Ovarian aging
Special Interest Group Reproductive Endocrinology

3 July 2011
Stockholm, Sweden
Ovarian aging

Stockholm, Sweden
3 July 2011

Organised by
Special Interest Group Reproductive Endocrinology
Contents

Course coordinators, course description and target audience  Page 5

Programme  Page 7

Introduction to ESHRE  Page 9

Speakers’ contributions

The genesis of the oocyte store: does it really stop in utero? - Claus-Yding Andersen (Denmark)  Page 17

Determinants of ovarian aging and premature ovarian failure - Richard Anderson (United Kingdom)  Page 18

Oocyte quality, genetics and metabolism - Helen Picton (United Kingdom)  Page 31

Is the oocyte the main determinant of embryo quality? - Ursula Eichenlaub-Ritter (Germany)  Page 43

Do ovarian reserve tests correlate with oocyte quality and natural fertility or simply numbers of oocytes available during ART? - Scott Nelson (United Kingdom)  Page 58

Preservation of fertility: oocyte or ovarian tissue freezing? - Dror Meirow (Israel)  Page 68

Hormone replacement therapy for Premature Ovarian Failure and the menopause – Melanie Davies (United Kingdom)  Page 79

Effect of postponing pregnancy on society as a whole: population impact, demand for/access to infertility treatment, financial implications – Siladitya Bhattacharya (United Kingdom)  Page 99

Upcoming ESHRE Campus Courses  Page 108

Notes  Page 109
Course coordinators

Adam Balen (United Kingdom)

Course description

The aims of this course are to provide an understanding of how oocytes are formed in the human ovary and then how they are lost. The attendee will leave with knowledge relating to the differences between embryo number and quality and how these can be determined. This is of key importance for advising women of their potential fertility and in the management of infertility. This course will cover how oocytes are formed in the ovary and determinants of their rate of loss. Detailed descriptions will be given of factors that influence oocyte quality and thereby potential fertility and how these may be quantified. The causes and management of premature ovarian failure will be described as will ways to preserve fertility by either oocyte or ovarian tissue cryopreservation. We will conclude with a socio-ethical talk on the effect of postponing pregnancy on society as a whole with respect to population impact, demand for or access to infertility treatment and its financial implications.

Target audience

Reproductive physicians, paramedicals, basic scientists and embryologists.
Scientific programme

Chair: Adam Balen (United Kingdom)

09.00 - 09.10 Introduction
09.10 - 09.40 The genesis of the oocyte store: does it really stop in utero? - Claus-Yding Andersen (Denmark)
09.40 - 09.50 Discussion
09.50 - 10.20 Determinants of ovarian aging and premature ovarian failure - Richard Anderson (United Kingdom)
10.20 - 10.30 Discussion
10.30 - 11.00 Coffee Break

Chair: Georg Griesinger (Germany)
11.00 - 11.30 Oocyte quality, genetics and metabolism - Helen Picton (United Kingdom)
11.30 - 11.40 Discussion
11.40 - 12.10 Is the oocyte the main determinant of embryo quality? - Ursula Eichenlaub-Ritter (Germany)
12.10 - 12.30 Discussion
12.30 - 13.30 Lunch

Chair: Richard Anderson (United Kingdom)
13.30 - 14.00 Do ovarian reserve tests correlate with oocyte quality and natural fertility or simply numbers of oocytes available during ART? - Scott Nelson (United Kingdom)
14.00 - 14.15 Discussion
14.15 - 14.45 Preservation of fertility: oocyte or ovarian tissue freezing? - Dror Meirow (Israel)
14.45 - 15.00 Discussion
15.00 - 15.30 Tea break

Chair: Frank Broekmans (The Netherlands)
15.30 - 16.00 Hormone replacement therapy for Premature Ovarian Failure and the menopause – Melanie Davies (United Kingdom)
16.00 - 16.15 Discussion
16.15 - 16.45 Effect of postponing pregnancy on society as a whole: population impact, demand for/access to infertility treatment, financial implications – Siladitya Bhattacharya (United Kingdom)
16.45 - 17.00 Discussion

17.00 Close
What is ESHRE?

ESHRE was founded in 1985 and its Mission Statement is to:

- promote interest in, and understanding of, reproductive science
- facilitate research and dissemination of research findings in human reproduction and embryology to the general public, scientists, clinicians and patient associations.
- inform policy makers in Europe
- promote improvements in clinical practice through educational activities
- develop and maintain data registries
- implement methods to improve safety and quality assurance

Executive Committee 2009/2011

<table>
<thead>
<tr>
<th>Chairman</th>
<th>Luca Gianaroli</th>
<th>Italy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chairman Elect</td>
<td>Anna Veiga</td>
<td>Spain</td>
</tr>
<tr>
<td>Past Chairman</td>
<td>Joep Geraedts</td>
<td>Netherlands</td>
</tr>
<tr>
<td></td>
<td>Jean François Guérin</td>
<td>France</td>
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<td></td>
<td>Timur Gürgan</td>
<td>Turkey</td>
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<td></td>
<td>Ursula Eichenlaub-Ritter</td>
<td>Germany</td>
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<td></td>
<td>Antonis Makrigiannakis</td>
<td>Greece</td>
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<td></td>
<td>Miroslav Stojkovic</td>
<td>Serbia</td>
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<td></td>
<td>Anne-Maria Sukkari</td>
<td>Finland</td>
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<td></td>
<td>Carlos Plancha</td>
<td>Portugal</td>
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<td></td>
<td>Françoise Shenfield</td>
<td>United Kingdom</td>
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<tr>
<td></td>
<td>Etienne Van den Abbeel</td>
<td>Belgium</td>
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<td></td>
<td>Jolienke Schoonenberg-Pomper</td>
<td>Netherlands</td>
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<tr>
<td></td>
<td>Veljko Vlašarjevic</td>
<td>Slovenia</td>
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<tr>
<td></td>
<td>Søren Ziebe</td>
<td>Denmark</td>
</tr>
</tbody>
</table>
### General Assembly of Members

- ESHRE Organisation
  - Executive Committee
  - Committee of Nat. Representatives
  - Central Office
  - ESHRE Consortia
    - EIM Consortium
    - PGD Consortium
  - Sub-Committees
    - Finance Sub-Committee
    - Comm. Sub-Committee
    - Publ. Sub-Committee
    - Task Forces
    - SIG Sub-Committee
    - SIG Coordinators

### ESHRE Journals

- **Human Reproduction** with impact factor 3.859
- **Human Reproduction Update** with impact factor 7.042
- **Molecular Human Reproduction** with impact factor 3.005

### Campus Activities and Data Collection

#### Campus / Workshops
- Meetings are organised across Europe by Special Interest Groups and Task Forces
- Visit [www.eshre.eu](http://www.eshre.eu) under CALENDAR

#### Data collection and monitoring
- European IVF Monitoring Group data collection
- PGD Consortium data collection
ESHRE Activities

- Embryology Certification
- Guidelines
- Position papers
- News magazine “Focus on Reproduction”

ESHRE COMMUNITY

RSS feeds for news in reproductive medicine

Since launch 12/2009: 1,360 Fans
Since launch 12/2009: 190 followers
(journalists, scientific organisations, patient societies, governmental bodies)

Retweets to MHR

ESHRE Membership (1/3)

TOTAL MEMBERSHIP*: 5 659 members

* as of July 2010
**ESHRE Membership (2/3)**

<table>
<thead>
<tr>
<th>Membership Type</th>
<th>1 yr</th>
<th>3 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordinary Member</td>
<td>€ 60</td>
<td>€ 180</td>
</tr>
<tr>
<td>Paramedical Member*</td>
<td>€ 30</td>
<td>€ 90</td>
</tr>
<tr>
<td>Student Member**</td>
<td>€ 30</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

*Paramedical membership applies to support personnel working in a routine environment such as nurses and lab technicians.
**Student membership applies to undergraduates, graduate and medical students, residents and post-doctoral research trainees.

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**ESHRE Membership – Benefits (3/3)**

1) Reduced registration fees for all ESHRE activities:
   - Annual Meeting
     - Ordinary € 480 (€ 720)
     - Students/Paramedicals € 240 (€ 360)
   - Workshops* All members €150 (€ 250)

2) Reduced subscription fees to all ESHRE journals – e.g. for Human Reproduction €191 (€ 573)

3) ESHRE monthly e-newsletter

4) News Magazine “Focus on Reproduction” (3 issues p.a.)

5) Active participation in the Society’s policy-making

*Workshop fees may vary

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**Special Interest Groups (SIGs)**

The SIGs reflect the scientific interests of the Society’s membership and bring together members of the Society in sub-fields of common interest:

- Andrology
- Psychology & Counselling
- Early Pregnancy
- Reproductive Genetics
- Embryology
- Reproductive Surgery
- Endometriosis / Endometrium
- Stem Cells
- Ethics & Law
- Reproductive Endocrinology
- Safety & Quality in ART
**Task Forces**

A task force is a unit established to work on a single defined task / activity

- Fertility Preservation in Severe Diseases
- Developing Countries and Infertility
- Cross Border Reproductive Care
- Reproduction and Society
- Basic Reproductive Science
- Fertility and Viral Diseases
- Management of Infertility Units
- PGS
- EU Tissues and Cells Directive

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**ESHRE – Annual Meeting**

- One of the most important events in reproductive science
- Steady increase in terms of attendance and of scientific recognition

**Track record:**

- ESHRE 2010 – Rome: 9,204 participants
- ESHRE 2009 – Amsterdam: 8,095 participants
- ESHRE 2008 – Barcelona: 7,559 participants

**Future meetings:**

- ESHRE 2011 – Stockholm, 3-6 July 2011

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**ESHRE 2011, Stockholm, Sweden**

**When:** 3 - 6 July 2011

**Where:** Stockholmsmässan, Mässvägen 1, Älvsjö, Sweden

**Chair of conference:** Kersti Lundin

**Hotel and Travel:**

MCI - Stockholm Office
Phone: +46 (0)8 54651500
E-mail: eshre@mci-group.com

For updates visit www.eshre.eu
ESHRE 2011, Stockholm

Keynote Lectures
Aneuploidy in humans: what we know and we wish we knew – Terry Hassold (USA)

Historical Lecture
A brave new world with a brave old humankind; quo vadimus – E. Diczfalusy (SE)

MHR Symposium – The paternal genome
Sperm chromatin packaging – B. Robaire (CDN)
The human sperm epigenome – B. Cairns (USA)

ESHRE 2011, Stockholm: Debates

This house believes that obese women should not receive treatment until they have lost weight
- Yes: Mark Hamilton (UK)
- No: Guido de Wert (NL) - TBC

Paramedical invited session: Should we pay donors?
- Yes: Herman Tournaye (BE)
- No: Laura Wiltens (UK)

Annual Meeting – Pre-Congress Courses

- PCC 1: The challenges of embryo transfer (Paramedical Group)
- PCC 2: The blastocyst: perpetuating life (SIG Embryology and SIG Stem Cells)
- PCC 3: From genes to gestation (SIG Early Pregnancy and SIG Reproductive Genetics)
- PCC 4: Lifestyle and male reproduction (SIG Andrology)
- PCC 5: Ovarian ageing (SIG Reproductive Endocrinology)
- PCC 6: The impact of the reproductive tract environment on implantation success (SIG Endometriosis/Endometrium)
- PCC 7: Adhesion prevention in reproductive surgery (SIG Reproductive Surgery)
### Annual Meeting – Pre-congress Courses

- **PCC 8:** Theory and practice update in third party reproduction  
  (SIG Psychology and Counselling)
- **PCC 9:** Ethical aspects of non-invasive prenatal diagnosis  
  (SIG Ethics & Law)
- **PCC 10:** Patient-centered fertility services  
  (SIG SQUART)
- **PCC 11:** Clinical management planning for fertility preservation in female cancer patients  
  (TF Basic Science and TF Preservation in Severe Disease in collaboration with the US OncoFertility Consortium)
- **PCC 12:** Opportunities for research in female germ cell biology  
  (TF Basic Science)
- **PCC 13:** Assisted reproduction in couples with HIV  
  (TF Fertility and Viral Diseases)
- **PCC 14:** Prevention of infertility – from preconception to post-menopause  
  (TF Reproduction and Society)
- **PCC 15:** Hot topics in male and female reproduction  
  (ASRM exchange course)
- **PCC 16:** Academic Authorship programme  
  (Associate Editors ESHRE journals)
- **PCC 17:** Science and the media, an introduction to effective communication with the media  
  (Communications SubCommittee ESHRE)

### Certificate of attendance

1. Please fill out the evaluation form during the campus
2. After the campus you can retrieve your certificate of attendance at www.eshre.eu
3. You need to enter the results of the evaluation form online
4. Once the results are entered, you can print the certificate of attendance from the ESHRE website
5. After the campus you will receive an email from ESHRE with the instructions
6. You will have TWO WEEKS to print your certificate of attendance
The genesis of the oocyte store: does it really stop in utero?

Claus Yding Andersen

Contribution not submitted by speaker
Determinants of ovarian aging and premature ovarian failure

Richard A Anderson

Ovarian ageing: learning objectives

• Background to ovarian ageing
• Environmental and genetic determinants
• Single-gene models
• Granulosa cell and oocyte contributions
• Is there potential for extending ovarian life?

Humans have a limited reproductive lifespan

Modified from A. H. Schultz (1969) The Life of Primates (20), 149
Age at menopause: health impact

- Early
  - Osteoporosis
  - Cardiovascular risk
- Late
  - Breast cancer

Human oocyte dynamics

Germ cell proliferation in ovary and testis
Synchronous division in germ cell cysts

Germ cells are connected by ring canals
Current model of follicular depletion

Age and reproductive success

Progressive loss of reproductive function
Environmental determinants of age at menopause

- Age at first childbirth
- Age at last childbirth
- Age at menarche
- Alcohol use
- Birth weight
- Clothing consumption
- Cognition
- Depression
- Diet
- Educational level
- Employment
- Ethnicity
- Exercising
- Height
- Height gain
- Height reduction diet
- History
- Marital status
- Menstrual cycle length
- Menstrual cycle irregularity
- Miscarriages
- Oral contraceptive use
- Physical activity
- Psychosocial stress
- Religion
- Siblings
- Smoking
- Smokers
- Sudden infant death syndrome
- Type 2 diabetes
- Year of birth
- Weight
- Weight gain
- Weight reduction diet

Total impact: 3%

Factors determining the age of menopause

Environmental factors

Women who smoke reach menopause 2 years earlier than non smokers

Premature ovarian failure

Etiology unknown in more than 90%

- other than surgery chemotherapy, radiotherapy and Turner syndrome
Genes and POF

- Familial cases
- 15-20% of cases

Approaches

- Genome scanning of familial cases
- Genome scanning of sporadic cases
- Candidate genes from animal models?

Premature Ovarian Failure (insufficiency)

- Autosomes
  - FSHR
  - FOXL3
  - GDF9
  - ATM
  - AIRE
  - NOBOX
  - GALT
  - EIF2B
  - NSY1
  - DMC1
  - Parathyroid responsive B1 gene
  - FIGLA
  - Progesterone receptor membrane component-1 (PGRMC1)

- X linked
  - X Monosomy
  - X,XX mosaicism
  - X ring
  - Triple X
  - X Deletions
  - X, autosome translocations
  - FMR1
  - BMP15 .......
**Genome-wide scanning**

- 165 Dutch families
- 9q21.3 and Xp21.3

GWAS in 2979 women

‘Loci at chromosomes 13, 19 and 20 influence age at natural menopause’

Stolk et al. Nature Genetics 2009, 41, 645

*Human studies on genetics of the age at natural menopause: a systematic review*

Voorhuis et al. Hum Reprod Update Jan 2010

‘…very few consistent associations were found’

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**Non-reproductive function in Bax-deficient mice**

Perez et al. 2007 Proc. Natl. Acad. Sci. USA 104, 5229

Absence of the proapoptotic Bax protein extends fertility and alleviates age-related health complications in female mice.

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**Follicle recruitment rate by age**


Follicle recruitment rate by age.
The growing follicle produces changing hormones

- AMH
- Inhibin B, oestradiol
- Primordial
- Preantral
- Antral
- Preovulatory

Changes in markers of the ovarian reserve with age

- FSH
- AMH
- Follicle count

In COS, AMH predicts no of oocytes (better than inhibin B)
Conveniently, AMH does not vary across the menstrual cycle

Seifer et al., 2002; Fanchin et al., 2003
 Prediction of menopause

50 women followed prospectively (Michigan Bone Health and Metabolism Study)
5 annual assessments
Mean initial age 42 yr

![Graph showing prediction of menopause]

The association of age at FMP with AMH and inhibin B profile

<table>
<thead>
<tr>
<th></th>
<th>β ± SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log AMH intercept</td>
<td>0.83 ± 0.38</td>
<td>0.035</td>
</tr>
<tr>
<td>Log AMH slope</td>
<td>0.75 ± 3.52</td>
<td>0.83</td>
</tr>
<tr>
<td>Log Inhibin B intercept</td>
<td>1.83 ± 1.77</td>
<td>0.31</td>
</tr>
<tr>
<td>Log Inhibin B slope</td>
<td>-0.07 ± 3.52</td>
<td>0.98</td>
</tr>
</tbody>
</table>

AMH nomogram with age

AMH in 9600 infertile women: Nelson et al Fertil Steril 2010
Poor responders = earlier menopause

<table>
<thead>
<tr>
<th>IVF poor responders</th>
<th>IVF normal responders</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median follow-up</td>
<td>% menopausal</td>
<td></td>
</tr>
<tr>
<td>Retrospective cohort</td>
<td>6 years 22</td>
<td>36/75 7</td>
</tr>
<tr>
<td>Retrospective cohort</td>
<td>5 years 18</td>
<td>18/65 16</td>
</tr>
<tr>
<td>Case control</td>
<td>7 years 12</td>
<td>2/26 7</td>
</tr>
</tbody>
</table>

Data from De Boer et al 2002, 2003; Nikolaou et al 2002; Lawton et al., 2003

Premature menopause in childhood cancer survivors

Relative risk 13.2
(95%CI 3.3-53.5)

Reduced ovarian reserve in childhood cancer survivors

FSH 15
E2 100
Inhibin B
AMH

Controls
Cancer survivors
All with regular menstrual cycles
Follicle activation and depletion in AMH ko

4 months

AMHR2–482 A>G genotypes and parity

Oocyte-dependent follicle activation

Normal at PD5
Widespread activation from PD5, few primordials by PD23
Excess follicles at transient stage
Germ stem cells in the ovary?


Ovarian regeneration?

Restoration of fertility in BMT recipients after busulphan/cyclophosphamide (CTx). All offspring from recipient germ cells.

Bone marrow-derived oocytes in recipients

Lee et al 2007 J Clin Oncol 3198
Bone-marrow infusion prolongs reproductive life

Also improvement in pup survival in older dams

Conclusions

• The ovary has a finite lifespan, shorter than any other major organ
• Evolutionary benefits vs individual detriment
• Major genetic component: irreversible?
• Prediction in the individual
• Stem oocytes: a real contributor?
**Oocyte Quality: Genetics and Metabolism**

Prof. Helen Picton  
Division Of Reproduction & Early Development  
Leeds Institute Of Genetics, Health and Therapeutics  
University of Leeds  
UK

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**Learning Objectives**

1. Define oocyte quality in health and infertility  
2. Discuss the dynamics of follicular fluid and granulosa markers of oocyte quality  
3. Evaluate molecular markers as indices of oocyte quality  
4. Assess cytogenetic markers of oocyte quality  
5. Explore the links between energy and protein metabolism and oocyte developmental competence

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**Genetic and Metabolic Markers Of Oocyte Quality**

Oocyte quality is defined as the ability of an egg to complete meiosis and undergo fertilization to produce a healthy embryo which has the potential to progress to the blastocyst stage *in vivo* or *in vitro* and/or implant to produce healthy offspring.
Follicular Fluid And Granulosa Cell Markers Of Follicle And Oocyte Development

- Nutrients
- Growth factors
- Hormones
- Oxygen tension
- Granulosa cells

1.5 mm Antral Follicle

Metabolic requirements change as:
1) Follicle size and vascularity
2) Follicle differentiation
3) Follicle exposure to LH/ hCG

18 mm Ovulatory Follicle

Genetic And Metabolic Markers Of Oocyte Development & Quality

1. Molecular Markers (Genomics)
2. Cytogenetic Markers
3. Metabolic Markers (Metabolomics)

- Species: Mouse, Cow, Human
  - In vivo animal and human studies
  - In vitro growth & maturation of oocytes
  - In vitro production of embryos

Strategies To Study Molecular Aspects Of Egg Quality

- Targeted molecular studies of known genes
- Expression analysis across all stages of egg development
  - Global screening: e.g. microarray analysis
  - Characterise: known and novel gene function
Microarray Analysis Of 8793 Gene Products In Single Vs Pooled (5) Human Oocytes

![Microarray Analysis Chart]

Summary Of Molecular Regulation Of Follicle Development & Egg Quality

- Two-way communication: Oocyte ↔ GCs via gap junctions
- Candidate granulosa & cumulus genes: e.g. Gremlin, BMPRIA, BMP-2, SERPINE2, SFRP5, HAS2, COX2, PTX3, EGF-R, TBC1D1, STX7, Ferredoxin 1
- Oocyte genes: e.g. GDF-9, BMP-15, BMP-6, G6PDH, Unknown?
- Unknowns?

Genetic And Metabolic Markers Of Oocyte Quality

1. Molecular Markers
   - Valuable & generates a lot of data
   - Destructive of cells of interest
   - Analyses often conducted on pooled eggs of “different quality”
   - Must be followed up by functional studies
   - No insight into chromosomal health of the gamete

2. Cytogenetic Markers

3. Metabolic Markers
Cytogenetic studies are highly relevant as oocyte quality is known to decline with advancing maternal age. This decline is due to:

1. Increased chromosomal error/aneuploidy in oocytes and embryos
2. Accumulation of mitochondrial deletions and reduced mitochondrial activity in oocytes

24-colour M-FISH On Human Oocytes

Oocyte Karyotype: 23,X +15cht,+19cht,+22cht
1st Polar Body Karyotype: 23,X,-15cht,-19cht,-22cht

(b) & (f) = DAPI
(a,c) & (e,g) = M-FISH
(c = Re-probing
*13
*18
*21
*X

Chylar et al., 2001

Analysis of Human Oocytes

IVF

IVM

ICSI-Rescue (GV to MII)

Molecular and metabolism assays and cytogenetic evaluation
after 30-36hrs of "true" IVM of GV oocytes to MII
after 12-16hrs of culture of ICSI GV/MI oocytes to MII

Genetic Analysis of Human Oocyte Karyotypes
By M-FISH or SKY

<table>
<thead>
<tr>
<th></th>
<th>IVF (n=50)</th>
<th>IVM (n=79)</th>
<th>ICSI-Rescue (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>45 (90%)</td>
<td>57 (72%)</td>
<td>24 (63%)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>4 (8%)</td>
<td>14 (22%)</td>
<td>14** (37%)</td>
</tr>
</tbody>
</table>

** 13% n<23, 21% n>23
True hypoploidy recorded for Chr 3, 8, 20, 22, X
1 oocyte with a balanced predvision of Chr 16, 1 diploid
Non-disjunction (+/- univalent) most frequent followed by predvision

Clyde et al., 2001

Analysis of Human Oocytes

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IVM

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1 oocyte with a balanced predvision of Chr 16, 1 diploid
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Clyde et al., 2001
Results of Human Oocyte Karyotyping Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient Age (yrs)</th>
<th>K-type No.</th>
<th>Tissue Type</th>
<th>Abnormal Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pellestor et al 2003</td>
<td>19-46</td>
<td>792</td>
<td>1379</td>
<td>IVF-FF</td>
</tr>
<tr>
<td>2. Sandelinas et al 2002</td>
<td>20-34</td>
<td>13</td>
<td>47</td>
<td>Donor</td>
</tr>
</tbody>
</table>

Aneuploidy rates increase with advancing maternal age and increasing FSH dose. Chromosomal errors induced by ART may compromised egg quality.

Genetic And Metabolic Markers Of Oocyte Quality

1. Molecular Markers
   Targeted molecular studies are valuable but invasive & destructive of the tissue under study.

2. Cytogenetic Markers
   Information on oocyte genetic health: – FISH studies are informative but time consuming; 1st polar body analysis by CGH array is likely to be a valuable tool to study impact of ovarian ageing on oocytes.

3. Metabolic Markers
   Non-invasive & sensitive at single oocyte level

Measurement Of Metabolism During Egg Development In Vitro

<table>
<thead>
<tr>
<th>Uptake From media</th>
<th>Production Into media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td>H₂O</td>
</tr>
<tr>
<td>Glucose</td>
<td>CO₂</td>
</tr>
<tr>
<td>Lactate</td>
<td>Lactate</td>
</tr>
<tr>
<td>Lipid</td>
<td>NH₄⁺</td>
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<tr>
<td>Amino acids</td>
<td>Amino acids</td>
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<tr>
<td>Oxygen</td>
<td>Enzymes</td>
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<tr>
<td></td>
<td>Growth factors</td>
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Influenced by:
- Cytogenetics
- Gene function
Evidence Of The Links Between Oocyte Metabolism & Oocyte Quality

*4 Species: mouse, sheep, cow & human*

---

**Pyruvate Consumption By Individual Oocytes Throughout Mouse Oocyte Development**

(a) Consumption per denuded oocyte

(b) Consumption per oocyte unit volume

Different letters are significantly different at p<0.05


---

**Energy Metabolism By Individual Human Oocytes From Infertile Patients With Polycystic Ovarian Syndrome (PCOS) Compared To Normal Controls**

(Harris et al 2010)
Metabolic Comparisons Of PCOS vs. Control Oocytes During IVM

1) No differences were detected in oocyte meiotic maturation or frequency of chromosome abnormalities (46%) ($P>0.2$) between 58 Control and 17 PCOS oocytes after 16-18 hrs of IVM.

2) Group G chromosomes were most likely to be involved in aneuploidy and predivision, for which there was an age-related increase ($P=0.035$). There was a trend for increased frequency of predivision in PCOS oocytes.

3) The PCOS aetiology did not influence oocyte pyruvate consumption but was significantly associated with increased glucose consumption and reduced lactate production.

(Harris et al 2010)

Protein Metabolism

Amino Acid Turnover

Physiological Functions Of Amino Acids

- Building blocks for protein synthesis
- Energy source
- Involved in nucleotide synthesis
- Osmolyte functions
- Antioxidant functions
- Involved in pH regulation (micro buffer function)
- Chelators- working as protection against oxidation
- Signalling molecule precursors
Measurement Of Amino Acid Turnover By Individual Pronucleate & Cleavage Stage Embryos

Evidence from mouse, pig, cow and human embryos shows that the non-invasive measurement of the turnover of key amino acids in spent embryo culture media by HPLC is predictive of embryo development to the blastocyst stage in vitro, pregnancy in vivo, DNA damage, embryo development after cryopreservation (Houghton et al 2002; Brison et al 2004; Stokes et al 2007; Sturmey et al 2010).

Furthermore, amino acid metabolism in human embryos is linked to embryo genetic health (Picton et al 2010).

Amino Acid Profiling As The Means To Select The Best Embryo

Philosophy of Approach

• The most viable preimplantation embryos are those with the lowest level of metabolism i.e. the "quiet embryos": overall metabolism, aa turnover and glycolysis

• Low metabolism is achieved by reducing the concentration of nutrients in culture media to the levels measured in the female reproductive tract, this encourages the embryo to use endogenous resources.

Can Measurement Of Amino Acid Turnover Be used To Measure Oocyte Quality?

1. Do developmentally competent oocytes have a distinct metabolic fingerprint?

2. Can the metabolic signature of an oocyte be linked to molecular &/or cytogenetic correlates of developmental competence?
MII oocytes stripped then cultured in 1 µl microdrops under oil for 6-8hr (80%MII)  

IVF/IVP

Methodology

Culture medium stored at -80°C for amino acid analysis

18 Amino acids measured by High Performance Liquid Chromatography (HPLC)

Amino acid profile as index of oocyte health

Molecular & Cytogenetic analyses of eggs & embryos

Zygote development tracked by immobilising cells onto 4 x 4 grid using CellTak™

Amino Acid Profile Of Bovine MII Oocytes According To Subsequent Blastocyst Development or Arrest In Vitro

Arrested (n=185) Developed (n=30)

* = p<0.05 ** <0.01

Amino Acid Profile

Human Oocytes Which Progress To MII Have a Different Amino Acid Profile To Oocytes That Fail To Complete Meiosis In Vitro

Picton et al., 2008

Human Oocytes Which Progress To MII Have a Different Amino Acid Profile To Oocytes That Fail To Complete Meiosis In Vitro

Picton et al., 2008
Mean Amino Acids Turnover By Individual Human Oocytes Is Related To Patient Age

-6
-4
-2
0
2
4
6
8
Net
Depletion
Appearance
Turnover

> 35 years (n=89)
< 35 years (n=80)

* p<0.05; ** p<0.001

Picton et al 2008

Summary Of Metabolic Analyses of Oocytes

- Amino acid consumption/production is significantly different between individual, developmentally competent bovine MII oocytes and those which fail to fertilise and/or arrest.
- Asparagine, glutamine, serine and phenylalanine turnover are potential markers of bovine oocyte developmental competence.
- Carbohydrate and amino acid metabolism by human oocytes are significantly linked to oocyte developmental competence, patient age, aetiology and gonadotrophin dose/treatment.

Gene Expression In Individual Human Oocytes & Cumulus In Relation To Oocyte Developmental Potential

(a) Oocyte
(b) Cumulus Cells
Summary Of Molecular, Cytogenetic and Metabolic Markers Of Oocyte Quality

1. Multiple assays of oocyte quality can be conducted on the same cell which has enabled us to link molecular, cytogenetic & metabolic markers of development.

2. Oocyte quality can be quantified by non-invasive assays of metabolism & oocytes of high quality have a metabolic signature which differs significantly from oocytes of low quality.

3. Oocyte quality in vitro and in vivo is characterised by oocyte and cumulus gene expression profiles which are themselves associated with oocyte developmental competence.

4. The manipulation of these indices of oocyte competence by exposure to different gonadotrophins in vivo will enable us to redefine ovarian stimulation protocols & improve oocyte quality.

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Page 42 of 116
ESHRE Precongress Course: Reproductive Endocrinology, Stockholm 2011

Is the oocyte the main determinant of embryo quality?

Prof. Dr. Ursula Eichenlaub-Ritter
University of Bielefeld
Gene Technology/Microbiology
Bielefeld
Germany

Objectives:
1. Provide an updated overview of relative contribution of oocyte and sperm to high quality embryo and some pathologies related to reduced embryo quality
2. To evaluate the impact of age on oocyte and embryo quality
3. To discuss some options which may become relevant to improve embryo quality

Pre-fertilization:
Relative Contribution and Relevance of Oocyte and Sperm for Embryo Quality:
- Genome
- Cytoplasm
- Organelles
- Epigenome

Post-fertilization:
Maternal and Paternal Pathologies affecting Embryo Quality
Suboptimal culture conditions
Sperm:
Paternal Genome
Activating Factors
Centrosome

Sperm Genome:
Aneuploidy on average much lower in the sperm compared to the oocyte and there is no pronounced paternal age effect (average 3-4% versus 20%, e.g. Martin et al., Am J Hum Genet, 1991)

Structural aberrations are more common in sperm than oocytes and DNA damage negatively impacts embryo quality, implantation and birth rate (Speyer et al., 2010, Hum Reprod)

However, the oocyte contains DNA repair enzymes and can thereby take care of lesions in sperm chromatin and zygote (Jaroudi et al., 2009). Repair capacity within the oocyte appears induced in advanced maternal age, possibly as a result of a compensatory mechanism to cope with stress (Girolami et al., 2010)

Cytoplasmic activating factors:
PLC-ζ essential for fertilization but not embryo development/quality
(Taylor et al., RBM Online 2010,20(4))

In contrast, maternal contributions like zona pellucida are involved in preventing polyspermy and polyploidy in the embryo but zona morphology does not appear to be affected by maternal age (Handyside et al., Hum Reprod 2011)

Epigenome:
Paternal chromatin important for regulation of transcriptional activity in zygote (Swain et al., Reproduction 2011), nucleosome packaging DNA sequence important, without this, epigenetic errors may become increased that lead to non-viable embryos (Miller et al., 2010, Reproduction)

But bi-maternal genomic embryos of the mouse are viable and bi-maternal females have an extended lifespan (Kono et al., Nature 2004, Kawahara et al., 2010). Reprogramming is not affected by maternal age (Francos et al., Aging Cell, 2011)

Sperm contribution: Cytoplasmic factors/Organelles
Paternal mitochondria degraded

Centrosome with basal body is essential for aster formation and pronuclear apposition as well as normal zygote bipolar spindle formation (e.g. Sanozaino et al., Hum Rep 2006)
But maternal products like Filia are essential to complement constraining mechanisms and maintain bipolar spindle formation and polarity in the embryo (Zheng and Dean, PNAS 2009). Mitotic errors are common in mammalian embryos (Fragouli and Wells, Cytogenet Genome Res. 2011) which potentially could relate to altered expression of mitotic genes in aged oocytes (Grondahl et al., 2010). Genes in GO 'microtubule cytoskeleton' are consistently altered with age although identity differs (e.g. Hamatami et al., 2004).

Schatten et al. Mol Hum Reprod. 2009

Teratozoospermia but not globozoospermia with failures; (Terada et al.: Tohoku J Ex Med 2010)

Contribution of the Oocyte to the Embryo:

Maternal Genome

Wast amount of cytoplasm containing activating factors (Maternal factors/ chromatin remodelling/ zygotic gene expression/ totipotency)

Housekeeping molecules: metabolism, secretion, translation

Organelles: ER, Golgi, mitochondria, ribosomes, cortical granules, zona pellucida, Cytoskeletal components (actin cytoskeleton/microtubules) cell cycle regulation membranes etc.

Maternal Genome:

History of oocyte is relevant: e.g. recombination at early meiosis in embryonic ovary (Pachierrotti et al., Environ. Res., 2007; Chen et al., PLOS Genet 2009):

First meiotic errors giving rise to trisomy 21 involve all recombination patterns and one distal chiasma points of high risk, second meiotic errors predominantly affect pericentromeric exchanger

Recombination and number of surviving oocytes is possibly affected by exposures of primary oocytes in embryonic ovary in utero (Susiarjo et al., PLOS Genet, 2007; Rodrigues et al., Reprod Toxicol. 2010)
Errors in chromosome segregation and premature separation of chromatids are a major cause of reduced oocyte quality and developmental potential, implantation failures, spontaneous abortions and chromosomal aberrations like Down syndrome in offspring.

![Graph showing age distribution of spontaneous abortions and stillbirths](image)

Errors in chromosome segregation are related to maternal age.

PB analysis by CGH revealed only 3% aneuploidy in oocytes of young (average 22 years) patients as compared to over 60% in oocytes of aged patients (Fragouli et al., RBM Online 2009)

Rate of aneuploidy is lower in blastocyst compared to cleavage stage (~30% mitotic aneuploidy and 33% mitotic aneuploidy; of the latter 15% aneuploid mosaics with aneuploidy in each cell). Euploidy have a better chance to develop to the blastocyst.

Errors in chromosome segregation and quality of oocyte/embryo may also relate to stimulation protocol:

Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo (Baart et al., Hum Rep 2007)

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Age-related nondisjunction may be based on loss of cohesion:

Homologues are attached to each other by cohesion between sister chromatids; chiasmata; chiasmata are resolved when cohesion between arms is released by proteolysis of phosphorylated Rec8 cohesin at anaphase I

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Anaphase II progression requires release from cytostatic arrest by EMI2 inhibitor downstream from fertilization triggered calcium release.

Calcium spike
CaMKII γ

Metaphase
Anaphase
Rec8 phosp.
c-mos      MAPK     p90rsk     EMI2       APC/C cdc20
Securin

Loss of cohesion during ageing prior to anaphase I by reduced Rec8, SMCs and/or Shugoshin (Lister et al., Curr Biol 2010; Chiang et al., Curr Biol 2010; Tachibana-Konwalski et al., 2010).

High risk for precocious loss of chiasmata and random segregation at meiosis I

Chromatid separation predominant cause of imbalance detected by PB analysis—aneuploidy linked to generalized oocyte disorganisation not in the shape of chromosome

Hierarchical cluster analysis of transcript patterns of human aneuploid oocytes containing either whole chromosome aberrations and chromatid aberrations suggest that there are subtle differences in expression associated with these types of errors (Fragouli et al., Mol Hum Rep 2010).
Oocyte:
Maternal Genome:
Age- and aneuploidy-related differentially expressed genes include those regulating cell cycle and spindle dynamics from the kinesin family of microtubule depolymerases.

‘History’ of oocyte is therefore relevant with respect to spindle function:
Observations in aged oocytes: aberrant spindles, congression failure, increased univalents/ chromatids: may be related to altered expression.


One example: Microtubule attachment is dynamic and aberrant (e.g. merotelic) attachments can be resolved by microtubule-depolymerizing kinesins (e.g. mitotic centromere associated kinesin, MCAK).

Vogt et al., Mol Hum Reprod, 2010

Gene expression in the oocyte is regulated by interactions between the oocyte and the somatic compartment (reviewed by Su et al., 2009). The majority of transcripts and protein are from the oocyte growth phase and contributed, used or degraded in early embryogenesis.

Therefore embryo quality relates to oocyte quality, which, in turn relates to follicular quality.

Dobran et al., Hum Reprod 2006: Majority of transcripts downregulated until day 3.
Particularly during maturation and early embryogenesis many mRNAs are recruited or degraded.


Ageing/depletion of follicle pool affects gene expression at the transcriptome level

Fragouli et al. (Mol Hum Reprod 2010) detected differences between transcriptomes of euploid and aneuploid human metaphase II oocytes

Cram and coworkers detected differences in abundance and also length of the poly(A) tail of mRNAs in aged human oocytes (reported at ESHRE Rome 2010)

Transcriptome profiling in human preimplantation embryos:
Two major transition phases (Zhang et al., 2009, PLOS One)

Expression of maternal genes

Upregulation of embryonic genes

Embryo quality: Depends on maternal factors plus variable environment (e.g. low oxygen, variable culture conditions) for upregulation of zygotic gene expression.
Analysis of oocyte proteome (mouse):
Already at GV stage many putative or known maternal factors present
(Wang et al., PNAS 2010):

- Oocyte growth phase derived factors are of relevance!

Base peak im chromatogram, nano-LC gradient

Analysis of oocyte proteome (mouse):
Already at GV stage many putative or known maternal factors present
(Wang et al., PNAS 2010):

Oocytes have to become transcriptionally quiescent before resumption
of maturation and this significantly affects developmental capacity of
the embryo after IVM and IVF (Bellone et al. Reproduction 2009).

Epigenetic modifications of histones (e.g. H3K4 trimethylation) by maternal
histone modifying enzymes (e.g. MLL2; Andrieu-Vieyare et al., PLOS Biol. 2010)
are essential for oocyte maturation and embryonic development

Maternal and paternal imprinting during gamete formation provides for monoallelic
expression in the embryo while resetting of global imprint patterns is essential to attain
a totipotent state

Reviewed by Trasler, Hum Repro Update 2004; Santos et al., Dev Biol 2005
No age-related changes in methylation of DNA of the differentially methylated regions of imprinted genes Snrpn, Kcnq1ot1, U2af1-rs1, Peg1, Igf2r and H19 were observed in embryos of aged mouse oocytes (Lopes et al., Hum Mol Genet 2009).

However, a significant percentage of old GV and MII mouse oocytes lacked typical histone modifications affecting chromatin conformation like H3K9me3, H3K36me2, H3K79me2 and H4K20me2 (Manosalva et al., Theriogenology 2009), and defective deacetylation of histones (H4K12) during human female meiosis appears to increase with maternal age and is correlated with misaligned chromosomes (van den Berg, Hum Reprod, advanced press, 2011).

The quality of the oocyte is predetermined by processes prior to resumption of maturation. Homeostasis and follicular environment are of relevance.

Spatial deposition and accumulation of molecules and cell organelles in oocytes define high quality.

For instance, oocytes with high developmental capacity possess aggregates of RNA-binding proteins implicated in maturation and differential recruitment/degradation of RNAs.

Acquisition of high-developmental potential coincides with formation of RNA-binding protein containing complexes containing RNA at the oolemma

(Flante et al., Biol. Reprod. 2010)

Functional organization of ooplasm appears to play a role in gene expression and acquisition of high developmental potential of mammalian oocytes (subplasmalemmal maternal complex, SMC and cytoplasmic lattices, CS)

Maternal factors: MATS6, FLIPPEP, FELIA, TLE6, PADI6

Influence of maternal age warrants studies.

(Material factors: FELIA, TLE6, PADI6)
Oocyte Proteome Markers and their spatial distribution in porcine oocytes

ATM (ataxia telanctasia mutated DNA protein kinase) in subplasmalemmal clusters in porcine oocytes

Kelch-like ECH-associated protein 1 (an adapter for ubiquitin-ligase CUL3), nuclear export factor CRM1 and ataxia-telangiectasia mutated protein kinase appear more abundant in high quality porcine oocytes (IVM with gonadotrophin) compared to low quality oocytes (IVM without gonadotrophin).

Powell et al., Proteomics Clin. App. 2010

Maternal mitochondria are determinants of oocyte quality and developmental potential of the embryo

1. Numbers (DNA copy numbers) may vary greatly and mutation in mtDNA of granulosa cells appear increased with age
2. Functional status (morphological and functional alterations and mutation during ageing) rather than numbers appears affected by age (e.g. ATP production)
3. Distribution (e.g. alterations in local ATP supply) can impact fertilization and spindle formation, metabolism, survival/cell death etc.

Reviewed by Eichenlaub-Ritter et al., Mitochondrion, 2010; Van Blerkom, Mitochondrion, 2010

Domains of mitochondria with high and lower inner membrane potential exist in mature oocytes and embryos

Functional state of mitochondria is influenced by cumulus

Examples of how metabolism of oocyte/follicle and oocyte quality affect embryo quality and developmental potential

High fat diet in mouse affects embryo development

Rosiglitazone, an insulin-sensitizing agent acting on expression of genes in lipid metabolism restores development of embryos from above, high fat diet mice.

Diet affects oocyte quality (via protection from ROS?): moderate caloric restriction initiated in rodents during adulthood sustains function of the female reproductive axis into advanced chronological age (Salesniemi et al., Ageing Cell 2008).

By contrast, high fat diet causes mitochondrial damage: reduced inner mitochondrial membrane potential in oocyte

Causes stress in endoplasmic reticulum

Increases ROS and mitochondrial calcium overload

Increased apoptosis

Reduced numbers of cells in inner cell mass/trophectoderm

Minge et al., Endocrinology 2008; 149:2646-2656
Lipid Metabolism and ß-Oxidation in Mitochondria affect Oocyte Quality and Embryo Quality

Matrix

Cytosol

Inhibition of CTPI by ETOMOXIR:
Significantly reduced rate of development to 2-cell, 4-8 cell and blastocyst stage
Dunning et al., Biol Reprod 2010

Novel method to assess redox regulation and status in mammalian oocytes

The thiol tripeptide glutathione (γ-glutamylcysteinylglycine, GSH) is the major intracellular redox buffer and represents a central element of the oocyte antioxidative system. The redox sensitive fluorescent fusion protein Mito-Grx1-
roGFP2 contains a mitochondrial signal sequence, the human glutaredoxin-1 (Grx1), an enzyme that interacts with the GSH/GSSG system, and roGFP2. Specific mRNA injection allows it to determine the mitochondrial glutathione dependent redox potential under physiological conditions.

modified scheme from Robert Norman, Robinson Institute, Adelaide

Eichenlaub-Ritter et al., Mitochondrion, 2010
Tatone et al., Hum Reprod, 2011
Highly reactive carbonyl compounds like methylglyoxal from glycolysis causing increased formation of advanced glycation end products and carbonyl stress are discussed in age-related accumulation of damage to DNA, membranes and mitochondria (Desai et al., Can J Physiol Pharmacol 2010; Tatone et al., Hum Reprod Update 2008).

We have shown that carbonyl stress by methylglyoxal exposure induces spindle aberrations, altered (prolonged) cell cycle kinetics, DNA damage, altered inner mitochondrial redox potential and contributes to ageing (Tatone et al., Hum Reprod 2011).

Cumulus from young females more efficiently than that from aged females protects from MG-induced meiotic arrest (Tatone et al., Hum Rep 2011)

Developing more efficient methods to obtain high quality metaphase II oocytes from IVM with young cumulus could be useful to establish heterologous systems in which aged oocytes mature under optimized conditions and protection from cumulus.
Is there room to improve oocyte and embryo quality?

Simulated physiological oocyte maturation (SPOM) is a novel in vitro maturation system that substantially improves embryo yield and pregnancy outcomes. Albuc et al., Hum Reprod 2010.

Pre-maturation period in cAMP modulator to increase cAMP in COC

IVM in presence of PDE inhibitor and FSH

Prolonged IVM

In bovine model: increased blastocyst rate and quality

In mouse model: blastocyst rate, implantation rate and fetal yield similar to IVF of in vivo matured oocytes

Is there room to improve oocyte and embryo quality?

Improve follicular/oocyte health- by diet, anti-oxidants, etc.??

Avoid age-related deterioration: social freezing- adverse influences of cryopreservation?

'Correct' age-related deterioration: total exchange of cytoplasm, nuclear donation or cytoplasmic transfer?
Conclusions I:

Oocytes are the main determinants of embryo quality but paternal contributions and pathologies have to be considered.

Oocyte 'history' largely determines on its quality and developmental potential. Accordingly, ovarian physiology, maternal age and some pathologic conditions are the predominant determinants of embryo quality in the human and this relates primarily to events in the follicle prior to or during resumption of maturation.

Since physiology is important, it may be improved by personalized treatment of the patient (e.g. diet, stimulation etc.).

Conclusions II:

There are novel options to improve oocyte IVM and thereby obtain good quality oocytes in animal studies producing embryos of high developmental capacity - studies in human are pending and it is unknown whether culture might improve quality of aged oocytes - loss of cohesion and aneuploidy may be inevitable.

Initial stages of embryo development appear specifically vulnerable to disturbances, e.g. while chromatin remodelling, zygotic gene activation and complex alterations in cellular homeostasis are taking place but it appears to be mainly the genetic and physiological status of the oocyte that determines embryo quality.

Optimization of the culture conditions may help to improve embryo quality while there are currently few options to compensate for intrinsic aberrations, particularly chromosomal aberrations, transmitted by the oocyte.

Thank you for your attention!
Do ovarian reserve tests correlate with oocyte quality and natural fertility or simply numbers of oocytes available during ART?

Scott Nelson
Muirhead Chair in Obstetrics & Gynaecology

Learning objectives

• Understand the role of AMH in assessing the ovarian reserve
• Appreciate the performance of AMH relative to other markers of ovarian reserve
• Be aware that AMH and age can be used together to stratify patients into prognostic groups for live birth
• Biomarkers have yet to be assessed in conjunction with novel prediction models of IVF success

Why do we need to know ovarian response?

"Just do IVF and see response"
Mild stimulation: one size fits all or does it?

- Lower live birth rates
- OHSS risk still exists
- Fewer embryos
- Programming of the cycle
- Individualised FSH-dosing algorithms not available

So what options do we have?

Paracrine Control

- Environmental Factors

Endocrine Control

- FSH and LH Dependent
- Dominant 10 mm Ovulatory
- Small 20 mm Sub-ovulatory

Recruitment

Growth

Menstrual cycle

>120 days

85 days

14 days

Primary alternative are endocrine markers

Primary alternative are endocrine markers

AMH in pre-antral and early antral follicles

Image courtesy of Hamish Fraser, MRC.
The new AMH Gen II assay

DSL antibody
Immunotech standards
Values ~40% higher

AMH is stable within and across menstrual cycles

AMH menstrual cycle stability is dependent on age

Values ~40% higher
Validated AMH age nomogram for DSL

Does AMH relate to ovarian response?
- Large prospective cohort of 340 women undergoing their first IVF cycle with a standard agonist approach: Prostap and 225 IU
- AMH strongly correlates with oocyte yield
- AMH distinguishes treatment categories
- FSH does not

AMH correlates with oocyte yield and is better than other predictors

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<td>(Nelson 2007)</td>
<td>340</td>
<td>0.71</td>
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<td>(Wunder 2008)</td>
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<td>(Gnoth 2008)</td>
<td>132</td>
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<tr>
<td>(Nardo 2008)</td>
<td>165</td>
<td></td>
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<td></td>
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<tr>
<td>(La Marca, et al. 2010)</td>
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</tbody>
</table>
AMH for poor response: CUT-OFF values

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>Study design</th>
<th>CUT-OFF value (ng/mL)</th>
<th>Sens (%)</th>
<th>Spec (%)</th>
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<tbody>
<tr>
<td>Van Rooij (2002)</td>
<td>68</td>
<td>Prosp</td>
<td>0.3</td>
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<tr>
<td>Muttukrishna (2004)</td>
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<td>Prosp</td>
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<td>Muttukrishna (2005)</td>
<td>108</td>
<td>Retro</td>
<td>0.2</td>
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<tr>
<td>Tremellen (2005)</td>
<td>75</td>
<td>Prosp</td>
<td>.11</td>
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<tr>
<td>Panarrubia (2005)</td>
<td>80</td>
<td>Prosp</td>
<td>0.69</td>
<td>53</td>
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<td>Ebner (2006)</td>
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<td>Prosp</td>
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<td>McIlveen (2007)</td>
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<td>Kwee (2007)</td>
<td>110</td>
<td>Prosp</td>
<td>1.4</td>
<td>76</td>
<td>86</td>
</tr>
<tr>
<td>Nelson (2007)</td>
<td>340</td>
<td>Prosp</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AMH <1.0 pmol/L only a few pregnancies to term


AMH - prediction of over-response

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>Study design</th>
<th>CUT-OFF value (ng/mL)</th>
<th>Sens (%)</th>
<th>Spec (%)</th>
<th>Prediction of hyper-response</th>
<th>Prediction of OHSS</th>
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</thead>
<tbody>
<tr>
<td>Kwee (2007)</td>
<td>110</td>
<td>Prosp</td>
<td>5</td>
<td>53</td>
<td>91</td>
<td></td>
<td></td>
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<tr>
<td>Nelson (2007)</td>
<td>340</td>
<td>Prosp</td>
<td>3.52</td>
<td>60</td>
<td>94.9</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>Lee (2008)</td>
<td>262</td>
<td>Prosp</td>
<td>3.36</td>
<td>90.5</td>
<td>81.3</td>
<td>√</td>
<td></td>
</tr>
</tbody>
</table>

Strengths:
- High number of patients
- Optimal classification of OHSS
- Only moderate to severe OHSS considered
- Limited to Chinese population
- Not clear definition of PCOS women included

Limitations:
- Limited to Chinese population
- Not clear definition of PCOS women included

AMH:
- Stable molecule
- Relatively stable across cycle
- Stable between cycles
- One assay on the market
- Age related nomogram – quadratic
- Strongly related to oocyte yield
- Can predict poor response
- Can predict excessive response
- Independent of age

How can we utilise this information?

So can we use AMH to inform IVF?
Prospective Evaluation of AMH based strategies

<table>
<thead>
<tr>
<th>Centre 1 (378)</th>
<th>AMH</th>
<th>Centre 2 (168)</th>
<th>AMH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>FSH Dose</td>
<td>Control</td>
<td>FSH Dose</td>
</tr>
<tr>
<td>Agonist</td>
<td>150</td>
<td>Agonist</td>
<td>150</td>
</tr>
<tr>
<td>Agonist</td>
<td>225</td>
<td>Normal Response</td>
<td>Agonist</td>
</tr>
<tr>
<td>Agonist</td>
<td>380</td>
<td>Reduced Response</td>
<td>Agonist</td>
</tr>
<tr>
<td>Agonist</td>
<td>375</td>
<td>Negligible Response</td>
<td>Agonist</td>
</tr>
</tbody>
</table>

- Risk of OHSS

- Lower oocyte yields in high responders

- Individualisation significantly improves clinical pregnancy rates

AMH dictated strategic approach

- **Antagonist Strategy**
  - "normalised" egg yields
  - Negligible excess responses
  - Low cryopreservation
  - High / maintained fresh CPR

- **Agonist Strategy**
  - Lower FSH dose has negligible impact on excess responses

**Control**

<table>
<thead>
<tr>
<th>AMH</th>
<th>FSH Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Responders (150IU daily)</td>
<td>( 150 ) / ( 225 )</td>
</tr>
<tr>
<td>Antagonist: FSH + LH</td>
<td>( 150 ) / ( 225 )</td>
</tr>
<tr>
<td>Agonist: HMG or FSH</td>
<td>( 225 ) / ( 300 )</td>
</tr>
<tr>
<td>Normal Responders</td>
<td>( 225 ) / ( 300 )</td>
</tr>
<tr>
<td>Reduced Responders</td>
<td>Minimal treatment burden e.g. flare</td>
</tr>
</tbody>
</table>

**Composite measures incorporating AMH**

AMH and age are independent predictors of oocyte yield

**AUROC 95% CI**

- Age: 0.60 (0.57, 0.64)
- AMH: 0.81 (0.78, 0.83)
- Age & FSH: 0.69 (0.69, 0.77)
- Age & AFC: 0.76 (0.72, 0.80)
- Age & AMH: 0.80 (0.76, 0.84)
- Age & AMH & AFC: 0.80 (0.74, 0.86)
- Age & AMH & AFC & FSH: 0.81 (0.75, 0.86)

**Table**

<table>
<thead>
<tr>
<th>AMH</th>
<th>Age</th>
<th>AMH &amp; Age</th>
<th>AMH &amp; FSH</th>
<th>AMH &amp; AFC</th>
<th>AMH &amp; Age &amp; FSH</th>
<th>AMH &amp; Age &amp; AFC</th>
<th>AMH &amp; Age &amp; AFC &amp; FSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
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<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>
Composite measures incorporating AMH

AMH and age are independent predictors of oocyte yield

Maximal value is from AMH

Never mind their age
Just measure AMH


AUROC 95% CI

Age 0.60 0.57, 0.64
AMH 0.81 0.78, 0.83
Age & FSH 0.69 0.69, 0.77
Age & AFC 0.76 0.72, 0.80
Age & AMH 0.80 0.76, 0.84
Age & AMH & AFC 0.80 0.74, 0.86
Age & AMH & AFC & FSH 0.81 0.75, 0.86


So utilising biomarkers?

- Individualise expectations of oocyte yield
- Individualise treatment strategies
- Improve safety of IVF
- Prospectively evaluate novel therapies
- A role independent of the classical biomarker date of birth?

What are our chances of having a baby?
Can biomarkers predict live birth?

- No – everything including age awful
- Limitation of ROC analysis

Yes if you think of “predict” in conventional terms of low medium, or high risk

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>AMH (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.4</td>
<td>0.05 (0.01 to 0.16)</td>
</tr>
<tr>
<td>0.4 – 2.8</td>
<td>0.18 (0.12 to 0.26)</td>
</tr>
<tr>
<td>≥2.8</td>
<td>0.29 (0.17 to 0.44)</td>
</tr>
</tbody>
</table>

Values in parentheses are 95% confidence intervals

Will biomarkers add to established risk estimates?

- 144,000 fresh IVF cycles
- Baseline characteristics
- Freely available
Summary

- Just do IVF is no longer an option
- AMH is easy and relatively stable
- AMH relates strongly to oocyte yield
- Accurate prediction of live birth is feasible
- Biomarkers can individualise expectations and treatment
- Biomarkers can improve outcomes and safety of IVF
Conflicts of interest?

- no commercial conflicts of interest
- author of publications on POF
- member of ESHRE POF guideline development group

Hormone replacement therapy for premature ovarian insufficiency and menopause

Melanie Davies
Consultant Gynaecologist
University College London Hospitals

Learning objectives

- know the immediate & long-term effects of hormone deficiency
- review evidence on benefit and risk of HRT in young women
- discuss the most appropriate form of HRT
  - types of estrogen and progestogen
  - dose
  - route of administration
  - role of testosterone
- plan follow-up and duration of therapy
**definition**

loss of ovarian function:
- before the age of 40
- age > 2SDs below mean for reference population
  (average age for Western populations 51)

terminology:
- "ovarian insufficiency" is preferred to "failure"

**prevalence**

- 1% of women < 40
- 0.1% of women < 30
- 0.01% of women < 20

**causation**

- environmental
- metabolic
- iatrogenic
- autoimmune
- genetic
- infective
- unknown
management

- make - and explain - diagnosis
- treat symptoms
- prevent long-term consequences
- address psychological needs
- treat infertility
- offer long-term follow-up and support

make the diagnosis!

- diagnosis is often delayed, even with classic symptoms of menopause
  Alzubaidi 2002

- ovarian insufficiency is often a fluctuating condition
  ovarian dysfunction precedes menopause

presentation

- amenorrhoea
- oligomenorrhoea
- menstrual dysfunction
- infertility
- oestrogen-deficiency symptoms

Coulam 1985, Anasti 1998
**symptoms**

- flushes
- night sweats and sleep disturbance
- vaginal dryness
- loss of libido
- stiffness and muscle pain
- mood changes
- fatigue
- poor concentration and memory

**diagnostic tests**

- elevated FSH levels in menopausal range (usually above 40IU/l) on at least two occasions a few weeks apart
- ultrasound not required for diagnosis
- no role for ovarian biopsy
- AMH may be useful

**assessment**

- FSH, LH, oestradiol (prolactin) (androgens) ?AMH
- thyroid function
- autoantibody screen
- karyotype (young patients)
- FRAXA screen
- pelvic ultrasound
- bone mineral density
pelvic ultrasound
- ovarian activity commonly seen in POI
- may be seen in primary amenorrhoea
- associated with higher BMD
- higher chance of pregnancy

bone density measurement
- methods of measurement
- baseline assessment at diagnosis
- serial follow-up

long-term risks
- life expectancy reduced
  - cohort of >12,000 women
  - 2 years less life expectancy if menopause <40
    - increased mortality ischaemic heart disease
    - reduced uterine and ovarian cancer
long-term risks

Mayo clinic cohort study – bilateral oophorectomy

- premature death
- cardiovascular disease
- cognitive impairment, dementia, parkinsonism
- osteoporosis & fractures
- ↓ psychological wellbeing
- ↓ sexual function

Shuster et al 2008

bone loss

- failure to achieve peak BMD
- increased loss
- fracture rate  OR 1.5 [1.2-1.8]


HRT prevents bone loss
little evidence on other Rx:
bisphosphonates
calcium + vitamin D
cardiac disease

- ischaemic heart disease increased in POF Lokkegaard 2006
- subclinical coronary artery disease $\times 2$ (OR 2.0, 95% CI: 1.2-3.4) after TAH+BSO modified by HRT within 5 years of oophorectomy Allison 2008
- vascular endothelial dysfunction with oestrogen-deficiency improved by HRT Kalantaridou 2004, Ostberg 2007

cognitive function

- oophorectomy associated with
  - increased risk of dementia linear trend with age at menopause
  - increased risk of Parkinsonism Rocca 2008

HRT

- which type?
- what dosage?
- what duration?

there are no satisfactory RCTs to determine the ideal dose, regimen or delivery system for young women
OCP vs HRT

- synthetic
- more potent
- Pill-free week
- like peer-group
- reminder of infertility
- free in UK

- physiological
- may be safer for long-term use
- continuous estrogen
- stigma of HRT
- not contraceptive
- UK prescription charge x2

HRT type

- cyclical or “no bleed” HRT?
- choice of progestogen?
  C19 e.g. norethisterone, norgestrel and levonorgestrel
  C21 e.g. dydrogesterone and medroxyprogesterone acetate
- route of administration?

HRT dosage

- standard HRT doses may be suboptimal
- monitor by symptoms and BMD
  (oestradiol levels useful only for implants)
- urogenital symptoms may need local Rx
  (vaginal moisturisers, topical oestrogen)
testosterone

- androgen levels ↓ in POI (half of testosterone supply from ovaries) Hartmann 1997
- reduced libido, sexual function, energy BMD
- worse in oophorectomised women
- replacement — patches (Intrinsa), implants
  s/e excess hair growth and acne
  Braunstein 2003, Shifren 2007

alternatives to HRT

- efficacy lower than HRT:
  o serotonin and noradrenaline re-uptake inhibitors
  o clonidine
  o gabapentin
- efficacy unproven:
  o progesterone transdermal creams
  o phyto-oestrogens (soy, red clover)
  o acupuncture
- safety unproven:
  o herbal preparations (black cohosh, dong quai)

Panay and Rees 2006

benefits and risks

- Women’s Health Initiative study and Million Women studies are not applicable to young women
- breast cancer: less common in POI, effect of physiological HRT
- ischaemic heart disease: HRT may benefit
- osteoporosis: clear benefit
HRT duration

until expected age of menopause

“In women who have experienced a premature menopause (due to ovarian failure, surgery or other causes) HRT may be used for treatment of menopausal symptoms and for prevention of osteoporosis until the age of 50 years. After this age, therapy for prevention of osteoporosis should be reviewed and HRT considered a second choice.”

MHRA 2007

psychological needs

- counsellor is key member of clinic staff
- information
  - from health professionals
  - from support groups
  - http://www.pofsupport.org/
  - www.daisynetwork.org.uk

fertility (1)

- HRT is not contraceptive!
- spontaneous pregnancy rate 5-10%
- miscarriage rate 20%
- prognostic factors:
  - recent diagnosis – short period of amenorrhea
  - fluctuating FSH
  - ovarian activity on ultrasound
  - POI due to autoimmunity or chemotherapy

Van Kasteren 1999
fertility (2)

- treatment strategies unproven:
  - stimulation after FSH suppression (OCP, GnRH-a)
  - corticosteroids
- review of >50 case reports, > 20 studies (poor quality)
  194 patients 3 pregnancies
  conclusion: no difference from background rate

Van Kasteren 1999

HRT for egg donation

summary

- POI is under-diagnosed – need improved awareness and information
- HRT effectively treats symptoms
- HRT can prevent long-term consequences
- HRT essential for egg recipients
- paucity of research on HRT in young women – cannot apply studies in older women
- watch out for the ESHRE guidelines!
Preservation of fertility: Oocyte or ovarian tissue freezing

Professor Dror Meirow
Fertility preservation Center, Sheba Medical Center, Sackler school of medicine, Tel Aviv University, Israel.

Learning Objectives

- Indications for fertility preservation.
- Fertility preservation options.
- Egg, embryo freezing; results, odds and cons.
- Ovarian tissue freezing; indications and results.
- Decision making; egg vs. ovarian tissue freezing.

Indications for fertility preservation

In Cancer patients
- Chemotherapy that causes ovarian injury.
- Pelvic irradiation.
- Ovarian surgery.
- Genetic- hereditary cancer gene mutation (BRCA).

Benign non- oncologic indications
- Chemotherapy.
- Ovarian surgery.
- Endometriosis – severe, ovarian involvement.
- Family planning, age related (social preservation).
Ovarian reserve assessment

- Age
- Hormone profile (FSH, E2) AMH
- AFC
- Previous IVF treatments

Consulting young female cancer patients - 
Risk assessment

- Ovarian reserve
- Toxicity risk

Evaluation of patients before 
Fertility preservation procedure

- Sterilization risk of future planned treatment.
- Age, family planning
- Ovarian reserve
- Time available – window for fertility preservation.
- Medical status – be aware of complications.

Only for cancer patients:
- Risk of ovarian cancer cells involvement.
- Estrogen sensitive tumors.
- Previous recent chemotherapy treatments.
Options for fertility preservation

- Egg, Embryo freezing
- Ovarian tissue freezing

Different approaches for fertility preservation
Currently used & experimental

- Prematuration follicles
- Early growth
- Mature eggs
- Stored ovarian tissue
- Follicle maturation: Not practiced
- Ovarian tissue transplantation
- In vitro Egg maturation
- Mature Egg Embryo freezing

Cryopreservation and Transplantation of ovarian tissue.
A realistic technique and dilemmas
## Ovarian tissue cryopreservation in cancer patients

**Advantages**
- Large number PMF survive freezing / thawing.
- Fast fertility preservation procedure.
- Well-adapted to children.
- Can prevent mutagenic effects of chemotherapy.
- Can produce many cycles of mature eggs after grafting.

**Disadvantages**
- Experimental (conditions, transplantation, outcome).
- Not economic - many PMF are lost.
- Risk of cancer cells.

Meirow D. 2011

## Storing ovarian tissue has been practiced during the last decade to preserve fertility.

Ovarian cryobanking as a strategy to preserve fertility
After first pregnancies in the sheep model.
Gosden, Baird, Denver, Meirow, Oktay

Ovarian tissue banking in patients with Hodgkin’s disease. Is it safe?
Meirow D et al. (Fertil. Steril. 1998)

A laparoscopic technique for obtaining ovarian cortical biopsies for fertility conservation in cancer patients.
Meirow D et al. (Fertil. Steril. 1999)

## Operation – laparoscopy/ Lap

When sterilization risk is minimal, is it justified?

Meirow D. 2011
Factors affecting procedure success

- Ovarian reserve
- Volume
- Thickness
- Freezing conditions
- Transplantation site
- Vascular bed

Factors affecting transplantation success

- Inhensic period
- Operative technique
- Environmental factors

Preparation for freezing- Tissue thickness

Human non-growing primordial follicles

1-2 mm

Freezing conditions

- Slow freezing: Gonen, Gook, protocols
- Medium: Libovitz, bovine, cryoprotectant - DMSO, PROH
- Serum: autologous, commercial
- Sucrose: +/- Survival of ~ 70% of follicles.
Freezing conditions

**Vitrification** - Kagawa protocol
- Thin slices 1mm cryotome
- Rapid cooling
- High follicle survival
- No reports on human pregnancies

Surgical grafting of ovarian tissue

**Successful sites**
- Orthotopic pelvis
- Ovary

**Failures**
- Arm
- Abdominal wall

Orthotopic Surgical grafting of ovarian tissue

**Publications:** Radford, Oktay, Donnez, Demeestere, Silber

**Indications:**
- Better option?
- No ovary
- Fibrosis vascular bed
Grafting ovarian tissue to the ovary

Meirow D. & Dor J. NEJM 2005

Donnez J. et al. 2008

Andersen Hum Reprod. 2008

---

Basal hormone levels

- FSH
- AMH
- Inhibin B

---

Children born after ovarian transplantation.
A review of 13 live births.

- Age at tissue collection 19-36
- Previous chemotherapy 40%.
- Endocrine results.
- IVF / Spontaneous pregnancy 50%.
- Pregnancy results- normal babies 100%.

Donnez et al 2011
Recovery of Endocrine function

Months post transplantation

Donnez et al 2011

Recovery of Endocrine function

Months post transplantation

Donnez et al 2011

single procedure- IVM + ovarian tissue

Fluid with immature eggs

Immature egg collection

IVM

OTCP

Embryo cryopreservation

NOT POST RECENT CHEMOTHERAPY (6mo)
Detection of Microscopic Metastases in Cryopreserved Ovaries.

- Cryopreservation
- Cancer cells?
- Sterilized cured patient
- Radio/chemotherapy

- Use the most sensitive techniques for detection.

---

Evaluation of ovarian tissue (CML)

- A 20-year-old female was diagnosed with CML.
- Ovarian tissue harvested for cryopreservation prior to bone marrow transplantation.
- Fragments of cortex were evaluated for MRD.

Philadelphia chromosome (reciprocal translocation t(9;22) is present in 95% of patients with CML.
Real-time PCR to detect CML cells

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
</table>

Real time PCR melting curves for Bcr-Abl

---

1. Bone marrow
2. Ovary 2000
4. Ovary 2004
5. Control
6. Positive control
7. No template
1. Marker

The tissue was not thawed.

---

The % of ovarian metastasis was 22.4%

Leukemia: 10.2% - 7.9%
Breast ca: 25.0% - 0%
Uterine ca: 13.3% - 0%
Lymphoma: 14.7% - 10.7%
pulmonary ca.: >24.8%
Gi ca. (gastric; colon): 54.2; 26.1%

---

Evidence of residual disease in cryopreserved ovarian cortex from female patients with leukemia

Evidence of residual disease in cryopreserved ovarian cortex from female patients with leukemia. (26 patients)

Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe. (18 patients)
Dolmans, Donnez et al. Blood 2010
Fertility preservation using

Embryo freezing

and

Egg freezing

Embryo freezing for fertility preservation

- Currently the most widely used method to preserve fertility worldwide.
- Cycle success rate – according with ovarian reserve.
- In cancer patients - thousands of patients worldwide.

IVF for benign conditions - Dilemmas

- No partner – Egg freezing or Donor sperm.
- Older patients - Low ovarian reserve.
- Adolescent patients
- Time needed before treatment.
- Patient’s health condition.
Egg Freezing for fertility preservation - Justified?

- Egg Freezing using vitrification method – high success rate.

<table>
<thead>
<tr>
<th>No of transfers</th>
<th>Frozen eggs 300 patients</th>
<th>Fresh cycles 300 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryos replaced</td>
<td>1.7 ± 0.7</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>39.9%</td>
<td>40.9%</td>
</tr>
<tr>
<td>Clinical pregnancy rate / transfer</td>
<td>55.4%</td>
<td>55.6%</td>
</tr>
</tbody>
</table>

A. Kobaj, A. Pellikaan et al. Hum Reprod. 2010

Egg Freezing for fertility preservation

- Older patients - Low ovarian reserve.
- Adolescent patients – Are ovarian stimulation & OPU justified?
- Family planning – Age 30-40y according with ovarian reserve.
- Time available before medical treatment.
- Patient’s health condition.

IVF in cancer patients - Dilemmas

- Time needed for IVF before chemotherapy.
- Age –children, aged patients- ovarian reserve.
- Patient’s health condition.
- No partner – Egg freezing or Donor sperm.
- Success rate in cancer patients.
- IVF post exposure to chemotherapy.
- Hormone sensitive tumors.

Meirow D. 2011

IVF in cancer patients – Dilemmas

- Time needed for IVF before chemotherapy.
- Age –children, aged patients- ovarian reserve.
- Patient’s health condition.
- No partner – Egg freezing or Donor sperm.
- Success rate in cancer patients.
- IVF post exposure to chemotherapy.
- Hormone sensitive tumors.

Meirow D. 2011
### Indications for IVF in cancer patients

E. Ginsburg  
Brigham & Women’s Hospital, USA, 2010

<table>
<thead>
<tr>
<th>Pre-therapy Diagnoses</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Cancer</td>
<td>16 (42.1)</td>
</tr>
<tr>
<td>Cervical Cancer</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Colorectal Cancer</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>Endometrial Cancer</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Hodgkin’s Lymphoma</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Leukemia, AML, ALL</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>Malignant Brain Tumor, Glioma</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>Myelodysplastic Syndrome</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Non-Hodgkin’s Lymphoma</td>
<td>2 (5.3)</td>
</tr>
<tr>
<td>Ovarian Epithelial Carcinoma</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>Systemic Sclerosis</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Systemic Lupus Erythematous</td>
<td>1 (2.6)</td>
</tr>
</tbody>
</table>

---

### Stimulation Protocols for cancer patients

- Minimal stimulation.
  - Minimizes OHSS risk
  - Fewer embryos banked
- "Standard Stimulation".
  - OHSS risk real: could delay cancer treatment
  - Higher E2 levels
  - More embryos banked
- Special protocols for Estrogen sensitive tumors.

### Ovarian Response to Ovulation Induction in Cancer

Knopman Fertil Steril 2009

<table>
<thead>
<tr>
<th></th>
<th>Cancer Cases (n=28)</th>
<th>Male Factor Controls (n=135)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y) Range</td>
<td>34 ± 5.1 (20-41)</td>
<td>35.4 ± 3.5 (20-41)</td>
</tr>
<tr>
<td>Day 3 FSH/ E2</td>
<td>7.5 ± 3.2/ 35 ± 15</td>
<td>8.3 ± 2.6/ 35 ± 15</td>
</tr>
<tr>
<td>Gonadotropin Dose</td>
<td>3.507 ± 1.012</td>
<td>3.306 ± 1.164</td>
</tr>
<tr>
<td>Peak E2 pg/mL</td>
<td>1,519 ± 712</td>
<td>1,393 ± 769</td>
</tr>
<tr>
<td>Embryos</td>
<td>14 ± 9</td>
<td>12 ± 7</td>
</tr>
</tbody>
</table>

Mean ± SD
**Ovulation Induction In Cancer Patients**

<table>
<thead>
<tr>
<th>study</th>
<th>controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>age</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>FSH</td>
<td>7.3</td>
<td>6.3</td>
</tr>
<tr>
<td>eggs</td>
<td>13</td>
<td>11.5</td>
</tr>
<tr>
<td>2PN</td>
<td>7.4</td>
<td>6.8</td>
</tr>
<tr>
<td>Stimulation days</td>
<td>10.5</td>
<td>9.0</td>
</tr>
<tr>
<td>FSH dose</td>
<td>4174</td>
<td>3416</td>
</tr>
</tbody>
</table>

controls: male factor, egg donor, oocyte cryo

Retrospective cohort study.

**Embryo yield after IVF in women undergoing fertility preservation before chemotherapy**

<table>
<thead>
<tr>
<th>Fertility Preservation</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>34 ± 5</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>stimulation days</td>
<td>11 ± 2</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>FSH (IU)</td>
<td>4,184 ± 1,791</td>
<td>3,487 ± 1,897</td>
</tr>
<tr>
<td>Peak E2 (pg/mL)</td>
<td>1,456 ± 1,063</td>
<td>2,098 ± 1,037</td>
</tr>
<tr>
<td>No. of mature oocytes retrieved</td>
<td>12 ± 8 (2–46)</td>
<td>14 ± 9 (0–62)</td>
</tr>
<tr>
<td>No. of embryos (2PN)</td>
<td>8 ± 5 (1–23)</td>
<td>7 ± 6 (0–42)</td>
</tr>
<tr>
<td>Fertilization, %</td>
<td>62</td>
<td>55</td>
</tr>
</tbody>
</table>

**Oocyte/ Embryo Banking: European Registry**

- Retrospective cohort study, 205 women, 70 ART centers 2007-9 (FertilPROTEKT network)
- Ages 18-40, mean 30.5
- Diagnoses: breast ca, lymphoma, Gynecol. malignancies, benign disease

**Results:**

- No response in 0.9%, no ER in 1.5%
- 125 women inseminated all eggs. In this group:
  - No of eggs: mean 11, median 10.5
  - Mean fertilization rate 61.3%
**IVF for fertility preservation in cancer Patients - Results**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Eggs</th>
<th>2PN</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oktay 2005</td>
<td>12.3</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>Knopman 2009</td>
<td>14 ± 9</td>
<td>12 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td>Quintero 2010</td>
<td>13</td>
<td>11.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Robertson 2010</td>
<td>12 ± 8</td>
<td>14 ± 9</td>
<td>6 ± 5</td>
</tr>
<tr>
<td>Lawrenz 2011</td>
<td>11.6 ± 7.7</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

**IVF time frame**

**Luteal Stimulation with GnRH antagonist**
- Embryo cryopreservation with IVF 24 pt.
- GnRH-ant concurrent with FSH simulation.
- Luteolysis within 4d (early luteal) or 2d (mid luteal).

<table>
<thead>
<tr>
<th></th>
<th>follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonadotropin used</td>
<td>More NS</td>
<td></td>
</tr>
<tr>
<td>Stimulation duration</td>
<td>10.6</td>
<td>11.4</td>
</tr>
<tr>
<td>Oocytes obtained</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>61</td>
<td>76</td>
</tr>
</tbody>
</table>

**Minimal Stimulation/ IVM Protocol**

<table>
<thead>
<tr>
<th></th>
<th>Shalom Paz 2010</th>
<th>Maman 2010 Luteal</th>
<th>Maman 2010 follicular</th>
<th>Sirowitzki 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cycles</td>
<td>31</td>
<td>5</td>
<td>13</td>
<td>215</td>
</tr>
<tr>
<td>Oocytes cycle</td>
<td>9.7 ± 6.4</td>
<td>12.8 ± 8.4</td>
<td>17.3 ± 13.5</td>
<td>8.9</td>
</tr>
<tr>
<td>Total MII in 48h</td>
<td>7.0 ± 7.6</td>
<td>9.5 ± 7.7</td>
<td>48%</td>
<td>58%</td>
</tr>
<tr>
<td>Maturation rate</td>
<td>48%</td>
<td>58%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>77.8%</td>
<td>69%</td>
<td>63%</td>
<td></td>
</tr>
<tr>
<td>Embryo stored</td>
<td>4.5 ± 2.7</td>
<td>2.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
IVM results for fertility preservation

- 215 cycles
- 1922 Oocytes (8.9/cycle)
- 1231 matured (64% 5.7/retrieval)
- 355 fertilized (29% 2.8/retrieval)

- Better results vitrifying mature rather than immature oocytes.
- Vitrification of in vivo mature + in vitro mature better results.

Fertility preservation post chemotherapy

ivf 40% of patients with Hematological malignancies had previous chemotherapy.
OTCP > 50% of patients with Hematological malignancies had previous chemotherapy.
Sheba experience

Ovarian reserve after chemotherapy for breast cancer Premenopausal survivors compared with controls.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Min-max</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFC</td>
<td>Controls</td>
<td>11</td>
<td>1 - 34</td>
</tr>
<tr>
<td></td>
<td>Survivors</td>
<td>5</td>
<td>0 - 12</td>
</tr>
<tr>
<td>AMH</td>
<td>Controls</td>
<td>1.8</td>
<td>0.3 - 6.3</td>
</tr>
<tr>
<td></td>
<td>Survivors</td>
<td>0.6</td>
<td>&lt;0.1 - 2.4</td>
</tr>
<tr>
<td>FSH</td>
<td>Controls</td>
<td>8.0</td>
<td>3.1 - 17.7</td>
</tr>
<tr>
<td></td>
<td>Survivors</td>
<td>11.6</td>
<td>3.3 - 24.5</td>
</tr>
<tr>
<td>Inh B</td>
<td>Controls</td>
<td>46.6</td>
<td>10.0 - 152.1</td>
</tr>
<tr>
<td></td>
<td>Survivors</td>
<td>24.3</td>
<td>10.0 - 91.8</td>
</tr>
<tr>
<td>E2</td>
<td>Controls</td>
<td>38.8</td>
<td>12.0 - 89.9</td>
</tr>
<tr>
<td></td>
<td>Survivors</td>
<td>126</td>
<td>14.4 - 806.0</td>
</tr>
</tbody>
</table>

20 pt. in each group
Ovarian tissue cryo-preservation
post recent Chemotherapy

2-3 months post chemotherapy

<table>
<thead>
<tr>
<th>Disease</th>
<th>Age</th>
<th>IVF</th>
<th>Eggs</th>
<th>Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Hodgkin’s D</td>
<td>21</td>
<td>0</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Hodgkin’s D</td>
<td>25</td>
<td>0</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Hodgkin’s D</td>
<td>25</td>
<td>0</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

Meirow D. 2011
Conclusions

Effects of chemotherapy on large follicles

- Immediate Apoptosis.
- Large follicles >> primordial follicles.
- DNA damage.
- Very low ovulation rate post exposure.
- High abortion and malformation rate.

We do not collect mature or immature eggs for fertility preservation in patients recently exposed to chemotherapy (up to 6 months).

ART and embryo / Egg freezing in breast cancer patients.

Safe protocols for ovarian stimulation.
**IVF in hormone sensitive tumors**

- Normal stimulation
- Aromatase inhibitor (Letrazole) reduce E2 levels.  
  (Oktay et al.)
- Tamoxifen blocks Estrogen receptors.  
  (Meirow et al.)

**Hormone sensitive - Breast cancer protocols**

- Cancer cells
- High E2
- Low E2
- Ovarian Stimulation FSH
- Aromatase inhibitor
- Good No. of eggs

**Conclusive points for discussion**
Effect of postponing pregnancy on society as a whole: population impact, demand for/access to infertility treatment, financial implications

Siladitya Bhattacharya
University of Aberdeen

Outline

- Delaying pregnancy – trends
- Impact on total fertility rates
- Clinical implications
- Social implications
- Costs and consequences of ART
- Summary
### Age and natural livebirth rate

<table>
<thead>
<tr>
<th>Length of exposure (months)</th>
<th>Starting age (yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>12</td>
<td>75</td>
</tr>
<tr>
<td>48</td>
<td>91</td>
</tr>
</tbody>
</table>

Leridon Hum. Reprod. 2004

### Women’s perceptions on delay

#### Perceptions of delaying childbirth

<table>
<thead>
<tr>
<th>Perceptions of delaying childbirth.</th>
<th>Sub fertile (n = 362)</th>
<th>Pregnant (n = 362)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>When did you try for your first (planned) pregnancy?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18 years</td>
<td>227 (62.7%)</td>
<td>273 (75.4%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>18-35 years</td>
<td>135 (37.3%)</td>
<td>88 (24.6%)</td>
<td></td>
</tr>
<tr>
<td>Did you use contraception before trying for your first pregnancy?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>219 (60.5%)</td>
<td>254 (70.6%)</td>
<td>0.04*</td>
</tr>
<tr>
<td>No</td>
<td>42 (11.6%)</td>
<td>73 (20.2%)</td>
<td></td>
</tr>
<tr>
<td>How many years did you use contraception for?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>118 (32.7%)</td>
<td>106 (29.7%)</td>
<td></td>
</tr>
<tr>
<td>6-9 years</td>
<td>91 (25.1%)</td>
<td>80 (22.1%)</td>
<td></td>
</tr>
<tr>
<td>&gt;9 years</td>
<td>154 (42.3%)</td>
<td>186 (52.3%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Do you feel you postponed trying for pregnancy until your circumstances were different?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>250 (72.3%)</td>
<td>185 (51.2%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>No</td>
<td>16 (8.8%)</td>
<td>96 (28.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Maheshwari et al. 2007

### Reasons for reproductive delay in women over 33 years

- **Relationships**: 74%
- **Other distractions**: 52%
- **Work or other training**: 34%

Infertility: mean female age at referral

Age and the cause of infertility

Age and the odds of unexplained infertility

<table>
<thead>
<tr>
<th>Age</th>
<th>Adjusted* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30 yrs</td>
<td>1</td>
</tr>
<tr>
<td>30 – 34 yrs</td>
<td>1.5 (1.3, 1.8)</td>
</tr>
<tr>
<td>35 – 39 yrs</td>
<td>1.8 (1.4, 2.2)</td>
</tr>
<tr>
<td>&gt;= 40 yrs</td>
<td>1.2 (0.9, 1.6)</td>
</tr>
</tbody>
</table>

*Adjusted for year of diagnosis
Consequences of delaying pregnancy

<table>
<thead>
<tr>
<th></th>
<th>24 mths spacing</th>
<th>Additional spacing for 1st birth</th>
<th>Additional spacing for 30 mths</th>
<th>Additional spacing for 69 mths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean fecundability</td>
<td>0.23</td>
<td>0.231</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Mean age at maternity</td>
<td>29.1</td>
<td>31</td>
<td>32.9</td>
<td>33.9</td>
</tr>
<tr>
<td>Mean no of children</td>
<td>2.004</td>
<td>1.900</td>
<td>1.766</td>
<td>1.766</td>
</tr>
<tr>
<td>Mean age at first pregnancy attempt</td>
<td>25.1</td>
<td>27.6</td>
<td>30.8</td>
<td>30.8</td>
</tr>
<tr>
<td>Mean time to first conception</td>
<td>9.5</td>
<td>10.5</td>
<td>10.1</td>
<td>10.1</td>
</tr>
<tr>
<td>Infertile</td>
<td>6.9%</td>
<td>10.1%</td>
<td>12.6%</td>
<td>12.6%</td>
</tr>
</tbody>
</table>

Leridon & Slama Hum. Reprod. 2008

Factors affecting the chances of live birth

<table>
<thead>
<tr>
<th></th>
<th>Adjusted Odds</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous pregnancy</td>
<td>1.8</td>
<td>1.2 – 2.7</td>
</tr>
<tr>
<td>Infertility &lt; 3 yrs</td>
<td>1.7</td>
<td>1.1 – 2.5</td>
</tr>
<tr>
<td>Female age &lt; 30 yrs</td>
<td>1.5</td>
<td>1.1 – 2.2</td>
</tr>
</tbody>
</table>

Collins et al.1995

Age and the outcome of IVF

Wang et al Hum. Reprod. 2007
Adjusted total fertility rates in Europe

Infertility (12 mths): married women 15-44 yrs
Prevalence of infertility in Grampian: a comparison*

<table>
<thead>
<tr>
<th></th>
<th>1988 survey**</th>
<th>2007 survey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women 46-50 yrs</td>
<td>N = 766</td>
</tr>
<tr>
<td>Primary infertility</td>
<td>7.9%</td>
<td>6.2%</td>
</tr>
<tr>
<td>Primary &amp; secondary infertility</td>
<td>1.7%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Secondary infertility</td>
<td>7.3%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Total infertility</td>
<td>15.2%</td>
<td>9.2%</td>
</tr>
</tbody>
</table>

*P < 0.05

** Templeton et al, 1990 BMJ

Europe: predicted population 2004-50

<table>
<thead>
<tr>
<th>10% decline</th>
<th>1-10% decline</th>
<th>1-10% increase</th>
<th>&gt;10% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baltic Republics</td>
<td>Slovenia</td>
<td>UK</td>
<td>Sweden</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>Portugal</td>
<td>France</td>
<td>Malta</td>
</tr>
<tr>
<td>Slovakia</td>
<td>Greece</td>
<td>Spain</td>
<td>Ireland</td>
</tr>
<tr>
<td>Poland</td>
<td>Austria</td>
<td>Cyprus</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>Finland</td>
<td>Luxembourg</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>Denmark</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rychtarikova, 2007

Delay vs other reasons for declining fertility

- Contraception
- Wish for smaller families
- Lack of support for child rearing
- Decreasing fecundity
- Reproductive delay
- Access to and uptake of fertility services
Interventions to increase fertility

- Income support
- Work related policies
- Access to ART

Projected value of spontaneous and IVF child born to a mother aged 35 yrs

ART after reproductive delay
Timing of IVF and livebirths

Age & the cost of IVF live birth

Cost of an IVF livebirth

Maheshwari et al, Fertil Steril. 2010

Reproductive delay: Summary

- Significant delay
- Social and economic reasons
- Impact on spontaneous and treatment assisted births
- Effect on total fertility rate & population growth
- Measures to address this: social and clinical
- Economic value of IVF
- Cost effectiveness of IVF in older women
Mark your calendar for the upcoming ESHRE campus workshops!

- Early pregnancy disorders: integrating clinical, immunological and epidemiological aspects
  23-26 August 2011 - Copenhagen, Denmark

- The management of infertility – training workshop for junior doctors, paramedics and embryologists
  7-8 September 2011 - St. Petersburg, Russia

- Basic genetics for ART practitioners
  9 September 2011 - Bucharest, Romania

- The whole man
  22-23 September 2011 - Sevilla, Spain

- Accreditation of a Preimplantation Genetic Diagnosis Laboratory
  3-4 October 2011 - Athens, Greece

- Human reproductive tissues, gametes and embryos: Innovations by science-driven culture and preservation systems
  9 October 2011 - Cairns, Australia

- Comprehensive preimplantation screening: dynamics and ethics
  13-14 October 2011 - Maastricht, The Netherlands

- Endometriosis and IVF
  28-29 October 2011 - Rome, Italy

- Endoscopy in reproductive medicine
  23-25 November 2011 - Leuven, Belgium

- What you always wanted to know about polycystic ovary syndrome
  8-10 December 2011 - Sofia, Bulgaria

www.eshre.eu
(see “Calendar“)

Contact us at info@eshre.eu