Individualized COS to optimize the quantity and quality of oocytes and embryos generated in ART cycles

DELEGATE WORKBOOK
April 1–2, 2011 | Paris, France

- Abstracts
- Key objectives
- Key messages
- Key slides
Scientific organizers

Carlo Alviggi
Department of Obstetrics and Gynecology
IVF Unit
University of Napoli “Federico II”
Napoli, Italy

William Blaine Schoolcraft
Colorado Center for Reproductive Medicine
Lone Tree, Colorado, USA

Scientific secretariat

Serono Symposia International Foundation
Salita di San Nicola da Tolentino 1/b
00187 Rome, Italy

Project Manager: Francesca Pellegrino
Tel.: +39 06 420413 511 - Fax: +39 06 420413 677
E-mail: info@seronosymposia.org

Associate Project Manager: Chloé Xilinas
Tel.: +39 06 420413 505 - Fax: +39 06 420413 677
E-mail: info@seronosymposia.org

Serono Symposia International Foundation is a Swiss
Foundation with headquarters in 14, rue du Rhône, 1204
Geneva, Switzerland

Organizing secretariat

Meridiano Congress International
Via Mentana, 2/B | 00185 Rome, Italy
Congress Coordinator: Federica Russetti
Tel.: +39 06 88595 209 - Fax: +39 06 88595 234
E-mail: f.russetti@meridiano.it

All Serono Symposia International Foundation programs are organized solely to
promote the exchange and dissemination of scientific and medical information.
No forms of promotional activities are permitted. All the Serono Symposia
International Foundation programs are made possible thanks to the unrestricted
Educational grant received from: Centre d’Esclerosi Multiple de Catalunya,
ComtecMed, Congrex Sweden, Congrex Switzerland, Cryo-Save, Datanalysis,
Esaote, European Society of Endocrinology, Fundación IVI, ISFP International
Society for Fertility Preservation, ISMH International Society of Men’s Health,
K.I.T.E., Merck Serono, Ministry of Health of the State of Israel, University of
Catania, Vall d’Hebron University Hospital.
Serono Symposia International Foundation Conference on:

Individualized COS to optimize the quantity and quality of oocytes and embryos generated in ART cycles

April 1–2, 2011, Paris, France

Serono Symposia International Foundation (SSIF) welcome you to Paris, where we have gathered a team of leading international experts to discuss the many factors that contribute to the complexity of infertility and how screening of the parameters that define the characteristics of infertility in couples can allow for personalization of treatment to optimize outcomes.

You will be able to claim CME credits for attending this event, which is accredited by the European Accreditation Council for Continuing Medical Education (EACCME).

It is hoped that during this conference, you will:

■ receive a comprehensive overview of factors affecting reproduction
■ be updated on markers and tests aimed to define individual infertility profiles
■ debate solutions to adapt treatment to deal with individual infertility characteristics.

In addition to the above learning objectives for the overall conference, we have devised learning objectives and messages for each individual session; these are detailed in this workbook. A number of slides from each presentation are also included to support the learning messages.

We anticipate that this programme will inspire thought-provoking and informative debate on many of the challenges encountered in individualizing COS. We look forward to your active participation.
A varied and stimulating scientific program has been planned, incorporating a range of different session types, including lectures, case studies, panel discussions and key pad voting. To assist you in orientation regarding each session type, please refer to the respective visual icon given alongside each session.

**Friday April 1, 2011**

**08:40** Welcome  
Robert Fischer (Germany); Jean-Daniel Baki (Switzerland)

**08:50** Introduction  
Carlo Alviggi (Italy); William Blaine Schoolcraft (USA)

<table>
<thead>
<tr>
<th>Session I-A</th>
<th>Sources of variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chairs: Carlo Alviggi (Italy), Jean-Noël Hugues (France)</td>
<td></td>
</tr>
</tbody>
</table>
| **09:00** Introduction to voting system and initial questions about Session 1-A  
Carlo Alviggi (Italy) |
| **09:15** L1: System inefficiency: the variable of variables  
Pasquale Patrizio (USA) |
| **09:35** L2: Influence of genetics on reproductive function  
Manuela Simoni (Italy) |
| **09:55** L3: The endocrine function in infertile women  
Ernesto Bosch (Spain) |
| **10:15** L4: Oocyte biochemistry  
Yves Ménézo (France) |
| **10:35** Coffee break |

**Case study**  
Chairs: Manuela Simoni (Italy), Jean-Noël Hugues (France)

| **11:00** Case study  
Carlo Alviggi (Italy) |
| **11:40** Case study voting and discussion |

**12:00** Lunch break

**Session I-B**  
Sources of variability

**Chairs: Jean-Noël Hugues (France), Carlos Simón (Spain)**

| **13:00** Initial questions about Session 1-B  
Carlos Simón (Spain) |
| **13:15** L5: Biological versus chronological ovarian age  
Frank J. Broekmans (The Netherlands) |
| **13:35** L6: The male factor  
Alberto Ferlin (Italy) |
| **13:55** L7: Influence of life habits and body weight on fertility profile  
Renato Pasquali (Italy) |
| **14:15** L8: Patients’ expectations: a further source of variability  
Michael M. Alper (USA) |

**Panel discussion**  
Chairs: Carlo Alviggi (Italy), Carlos Simón (Spain)

| **14:35** PD1: Sources of variability  
Panelists: M.M. Alper, E. Bosch, F.J. Broekmans, A. Ferlin, Y. Ménézo, R. Pasquali, P. Patrizio, M. Simoni |
Saturday April 2, 2011

Session II  | Screening the variability

| Chairs: Yves Ménézo (France), Pasquale Patrizio (USA) |
| --- | --- |
| 15:20 | **L9:** Hormonal biomarkers predictive of response  
Scott M. Nelson (UK) |
| 15:40 | **L10:** Ultrasound test for ovarian response prediction  
Frank J. Broekmans (The Netherlands) |
| 16:00 | Coffee break |
| 16:30 | **L11:** Oocyte quality: when and how to measure it  
Nathalie Lédée (France) |
| 16:50 | **L12:** Endometrial receptivity and embryo-endometrial cross-talk: monitoring methods  
Carlos Simón (Spain) |

Panel discussion  
**Chairs:** Yves Ménézo (France), Pasquale Patrizio (USA)

| 17:10 | **PD2:** Screening the variability  
Panelists: F.J. Broekmans, N. Lédée, S.M. Nelson, C. Simón |
| 18:10 | End of first day |

Session III  | Managing the variability

| Chairs: Bruno Salle (France), William Blaine Schoolcraft (USA) |
| --- | --- |
| 09:00 | Questionnaire on managing variability  
William Blaine Schoolcraft (USA) |
| 09:15 | **L13:** Algorithm to predict ovarian response  
Renato Fanchin (France) |
| 09:35 | **L14:** Treatment individualization  
Carlo Alviggi (Italy) |
| 09:55 | Questions and answers on L13 and L14 |
| 10:15 | Coffee break |
| 10:45 | **L15:** Selecting the best sperm  
Mona Bungum (Sweden) |
| 11:05 | **L16:** Proteomics for embryo selection  
William Blaine Schoolcraft (USA) |
| 11:25 | **L17:** Genomics for embryo selection  
Santiago Munné (USA) |
| 11:45 | Questions and answers on L15–L17 |

Panel discussion  
**Chairs:** Ernesto Bosch Aparicio (Spain), Bruno Salle (France)

| 12:15 | **PD3:** Managing the variability  
Panelists: C. Alviggi, M. Bungum, R. Fanchin, S. Munné, W.B. Schoolcraft |
| 12:45 | Closing remarks |
| 13:00 | End of meeting |
| 13:00 | Lunch |
Dr Pasquale Patrizio
Yale University Fertility Center, New Haven, Connecticut, USA

Dr Pasquale Patrizio is a board-certified specialist in Obstetrics and Gynecology and Reproductive Endocrinology and Infertility. He is Professor of Obstetrics/Gynecology and Director of the Yale Fertility Center, New Haven, CT, USA.

Dr Patrizio received his MD (summa cum laude) from the University of Napoli, Napoli, Italy, and completed two residencies in Obstetrics and Gynecology (Napoli) and Andrology (Pisa). After moving to the USA, he completed a residency in Obstetrics and Gynecology at the University of California, Irvine and a fellowship in Reproductive Endocrinology and Infertility at the same university. He was then recruited as Associate Professor to the faculty of the University of Pennsylvania, Philadelphia, PA, USA, to establish and direct the Male Infertility Program. In August 2003, he completed a Master in Bioethics (MBE) at the University of Pennsylvania and became certified as a High Complexity Laboratory Director (HCLD). He joined the Yale faculty in his present position in January 2004.

Dr Patrizio has lectured throughout the world on the topics of IVF, male infertility and ICSI, garnering a number of awards, and serves as a committee member of important national and international professional organizations, including the ASRM, the American Society of Andrology (ASA) and the International Society for Fertility Preservation (ISFP). He is also a Fellow of the AGOS and of the International Academy of Human Reproduction, and secretary of the ISFP. Additionally, Dr Patrizio is associate editor of Journal of Assisted Reproduction and Genetics and a member of the editorial board of American Journal of Obstetrics and Gynecology, Reproductive BioMedicine Online, Journal of Experimental & Clinical Assisted Reproduction and www.IVF-worldwide.com.

Dr Patrizio’s main areas of interest include IVF, ICSI, epididymal and testicular sperm retrieval and freezing, genetics of female and male infertility, PGD, oocyte and embryo freezing, oocyte donation and surrogacy, fertility preservation for cancer patients, and development competence of oocytes and embryos.

He has authored a prominent ART textbook and 320 scientific papers, book chapters and abstracts, including 105 peer-review publications.
ABSTRACT

L1: System inefficiency: the variable of variables
Pasquale Patrizio

The process of IVF is highly inefficient. Only a small percentage (~20%) of embryos produced and transferred result in a live birth, and an even smaller percentage (~5%) of oocytes retrieved result in a live birth. To fully appreciate progress and efficiency in IVF, it is crucial to understand this important biological variable (the variable of the variables!) and measure ART success against the newly proposed metric of embryo and oocyte wastage.1,2

[Definition of Embryo wastage = Embryos transferred/Infants delivered × 100]
[Definition of Oocyte wastage = Oocytes retrieved/Infants delivered × 100]

Using the US summary statistics for ART cycles using fresh, non-donor oocytes and embryos, we calculated the percentage of embryos wasted each year from 1995 to 2008. Trends over time and among different age groups were evaluated for percent embryos wasted, the average number of embryos transferred, and the delivery rate per transfer. Further, we analysed the correlations between oocytes retrieved and used, and embryos transferred. The percentage of embryos wasted significantly decreased from 1995 to 2008, from 90% to 80% (P=0.01). Over this period, the mean number of embryos transferred per year positively correlated with the percentage of embryos wasted. Interestingly, despite a reduction in the mean number of embryos transferred, the percentage of transfers leading to a delivery has remained stable at about 35%.

When analysed by age, the rate of embryo wastage for women aged over 40 years was stable at approximately 95%. Furthermore, these women also have a correspondingly low rate of multiple pregnancy per cycle started (2.5% and 1.6% for women aged 41–42 years and 43–44 years, respectively). These data underscore the low reproductive efficiency of oocytes in women aged over 40 years and the very low probability of a multiple-gestation live birth, despite the high number of embryos routinely transferred.3

Oocyte wastage is also very high, with 95% of the oocytes in patients aged younger than 37 years, 97% for women aged 38–40 years, and 99% for women aged 41–42 years failing to produce viable births. Even the use of oocytes from donors confirmed a highly inefficient ART system. A total of 130 oocyte retrievals from the best-prognosis oocyte donors, i.e. donors who consistently procured pregnancies after each donation, yielded 2470 oocytes. The total live babies born (LBB) per oocyte retrieved and per embryo transferred was 7.3% and 24.6%, respectively.4 A total of 61 oocyte retrievals from the standard donors yielded 1044 oocytes. In this group, the total LBB per oocyte and per embryo transferred was significantly less, at 5.0% and 16.6%, respectively.

Despite advances in ART, the great majority of embryos transferred still do not result in a live birth. However, embryo wastage rates have decreased from a high of approximately 90% in 1995 to current rates near 80%. This correlated with a decrease in the mean number of embryos transferred from 3.9 in 1995 to 2.4 in 2006 and thereafter. Embryo and oocyte wastage rates may be decreased by transferring fewer embryos and by improving our ability to select and identify oocytes with the greatest potential for producing a viable, competent embryo.5,6

Recent results found statistically (P<0.05) significant differences in mRNA transcripts between normal and aneuploid oocytes for a set of 327 genes, indicating that aneuploidy is associated with altered expression levels of a subset of genes. A link between mRNA transcript numbers and age was also observed. Some of the highlighted genes produce proteins involved in spindle assembly and chromosome alignment, which parallel one of the most important defects affecting oocyte aneuploidy, closely related with advancing maternal age. In the future, better characterization of oocyte genes and their transcripts could serve as targets for non-invasive oocyte aneuploidy assessment.6

References
Learning objectives

- Understand that the overall success rates of IVF are low.
- Understand the possible reasons for the low success rates with IVF.

Topics
- Embryo Wastage
- Oocyte Wastage
- The “Super Donors”
- Pregnancy and Multiple rates in 40 or older
- New Genetic tools to identify competent Oocytes and Embryos:
  - CCOGE projects (genomics-transcriptomics)

High Rates of Embryos Wastage in ART

- To determine the percentage of embryos produced in ART and transferred that do not produce a Live Birth

**Embryo Wastage =**

\[
\text{Embryo Wastage} = \left( \frac{\text{Embryos Transferred}}{\text{Infants delivered}} \right) \times 100
\]


<table>
<thead>
<tr>
<th>Year</th>
<th>Fertilized Eggs Transferred</th>
<th>Transfers Live Birth</th>
<th>Successful Transfers</th>
<th>Miscarriages</th>
<th>Live Birth Rate</th>
<th>Overall Success %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>3.9</td>
<td>31,794</td>
<td>9,780</td>
<td>11,479</td>
<td>86.8</td>
<td>51.0</td>
</tr>
<tr>
<td>1996</td>
<td>4.0</td>
<td>35,859</td>
<td>9,780</td>
<td>11,479</td>
<td>86.8</td>
<td>51.0</td>
</tr>
<tr>
<td>1997</td>
<td>3.0</td>
<td>41,200</td>
<td>9,780</td>
<td>11,479</td>
<td>86.8</td>
<td>51.0</td>
</tr>
<tr>
<td>1998</td>
<td>3.5</td>
<td>47,529</td>
<td>13,952</td>
<td>21,583</td>
<td>87.1</td>
<td>31.1</td>
</tr>
<tr>
<td>1999</td>
<td>3.2</td>
<td>51,149</td>
<td>13,952</td>
<td>21,583</td>
<td>85.0</td>
<td>31.0</td>
</tr>
<tr>
<td>2000</td>
<td>3.1</td>
<td>59,014</td>
<td>16,830</td>
<td>26,284</td>
<td>85.7</td>
<td>31.0</td>
</tr>
<tr>
<td>2001</td>
<td>3.1</td>
<td>66,363</td>
<td>20,710</td>
<td>30,411</td>
<td>86.9</td>
<td>33.4</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Year</th>
<th>Ave. No. Fertilized Eggs Transferred</th>
<th>Transfers Live Birth</th>
<th>Successful Transfers</th>
<th>Miscarriages</th>
<th>Live Birth Rate</th>
<th>Overall Success Rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>2.8</td>
<td>67,177</td>
<td>34,328</td>
<td>82.1</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>2.8</td>
<td>71,437</td>
<td>35,876</td>
<td>82.3</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>2.7</td>
<td>73,567</td>
<td>35,586</td>
<td>82.2</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>2.6</td>
<td>75,169</td>
<td>35,604</td>
<td>81.6</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>2.4</td>
<td>76,981</td>
<td>37,596</td>
<td>79.5</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>2.5</td>
<td>78,460</td>
<td>39,491</td>
<td>79.9</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>2.4</td>
<td>81,156</td>
<td>39,489</td>
<td>80.5</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>

High Rate of Embryo Wastage in Women Aged 41 - 42

- Bronner et al. ARBM, 2009
During IVF, only about 20% of embryos produced and transferred, and only about 5% of oocytes retrieved, result in a live birth.

Because the overall efficiency of IVF is poor, the success of ART should be measured by consideration of wastage:
- Embryo wastage = embryos transferred/infants delivered × 100
- Oocyte wastage = oocytes retrieved/infants delivered × 100

Despite a reduction in the mean number of embryos transferred, the percentage of transfers leading to a delivery has remained stable (about 35%).

Embryo and oocyte wastage rates may be decreased by transferring fewer embryos and by improving the ability to select and identify oocytes with the greatest potential for producing a viable, competent embryo.
Professor Manuela Simoni
Department of Medicine, Endocrinology, Metabolism and Geriatrics
University of Modena and Reggio Emilia, Modena, Italy

Professor Manuela Simoni is full Professor for Endocrinology at the Department of Medicine, Endocrinology, Metabolism and Geriatrics at the University of Modena and Reggio Emilia, Italy.

Professor Simoni obtained her MD in 1982 and her specialization in Endocrinology and Metabolism in 1985 at the University of Modena. In 1991 she obtained a PhD in Endocrinology and Metabolism. Between 1990 and 2007 she worked at the Institute of Reproductive Medicine of the University of Münster, Germany, where she was Professor for Endocrinology and Molecular Biology of Reproduction.

Her current research interests are gonadotrophin and androgen action, testicular function, genetics of male infertility, endocrinology, and the pathophysiology of reproduction. She is a member of several societies, including the European Academy of Andrology and the European Society of Endocrinology and serves on the editorial boards of several journals in the fields of endocrinology and reproduction.

References
The gonadotrophins LH and FSH are fundamental for gonadal maturation (at puberty) and function (in adulthood). Gonadotrophins act by binding to specific receptors, the FSHR and the LHCGR. Both receptor genes are characterized by a large number of SNPs (210 for LHCGR and 902 for FSHR, as listed in the NCBI SNP database), most of them located in intronic regions and of unknown heterozygosity rate. Some SNPs, particularly those that are non-synonymous and located in exons, have been studied in association with gonadal function.

Concerning FSHR, the SNPs at nucleotides 919 and 2039 in exon 10 are very common (heterozygosity: 0.469) and result in the amino acid transition Thr–Ala at codon 307 and Asn–Ser at codon 680, respectively. In the Caucasian population, the two SNPs are mostly in linkage disequilibrium, with the Thr307–Asn680 variant covering 55% and the Ala307–Ser680 variant covering 45% of the alleles. The other two possible combinations represent <1% of all alleles in Caucasians, but are more frequent in the Far East. In addition, for a G/A SNP in the promoter region at position −29, the G allele covers 75% and the A allele covers 25% of the alleles in Caucasians, while the distribution is equal (50%) in Indonesians. The SNPs of FSHR do not have any apparent functional effect in vitro, but do influence receptor activity in vivo, at least in women. The Ala307–Ser680 variant is associated with higher basal serum FSH levels and lower sensitivity to FSH stimulation in women with normal ovarian function undergoing COS for assisted reproduction and during normal menstrual cycles. However, these two SNPs apparently do not influence serum FSH levels and semen parameters in men with normal or reduced spermatogenesis. When the haplotypes resulting from the SNPs in exon 10 and from a common SNP at position −29 in the promoter region are considered, the two allelic variants A/Ala–Ser and G/Thr–Asn showed a statistically significant different distribution between controls and men with non-obstructive azoospermia, suggesting that the FSHR genotype might constitute a risk factor for spermatogenetic failure.

The polymorphisms in exon 1 and exon 10 of LHCGR have been studied in much less detail. For exon 1, a 6 bp insertion results in the addition of two amino acids. This gives rise to two types of LH receptor with relative frequencies of 0.37 and 0.63 in the general population. An association between an earlier age of onset and a poorer outcome of breast cancer has been reported recently. We performed a retrospective, case–controlled study comprising 278 patients with maldescended testes, 277 infertile men without maldescensus testis and 271 controls with normal sperm concentrations. The insLQ polymorphism in exon 1 (rs4539842) and the N291S SNP in exon 10 (rs12470652), showing increased receptor sensitivity in vitro, were not differently distributed between patients and controls. The S312N SNP in exon 10 (rs2293275) was significantly less frequent in men with maldescended testes than in controls. This difference was confirmed when infertile men with and without maldescensus testis were considered together. From these data we concluded that, in men with maldescensus testis, a high LH drive maintains normal levels of testosterone, but this LH resistance is not associated with any particular LHCGR genotype. A significant association with the S312N polymorphism in exon 10 of LHCGR is correlated with the spermatogenetic damage rather than with the maldescensus testis itself. Either the LHCGR itself or another genomic region linked to this SNP, possibly the germ cell–specific ALF gene transcribed from the same genomic region in the opposite direction, is a risk factor for male infertility.

Beside SNPs, several splicing variants of the gonadotrophin receptors exist and might modulate gonadotrophin action. A new, primate-specific, regulatory exon within LHCGR has been identified. This exon is located within intron 6 and shows characteristics of a composite exon acting either as a terminal exon, giving rise to a putative truncated LHCGR protein consisting only of major parts of the extracellular domain, or as an additional internal exon of the full mature LHCGR transcript. In this case, owing to the presence of premature termination codons within the exon, the LHCGR transcripts are a potential target for non-sense-mediated decay. Mutations within this novel exon result in disorders of sexual differentiation. Three SNPs in this novel exon [which has been called exon 6A] have been found and are currently being investigated for their role in androgenization and maldescensus testis. In perspective, it will be interesting to analyse whether polymorphisms in exon 6A are associated with response to LH in COS.
Learning objectives

- The importance of genetics in assessing and managing reproductive function.
- How association studies can provide markers of fertility potential that physicians can use to maximize outcomes of ART.

**What do genetic association studies show?**

- **Direct association:** the genotyped SNP is responsible for the phenotype
- **Indirect association:** the genotyped SNPs are not responsible for the phenotype but are in LD with the causal SNP

*Cave!* A statistically significant association can be due to chance or bias

**Physiological menstrual cycle: higher serum FSH is in Ser<sup>680</sup> carriers**

Gree et al., JCEM, 2003; Urenov & Simon, TED, 2009

**AMH and AMH<sub>II</sub> polymorphisms are associated with estrogen levels and modulate FSH sensitivity**

Two independent cohorts of women with normal ovarian function

Keener et al., Hum. Reprod., 2007

**Poor response to COS is a polygenic trait involving FSHR, ER1 and ER2**

De Castro et al., Pharmacogenetica 14:285, 2004

Pharmacogenetic COS approach

Sehr et al., Pharmacogenet. Genomics, 15:181, 2005
Genetic markers and ovarian response to gonadotropins

- **FSHR**
  - confirmed
  - mechanism unknown
- **AMH – AMHR2**
  - to be independently verified
- **MTHFR**
  - unclear
- **ER1, ER2**
  - to be independently verified

Conclusions: **LHR polymorphisms and testis function**

- The mild LH-resistance of men with maldescended testes is weakly associated with the LHR genotype
- The 312Asn SNP in exon 10 is associated with the “infertility” phenotype
- Association with LHR or ALP?

Part 1: Summary and Conclusions

- The **FSHR** genotype determines the ovarian response to FSH
- Ovarian sensitivity to FSH is a polygenic trait
- Prospective studies should evaluate the usefulness of the FSHR genotype
- Powerful, multicenter, retrospective studies should evaluate the genetic factors involved in poor response and OHSS

Non-synonymous SNPs in LHR

- **Exon 1**
  - **LHR1**
    - MAP: 0.30
  - **LHR2**
    - MAP: 0.20
- **Exon 11**
  - **LHR3**
    - MAP: 0.45

Outlook: a role for a novel genomic element of the LHR in the ovarian response to LH?

Mutations in a Novel, Cryptic Exon of the Luteinizing Hormone/Chorionic Gonadotropin Receptor Gene Cause Male Pseudohermaphroditism

Key learning messages

- The gonadotrophins, LH and FSH, act by binding to specific receptors, the FSHR and the LHGR. Genes for these receptors are characterized by a large number of SNPs.
- Genetic studies have shown direct and indirect associations of genotyped SNPs with infertility.
- Polymorphisms in FSHR are linked to differences in ovarian response to FSH, but ovarian sensitivity is a polygenic trait also involving oestrogen receptors 1 and 2.
- Powerful, multicentre, retrospective studies should evaluate the genetic factors involved in poor response and OHSS.
- In men with maldescended testes, the presence of LHR polymorphisms (exon 6A) has been associated with mild LH resistance.
  - Future studies may focus on the association of the LHR polymorphism with response to LH in COS.
Dr Ernesto Bosch
Instituto Valenciano de Infertilidad, Valencia, Spain

Dr Ernesto Bosch completed his MD at the University of Valencia, Valencia, Italy in 1992 and from 1993 to 1997, worked as a Specialist in Obstetrics and Gynaecology at Hospital La Fe, Valencia. Dr Bosch trained in Human Reproduction at the Hospital of the University of Pennsylvania, Philadelphia, PA, USA, in 1997. In 1999, he completed his PhD, with cum laude qualification, at the University of Valencia. In January 2000, Dr Bosch joined the team at the Human Reproduction Unit of the Instituto Valenciano de Infertilidad in Valencia, and in 2008 he obtained the title of Master in Research on Health Sciences, by the Autonomous University of Barcelona, Spain.

Dr Bosch has published 25 papers and written more than 20 book chapters on the subject of IVF. He has given over 50 lectures at international meetings around the world and is a regular reviewer for Human Reproduction, Fertility & Sterility, Reproductive BioMedicine Online and Reproductive Biology and Endocrinology. He is a member of the Special Interest Group on Reproductive Endocrinology of the Spanish Fertility Society, and has received the Scientific Program Prize Paper Award at the 2008 Annual Meeting of the ASRM. In May 2010, Dr Bosch was appointed Medical Director of the Human Reproduction Unit of the Instituto Valenciano de Infertilidad in Valencia.
L3: The endocrine function in infertile women
Ernesto Bosch

The reproductive function in mammalian females is controlled by a perfect interplay between the hypothalamus, the pituitary and the ovaries. In response to an adequate pulsatility of GnRH by the arcuate nuclei of the hypothalamus, the anterior pituitary produces both gonadotrophins – FSH and LH – and both gonadotrophins interact with the ovary for folliculogenesis, ovulation and corpus luteum maintenance. However, through a feedback mechanism, the production of oestrogen and progesterone by the ovary controls the function of the whole axis, together with a number of autocrine and paracrine factors that are also involved.

The alteration of the hypothalamus–pituitary–ovary axis at any point may cause infertility because of the ultimate impairment of ovarian function. Although the effects at the hypothalamic level may lead to hypogonadotrophic hypogonadism and, therefore, to anovulation, changes in the pulsatility of GnRH may cause anovulation also, despite the presence of normal gonadotrophin levels. However, the senescence of the ovary leads to a lack of response to gonadotrophin stimulus, and therefore to ovarian dysfunction with high gonadotrophin levels.

Female infertile and/or subfertile patients may present variations on their endocrine profile at any of the above described levels. The protocols for ovarian stimulation cannot ignore these individual profiles, as the needs might be completely different from one type of patient to another. Today, clinicians have the chance of individualizing the combination of gonadotrophins for ovarian stimulation. To optimize the outcome, it is crucial to thoroughly evaluate the particular starting point of each patient and also the different response to the diverse approaches, in order to choose the best protocol for each type of patient.
Learning objectives

- Understand the important endocrine alterations that occur in female infertility and their causes.
- Understand the role of exogenous gonadotrophins in ART.
Infertility may be caused by alteration of the hypothalamus–pituitary–ovary axis at any point that leads to impairment of ovarian function.

- At the hypothalamic level, alterations may lead to hypogonadotrophic hypogonadism.
- Changes in the pulsatility of GnRH may cause anovulation even when gonadotrophin levels are normal.
- At the level of the ovary, senescence leads to a reduction in response to gonadotrophin stimulation, leading to ovarian dysfunction and high circulating gonadotrophin levels.
- Assessment of the specific endocrine profile of each patient can enable greater individualization of treatment protocols used for ovarian stimulation.
Professor Yves Ménézo
IVF Centre Eylau La Muette, Paris, France

Professor Yves Ménézo is a Scientific Advisor for both UNILABS, Geneva, Switzerland, and Laboratoire d’Eylau, Paris, France.

After gaining his doctorate, Professor Ménézo furthered his education, gaining the titles of Dr Sci and TC [ABB], before becoming a Senior Clinical Embryologist (ESHRE).

From 1987 to 1993, Professor Ménézo worked as Associate Professor at the Department of Animal Science, Louisiana State University, LA, USA, and from 1999 to 2001, was the Chairman of Alpha.

He has published over 300 scientific articles, including journal publications, book chapters and congress proceedings, and also has roles on the editorial boards of major journals specializing in fertility. He has won several awards, including a gold medal from the Department of Obstetrics and Gynaecology at the Instituto Universitario Dexeus, Barcelona, Spain in 2001 and the Paques Salat-Baroux prize from the French Academy of Medicine in 2004.
Oocyte biochemistry

Yves Ménézo and Elisabetta Tosti

The oocyte has a pivotal role during preimplantation embryogenesis totally under maternal control until maternal to zygotic transition (MZT). Oocyte competence is a measure of the ability of an oocyte to produce embryos with high developmental potential; however, oocyte competence is poorly defined, although it is improved by some growth factors and interleukins. Messenger RNAs and proteins are stored during oocyte growth and maturation, and the quality of this storage will define the competence of the early embryo as there is an unavoidable turnover of these reserves starting immediately before ovulation. The DNA repair capacity of the oocytes is particularly important as it controls the integrity of the chromatin: several hundred thousand lesions have to be repaired during the first 24 hours, immediately before and after the S-phase, particularly if we consider both the maternal and the paternal genome. Lack of repair of chemically modified [oxidized] bases, errors of methylation, DNA bridges etc. may have severe consequences: compounded damage and errant transcripts are sources of cell death and/or mutagenesis leading to cancer. DNA methylation, linked to imprinting, is one of the epigenetic processes that has to be precisely controlled. Regulation of polyadenylation of mRNA has two major effects: (i) it allows translation with accurate timing and (ii) it prevents a too rapid degradation of the important mRNAs coding for the genes involved in intermediate metabolism and housekeeping, which allows genomic activation to be achieved at the proper time. A too early depletion of maternal mRNAs and proteins will lead to developmental arrests at the time of MZT. The quality of the endogenous pool of small, intermediate metabolites is also very important. After oocyte activation, there is an instant increase and mobilization in glutathione (necessary for sperm head swelling) associated with cysteine availability. The impact on further embryonic development is immediate: increased blastocyst formation rate and increased cell number per blastocyst formed. Methionine will be used for imprinting following formation of S adenosyl methionine. Folic acid is used for the recycling of homocysteine, which is necessary for a correct imprinting process and the synthesis of thymidine. It is common knowledge that maternal age impairs all the biochemical parameters.

The major problem with evaluating the quality of oocytes is the scarcity of biological material at the limit of all the analytical techniques. Moreover, at present, it is impossible to have access to non-invasive technologies.

Reference
Learning objectives

- Understand the importance of the biochemical aspects of oocyte maturation in infertility and how this can be used to enhance ART.
- Understand the importance of DNA repair mechanisms in human oocytes.

![Composition of culture medium impacts on mRNA accumulation and microRNA processing](image1)

![Effect of external methionine on glucose uptake and incorporation into proteins by mouse preimplantation conceptuses](image2)

![Glucose Lactate, Pyruvate: Anabolism, Catabolism](image3)

![Hyperglycemia, Apoptosis and sex ratio (Role of X-linked Apoptosis inhibitor (XIAP))](image4)
Key learning messages

- Oocyte competence is defined as the ability to produce embryos with high development potential and is an important aim in ART.
- Early embryo competence relates to the quality of mRNA and protein storage during oocyte growth and maturation.
- Important regulators of competence include the DNA repair capacity of oocytes; lack of repair may lead to cell death, mutagenesis or carcinogenesis.
- DNA methylation needs to be precisely controlled and regulation of the polyadenylation of mRNA allows for accurate translation and limited degradation of mRNAs important for coding of genes involved in timely genomic activation.
- The quality of the endogenous pool of small intermediate metabolites, such as glutathione, cysteine, methionine, homocysteine and thymidine, is also important.
- Evaluation of oocyte biochemistry and competence is currently hampered by lack of non-invasive technologies and scarcity of biological material.
Dr Frank J. Broekmans
Department of Reproductive Medicine, University Medical Center Utrecht, Utrecht, the Netherlands

Dr Frank Broekmans is Professor in Reproductive Endocrinology and Surgery, and Head of Reproductive Medicine at the University Medical Center, Utrecht, the Netherlands. He is also Chairman of the Dutch–Flemish Society for Fertility Studies.

Graduating from the Faculty of Medicine at the VU Medical Center, Amsterdam, in 1983, Dr Broekmans later became Consultant OBGYN in 1990, completed a Fellowship in Reproductive Medicine in 1993, and completed his PhD in 1995.

Dr Broekmans’ scientific career has been devoted to the field of female reproductive ageing. His main research interests are ovarian ageing and dysfunction, as well as methods of testing ovarian reserve, including the use of anti-Müllerian hormone, inhibin B and antral follicle count.

Dr Broekmans has held an Associate Editorship for Human Reproduction between 2006 and 2009 and has published over 110 peer-reviewed scientific papers, contributed to seven book chapters, and presented over 80 invited lectures at various international meetings.
Several reasons exist for wishing to have advance notice of the timing of menopause. There is a strong relationship between the onset of menopause and natural infertility levels some 10 years earlier, so long-term prediction of menopause onset may help women to plan better their attempts to have children. If premature or early menopause could be predicted, preventive measures could be taken to minimize the related long-term health risks. Incipient ovarian failure in terms of the current inability to create a viable ongoing pregnancy is another issue that has been the subject of numerous studies, particularly directed in the field of assisted reproduction.

The process of ovarian ageing consists of the gradual decline in number and quality of the remaining follicles and oocytes in both ovaries at a given age. Decline in follicle numbers dictates the occurrence of irregular cycles and menopause, while decay in the quality of oocytes results in decreasing fertility, defined as the capacity to conceive and give birth to a child. There is substantial individual variation in the onset of menopause, which varies roughly between 40 and 60 years, with a mean age of 51 years. Following this same pattern, the rate of decline in fertility may vary considerably between women of the same age. These notions underline the need for tests [ovarian reserve tests, ORTs] that describe future and current ovarian reserve status.

Among these tests are calendar age, family history, AMH, poor response in IVF, basal FSH, AFC and genetic variation for long-term prediction. From recent long-term follow-up studies, only AMH has provided some individual value in the prediction of time to menopause. The application of genetic profiles has so far been hampered by inconsistent findings and only very small effects of the associated variation on age at menopause.

Assessment of ovarian reserve status in patients undergoing ART has been demonstrated as being inadequate when pregnancy prospects are concerned. From recent individual patient data analysis, it has been demonstrated that ORTs do not add to the prediction based on female age alone. Response to COS has appeared to be highly predictable, particularly when AMH levels and AFC are included. Management options for predicted poor and high responders are still under study and debate. The combination of poor response in a first ART cycle and an abnormal AMH or AFC test result may identify a group of patients who have such a poor prognosis that it may be better for further treatment to be refused.
Learning objectives

- Appreciate the variability in the lifecycle of the oocyte and the mechanisms involved in ovarian ageing.
- Understand the importance of biological age in predicting the outcome of ART.

Questions

- What do we know about mechanisms of Ovarian Ageing?
- What are the implications of this Ageing process?
- Are reliable Markers available for estimating Ovarian Age?
- Can these Markers help us in Clinical Conditions?
- Conclusions

Reproductive Ageing – Ovarian Ageing

Or CNS Ageing?

Questions

- What do we know about mechanisms of Ovarian Ageing?
- What are the implications of this Ageing process?
- Are reliable Markers available for estimating Ovarian Age?
- Can these Markers help us in Clinical Conditions?
- Conclusions
The age of onset of menopause is variable, generally occurring between the ages of 40 and 60 years. The rate of decline in fertility (reduction in ovarian reserve) varies considerably among women of the same age. Determination of biological age, in conjunction with chronological age, can assist in determining the prognosis of ART. There is a range of proposed predictors of ovarian reserve, including basal FSH, AMH, poor response to IVF, age, family history and the direct measurement of AFC. To date, only AMH has provided value in predicting the time to menopause. An abnormal AMH or AFC test and a poor response to a first ART cycle can identify a group of patients who have a poor prognosis and should be advised to cease treatment. More research is needed into earlier, reliable indicators of ovarian ageing.
Dr Alberto Ferlin
Department of Histology Microbiology and Medical Biotechnologies, University of Padova, Padova, Italy

Dr Alberto Ferlin is Assistant Professor of Clinical Pathology at the Department of Histology, Microbiology and Medical Biotechnologies, University of Padova, Padova, Italy. He completed his MD in 1995, and specialized in Endocrinology and Metabolism in 2000. In 2005, he earned his PhD in Endocrinological and Haematological Sciences.

Dr Ferlin has authored more than 300 publications and has been an invited speaker in 30 international congresses and 60 national congresses. He has won 12 scientific awards in national and international congresses, and is a member of the scientific committee or president of more than 20 national and international congresses.
Despite spermatogenesis being among the most finely regulated processes in the body, few genetic tests are currently used routinely in infertile males. These tests include analysis of karyotype, Yq microdeletions, CFTR and androgen receptor gene mutations, which collectively account for 15–20% of male infertility. ART allows the transmission of infertility-related genetic anomalies. Very little is known about the pathogenic mechanism leading to spermatogenesis disruption in many of these patients, particularly those carrying Yq microdeletions.

A recent field of research of our group has focused on the identification of molecular pathways leading to spermatogenic damage in men with AZFc microdeletions and idiopathic infertility. Testicular gene expression profiling carried out by microarray assay revealed that all the AZFc-deleted samples clustered together and showed a down-regulation of several genes [331] related to spermatogenesis. Interestingly, some idiopathic patients clustered together with the AZFc-deleted patients, suggesting that several forms of infertility can be triggered by a common pathogenic mechanism that is likely related to alterations in testicular mRNA storage. Our data suggest that a lack of testicular DAZ gene expression may be the trigger of such a mechanism and DAZ gene dysfunctions could therefore account for a larger number of previously thought ‘idiopathic’ infertility cases. A second line of research includes the contribution of genetic polymorphisms to male infertility and, more importantly, as markers for a pharmacogenomic approach to the treatment of infertile males. In this light, we demonstrated the diagnostic and therapeutic validity of polymorphisms in the FSH receptor and FSH beta genes. These results, taken together with the most recent research published on the genetics of male infertility are shedding light on novel molecular events involved in ‘old’ causes of male infertility and are suggesting new pathogenetic mechanisms of spermatogenesis disruption. It is possible that new genetic tests could be introduced in clinical practice in the near future as diagnostic, susceptibility or pharmacogenetic tests.
Learning objectives

- Understand the importance of recognition that the male factor is a major contributor to infertility and can influence outcomes of ART.
- Be aware of the altered molecular pathways that can lead to male infertility.
The male factor is an important contributor to infertility and there should be a greater emphasis on the assessment of genetic anomalies in men to prevent these being transmitted during ART.

Alterations in testicular mRNA storage can trigger several forms of male infertility previously thought to be idiopathic.

Testicular gene expression profiling is a valuable tool for the assessment of the molecular pathways leading to spermatogenic damage.

Genetic polymorphisms also contribute to male infertility and may be markers for a pharmacogenomic approach to treatment of infertile males.

Sperm integrity testing may have a diagnostic/prognostic role in subgroups of infertile couples in the future.
Professor Renato Pasquali

Department of Clinical Medicine, St. Orsola-Malpighi Hospital, University Alma Mater Studiorum, Bologna, Italy

Professor Renato Pasquali is Professor of Endocrinology and Director of the Division of Endocrinology of the St. Orsola-Malpighi Hospital, Bologna, Italy. Moreover, he heads the School of Specialization in Endocrinology and Metabolism at the University Alma Mater Studiorum of Bologna, Italy.

Professor Pasquali’s scientific activity pertains to the pathophysiology and treatment of PCOS, and the endocrinology of obesity (sex hormones, the hypothalamic–pituitary–adrenal axis, and the endocannabinoid system).

He has authored 205 original papers and review articles published in international journals and 16 book chapters in international textbooks. He is also a member of numerous national and international scientific societies and on the editorial boards of many international journals.
Obese women may be affected by fertility-related disorders. The relationship between excess body fat and reproductive disturbances appears to be stronger for early-onset obesity, particularly during adolescence. Moreover, obesity in women can increase risk of miscarriage and impair the outcome of ART. The main factor implicated in the association between obesity and fertility-related disorders is insulin excess, which accompanies insulin resistance. Hyperinsulinaemia may, in fact, be directly responsible for the development of androgen excess, through its effects in reducing SHBG synthesis, and in stimulating ovarian androgen production rates. Androgen excess, in turn, represents one of the major factors leading to altered ovarian physiology and associated ovulatory disorders. Obesity-associated hyperleptinaemia may represent an additional factor involved in anovulation, not only through the induction of insulin resistance, but also through a direct impairment of ovarian function.

The paradigm of PCOS clearly depicts the independent effects of obesity on fertility and reproduction. The prevalence of obesity in women with PCOS appears, in fact, to be much larger than that expected in the general population. Mechanisms by which obesity influences the pathophysiology and clinical expression of PCOS, particularly androgen excess and altered folliculogenesis, are complex and not completely understood, although available data point to the relevant roles of insulin excess and insulin resistance-related adipokine dysregulation. Clinically, the phenotypic presentation of PCOS largely depends on the presence of obesity, which accounts for worsened hyperandrogenism, metabolic alterations and ovulatory performance. Obesity may also affect ART outcomes, and often pregnancy outcomes. Interestingly, treatment of obesity by lifestyle intervention (and insulin sensitizers, if appropriate) may improve infertility in women with PCOS. In most women, a partial or complete recovery from the PCOS phenotype can be achieved, which adds a new perspective to the impact of obesity on the pathophysiology of PCOS, and suggests that it may play a causative role in this disorder in susceptible individuals, through still undefined mechanisms.
Learning objectives

- Understand the impact of the main lifestyle-related factors on the fertility profiles of couples attempting to conceive either naturally or by assisted methods.
- Awareness of the need for a multidisciplinary approach to modify lifestyle.

**Potential adverse effects of obesity on fertility in women**
- Precocious menarche
- Irregular cycles, oligo/amenorrhea
- Chronic anovulation
- Increased risk of miscarriages
- Decreased conception rates after ART
- Increased morbidity in pregnant women
- Worsened outcomes of preterm deliveries
- Pathophysiologic implication in determining PCOS and the associated metabolic abnormalities (insulin resistance, etc.)

**Obesity and sex hormones**

- Altered regulation of androgen/estrogen secretion, transport, metabolism, action, and regulation
- Different phenotypes of obesity
- The sex hormone balance
- Role in determining the phenotype, fat distribution, and the association with metabolic abnormalities (i.e., insulin resistance, etc.)

**Leptin and reproduction**

- Leptin is a messenger of energy stores to the brain and also regulates gonadal function and reproduction
- Leptin regulates GnRH (high receptor expression in the hypothalamus) and anterior pituitary cells express leptin, whereas it stimulates LH (and FSH)
- Leptin deficient mice are obese, insulin resistant and hypofertile, and treatment with r-leptin improves the phenotype
- ESTR induce leptin PR, whereas ANDR suppress it
- However, whether leptin is involved in the regulation of the HPG axis in obesity has been poorly investigated

**Symbols of fat and fertility**

- The “Venus” from Lespugue (Haute-Garonne, France) made from mammoth tusk and found by Saint Perier in 1922 (height 15 cm)
Key learning messages

- Obesity is associated with fertility-related disorders in women. The main factor is likely to be insulin excess, which may cause androgen excess, leading to altered ovarian physiology and ovulatory disorders.

- Infertility is more common in women who had early onset of obesity, particularly in adolescence.

- Hyperleptinaemia associated with obesity can cause anovulation via direct effects on ovarian function.

- Obesity is more prevalent in women with PCOS due to insulin excess and insulin resistance-related adipokine dysregulation.

- ART outcomes are altered by obesity, making the management of life habits and body weight of utmost importance in women undergoing treatment.
Dr Michael Alper
Boston IVF, Waltham, and Harvard Medical School, Boston, Massachusetts, USA

Dr Michael Alper is co-founder and Medical Director at Boston IVF, which has one of the USA’s largest and most experienced groups of reproductive endocrinologists.

Dr Alper completed his undergraduate and medical school training at McGill University in Montreal, Canada. After completing his internship at the University of Toronto, he pursued his residency in Obstetrics and Gynecology at Beth Israel Hospital in Boston, MA, USA. After completing his training in OBGYN, he completed 2 years of research at the University of Ottawa, followed by a fellowship in reproductive endocrinology at the Beth Israel Hospital/Harvard Medical School. Dr Alper is on staff at the Beth Israel Deaconess Medical Center and is an Associate Clinical Professor of OBGYN and Reproductive Biology at Harvard Medical School.

Dr Alper’s scientific focus is on the integration of technology, patient service and quality care.

Dr Alper has authored several textbooks and many articles in peer-reviewed publications, and has lectured extensively on many areas of reproductive medicine.
L8: Patients’ expectations: a further source of variability
Michael M Alper

Infertility treatment can be quite involved for patients. Patients often report dissatisfaction with many aspects of IVF treatment (particularly if not pregnant) and much of the focus surrounds communication issues and setting expectations. Medications and their associated complications, as well as the method of administration, are often cited as particularly concerning to patients. Also, patients often fail to fully understand the statistics of IVF and have unrealistic expectations for success.

Studies clearly demonstrate that the physical and emotional burdens of IVF treatment are associated with a very high drop-out rate. Studies demonstrate that as many as 40–50% of patients completing any given cycle do not return for further treatment. Numerous factors play a role in patients discontinuing treatment, which include financial concerns, stress and physical burdens. It is in the best interests of patients and those providing care to develop a strategy to address patient drop-out in order to maximize the likelihood of IVF being an effective process. IVF programmes need to ensure that the education and counselling process is effective, have effective communication, and have the appropriate mental health team available for patients in need of such services. It is also clear that an important challenge for all of us who care for patients undergoing IVF is to be certain that we use the least intrusive [but effective] treatment protocols that have the least impact on the lives of our patients. Of course, IVF technology is the key to our success, but our patients must return to us for treatment if we are to help them.

Reference
Learning objectives

- Understand the influence of inadequate patient expectations on adherence to treatment cycles.
- Understand the impact of patients’ adherence to treatment cycles on the overall success of assisted reproductive strategies.
- Be equipped with a range of strategies to enhance adherence rates and thus improve pregnancy rates.
Key learning messages

- Patients can drop out of infertility treatment at any stage.
- Communication issues and a mismatch of expectations can lead to a high level of psychological burden, which is the most common factor involved in dissatisfaction with IVF.
- As many as 40–50% of patients undergoing any cycle do not return for subsequent treatment.
- Employing strategies to minimize patient drop-out from treatment cycles will maximize the likelihood of success with IVF. These can include the use of:
  - more comprehensive education and counselling by a dedicated specialist team, including IVF nurses
  - the least intrusive, simplified treatment protocols.
Professor Scott M. Nelson
University of Glasgow, Glasgow, UK

Professor Scott Nelson obtained a BSc in immunology from the University of Glasgow, Glasgow, UK, in 1994. After commencing clinical work in obstetrics and gynaecology, he furthered his education, gaining a PhD at the University of Dundee, Dundee, UK, graduating in 2003. In 2005 he was appointed as a Clinician Scientist by the University of Glasgow, with progression to the Muirhead Chair of Obstetrics and Gynaecology in August 2008.

Professor Nelson’s research focuses on several key endocrine and metabolic pathways and their role in determining pregnancy and long-term maternal and offspring outcomes.
Recent studies have indicated that AMH constitutes an important novel measure of ovarian reserve, with the current literature indicating that AMH is a superior marker for predicting ovarian response over either age of the patient, day 3 FSH, oestradiol, inhibin B or AFC. Consistent with being strongly correlated with oocyte yield, AMH is a useful clinical marker for the prediction of both poor- and hyper-responses to COS. The primary utility of a highly discriminatory biomarker like AMH is in the individualization of patients’ expectations and optimization of treatment strategy prior to the first IVF cycle. In addition to reflecting the quantitative ovarian response, several studies, although not all, have found a significant positive correlation between AMH levels and oocyte quality and embryo morphology. With respect to the prediction of live birth after COS, several studies have now shown that models that include AMH allow accurate discrimination of the probability of success. This further enhances the utility of AMH in the setting of initial treatment planning.
Learning objectives

- Confirm the role of ovarian reserve testing using AMH.
- Provide evidence for the biomarkers of most value in predicting response to ART, with recommendations for their use in practice.

---

The new AMH Gen II assay

| CSL antibody Immunoradiometric standards | Values ~40% higher |

---

AMH correlates with oocyte yield and is better than other predictors

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>Fourth-oocytes</th>
<th>Fifth-oocytes</th>
<th>Sixth-oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sidel (2000)</td>
<td>407</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Ivanov (2000)</td>
<td>153</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Ciarleglio (2000)</td>
<td>90</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Sturis (1999)</td>
<td>106</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Dudgeon (2000)</td>
<td>60</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Ellis (2000)</td>
<td>56</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Frattini (2000)</td>
<td>35</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Listeria (2000)</td>
<td>80</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Hassan (2000)</td>
<td>110</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Epping (2001)</td>
<td>22</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Nas (2001)</td>
<td>54</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Mosier (2001)</td>
<td>176</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Davis (2000)</td>
<td>100</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>

---

Validated AMH age nomogram for DSL

---

AMH - prediction of over-response

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>Study design</th>
<th>AMH values (range)</th>
<th>Success (%)</th>
<th>Number (%)</th>
<th>Prediction of over-response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kang (2007)</td>
<td>110</td>
<td>Prog</td>
<td>0.45-2.00</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Nelson (2007)</td>
<td>300</td>
<td>Prog</td>
<td>0.45-2.00</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Lim (2004)</td>
<td>252</td>
<td>Prog</td>
<td>0.45-2.00</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>

Strengths:
- High number of patients
- Optimal classification of OHSS
- Only moderate to severe OHSS considered

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

- The most important measure of ovarian reserve is AMH.
- AMH is also an important predictor of ovarian response, being more predictive than age, day 3 FSH, oestradiol, inhibin B or AFC.
- AMH can be used to predict poor- or hyper-response to COS, thus allowing for greater individualization of treatment and alignment of the patient’s expectations prior to the first IVF cycle.
- Some scientists have shown that AMH levels correlate well with oocyte quality and embryo morphology.
- AMH can be added to models that allow discrimination of the probability of live birth after COS.
Dr Frank J. Broekmans
Department of Reproductive Medicine, University Medical Center Utrecht, Utrecht, the Netherlands

Dr Frank Broekmans is Professor in Reproductive Endocrinology and Surgery, and Head of Reproductive Medicine at the University Medical Center, Utrecht, the Netherlands. He is also Chairman of the Dutch–Flemish Society for Fertility Studies.

Graduating from the Faculty of Medicine at the VU Medical Center, Amsterdam, in 1983, Dr Broekmans later became Consultant OBGYN in 1990, completed a Fellowship in Reproductive Medicine in 1993, and completed his PhD in 1995.

Dr Broekmans’ scientific career has been devoted to the field of female reproductive ageing. His main research interests are ovarian ageing and dysfunction, as well as methods of testing ovarian reserve, including the use of anti-Müllerian hormone, inhibin B and antral follicle count.

Dr Broekmans has held an Associate Editorship for Human Reproduction between 2006 and 2009 and has published over 110 peer-reviewed scientific papers, contributed to seven book chapters, and presented over 80 invited lectures at various international meetings.
COS for IVF aims to produce multiple embryos of high quality to make the chances of an ongoing pregnancy occurring as high as possible. Two distinct problems may arise when performing COS: a too low response with inherent reduction in pregnancy rates and a too high ovarian response with the threat of OHSS developing. Both outcomes of the stimulation also bear the risk of cycle cancellation. Therefore, prediction of the outcome response in terms of number of oocytes is of great importance. In addition, prediction of the outcome of the ART cycle or sequence of cycles in terms of ongoing pregnancy is another feature that could allow clinicians to select patients for whom ART using their own oocytes may not be the proper choice.

Two issues are key in predicting the outcome of ovarian response. First, the relationship between FSH dose level and ovarian response in terms of number of dominant follicles growing has not been clearly established. So far, the evidence has shown that the dose–response curve is very steep, indicating that with only small increases in FSH level, single follicle growth is turned into maximal ovarian response. This implies that in the vast majority of patients, the dosage administered (150 IU or higher) will provide maximal stimulation of the ovaries. Only factors such as high BMI or the presence of specific FSH receptor variants may contribute to the creation of FSH levels that lead to submaximal stimulation of the ovaries. Second, the use of maximal stimulation dosages as a rule implies that the ovarian response is not principally dependent upon the dose of FSH applied, but on the size of the cohort of the FSH sensitive antral follicles present in the ovaries at the time of stimulation. The antral follicle cohort size is fully determined by the ovarian reserve status of the individual woman and expressed by female age and possibly by ovarian reserve tests. These two factors are therefore the most important tools for response prediction.

Predicting the outcome of ongoing pregnancy necessitates having a marker that somehow depicts the decline in the number of follicles and, at the same time, the level of competence for oocytes to develop into a normal embryo. As most markers only relate to the quantity of follicles, and the relationship between quantity and quality of oocytes is not fully understood, tools that correctly recognize very poor prognosis cases in ART have been difficult to identify.

In poor response prediction, several ovarian reserve tests (FSH, AFC, AMH) have been shown to be accurate predictors. However, the added value to female age can be considered doubtful. Moreover, if a poor response is predicted, no clear options for altered management other than counselling are available. In particular, the application of higher dosages of FSH in predicted poor responders is not evidence based. Applying ovarian reserve tests in actual poor responders in the first IVF cycle may, however, help to classify the nature of the poor response and estimate the remaining chances for ongoing pregnancy. Especially if no other signs of reduced reserve are present, prognosis is fairly good and continuation of IVF treatment is justified.

Hyper-response to ovarian stimulation is increasingly considered as a condition in which low quality or immature oocytes are added to a basal number of best quality oocytes. Prevention of hyper-response has been mainly based on patient profiles, such as very young age and PCOS, as well as the general use of modest dosing schemes not exceeding 200 IU in first cycles. Recent review studies have shown that the correct prediction of hyper-response is possible by using tests such as the AFC or AMH. However, it is currently not known whether effective strategies for managing predicted hyper-responders can be developed.

Identification of cases with poor prognosis relating to their chances of ongoing pregnancy has been notoriously difficult using quantity markers such as basal FSH, AMH and the AFC, particularly if female age is initially used as a baseline test. The added value of knowing female age in individual data analysis [IMPORT (Individual Meta-analysis of Patient data for Ovarian Reserve Testing) study group] has been shown to be absent. This implies that routine screening of patients undergoing ART prior to starting treatment should be limited to response profiling.
Learning objectives

- Understand the clinical application of AFC testing using ultrasound imaging to predict ovarian response to ART in individual cases.
- Be aware of the positive predictive value of different techniques and their practical application.

Questions

- What is Ovarian Reserve?
- What is the Aim of Ovarian Reserve testing?
- Ultrasound marks Ovarian Reserve?
- What does Ultrasound offer in OR Testing?
- Conclusions

Questions

- What is Ovarian Reserve?
- What is the Aim of Ovarian Reserve testing?
- Ultrasound marks Ovarian Reserve?
- What does Ultrasound offer in OR Testing?
- Conclusions

Aim of Ovarian Reserve assessment

To identify cases with

Severely diminished or with

Still adequate

ovarian reserve
Assessment of ovarian reserve can support the individualization of COS and allow prediction of better outcomes in ART.

Ultrasound can be used to assess ovarian reserve by measuring AFC, ovarian volume and ovarian blood flow.

AFC and ovarian volume decline with age and can be used to estimate ovarian capacity and to predict a poor response to IVF, but are not predictive of non-pregnancy.

AFC can be used for poor responder typing and as an indicator of ovarian capacity for women aged over 40 years.
Dr Nathalie Lédée studied Medicine, then Obstetrics and Gynaecology at the University of Paris VI, Paris, France. She earned her MD in 1993 and subsequently specialized in Reproductive Medicine. Dr Lédée furthered her education by completing a masters degree at McGill University, Montreal, Quebec, Canada, and in 2003, a PhD in Reproductive Immunology.

Dr Lédée now heads a team of researchers at the Unit of Medical Research (University of Paris XI and INSERM unit 782). From 2004 to 2008, she was Workpackage Leader in the European network EMBIC focusing on embryo implantation.

Dr Lédée’s main field of research focuses on the involvement of cytokines/chemokines in the process of embryo implantation.
L11: Oocyte quality: when and how to measure it
Nathalie Lédée

Due to the predominant role of maternal factors during early embryo development, oocyte quality remains the main factor limiting the success of ART in human fertility. ART teams are currently able to evaluate routinely the overall ovarian reserve of a patient (hormonal dosages, AFC), but fail to define individual oocyte quality and competence, and subsequent reliable parameters despite extensive research.

Here we will detail some emerging strategies aiming to define oocyte quality during IVF. Most strategies are based on either cumulus cell analysis of their transcriptomics profiles or proteomic analysis of individual follicular fluids (FF). After an overview of the current knowledge, we plan to focus on G-CSF, the third member of the CSF family. Indeed, G-CSF quantified in individual FF appeared in three distinct experiments measuring simultaneously 26 cytokines/chemokines and growth factors1–3 as the non-invasive immune biomarker of oocyte competence able to predict the potentiality of subsequent birth of the corresponding embryo.

Based on such a result, we will present the first proof-of-concept (POC) study aiming to detail the potential impact of FF G-CSF assessment on subsequent embryologists’ decisions to either transfer, freeze or destruct corresponding embryos and the consequences on subsequent pregnancy rates. FF G-CSF quantification has been performed in 563 FF samples by bead-based assay (119 fresh/transferred; 276 frozen; 131 destructed) among 79 patients. A combination of both FF G-CSF and embryo morphology (fitting equation) appears to be more discriminant than morphology or FF G-CSF quantification alone to predict subsequent pregnancy. Forty percent of the patients would have had benefit of such an approach as it would have modified the choice of the embryo to transfer, with an hypothetical increase of 10% for the pregnancy rates at the first fresh transfer. Moreover, half of embryos frozen seem to have no potentiality to implant whereas 10% of destructed embryos seem to have implantation potential. Using FF G-CSF combined with embryo morphology, all cases at high risk of multiple pregnancy would have been identified in the present study.

Based on this POC study, we will discuss problems related to an effective prospective application, i.e. the method for individual FF collection as well as the methodology required for a reliable assessment of FF G-CSF.

References
Learning objectives

- Understand the problems in oocyte quality assessment.
- Know the markers used to measure oocyte quality in studies utilizing ovarian stimulation.
- Know how to interpret outcomes of studies assessing effects of ovarian stimulation on oocyte quality, in consideration of the robustness of the markers.
- Recognize the most reliable markers of oocyte quality for use in clinical practice.
The assessment of individual oocyte quality and competence provides important information; oocyte quality is the main factor limiting success rates in ART.

Emerging techniques for defining oocyte quality include those based on cumulus cell analysis of transcriptomic profile or proteomic analysis of individual follicular fluids.

Quantification of G-CSF in follicular fluids together with embryo morphology assessment appeared to be a more discriminant measure of oocyte quality than either procedure used alone.

Studies have estimated that the use of follicular fluid G-CSF monitoring together with morphological assessment could enhance pregnancy rates from first transfer by 10% and accurately predict all cases at a high risk for a multiple pregnancy.
Dr Carlos Simón
Instituto Valenciano de Infertilidad (IVI), Prince Felipe Research Center (CIPF), Valencia, Spain

Dr Carlos Simón is a board-certified and full Professor of Obstetrics and Gynecology at the University of Valencia, Valencia, Spain, and Scientific Director of both the Fundación IVI and the Prince Felipe Research Center, Valencia.

Since 1991, Dr Simón’s basic and clinical research has contributed to the advance of reproductive medicine, specifically in the understanding of human endometrial receptivity, embryo viability, embryonic implantation and endometriosis. Since 2001, Dr Simón has expanded his research into the field of stem cells, resulting in the derivation, characterization and registration in the Spanish National Stem Cell Bank (BNLC) of 10 human embryonic stem cell lines.

As Principal Investigator, Dr Simón’s work has been funded through 10 projects sponsored by the Spanish Government, five by the Valencian Government, including a PROMETEO (granted to prestigious scientists), and 14 projects by international organizations, American universities and private companies. He has been the Director for 17 doctorates, all of whom qualified with cum laude, including four PhD awards of excellence and one European PhD. As inventor, his research has originated 11 patent applications, leading to the creation of three Biotechnology companies (iGenomix, Embryomics and Stemlifeline).

Dr Simón has published a total of 264 papers in international peer-review journals and is an Editor of 14 books.
The endometrium is a hormonally regulated organ that is non-adhesive to embryos throughout most of the menstrual cycle in humans and other mammals. Endometrial receptivity refers to a hormone-limited period in which the endometrial tissue acquires a functional and transient ovarian steroid-dependent status allowing blastocyst adhesion. Functional genomic studies of the human endometrium in natural cycles have demonstrated that endometrial receptivity is an active process involving up- and down-regulation of hundreds of genes.\textsuperscript{1,2} Since the refractory endometrium represents the opposite part of the spectrum, researchers have investigated the gene expression profile in conditions that will render a receptive endometrium non-refractory, such as that induced by the presence of an inert IUD.\textsuperscript{3} The gene expression pattern of endometrium in COS has been addressed specifically at the time of implantation at day LH+7.\textsuperscript{4}

To increase further our understanding of the pathways governing endometrial receptivity, we have expanded these studies by comparing the gene expression profile of the human endometrium throughout the early-mid secretory phase in natural cycles. We have analysed endometrial samples collected from healthy fertile cycling ovum donors (aged 23–39 years) who underwent natural cycles (n=25) at days LH+1, LH+3, LH+5, LH+7 and LH+9 (n=5 per timepoint); no progesterone supplementation was administered. RNA was extracted and labelled cDNA was hybridized onto the GeneChip HG_U133A (Affymetrix) for comparisons.\textsuperscript{5}

Based on these previous results, we have created a genomic tool named endometrial receptivity array (ERA), which comprises a customized microarray, and a bioinformatic predictor for endometrial dating and to detect pathologies of endometrial origin. The ERA included 238 selected genes and the transcriptomic signature was defined by 134 genes. The predictor showed a specificity of 0.8857 and a sensitivity of 0.99758 for endometrial dating, and a specificity of 0.1571 and a sensitivity of 0.995 for pathological classification.\textsuperscript{6} This diagnostic tool can be used clinically in reproductive medicine and gynaecology. The transcriptomic signature is a potential endometrial receptivity biomarkers cluster.

References
Learning objectives

- Understand the differences in endometrial receptivity between natural and COS cycles.
- Be aware of the utility of endometrial monitoring tests in optimizing embryo implantation following COS.

Key learning messages

- A full understanding of defects in endometrial receptivity in infertile women is an important consideration when considering COS.
- Endometrial receptivity is an active process involving up- and down-regulation of hundreds of genes.
- The endometrial receptivity array is a genomic tool that comprises a customized microarray and a bioinformatic predictor for endometrial dating, and can be used to detect endometrial pathologies.
Professor Renato Fanchin
ClamART, Center for Reproductive Medicine, Hospital Antoine Béclère, Clamart, France

Professor Renato Fanchin is Chief of the Division of Reproductive Medicine, Department of Gynaecology and Obstetrics, Antoine Béclère Hospital, Clamart, France. He specialized in Obstetrics and Gynaecology and Reproductive Medicine in France, and obtained his PhD from University of Paris-Sud 11 in 2005.

Professor Fanchin has been invited to speak at over 110 international meetings, is a full member of the Society for Gynaecologic Investigation, and was awarded the Society for Assisted Reproductive Technology Prize Paper by the ASRM in 1999. He is an active member of the INSERM unit 782. His current research interests include the assessment of ovarian follicular status and folliculogenesis.

Professor Fanchin, along with his colleagues, has published over 150 peer-reviewed articles in international journals and books.
Although the regulatory mechanisms determining the extent of FSH sensitivity of individual antral follicles remain to be elucidated, adequate responsiveness to FSH is characteristic of healthy and differentiated granulosa cells.\textsuperscript{1,2} Indeed, granulosa cells displaying a proper reactivity to FSH are not only endowed with functional FSH receptors, but are also able to execute properly a cascade of specialized tasks, such as signal transduction, steroidogenesis, and cell proliferation and differentiation. This physiological context suggests that responsiveness to FSH of antral follicles may constitute a marker of their health and reproductive competence, and it leads us to hypothesize that patients endowed with a large proportion of FSH-responsive antral follicles should be prone to become pregnant after ART.

Conclusive evidence of such a relationship, however, is still lacking. Some investigators have attempted to address this issue by merely analysing the strength of ovarian response to COS.\textsuperscript{3-6} Unfortunately, the number of pre-ovulatory follicles obtained at the end of COS is not a reliable reflection of antral follicle sensitivity to FSH, as it is greatly influenced by the number of small antral follicles available before treatment. This contingency constitutes a possible explanation for the inconstant relationship between the absolute counting of growing follicles obtained in COS and IVF-ET outcome.

In an effort to evaluate objectively antral follicle responsiveness to exogenous FSH, we started a series of investigations based on the use of Follicular Output RaTe (FORT). This innovative index is assessed by the ratio between the number of preovulatory follicles obtained in response to FSH administration and the pre-existing pool of small antral follicles; by design, it is not influenced by the number of small antral follicles before FSH treatment.

Our presentation will focus on parameters that influence ovarian response to COS and the contribution of FORT to understanding ovarian physiology and follicle responsiveness to FSH as well as its relationship with AMH, FSH-R polymorphisms and reproductive competence of follicles.

References
Learning objectives

- Understand the reasons why using an algorithm to predict ovarian response can be useful.
- Get an overview of results from studies testing suggested means of ovarian response prediction in patients.

Key learning messages

- Measurements of ovarian response to stimulation provide important information to allow for individualization of IVF cycles.
- Good antral follicle FSH sensitivity may be a marker of reproductive competence and thus potentially indicative of pregnancy chances following ART.
- The number of preovulatory follicles obtained following COS is not a reliable reflection of antral follicle sensitivity to FSH as it is influenced by the number of small antral follicles available before treatment.
- Further information can be provided by assessment of the Follicular Output RaTe (FORT), which objectively evaluates antral follicle responsiveness to exogenous FSH by using the ratio between the number of preovulatory follicles obtained in response to FSH administration and the preexisting pool of small antral follicles.
Dr Carlo Alviggi

Department of Obstetrics and Gynaecology, IVF Unit, University of Napoli “Federico II”, Napoli, Italy

Dr Carlo Alviggi works as Specialist in Reproductive Medicine at the Fertility Unit of the University of Naples “Federico II”, Naples, Italy. Since 2006, he has been working as Assistant Professor in the same unit.

His current research interests are the role of LH in folliculogenesis, the use of LH-containing drugs in patients undergoing COS for IVF, the pathogenesis of pelvic endometriosis, and the genetics of human reproduction.

Dr Alviggi has published extensively and has been invited to lecture at over 40 international meetings dealing with reproductive medicine and gynaecological endocrinology. He has also served as ad hoc reviewer for international journals of these fields and has participated in several national and international (Phase II/III) multicentre, prospective, randomized trials.
L14: Treatment individualization

Carlo Alviggi and Pasquale De Rosa

Despite the introduction of new drugs enabling the development of a variety of stimulation protocols, many patients worldwide currently receive identical treatment. Predictive biomarkers could be used to facilitate treatment decisions and to tailor therapy to increase the chances of achieving pregnancy while reducing stimulation burden and cancellation rates as well as treatment-related complications such as multiple pregnancies and OHSS.

A number of predictive variables for ovarian response have been identified, including hormonal, functional and genetic biomarkers. Among the hormonal biomarkers, AMH has been shown to have the highest predictive value. AMH is produced by the granulosa cells of early developing follicles and plays a crucial role in regulating the progression of smaller pre-antral follicles. It also modulates the activity of FSH in antral follicles during the FSH-dependent growth stage.

AFC is a well-known functional biomarker that is used to predict ovarian response to stimulation. It is also an important factor in determining the optimal starting dose of FSH for ART. However, the use of AFC could be limited by the variability in technical methods used to count and measure antral follicles.

Although hormonal and functional biomarkers are useful tools for predicting ovarian response, genetic factors also need to be taken into consideration. For example, a subgroup of patients with a hypo-response to recombinant FSH has recently been identified, comprising young, normogonadotrophic women with normal AFC and good prognosis. Such patients have an apparent hypo-sensitivity to FSH. This results in a need for longer stimulation periods and higher total doses of FSH, and leads to poor treatment response and low pregnancy rates. The pathogenesis of this phenomenon is still unknown. Preliminary data have shown that woman with ovarian hypo-sensitivity to exogenous FSH may benefit from LH supplementation. In addition, it has been found that hypo-response to FSH is associated with an increased frequency of a common and less bioactive LH polymorphism (v-LH [Trp8Arg/Ile15/Thr]).

A polymorphic variant of the FSH receptor (FSH-R) in which Asn at position 680 is replaced by Ser has been associated with higher FSH basal levels and increased number of antral follicles during the early follicular phase. Recent studies have also proven that this common polymorphism is associated with a higher consumption of exogenous FSH during ovarian stimulation for IVF/ICSI cycles.

These lines of research reinforce the hypothesis that ovarian resistance (hypo-response) to exogenous FSH can be related to specific gene polymorphisms. In addition, these data support the idea of a tailored administration of gonadotrophins based on a pharmacogenomic approach.

The future will, potentially, hold a combination of hormonal, functional and genetic biomarkers that will be utilized to define ovarian reserve, and tailored COS protocols that will provide the right treatment for the right patient with the highest safety and efficacy outcomes.
Learning objectives

- Understand the role of supplemental gonadotrophins in ART protocols.
- Understand and identify future areas for research that may help to tailor existing treatment regimens.

How can we predict ovarian response?

- **Age**
- **Biomarkers**
  - Hormonal Biomarkers: FSH, Inhibin B, AMH
  - Functional Biomarkers: Antral Follicle Count (AFC)
  - Genetic Biomarkers: Single Nucleotide Polymorphisms for FSH-R/LH-R/AMH-R

Patients are the main variable associated with response to OS

The patient individual factors of response to stimulation are:
- Demographics and anthropometrics (Age, BMI, Race)
- Health status
- Cause of Infertility
- Years of infertility
- Nutrition

What we really need to know is, how to define the right individual treatment for the right patient to reduce cancellations due to OHSS and Poor response, while increasing the chances of achieving pregnancy.

AMH based strategies in ART

Individualized treatments: relevance of FSH dose

- Selecting the optimal dose of r-hMG to achieve an acceptable number of oocytes in women who have already failed to respond to baseline gonadotropin stimulation has been proposed.
- Individualized dosing is essential in women of advanced maternal age to avoid the use of the CONCEPT dosing algorithm. This is particularly important for women who have already failed to respond to baseline gonadotropin stimulation and where a continuous FSH dose does not achieve clinical pregnancy.

Clinical pregnancy rates/number started

- 11.1% (79 FSH)
- 11.7% (100 FSH)
- 16.3% (150 FSH)
- 20.0% (250 FSH)
- 30.0% (350 FSH)
- 30.0% (400 FSH)
The individualization of stimulation protocols to increase the chances of pregnancy is not yet common practice.

A range of variables have been identified (hormonal, functional and genetic) that allow for the prediction of ovarian response.

- These give important information to allow treatments to be tailored to increase pregnancy rates while reducing stimulation burden, cancellation rates and treatment-related complications.

- Of the hormonal biomarkers, AMH has the highest value in predicting outcomes.

- The functional biomarker of AFC can be used to predict response to ovarian stimulation and for determining the optimal starting dose of FSH for ART.

- Genetic factors must also be considered; it has been shown that hyposensitivity to FSH or LH can be related to specific gene polymorphisms of their receptors.

Key learning messages

- The individualization of stimulation protocols to increase the chances of pregnancy is not yet common practice.

- A range of variables have been identified (hormonal, functional and genetic) that allow for the prediction of ovarian response.

- The future must hold a combination between hormonal, functional and genetic biomarkers to secure the right treatment for the right patient.

- Why?
  - Normal SNP profile for FSH-R/LH-R/H gene with low AMH/AFC, no dose will compensate
  - Bad FSH profile for FSH-R with normal AMH/AFC, increase the dose of FSH
  - Bad SNP profile for FSH-R/LH-R with normal AMH/AFC, increase dose of FSH and avoid LH
Dr Mona Bungum
Reproductive Medicine Centre (RMC), Skåne University Hospital, Malmö, Sweden

Dr Mona Bungum is Laboratory Director at the Reproductive Medicine Centre of Skåne University Hospital, Malmö, Sweden.

Dr Bungum studied laboratory medicine and embryology in Norway and the UK, and in 2008, defended her PhD thesis on the issue of sperm DNA integrity testing at Lunds University, Malmö, Sweden.

During her career, Dr Bungum has set up IVF laboratories in Norway, Denmark and Sweden.
L15: Selecting the best sperm
Mona Bungum

The selection of a normal, vital spermatozoon is a prerequisite for achieving fertilization and embryo development both in vivo and in vitro. Any spermatozoon contributing to IVF has to perform almost perfectly and overcome a series of hindrances imposed by the female reproductive tract. This implies that during formation and maturation, the spermatozoa should not incur any morphological, metabolic, immunological or genetic abnormalities. Unfortunately, existing laboratory tests of semen quality cannot approach the efficacy of the female reproductive tract and predict the odds of spermatozoa entering the oocyte.

Although the traditional parameters for semen analysis (concentration, motility and morphology) recommended by the WHO give considerable information and are a ‘gold standard’ in the management of male infertility, it has become apparent that none of these parameters is sufficient for the determination of male fertility capacity or identification of the ‘perfect sperm’. The WHO parameters address only a few aspects of sperm quality and function, and thus the discriminative power in relation to fertility in vivo as well as in vitro is quite low.

During the last decade, assessment of sperm DNA integrity has emerged as a new biomarker of semen quality that may help in the discrimination between infertile and fertile men, and in predicting pregnancy outcome in ART. The sperm chromatin structure assay (SCSA) parameter DNA fragmentation index (DFI) has been shown to be an independent predictor of success in first pregnancies and in couples undergoing IUI. More contrasting data exist regarding the role of sperm DNA fragmentation in relation to fertilization, pre-embryo development and pregnancy outcome in IVF and ICSI.

ART is used increasingly, but success rates remain suboptimal. Possible explanations to this may be the lack of methods to identify the most effective ART treatment in a given couple or the lack of methods to allow the selection of viable spermatozoa exhibiting low levels of DNA damage for ART.

Similar to the improvement in methods of sperm analysis, new and promising methods of sperm sorting and sperm selection for ICSI have been suggested. A variety of sperm preparation methods have been shown to reduce the number of sperm with DNA fragmentation. However, to date, no reliable approach to completely isolate sperm with normal DNA integrity has been developed. Recently, techniques such as intracytoplasmic morphologically selected sperm injection (IMSI) using high magnification microscopy and different commercially available devices to select normal sperm for ICSI have been introduced. However, evidence of improved fertilization and pregnancy rates is not yet apparent.
Learning objectives
- Understand the relative importance of the different sperm assessment techniques and how to use these in daily practice.
- Be aware of the key findings of studies evaluating newer sperm assessment techniques.

Diagnosis of male infertility

- Conventional sperm analysis
  - Criteria for sperm concentration, motility and morphology (WHO, 2010)
  - Subjective (Auper et al., 2000)
  - Poorly standardized (Jorgensen et al., 1997)
  - Not powerful predictors of fertility (Bondy et al., 1998; Gauzzi et al., 2001)

<table>
<thead>
<tr>
<th>Fertile range</th>
<th>Intermediate range</th>
<th>Subfertile range</th>
</tr>
</thead>
<tbody>
<tr>
<td>sperm conc. (x10^9/ml)</td>
<td>&gt;40</td>
<td>12.5-40</td>
</tr>
<tr>
<td>sperm motility (%)</td>
<td>&gt;63</td>
<td>32-63</td>
</tr>
<tr>
<td>sperm morphol. (%)</td>
<td>&gt;12</td>
<td>9-12</td>
</tr>
</tbody>
</table>

From Gauzzi et al., N Engl 2001

Additional tests used
- Capacitation tests
- Zona-free hamster penetration assay
- Membrane integrity tests
- Antisperm antibodies
- Vital staining
- Biochemical analysis
- Peroxidase staining

Most assays only test one single sperm attribute

Lack of methods
- To find the underlying causes of male infertility/subfertility
- To evaluate male (and couple) fertility potential
- To identify the most effective ART treatment in a given couple
- To identify the ‘perfect sperm’

Sperm DNA integrity methods
- SCSA (Sperm Chromatin Structure Assay) (Fentem et al., 1992)
- TUNEL assay (apoptotic DNA fragmentation: transferase dUTP Nick End Labeling) (Gupta et al., 1993)
- Comet assay (single cell gel electrophoresis)
- Alkaline (pH 13.0): Singh et al., 1988
  - Neutral (pH 8.5): Singh & Mistry, 1998
- Sperm Chromatin Disruption (SCD) (Fentem et al., 1992)

Correlate only to a certain degree (r=0.1-0.3)
Although the WHO has defined conventional semen parameters that are considered the gold standard in male fertility management, these parameters are insufficient for the determination of male fertility capacity or identification of the 'perfect sperm'.

New biomarkers of semen quality have emerged, including sperm DNA integrity testing using the SCAS parameter DFI, which has been shown to be an independent predictor of success in first pregnancy planners and in IUI.

More research is needed to determine the role of the new biomarkers for predicting pre-embryo development and pregnancy outcome in IVF.

New and promising methods of sperm sorting and sperm selection are available

- IMSI has been developed to select normal sperm for ICSI.
Dr William Blaine Schoolcraft
Colorado Center for Reproductive Medicine, Lone Tree, Colorado, USA

Dr William Schoolcraft is the founder and Medical Director of the Colorado Center for Reproductive Medicine, Lone Tree, CO, USA. He completed his medical training at the University of Kansas and finished his residency in Obstetrics and Gynecology in 1983 at the UCLA School of Medicine in Los Angeles, CA, USA. In 1983, he began a private practice in the field of obstetrics, gynaecology and infertility in Denver.

Dr Schoolcraft is an award-winning researcher and has authored *If at First You Don’t Conceive: A Complete Guide to Infertility from One of the Nation’s Leading Clinics* and numerous articles on infertility.
Embryo selection is a critical component of ART. Current selection methods are based on detailed embryo morphology. Although relatively successful, morphology has limitations, with more than 70% of embryos produced in vitro arresting in culture or failing to implant. ART would benefit from a non-invasive quantitative method of embryo assessment.

Proteomic technologies performed in our laboratory have already begun providing evidence that developmentally competent embryos possess unique protein profiles that could be utilized for selection purposes. Knowledge of the human embryonic proteome is very limited, due primarily to the restricted template, low protein concentration and deficient platform sensitivity. The protein secretome is, therefore, of particular interest in ART and is defined as those proteins that are produced by the embryo and secreted at any given time into the surrounding environment [microdrop of culture media]; the embryonic protein secretome is potentially transient and dynamic. To date, investigation of the embryonic protein secretome has been challenging, but recent developments in platform sensitivity of mass spectrometry and protein microarrays hold great promise.

Characterizing the embryonic protein secretome has the potential to increase our knowledge of the biological processes involved in human embryonic development. Further focus correlating the embryonic protein secretome with implantation potential could lead to the development of a non-invasive quantitative assessment for embryo viability. The ability to select the most viable embryo in a cohort could result in improved IVF outcomes and will allow for routine single embryo transfer, while maintaining or improving pregnancy rates.
Learning objectives

- Understand the potential role of proteomics as a tool to assess embryo viability.
- Be aware of the potential markers of embryo viability identified by proteomic analysis of culture medium.
The assessment of morphology alone cannot reliably predict the viability of an embryo. Proteomic technologies are providing evidence that developmentally competent embryos possess unique protein profiles that could be utilized for assessment purposes. The embryonic protein secretome is defined as those proteins produced by the embryo and secreted into the surrounding environment. It can be studied non-invasively using mass spectrometry and protein microarrays. Secretome studies will assist in characterizing the dialogue between the developing embryo and its maternal environment. Further studies correlating the embryonic protein secretome with implantation potential could lead to the development of a non-invasive quantitative assessment for embryo viability.
Dr Santiago Munné is the Founder and Director of Reprogenetics. Originally from Barcelona, Spain, he gained his PhD in Genetics from the University of Pittsburgh, PA, USA, and in 1991 joined Cornell University Medical College, New York, NY, USA. There he developed the first preimplantation genetic diagnosis (PGD) test to detect embryonic numerical chromosome abnormalities to avoid Down’s syndrome and other abnormalities. This and his following work were recognized with two consecutive prizes in 1994 and 1995 from the Society for Assisted Reproductive Technology (SART).

In 1995, Dr Munné became Director of PGD at the Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center, Livingstone, NJ, USA. Here he developed the first test to detect chromosome translocations in human embryos. For this work he obtained the General Program Prize of the ASRM (1996). Following on his research on PGD of aneuploidy, his group also demonstrated a significant decrease in spontaneous abortions after PGD in women aged ≥35 years undergoing IVF and PGD, which was again recognized in 1998 with the Prize Paper of SART. In the 2005 ASRM meeting, Dr Munné’s team also obtained the Prize Paper for their work on obtaining chromosomally normal stem cells from trisomic embryos.

Dr Munné’s research activity is focused on developing new PGD techniques, understanding the impact of chromosome abnormalities in human reproduction, and the production and study of genetically defined stem cells.

Dr Munné has authored over 200 publications and is a frequent lecturer, both nationally and internationally, on his team’s work, and in the field of preimplantation genetics and genetic abnormalities in embryos. He is also a member of the Board of Directors of the Preimplantation Genetic Diagnosis International Society (PGDIS) and was the President of the organizing committee for 2008.
In the last few years, the screening of embryos for chromosome abnormalities and gene defects, known as PGD, has been invigorated by the introduction of microarray-based testing methods. These methods allow for the simultaneous analysis of all 24 chromosome types, as well as carrier status chips to detect hundreds of mutations. Preliminary data have shown an increase in implantation rates and pregnancy rates after microarray-based testing.

Several methods are used currently to analyse the whole genome of single cells for genetic diagnosis, these being array Comparative Genome Hybridization (aCGH), quantitative PCR and SNP microarrays. The first two analyse directly for all chromosome aneuploidies. SNP arrays allow for more information and the potential for insight into the parental origin of aneuploidy and uniparental disomy, as well as gene defects.

Each technique, however, has pros and cons. For chromosome abnormalities, aCGH has provided the lowest error rates with embryos biopsied on Day 3. The usefulness of SNP arrays for detecting the origin of the aneuploidy is questionable when 50% of abnormalities are post-meiotic in origin (not originated by any parent) and 90% of meiotic abnormalities are maternal. Similarly, the simultaneous analysis of gene defects and chromosome abnormalities is not without some trade off. For example, the DNA amplification used for aCGH cannot amplify linked markers, so direct analysis of a mutation may have a high error rate due to undetected allele dropout. However, SNP array data at the single cell level are too noisy to read mutations. Haplotyping is, therefore, necessary, but this requires an affected child to have been identified previously and this is not always possible.

In the near future, sequencing may provide a better way to provide direct mutation analysis plus linked marker analysis simultaneously with chromosome analysis.
Learning objectives

- Understand the role that genomics play in embryo selection.
- Understand the advantages and disadvantages that the techniques listed have for embryo selection.

<table>
<thead>
<tr>
<th># of probes</th>
<th>probe size</th>
<th>genome covered</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCGH 4,000</td>
<td>150,000 kb</td>
<td>600.0 Mb (25%)</td>
</tr>
<tr>
<td>SNPs 300,000</td>
<td>50 kb</td>
<td>1.5 Mb (&gt;0.1%)</td>
</tr>
</tbody>
</table>

The Majority of Embryos with ‘Good’ Morphology are Chromosomally Abnormal

Array CGH Validation Reanalysis by FISH

Prognosis by Age and Ovarian Response
The use of microarray-based testing for PGD diagnosis has increased implantation and pregnancy rates. The methods employed include those that directly detect all chromosome aneuploidies, such as array comparative genome hybridization and quantitative PCR. SNP microarrays can be used to detect gene defects and can give information about the parental origin of aneuploidy and uniparental disomy. Gene sequencing may enhance the analysis of mutations, linked markers and chromosomes in the future.
Disclosure of faculty relationships

Serono Symposia International Foundation adheres to guidelines of the European Accreditation Council for Continuing Medical Education (EACCME) and all other professional organizations, as applicable, which state that programs awarding continuing education credits must be balanced, independent, objective, and scientifically rigorous. Investigative and other uses for pharmaceutical agents, medical devices, and other products (other than those uses indicated in approved product labeling/package insert for the product) may be presented in the program (which may reflect clinical experience, the professional literature or other clinical sources known to the presenter). We ask all presenters to provide participants with information about relationships with pharmaceutical or medical equipment companies that may have relevance to their lectures. This policy is not intended to exclude faculty who have relationships with such companies; it is only intended to inform participants of any potential conflicts so participants may form their own judgments, based on full disclosure of the facts. Further, all opinions and recommendations presented during the program and all program-related materials neither imply an endorsement, nor a recommendation, on the part of Serono Symposia International Foundation. All presentations solely represent the independent views of the presenters/authors.

The following faculty provided information regarding significant commercial relationships and/or discussions of investigational or non-EMEA/FDA approved (off-label) uses of drugs:

- **Michael M. Alper** Declared being a member of EMD Serono Advisory Board
- **Carlo Alviggi** Declared no potential conflict of interest
- **Jean-Daniel Baki** Declared no potential conflict of interest
- **Ernesto Bosch** Declared honoraria or consultation fees from Merck Serono and Ferring Pharmaceuticals
- **Frank J. Broekmans** Declared scientific grant from Dutch Government, honoraria or consultation fees from Merck Serono and a member of the board for Ferring
- **Mona Bungum** Declared no potential conflict of interest
- **Renato Fanchin** Declared no potential conflict of interest
- **Alberto Ferlin** Declared no potential conflict of interest
- **Robert Fischer** Declared honoraria or consultation fees from and member of SSIF Scientific Committee
- **Jean-Noël Hugues** Declared honoraria or consultation fees from Merck Serono
- **Nathalie Lédée** Declared grants and contracts from Merck Serono
- **Yves Ménézo** Declared honoraria or consultation fees from Unilabs, Nurilla and stakeholder in Medicult
- **Santiago Munné** Declared stakeholder in Reprogenetics
- **Scott M. Nelson** Declared no potential conflict of interest
- **Renato Pasquali** Declared no potential conflict of interest
- **Pasquale Patrizio** Declared participation in a Speaker’s Bureau for EMD Serono
- **William Blaine Schoolcraft** Declared member of a board of other similar group for Merck Serono
- **Carlos Simón** Declared no potential conflict of interest
- **Manuela Simoni** Declared grants and contracts and honoraria or consultation fees from Merck Serono
The following faculty has provided no information regarding significant relationship with commercial supporters and/or discussion of investigational or non-EMEA/FDA approved (off-label) uses of drugs as of October 29, 2010

Bruno Salle