Ovarian stimulation and consequences for oocyte/embryo quality

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Presentation outline

• Follikel development and oocyte quality
• Embryo quality assessment
  • Morphology and development
  • Chromosomal constitution
• Effect of ovarian stimulation approaches
  • GnRH agonist versus GnRH Antagonist
  • Recombinant FSH versus hMG
• New techniques in embryo quality assessment

What is a good oocyte/embryo?

• Competent to undergo fertilization
  • Chromatin remodeling
  • DNA repair
• Supports timely completion of cleavage divisions
• Reliably segregates chromosomes
  • Spindle formation
  • Checkpoint functions
• Activates the embryonic genome (8 cell stage)
  • Chromatin remodeling
  • Establishment of genomic imprinting
How to assess embryo quality?
- The classical approach -

- Implantation potential, ongoing PR and live birth
- Morphology and development:
  - Assessment of pronucleate embryos
  - Timing of cleavage
  - Assessment on day 3 after fertilization
  - Development to the blastocyst stage

The perfect embryo
(based on morphology and development)

Successful implantation after SET in 49% of patients ≤36 yrs

At least 50% of embryos are chromosomally abnormal

Day 3: cleavage stage and chromosome abnormalities

- 662 patients, 916 cycles
- Poor prognosis patients
- PGS on day 3
- XY, 13, 14, 15, 16, 18, 21, 22
- Cleavage stage assessment

Papanikolaou et al., NEJM, 2006

Magli et al., Fertil Steril, 2007
Day 3: fragmentation and cell number

Development to the blastocyst stage and chromosomal abnormalities

- 148 patients, 148 cycles
- patients ≥37 years
- IVF and ICSI
- PGS on day 3, two cells
- XY, 13, 16, 18, 21, 22
- Assessment of blastocyst development

Staessen et al., Hum Reprod, 2004

FISH diagnosis on day 3 and development on day 5
Randomized comparison of two ovarian stimulation approaches

- Determine the incidence of aneuploidy and mosaicism in embryos from younger IVF patients
- Can PGS be used as an extra parameter to assess embryo quality?

Comparison of stimulation approaches

Randomization of 111 patients:

- Conventional
  - FSH 150 IE/day
  - Ultrasound
  - rFSH 150 IE/day
  - Antagonist
  - Ultrasound
  - 2 wks

- Mild
  - Agonist
  - rFSH 225 IE/day
  - OPU
  - ET
  - ET

Preimplantation genetic screening

- Fertilization
- Day 3: embryo biopsy
- FISH analysis
- Diagnosis on one or two blastomeres
- Transfer of chromosomally normal embryos
Fixation and analysis of blastomeres

Method using HCl/Tween and Methanol/Acetic acid

First round of FISH: chromosomes 1, 7, 15, X & Y

Second round of FISH: chromosomes 13, 16, 18, 21, 22

Baart et al., Hum Reprod, 2004

PGS Diagnosis in young IVF patients

Analysis of 265 embryos:

Diagnosis based on: one blastomere two blastomeres

PGS Diagnosis for statistical analysis

Analysis of 265 embryos:

Diagnosis based on: first blastomere biopsied
Lower aneuploidy rate after mild stimulation

<table>
<thead>
<tr>
<th>% abnormal embryos / diagnosed</th>
<th>Stimulation protocol</th>
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<tbody>
<tr>
<td>60%</td>
<td>Conventional</td>
</tr>
<tr>
<td>40%</td>
<td>Mild</td>
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<tr>
<td>30%</td>
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N = 33 40  
P = 0.016

Good quality embryo rate per patient: 35% 51%  
P = 0.04

Average number per patient

What could it mean to the embryologist?

Conventional ovarian stimulation:

Mild ovarian stimulation:
What could it mean to the embryologist?

Conventional ovarian stimulation:

Mild ovarian stimulation:

Chromosomal mosaicism after analysis of two cells

Conventional stimulation (98 embryos)

Mild stimulation (96 embryos)

Rate of mosaic embryos per patient:

65% 37% P= 0.004

Effect of LH/hCG containing gonadotropins

- Retrospective analysis
- recFSH vs. hMG
- Long agonist protocol
- PGS analysis on day 3
- Reduction in the number of oocytes
- Similar no. of normal embryos (3.1 vs. 3.3)

Weghofer et al., Hum Reprod, 2008
PGS and embryo quality: conclusions

- Ovarian stimulation has an impact on the proportion of aneuploid/mosaic embryos
- PGS provides an additional marker of embryo quality
- PGS is invasive, costly and time consuming

New techniques in embryo quality assessment

- RNA purification
- DNA microarray
- Cytogenetics FISH/CGH
- Embryo biopsy
- Culture medium
- Follicular fluid
- Metabolomics/Proteomics
- Embryo biopsy
- Realtime PCR
- Genomics
- Zona pellucida
- Cumulus cells
- Culture medium

Freeze 'm all?

- Improvements in cryopreservation protocols
- Single embryo transfer in the natural cycle
- Sequential thawing and transfer of all embryos
- Timing of transfer?
Conclusions

- Assessment of chromosome constitution provides an additional marker for embryo quality
- Ovarian stimulation has an impact on the proportion of good quality embryos
- Ovarian stimulation should not aim at maximizing oocyte yield but at optimizing embryo quality
- Further improvements in embryo quality assessment are needed

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