval.

Material and methods;
Ovulation was induced in 226 unexplained infertility patients, who had three previous failed cycles, with 100 mg clomiphene citrate (CC) from days 3 to 7 of the cycle. Human chorionic gonadotrophin (HCG) injection (10,000 IU i.m.) was given and timed intercourse was recommended when a leading follicle reached >17 mm and serum oestradiol exceeded 200 pg/ml.

Results;
A receiver operating characteristic (ROC) curve showed that endometrial thickness >11.60 mm was associated with the lowest, while values >5.50 mm were associated with the highest chance of pregnancy. An endometrial thickness of 7.05 mm showed the best sensitivity and specificity. Patients with endometrial thickness <7.05 mm (n = 98) had significantly more clinical pregnancies (28.6 versus 8.9%), fewer days until HCG injection, thicker endometrium, higher serum progesterone measured on days 20-22 and more triple layer endometria than patients with endometrial thickness > or =7.05 mm (n = 56).

Conclusions;
It is concluded that endometrial thickness range of 5.50-8.25 mm and triple layer endometrium are highly predictive for pregnancy in patients with unexplained infertility induced with CC after repeated failures. Endometrial thickness of 11.60 mm was associated with a low chance of pregnancy.
Effect of artificial oocyte activation using Ionomycin on ICSI outcome

Shahnaz Razavi¹, Shirin Reisi¹, Mohamad Hosein Nasr Esfahani²
1 Department of Anatomy, Isfahan Medical University
2 Royan Institute, (Isfahan campus), ACECR

Introduction;
To evaluate efficiency of Ionomycin on fertilization and cleavage rates, embryo development, and pregnancy rate after ICSI.

Material and methods;
Semen samples were collected from 87 couples with male factor etiology referring to Fertility and Infertility center for ICSI treatment. After oocyte collection, the oocytes were randomly divided into two groups: control and artificial oocyte activation (AOA). The injected oocytes in the control group were cultured in G1. The remaining oocytes were chemically activated by exposure to 10µM Ionomycin for 10 minutes. Around 16-18 hours after ICSI, fertilization was assessed by presence of pronuclei. The percentage of cleavage and high quality embryo were calculated 48 and 72 hours after ICSI. Clinical pregnancy also was determined based on ultrasound observation of fetal heart beat.

Results;
There are significant differences in the mean of fertilization, cleavage rates 72 hours after ICSI between artificial oocyte activation (AOA) and control groups (P<0.001). In patients who had no fertilization in the control group and all the embryos for transfer were derived from AOA group, two pregnancies were recorded. In the patients who had poor fertilization rate (1-33%) in the control group (14.30%), there was a significant increase in mean fertilization rate (58.31%) due to AOA.

Conclusions;
It can be concluded that in cases artificial oocyte activation may improve fertilization, cleavage rates and embryo quality which in turn affect the implantation and pregnancy rate.
P-023

Zeta sperm selection: A suitable method for recovery of sperm with low DNA fragmentation and protamine deficiency

Shahnaz Razavi, Shirin Reisi
Department of Anatomy, Isfahan Medical University

Introduction;
Currently selection of human sperm for ICSI is based on motility and morphology but the sperm shape is an inadequate parameter and other procedures should be used for selection of normal sperm. Therefore the aim of this study was to compare the efficiency of Zeta method with Density gradient centrifugation (PurSperm) for separation of sperm with normal chromatin structure.

Material and methods;
Semen samples were obtained from 63 patients referred to Isfahan Fertility and Infertility Center. Sperm recovered from Zeta method and Density gradient centrifugation (DGC) procedures were evaluated with respect to control (neat semen) group for protamine deficiency, by using Chromomycin A3 (CMA3) staining and for DNA integrity using three different techniques including: 1) sperm chromatin dispersion test (SCD), 2) Acridine orange (AO), and 3) TUNEL assay.

Results;
The results show that the percentage of CMA3 positive sperm have significantly reduced in Zeta and DGC procedures compared to the neat semen. In addition, using three different techniques, the percentage of DNA damaged sperm have significantly reduced in both procedures compared to the neat semen (control) (P< 0.001) but, the percentage of DNA damaged sperm is significantly lower in the Zeta procedure compared to the DGC procedure with respect to the three tests. The results indicate that the efficiency of Zeta method to separate sperm with normal protamine and intact DNA was higher than DGC procedure.

Conclusions;
It can be concluded that Zeta method is more efficient to recover sperm with minimal DNA damage; however, this method has its own limitations.
P-024

Effects of antisperm antibodies on fertilization, cleavage and pregnancy rate in infertile couples undergoing In-vitro Fertilization at a selected centre in Sri Lanka

Varuni Tennakoon1, Surangi G. Yasawardene2, Deepal S. Weerasekera1, James W. Catt3
1  Prarthana, Centre for IVF
2  Department of Anatomy, Faculty of Medical Sciences, University of Sri Jayawardenepura
3  Optimal IVF

Introduction;
The presence of antisperm antibodies (ASA) on spermatozoa has shown to cause infertility in human. These antibodies are postulated to interfere with the fecundity process through various mechanisms such as interference with sperm transport within the female genital tract, alteration of sperm capacitation or acrosomal reaction, interference with fertilization by affecting the sperm binding to zona pellucida, sperm penetration of the zona pellucida, zona reaction, gamete fusion, embryo cleavage, and embryo development.
The formation of ASA may be a consequence of rupture in the blood–testis barrier, obstruction, inflammation and trauma of the genital tract and reduced immunosuppressive activity in males and mechanical or chemical disruption of the mucosal layer of the female genital tract, inadequate seminal plasma immunosuppression and deposition of semen on sites other than vagina in females. ASA can be found in serum, semen, female reproductive tract secretions (oviductal, uterine or cervical-vaginal) and in follicular fluid.
The reported three major structural types of antisperm immunoglobulins are IgA, IgG and IgM; IgM is a larger molecule rarely detected alone or combined with IgA/IgG. Each of the three types can be bound to the whole sperm surface or selectively to the head, midpiece or tail of the spermatozoa when assessed by the mixed agglutination reaction (MAR) technique.
Although many studies are reported in demonstrating the impact of ASA on fertilization, cleavage and pregnancy outcome in world literature, in Sri Lanka the data relating to this issue in humans is scarce.
The aim of this study was to evaluate those issues in our own Sri Lankan setup, compare our findings with those already published in the literature and provide valuable insights for the investigation and management of infertile couples.

Material and methods;
This prospective analytical study was conducted on infertile couples undergoing In-vivo Fertilization (IVF) procedure at ‘Prarthana’, Centre for IVF, 1175, Cotta Road, Rajagiriya, Sri Lanka, from 01.01.2006 to 01.01.2009.
The ASA were detected using MAR latex bead test (SpermMAR, Fertipro NV, Belgium). In the male partner spermatozoa, seminal plasma and serum were examined for ASA isotypes of IgA and IgG. In the female partner, serum and follicular fluid were examined for both ASA isotypes. The direct ‘SpermMAR’ test was performed on spermatozoa and indirect test was performed on seminal plasma, serum and follicular fluid to elicit ASA. The percentage of motile sperm exhibiting latex bead binding was calculated. A test with >30% of the sperms with bound beads was considered ‘positive for ASA’. Any one of the partners showing ASA positivity to one or more of the test samples were taken as a ‘couple positive for ASA’.
A total of 172 couples were investigated; each underwent a single cycle of IVF-ET (embryo transfer). Average age of female partners was 34 years (ranging from 25 to 40); whereas average age of male partners was 37.7 years (ranging from 25 to 47). Female partner underwent controlled ovarian hyperstimulation to obtain a cohort of oocytes for IVF. In 172 IVF cycles, the mean number of oocytes recovered per female was 6.2. Oocytes were recovered trans-vaginally under ultra sound guidance. The preovulatory oocytes in the metaphase II and late metaphase I stages of development were considered in determining the fertilization rate. Fertilization of the oocyte by the sperm was confirmed, if the cell had 02 pronuclei and 02 polar bodies. Day 03 cleavage of the embryo was observed; embryos with 6-8 evenly placed cells with no or mild to moderate fragmentation and cytoplasmic granularity were considered as well cleaved embryos.
Following IVF-ET, the clinical pregnancies were confirmed at 08 weeks of gestation by an ultra sound scan revealing a foetus with heart beat.
Two sample proportion z test and Fisher’s exact test were used for statistical analysis.

Results;
Out of the 172 couples, 48 were positive for ASA while 124 were negative for ASA. The total number of oocytes available for insemination was 258 in ASA positives and it was 808 in ASA negatives. The total fertilization rates of ASA positives and ASA negatives were 69.38% (179/258) and 58.54% (473/808) respectively. And the total cleavage rates were 53.63% (96/179) and 61.73% (292/473) in ASA positives and negatives respectively. It was observed that in ASA positives the total fertilization rate was significantly higher (P-value=0.001) than
that of the ASA negatives. When comparing the total cleavage rates, it was observed that ASA positives had a significantly lower (P-value=0.037) cleavage rate than that of ASA negatives. Therefore, this data shows that although higher fertilization rates are seen in ASA positives, the cleavage rates are significantly lower than the ASA negatives, supporting the fact that ASA have negative effects on post fertilization events.

Following IVF-ET, a total of 08 clinical pregnancies were observed among ASA positives (16.7%; 8/48) and a total of 28 clinical pregnancies were observed among ASA negatives (22.6%; 28/124). Hence, it was observed that ASA positives have a proportionately lower pregnancy rate than ASA negatives in IVF. Thus, it is suggested that ASA may have deleterious post fertilization effects on developing pre implantation embryo.

Conclusions;
Numerous studies have shown a negative association between ASA positives with fertilization and cleavage rates. In this study, fertilization was not affected by the presence of ASA. In fact ASA positives showed a significantly higher fertilization rate (P-value=0.001) than ASA negatives. However, the cleavage rate of embryos in ASA positives was significantly lower (P-value=0.037) than that of ASA negatives. Therefore our data indicate that the antibodies to sperm surface antigens may inhibit early cleavage of oocytes although it does not affect fertilization. These findings may suggest that these antigens may constitute an extranuclear cleavage signal for early division of fertilized zygotes. Several studies have demonstrated a low implantation rate in ASA positives, although some studies in contrast, have showed no correlation between presence of ASA and pregnancy rate. This study shows a proportionately low pregnancy rate in ASA positives than in ASA negatives. During embryo development and perhaps particularly around the time of blastocyst hatching, ASA may have an opportunity to bind to cross-reacting embryonic antigens and may cause embryo degeneration or possibly block implantation. However, more studies with large number and control groups need to be conducted in this regard to achieve conclusive data.

In conclusion, the present study reveals that presence of ASA affect cleavage rate of embryos significantly, although it does not affect the fertilization rate of oocytes. Presence of ASA also shows a negative influence on pregnancy rate. Future studies are needed to understand the specificities of ASA that contribute to infertility, to alleviate current controversies in research conclusions and to provide proper treatment modalities for infertile couples.
Influence of immunoglobulin isotype and sperm surface location of antisperm antibodies on fertilization, cleavage and pregnancy rate in human - A Sri Lankan study

Varuni Tennakoon¹, Surangi G. Yasawardene², Deepal S. Weerasekera¹, James W. Catt³

¹ Prarthana, Centre for IVF
² Department of Anatomy, Faculty of Medical Sciences, University of Sri Jayawardenepura
³ Optimal IVF

Introduction;
Research in antisperm antibodies (ASA) began in 1899 when Landsteiner initially reported that sperm could be antigenic if injected into a foreign species. Since then ASA in infertility has been largely investigated and has led to the possibility that naturally occurring male and female ASA could serve as one mechanism for human infertility. ASA have been theorized to negatively impact fertility by affecting sperm motility, cervical mucus penetration, gamete fusion and potentially even the first steps of embryo development.

Several hypotheses have been put forward for ASA formation. Disruption of blood–testis barrier, obstruction of the genital tract and reduced immunosuppressive activity could lead to ASA formation in males. In females mechanical or chemical disruption of the mucosal layer of the female genital tract, inadequate seminal plasma immunosuppression and deposition of semen on sites other than vagina are some of the causes for formation of ASA.

The reported three major structural types of antisperm immunoglobulins are IgA, IgG and IgM; IgM is a larger molecule and rarely detected alone or combined with IgA/IgG. Each of the three types can be bound to the whole sperm surface or selectively to the head, midpiece or tail of the spermatozoa when assessed by the mixed agglutination reaction (MAR) technique.

Relatively less number of reports have addressed the issue of the impact of immunoglobulin isotype and topographical location of ASA on the sperm surface on the result of fertilization, cleavage and pregnancy rate in human.

The aim of this study was to re-evaluate those issues in our own Sri Lankan setup, compare our findings with those already published in the literature and provide valuable insights for the investigation and management of infertile couples.

Material and methods;
This prospective analytical study was conducted on infertile couples undergoing In-vivo Fertilization (IVF) procedure at ‘Prarthana’, Centre for IVF, 1175, Cotta Road, Rajagiriya, Sri Lanka, from 01.01.2006 to 01.01.2009.

The ASA were detected using MAR latex bead test (SpermMAR, Fertipro NV, Belgium). In the male partner spermatozoa, seminal plasma and serum were examined for ASA isotypes of IgA and IgG. In the female partner, serum and follicular fluid were examined for both ASA isotypes. The direct ‘SpermMAR’ test was performed on spermatozoa and indirect test was performed on seminal plasma, serum and follicular fluid to elicit ASA. The percentage of motile sperm exhibiting latex bead binding was calculated. A test with >30% of the sperms with bound beads was considered ‘positive for ASA’. The isotype of the ASA was observed. Furthermore, the site of attachment of ASA was also considered: binding to the head, midpiece, tail or to the whole sperm as well as to more than one region was indicated.

The study group consisted of 48 couples; each underwent a single cycle of IVF-ET (embryo transfer). One of the partners in each couple was positive for ASA in one or more of the test samples. Average age of female partners was 33 years (ranging from 26 to 40); whereas average age of male partners was 36.1 years (ranging from 26 to 45). Female partner underwent controlled ovarian hyperstimulation to obtain a cohort of oocytes for IVF. In 48 IVF cycles, the mean number of oocytes recovered per female was 5.4. Oocytes were recovered trans-vaginally under ultra sound guidance. The preovulatory oocytes in metaphase II and late metaphase I stages of development were considered in determining the fertilization rate. Fertilization of the oocyte by the sperm was confirmed, if the cell had 02 pronuclei and 02 polar bodies. Day 03 cleavage of the embryo was observed; embryos with 6-8 evenly placed cells with no or mild to moderate fragmentation and cytoplasmic granularity were considered as well cleaved embryos.

Following IVF-ET, the clinical pregnancies were confirmed at 08 weeks of gestation by an ultra sound scan revealing a foetus with heat beat.

Two sample proportion z test and Fisher’s exact test were used for statistical analysis. The fertilization rate and the cleavage rate of ASA negative couples who underwent the same procedure during the same period of time in ‘Prarthana’ IVF laboratory were considered as the ‘standards’ to compare with the same rates of this study group. The standards for the fertilization rate was 58.54% (473/808) and for the cleavage rate was 61.73% (292/473).

Results;
Out of the 48 ASA positive couples, 19(39.58%), 13(27.08%) and 16(33.34%) demonstrated IgA, IgG and both IgA+IgG respectively. It was observed that fertilization rate of oocytes were proportionately higher with IgA (82.76%; 24/29) and
IgG (70.75%; 75/106) compared to ‘standards’ (58.54%) though there was no statistically significant difference. Likewise the cleavage rates were proportionately lower with IgA (45.83%; 11/24) and IgG (49.33%; 37/75) isotypes compared to ‘standards’ (61.73%). In IgA+IgG fertilization rate (65.04%; 80/123) and cleavage rates (60%; 48/80) had a marginal difference with that of ‘standard’ values. IgA isotype had the highest fertilization rate and the lowest cleavage rate. Hence it was evident that IgA isotype may be responsible for more deleterious effects on cleaving embryos.

A total of 08 clinical pregnancies were observed following IVF-ET. Out of the 08 pregnancies, 02, 03 and 03 couples were positive for IgA, IgG and both IgA+IgG. It was observed that couples with IgG isotype of ASA achieved the proportionately highest pregnancy rate (23.08%; 3/13) while couples with IgA isotype of ASA had the lowest pregnancy rate (10.53%; 2/19).

Among ASA positives, 10 (20.83%), 24 (50%), 11 (22.92%), 03 (6.25%) had head, tail, midpiece+tail and whole sperm bound ASA respectively. The fertilization and cleavage of couples with whole sperm bound ASA were not considered for statistical analysis due to the availability of less number of (09) oocytes. The fertilization rates of head, tail and midpiece+tail bound ASA were 69.09% (76/110), 67% (67/100), and 84.62% (33/39) respectively. And that of the cleavage rates were 51.32% (39/76), 61.12% (41/67) and 39.39% (13/33) respectively. It was observed that couples who had head and midpiece+tail bound ASA had a proportionately and significantly lower (P-value=0.018) cleavage rates respectively than that of the “standard”.

Out of 08 pregnancies, most number of pregnancies (n=5) was observed when ASA was bound to the tail of the spermatozoa. There were no pregnancies achieved when ASA was bound to the head of the spermatozoa. Two pregnancies were observed with ASA bound to midpiece+tail and one pregnancy was observed with ASA bound to whole surface of spermatozoa. Thus, it is suggested that some head bound ASA may have more deleterious post fertilization effects on developing pre implantation embryo.

Conclusions;

Some ASA cause infertility or contribute significantly to infertility in humans. Although there is a strong body of evidence that in humans and in other species at least some antibodies that bind to sperm antigens can cause infertility, it is still a debated matter. This is mainly due to the fact that a significant percentage of fertile couples have detectable ASA, and clearly showing that these antibodies have not all disrupted fertility. In this study, the fertilization rates of oocytes were not affected by the isotype of ASA. Couples having the IgA isotype of ASA posses the lowest cleavage rate as well as the pregnancy rate. Other studies have shown the influence of IgA isotype on fertilization, cleavage and pregnancy rate. In contrast, some studies have failed to show any correlation. In this analysis, fertilization of oocytes were not affected by the site of binding of ASA on spermatozoa. Presence of ASA on head region and midpiece+tail of spermatozoa reduce cleavage rate of embryos remarkably and significantly respectively. Head bound ASA appear to have most deleterious effects on early embryonic development and hence pregnancy rate. Head status of spermatozoa is assumed to play a key role in fertilization and cleavage because of sperm’s plasma membrane receptors and its internal nuclear content. The midpiece+tail on the other hand may have high density of ASA on spermatozoa surface which affect cleavage rate.

In conclusion, this study reveals that IgA isotype and head bound ASA on spermatozoa has the most deleterious clinical effects on cleaving embryos and in early implanting embryos. when the density of ASA bound to spermatozoa is high (as in midpiece+tail), it can have negative effects on cleavage of embryos. Nonetheless, extensive studies involving many IVF centres with large number of subjects are in need to investigate the magnitude of this effect.
P-026

TREATMENT OF CERVICAL INSUFFICIENCY ABORTION BY AUTOLOGOUS HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS, MODERN TREND

Ali Farid. Ali ¹, Laila Farid ², Sanaa Mohamed Ali ³, Ahmed Abd el shafy ²

¹ Heliopolis research center
² Ain shams university
³ Kafr EL-Sheikh University

Introduction;
To test the Efficacy of outpatient intracervical injection of peripheral blood mononuclear cells in the treatment of habitual abortion cervical insufficiency.

Material and methods;
Preparation of peripheral blood mononuclear cells (PBMC), transvaginal ultrasound, IL-8, collagenases in the cervical mucus, Aquaporins in the cervical smears before PBMC injection at the time of the delivery. Primary outcome was delivery of full term fetus.

Results;
Full term delivery occurred in 48 cases (96%), abortion 1 case (2%), Preterm delivery 1 case (2%), vaginal delivery in 40 cases (80%), cesarian section in 9 cases (18%). No fetal or maternal complication, were reported.

Conclusions;
Treatment of cervical insufficiency habitual abortion by cervical injection of autologus human peripheral blood mononuclear cell (PBMCS) is safe, effective, and cheap with positive fetal effect and no fetomaternal complications, but more cases and randomization is needed before elucidation the effectiveness of the procedure.
P-027

Yeast Beta Glucan inhibit development of experimental endometriosis and induces apoptosis of endometrial stroma

Ali Farid. Ali 1, Laila Farid 2, Ahmed Abd el shafy 2
1 Heliopolis research center
2 Ain shams university

Introduction;
Yeast beta glucan as an antitumor, antiproliferative, antiangiogenic, antiinflammatory, cholesterol lowering effect, this study evaluate yeast beta glycan on mouse model of endometriosis and on culture of human endometrial cells.

Material and methods;
Human proliferative phase endometrial biopsy were established as organ culture or used for human endometrial stromal cells to established endometriosis in ovariectomized mouse, endometrial tissues were maintained in Oestradiol for 24 hours and subsequently injected intraperitoneal. Mice randomly assigned to receive yeast beta glycan and saline (study group n=20, control group n=20). The animal were then sacrificed and endometrial implants were measured and stained (tunnel for endometriosis).

Results;
Mice treated with beta glucan detect a significant lower number of endometrial implants rather then control p<0.01, tunnel staining for implants in the study group is more than control group p<0.01.

Conclusions;
Yeast beta glucan decrease endometriosis as well as induce apoptosis in human endometrial stromal cells, this will open a novel treatment for endometriosis.
Comparison of $\alpha_6$ integrin expression in mouse embryonic stem cell derived germ cells differentiated in STO co-culture system

Zohreh Makoolati¹, Mansoureh Movahedin², Mehdi Forouzandeh-Moghadam²

¹ Tarbiat Modares University
² Tarbiat Modares University, Anatomical sciences department

Introduction;
The aim of this study was to evaluate the expression of $\alpha_6$ integrin, upon germ cell differentiation from mouse embryonic stem cells (ESCs) in simple and STO co-culture systems.

Material and methods;
ESCs were cultured in DMED containing 20% fetal bovine serum (FBS) for 1 day in order to embryoid body (EB) formation (the first step of germ cell induction) and then cultured 4 days in STO co-culture systems both in the presence or absence of 5 ng/ml BMP4 (the second step of germ cell induction). The expression of $\alpha_6$ integrin that express during germ cell development was calculated in the first and second steps of germ cell induction by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) method.

Results;
Quantitative PCR results showed that $\alpha_6$ integrin was expressed in a higher significant rate in STO co-culture group in the presence of 5ng/ml BMP4 concentration relative to 1-day-old EB and STO co-culture group.

Conclusions;
RT-qPCR was used to estimate the level of germ cell gene expression. The results confirmed that addition of 5ng/ml BMP4 in STO co-culture system improve the differentiation of mouse germ cells from ESCs.
Case Report: Efficacy of Mild stimulation and single vitrified blastocyst transfer of Preimplantation Genetic Diagnosis (PGD) -Why do we need so many oocytes?-


Kato Ladies Clinic

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 (GnRH 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 [9](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-030

New strategy of ovulation induction for poor responders

Kohzo Aisaka¹, H. Hiraike¹, Y. Ikezuki⁴, S. Obata¹, O. Hiraike², H. Mori³

1 Hamada Hospital, Tokyo
2 University of Tokyo
3 Teikyo University

Introduction:
It is well known that there are some patients who cannot respond properly by the exogenous administration of gonadotropin preparations. In addition, it is reported that plasma estrogen levels can modify the activities of gonadotropin receptors. Present study was performed to elucidate whether exogenous administration of estrogen preparation with Gn-RH agonist was effective to improve the response of gonadotropin administration in the patients of gonadotropin resistance syndrome.

Material and methods:
Thirty-three patients who have resistance to gonadotropin therapy were subjected (35.4+-3.2 years old). All of them had experiences of previous treatment of hMG or rec FSH (up to 600iu/day), and could not respond to the treatment (plasma estradiol levels, before: 15.3+-5.1, after: 39.6+-15.9 pg/ml). Then, exogenous estrogen (estradiol 5mg/day, Estrace tablets) was administered for 2 weeks with Gn-RH agonist (buserelin nasal splay). After that, rec FSH preparation (Follstim, 150iu/day) was administered to the subjects with estrogen preparations in gradual increasing method up to 600iu/day, and the follicular growths were observed by the transvaginal ultrasonic scanner. Then, 10000iu of hCG injection was performed when the matured follicles (diameter over 18mm) were observed, and the proper luteal support (administration of progesterone vaginal tablets, 60mg/day, for 2 weeks) was also done.

Results;
Plasma FSH (before estrogen + Gn-RH agonist administration: 43.1+-10.5, after: 7.3+-3.1mlU/ml) and LH (before: 24.6+-8.8, after: 5.8+-2.6mlU/ml) decreased significantly by the exogenous estrogen and Gn-RH agonist administration (p<0.001). Plasma estradiol levels increased up to 247.3+-42.5 pg/ml after exogenous estrogen administration (p<0.001, compared to the levels before treatment). Twenty-seven cases out of 33 (total 102 cycles) could get mature follicles (over 18mm, 4.0+-1.1 /case). Total dose of rec FSH preparation was 2375.6+-239.0iu/case, and plasma estradiol level before hCG injection was 2220.5+-472.2pg/ml. Twelve cases could get pregnancies during this study. No severe side effect, such as ovarian hyper stimulation syndrome, was observed.

Conclusions;
It was suggested that gonadotropin receptors might be activated by the administration of exogenous estrogens, and the controlled ovarian stimulation using rec FSH with Gn-RH agonist after exogenous estrogen priming was effective for the treatment of the intractable ovulatory disturbances.
Successful outcome in high survival rate and pregnancy rate after Single Embryo Transfer (SET) using cryotop vitrification method. –A clinical analysis for three years.

Tsuyoshi Okubo, Teruaki Hayashi, Tomohiro Sueyoshi, Junichiro Fukuda, Masahige Kuwayama, Keiichi Kato, Osamu Kato

1 Shimbashi yume clinic
2 Kato ladies’ clinic

Introduction;
The multiple pregnancy rate of IVF treatment is higher than that of natural pregnancy, because of the tendency to transfer multiple embryos.
To relieve the burden during the neonatal period, the multiple pregnancy rate in IVF cycles must be controlled to a level consistent with natural pregnancy.
SET may be the most effective solution for preventing multiple pregnancy.

Material and methods;
The study in our clinic was conducted from May 2007 to December 2009 in as the minimal stimulation IVF cycles.
All of the patients were treated with the minimal stimulation IVF cycles using clomiphene citrate and recombinant FSH.
Administration of 50 mg clomiphene citrate was initiated on cycle day 3 and then 50-150 IU recombinant FSH was injected every other day from day 8.
When the size of the dominant follicle and the estradiol concentration reach the predefined values, gonadotropin-releasing hormone agonist was administered to induce follicular maturation.
Oocytes were then retrieved at aspiration 30-35 h after nasal GnRHa spray.
Then matured oocytes were inseminated by conventional IVF or ICSI procedure.
When the blastocyst developed over 170 μm diameter, they were vitrified by the Cryotop vitrification method (Kuwayama, 2006) on day 5 to 7.
A total of 2542 cycles, only one frozen-thawed blastosyst was transferred to the patient using the Cryotop thawing method.

Results;
The mean number of oocytes retrieved was 2.8 per OPU cycle.
The number of patient cryopreserved blastocyst by Cryotop vitrification method was 2684 and mean age of patient was 37.3±4.3 years old.
Of all 2684 IVF-ET cycles initiated, the rate for blastocyst survival was 95.1%(2553/2684).
And of all 2542 frozen-thawed blastocyst transferred, the rate for clinical pregancty was 53.3%(1356/2542).
The rate for ongoing pregnancy and multiple pregnancy were 50.8%(1291/2542) and 0.6%(8/1356) , respectively.

Conclusions;
SET has a significantly higher pregnancy rate than standard ET at IVF centers both in Japan and overseas, and the multiple pregnancy rate is only 0.6%.
SET also has a significantly lower multiple pregnancy rate than standard ET at IVF centers both in Japan and overseas.
SET has higher pregnancy expectations, and moreover can reduce multiple pregnancies.
Effects of new protocol for estrogen and progesterone replacement with FSH/HMG simulation on patients with premature ovarian failure: a report of a case in whom fertility was successfully induced.

Yodo Sugishita¹, Mari Watanabe², Nobuhito Yoshioka², Juichiro Saito², Midori Tamura²
Bunpei Ishizuka²

1 Department of Obstetrics and Gynecology, St. Marianna University School of Medicine
2 St. Marianna University School of Medicine

Introduction;
Objective: To estimate a new protocol of ovarian stimulation with sex steroids and gonadotropins in women with premature ovarian failure (POF).
Design: POF is a syndrome clinically defined by ovarian dysfunction before the age of 40, which is characterized by a hypergonadotrophic status and by amenorrhea. POF is not a rare condition: its incidence rate is as great as 1% in women under the age of 40, and 0.1% in women under the age of 30. The diagnosis of POF is made with a serum FSH level over 40mIU/mL. The ovulation induction protocols for women with POF have not been successful.

Material and methods;
Materials and Methods: Informed consent was obtained from all patients. During 3 menstruation periods, serum FSH levels were controlled to be below 7mIU/mL by administrations of estradiol and progesterone. In addition, vitamin E, eicosapentaenoic acid and L-arginine were administrated to improve ovarian bloodflow. Patients have controlled ovarian stimulation for 3-4 weeks. If follicles with over 8mm in the diameter by ultrasounds, oocyte was picked up (OPU) by IVM-IVF (in vitro maturation-in vitro fertilization) or IVF needle. Oocytes at GV, M Ⅰ or M Ⅱ stage were cultured in IVM or IVF medium respectively for suitable durations. Thereafter, the oocyte was fertilized by insemination or ICSI, cultured for 3 days and then vitrified. After some menstrual periods, embryo was transferred to patients under the controlled hypogonadrophic status by estradiol and GnRHagonist.

Results;
Results: Thirty of 112 patients had ovarian follicles above 8mm in diameter. In 151 cycles on 30 patients, OPU was performed. 19 oocytes were picked up from 13 patients. Three oocytes were cultured as IVM and other oocytes were cultured as IVF. 8 embryos were vitrified. So fer, two embryos were transferred and one patient conceived.

Conclusions;
Conclusions: It is suggested that our new protocol is effective for POF patients who are eager to conceive by their own oocytes, although further improvements are necessary in the method. The control of serum FSH levels during more than 3 menstruation cycles may enhance responsiveness of follicles to gonadotropins in patients with POF.
Heterotopic cesarean scar pregnancy: Case report

Wai Leng Wong, Lee SL, Ho TH, Tan HK
Singapore General Hospital

Introduction;
Heterotopic cesarean scar pregnancy is a very rare occurrence especially in a low-risk patient who had not undergone assisted reproduction.

Material and methods;
Patient was a 41 year old Chinese female, gynae code 41/14/0/2. Her obstetric history included two elective LSCS (lower segment cesarean section) in 1995 and 1998. She had regular 30 day cycles, 7 day flow every 30 days, with no complaint of dysmenorrhoea and menorrhagia.
She presented in our department with persistent per vaginal bleeding after termination of pregnancy in another hospital. An ultrasound report from her previous scan noted an intracervical gestational sac which was not low-lying. Ultrasound examination which was performed in our department revealed a heterogeneous mass measuring 3.4 x 3.2 x 4.1 cm in LSCS scar; the posterior part of mass was noted to encroach endometrial cavity, no myometrium was seen anterior to mass. Colour and Power Doppler detected rich vascularity. Large branches of both uterine arteries were noted to supply the mass. Upper endometrial cavity was empty. Her beta HCG was 9893.
MRI of the pelvis revealed a 5.1 x 4.3 x 4.5 cm complex hypervascular solid and cystic mass with multiple vascular channels at lower segment of uterus in the myometrium and overlying the cervical canal. Part of the mass appeared in close contact with bladder dome and urinary bladder serosal involvement should be considered.
Pelvic angiogram and embolisation of both uterine arteries were performed. An arteriovenous fistula was noted arising from right distal uterine artery and was successfully occluded. Two weeks after embolisation, the mass measured about 4 cm and beta HCG was 1668.

Results;
She was on follow-up and the mass shrunk to 1.3 cm after six months.

Conclusions;
Ultrasound serves as a useful examination for monitoring the reduction of a heterotopic LSCS scar pregnancy.
Heterotopic pregnancy following assisted reproduction and embryo transfer

Lay Kieng NG, Lee SL, Hemashree R, Yu S L
Singapore General Hospital

Introduction;
Heterotopic pregnancy is a rare occurrence in the Centre for Assisted Reproduction in our department. The centre has been in existence for twenty-one years and there is a collection of about five cases of heterotopic pregnancy to date.

Material and methods;
We present a case which we encountered last year

Results;
Patient was a 31 year old Indian female, gynae code 31/5/0/0, who was on follow up in our Centre for Assisted Reproduction. Her medical history include hypothyroidism and was on thyroxine treatment. Her pre - IVF pelvic scan was normal. She was on regular cycles of clomid and folic acid. She underwent IVF Trial cannulation and subsequently three embryos were implanted. Ultrasound scan at 6.7 weeks menstrual age noted two viable embryos of 6.3 weeks gestation with FHR of 115 bpm and 112 bpm respectively. A 2 cm clear cyst was seen in right ovary and two corpora lutea was seen in left ovary. There was no fluid or other obvious mass seen in pelvis. Nine days later she was found by her husband to be unresponsive at home. She complained of severe abdominal pain for one day, had diarrhoea but no per vaginal bleeding. Her blood pressure was unrecordable when she arrived at the hospital. She was resuscitated. An ultrasound examination revealed two intrauterine viable embryos. There was also pelvic hemorrhage of determinate source. Retrospective review of images performed previously revealed a probable soft tissue mass adjacent to right ovary. Urgent diagnostic laparoscopy was arranged. Open laparoscopy was performed. Intraoperatively, there was a right ruptured tubal ectopic with hemoperitoneum. Postoperatively, an ultrasound scan revealed absent cardiac activity in both twins

Conclusions;
Assisted reproductive technology is associated with an increased risk of heterotopic pregnancy (HP). Therefore, an early thorough examination is essential to exclude the presence of HP.
The chromosomal constitution of embryos developing from tri-pronuclear zygotes during assisted reproductive technology

Saori Maruyama1, Naoki Aoyama2, Yasuhiro Mchikura3, Hiroshi Ishikawa1, Hrokazu Usi1, Osamu Kato2, Makio Shozu1
1 Chiba University
2 Kato Ladies’ Clinic
3 Towako Ladies’ Clinic

Introduction;
Tripronuclear (3PN) oocytes are observed in 4% of all zygotes during ART, and some of these develop into normal-appearing embryos. The formation mechanisms of a total of three pronuclei have not yet been fully characterized. To clarify the origin and fate of 3PN oocytes, we analyzed the chromosomal constitution of the 3PN zygotes on days 1 and 2.

Material and methods;
Twenty-four and twenty-seven 3PN embryos from c-IVF and ICSI, respectively, were cryopreserved by the vitrification method on day 1 during ART. After thawing, the zygotes were stained with Hoechst 33258 dye and the number of polar bodies and pronuclei were determined. Half of these were analyzed by fluorescent in situ hybridization (FISH) using DNA probes for chromosomes 13, 18, 21, X and Y. The other half of the zygotes were incubated for 20 h, and the resulting day 2 embryos were similarly analyzed. Ploidy was determined when the number of FISH signals was identical for at least three loci, and the cells were otherwise regarded as aneuploid.

Results;
Among 16 zygotes derived from c-IVF derived zygotes, ploidy was successfully determined for 12 zygotes. Eleven zygotes were triploid and one was diploid. The triploid oocytes possessed two polar bodies each, thus suggesting that they were of diandric origin (fertilized by two haploid spermatozoa). After 20 h incubation, 6 out of 10 c-IVF-derived 3 PN embryos developed into the two-cell stage, among which the FISH analysis identified three triploid and two diploid embryos. In contrast, the FISH analyses of 16 ICSI-derived zygotes identified 8 triploid and 7 diploid embryos at day 1. All triploid embryos possessed only one polar body each, thus suggesting a failure in the second polar body extrusion. Out of 8 diploid embryos, five each possessed two polar bodies and four showed pronuclei unequal in size (aniso-pronuclei). After 20 h incubation, 6 out of 11 ICSI-derived embryos developed into the two-cell stage. The FISH analyses identified one diploid, three triploid, and one hexaploid zygote. The ratio of diploid to triploid zygotes was not significantly different from the ratio on day 1, thus suggesting that ICSI-derived 3PN embryos maintained chromosomal constitution at least through the first cleavage stage. Aneuploidy was more frequent in ICSI-derived embryos (5/13) than in c-IVF-derived embryos (2/12) on day 1, and the frequency of aneuploidy did not change after the first cleavage as in the case of c-IVF-derived embryos.

Conclusions;
Our results suggest that the mechanisms that give rise to 3PN zygotes are different between c-IVF and ICSI. c-IVF 3PN zygotes are primarily diandric and are derived from the fertilization of two haploid spermatozoa, while ICSI-derived 3PN zygotes are of digynic origin. The latter are derived from two different mechanisms: namely, a failure in extrusion of the second polar body, which gives rise to a triploid embryo, or the bi-nucleation of a female pronucleus, which thus gives rise to a diploid embryo.

The chromosomal cleavage of developing 3PN embryos also differed between cIVF and ICSI. Half of the cIVF-3PN zygotes lost one set of chromosomes to become diploid, while the other half maintained diandric triploidy throughout the first cleavage. In contrast, both the ploidy and frequency of aneuploidy did not change in the ICSI-3PN embryo after the first cleavage.
Utility of natural cycle ART

Masanori Ochi, A. Kuwahata, T. Wada, M. Kamihata
Ochi Yume Clinic Nagoya

Introduction;
Patients of regular menstrual cycle who were less than 37 years, were able to develop a follicle during the cycle, although ovulation induction has been improved owing to many oocytes retrieval since GnRHagonist and GnRHantagonist was saled. Therefore, in this study, we returned in the basis of a follicle development and showed results that we performed single oocyte retrieval and single embryo transfer which weren’t used ovulation inducing agent.

Material and methods;
Between January 2008 and December 2009, we studied 232 cycles which were less than 37 years, menstrual cycle lengths of 26-32 days, the first ART in our clinic in spite of their past history of ART. Ovulation inducing agent weren’t used at all. Oocyte was retrieved 34 h after GnRHa was sprayed when luteinizing hormone (LH) wasn’t surged, Estradiol (E2) was more than 150 pg/ml, and the follicle of 17 mm in diameter. Oocyte was retrieved after confirmed LH surg lowing when LH was surged. IVF, rescueICSI, ICSI was performed following our adaptation. The early embryo transfer and the blastocyst transfer was performed in principle by fresh without vitrified.

Results;
Oocyte retrieval was performed 232 cycles. The breakdown was; metaphase II (MII): 141 cycles, metaphase I (MI): 21 cycles, germinal vesicle (GV): 14 cycles, degeneration: 16 cycles, oocyte unretrieval: 41 cycles, respectively. IVF, rescueICSI, and ICSI was performed 162 cycles of MII and MI. The breakdown was; IVF: 92 cycles, rescueICSI: 35 cycles, ICSI: 35 cycles. The breakdown of fertilization cycles was; normal fertilization cycle: 146 cycles, abnormal fertilization: 12 cycles, unfertilization: 4 cycles. The early embryo transfer and the blastocyst transfer was performed by fresh 135 cycles except 2 cycle of uncleaved and 9 cycles of undeveloped to blastocyst until day 5 (day 0 = the day of IVF, rescueICSI, and ICSI). The patients of 54 cycles became pregnant (40.0%). Of those, 7 cycles was abortion (13.5%). Undeveloped to blastocyst until day 5 of 7 cycles was vitrified, the blastocyst was transfered under hormone replacement therapy at a later date. The patients of 4 cycles became pregnant (57.1%). Of those, 1 cycle was abortion (25%).

Conclusions;
These results were hardly possible that the superior oocyte was retrieved from patient of regular menstrual cycle who was less than 37 years in condition of natural cycle. Moreover, Our results indicated that oocyte retrieval in condition of natural cycle which was the regular menstrual cycle and less than 37 years gave higher implantation rate because hormone condition was normal. Therefore, we thought that a single oocyte retrieval and a single embryo transfer without ovulation inducing agent should be tried.
The Effectiveness of Clomiphene Citrate in Suppressing the LH Surge in the Minimal Stimulation IVF Protocol

Satoshi Kawachiya, Kato K, Osada H, Takehara Y, Kato O
Kato Ladies Clinic

Introduction;
The standard protocol for IVF is long/short protocol or FSH/antagonist protocol. These protocols, however, have various complications, for example, ovarian hyperstimulation syndrome. Natural cycle IVF is one option for IVF but not so easy to retrieve egg because we can’t control natural LH surge. A minimal stimulation protocol using clomiphene citrate (CC) is known for patient friendly IVF protocol. Clomiphene citrate (CC) itself has antagonistic effect for LH. So we can avoid premature LH surge with CC. Our study shows that CC can suppress the LH surge.

Material and methods;
A total of 543 cycles in patients of 25-39 years old with a regular menstrual cycle, from which oocytes were retrieved during the CC cycle from January to October, 2005, were included in this study. 50 mg CC was administered from cycle day 3 and 75 IU HMG/FSH was administered every other day from day 8 until the leading follicle developed to 18 mm in diameter. Administration of CC was then stopped, and 300µg GnRH-agonist (buserelin) given for maturation trigger. Oocytes were retrieved 25-36 h following the administration of the GnRH-agonist. Serum estradiol and LH levels at the day of maturation trigger were measured and compared to those of the natural cycle protocol group (n=201) recruited in the same institution in order to evaluate the efficacy of CC in LH surge suppression. The oocyte retrieval rate and pregnancy rate of the minimal stimulation cycles were also examined.

Results;
The mean number of oocytes retrieved was 1.83 in CC cycle. The serum estradiol and LH levels were 212-2296 pg/mL (mean: 755.5 pg/mL), and 2.8-33 mIU/L (mean: 8.4 mIU/L), respectively. In these 543 cycles, only 27 cycles (5.0%) showed premature LH surge. In the natural cycle protocol control group, the mean serum estradiol level and the mean serum LH level was 338.4 pg/mL and 13.6 mIU/L, respectively. These result shows that the LH level in the minimal stimulation cycle with CC was significantly lower than that of the control group (P<0.001). Of 543 cycles in which the stimulation was initiated, the number of cycles in which the ovulation had occurred before oocyte retrieval was only 15 cycles (2.8%), demonstrating a significantly lower rate compared to that of the control group (38 cycles; 18.9%; P<0.001

Conclusions;
This study suggests that CC is sufficiently effective in suppressing the LH surge in the minimal stimulation IVF protocol. Mini IVF protocol with CC, which can control the LH surge without the use of GnRH-agonist or GnRH-antagonist, is very useful protocol for IVF-ET.
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LAST DATE FOR
EARLY BIRD REGISTRATION
16 August, 2010

&

LAST DATE FOR
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30 September, 2010
Some Key Sessions on cutting edge topics in ART

- IVM as the cornerstone of mild ART: Evolution or revolution?
- Mild Stimulation & modified natural cycle IVF
- Stem cells in Reproduction and Regenerative medicine
- Proteomics and metabolomics – are we in for a potential overhaul in the non-invasive assessment of the embryo?
- New concepts in fertility preservation and procreative liberty: widening the playing field for the 21st century?
- Advanced ultrasound in the optimization of safe and mild ART
- PCOS and patient friendly strategies – current status
- Vitrification of oocytes and embryos: achievements, promise and possibilities
- From gamete to embryo implantation: emerging technology in ART
- The role of Minimally invasive surgery to improve outcome in ART
- 2 exciting debate sessions where world leaders face off on current controversies in ART

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02 Optimization of COS – hyper responders to poor responders
03 A-Z of embroylo Laboratory to improve ART outcome (including interactive workshops on vitrification and embryo scoring)
04 Minimally invasive fertility enhancing surgery to improve ART outcome (including live demonstration)

* Limited Seats
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<td>PRE CONGRESS PG COURSE ONLY</td>
<td>2500 150 1000 75</td>
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