POR: stimulation and oocyte quality

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Learning Objectives

- Definition of a poor ovarian response (POR)
- Identification of poor responders (PORs)
- Is oocyte quality affected in poor ovarian response and in poor responders?
- Stimulation strategies tested for PORs (review of the literature and SISMER strategy)

ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria

A.P. Ferraretti1, A. La Marca2, B.C. J.M. Fauser3, B. Tarlatzis4, G. Nargund5, L. Gianaroli6, and on behalf of the ESHRE working group on Poor Ovarian Response Definition

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Poor ovarian response to COH

- Poor responders women (recurrent POR)

When an ovarian response is poor?

- When patient is exposed to a significantly lower PR compared to women from whom "more" oocytes are retrieved

Poor ovarian response

- Cycle cancelled because of <3 growing follicles or
- Collection of ≤3 oocytes collected

...in response to conventional stimulations
Terminology of ovarian stimulation in ART (ISMAAR Consensus Group)

Conventional COH:
- down regulation with GnRH agonist
- GnRH antagonist
- flare-up
and conventional starting doses of FSH or HMG (150-400 IU/day) according to age

Mild IVF:
fixed low doses of FSH or HMG ± CC (and antagonist)

Incidence of Poor Ovarian Response in the first cycle according to age
(SISMER - 2847 patients – Conventional protocols)

Poor ovarian response

[Graphs and data]
Pregnancy rate per cycle in relation to the number of collected oocytes (6678 cycles)

Cut-off point of statistically significant difference

Poor responders in the first cycle entering a second cycle were stimulated with higher starting dose of Gn

Cumulative ongoing PR after 3 cycles
*Second cycle in previous POR*

- Patients with an intrinsic capability to recruit few follicles and to produce few eggs for their age

*Poor responders in the first cycle entering further cycles*

- Patients with risk of POR after two previous cycles

*Who are the poor ovarian responders?*

- Patients with an intrinsic capability to recruit few follicles and to produce few eggs for their age
Risk factors for POR

- Age
- Genetic factors related to premature ovarian ageing
- Previous ovarian surgery
- Chemotherapy
- Pelvic infection (?)

Risk factors for POR

- Altered Ovarian Reserve Tests (ORTs)

Accuracy of the three best ORTs

Accuracy is moderate to predict quantitative response and is low for the qualitative aspects (unless very high thresholds are used)

AFC and AMH have the best sensitivity and specificity.

Even the best ORT is associated with 20-25% false positive results

The use of more than one tests does not improve the performance given by a single test

Risk factors for POR

Two previous POR (with conventional and maximal stimulations)

ESHRE Consensus on POR definition

At least two of the following three features have to be present:

- Advanced maternal age (> 40yrs) or any other risk factor for POR
- One previous POR (≤ 3 oocytes with conventional stimulation protocol)
- An abnormal ORT (preferably AMH or AFC)

Two episodes of POR to maximal stimulation are sufficient to define a patient as poor responders in the absence of advanced maternal age or abnormal ORT

Estimation of Poor Responders

<table>
<thead>
<tr>
<th>Age</th>
<th>Incidence of PORs</th>
<th>Number of ART cycles/year in Europe (EIM data)</th>
<th>Number of PORs</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td>2%</td>
<td>~50,000</td>
<td>1,000</td>
</tr>
<tr>
<td>30-35</td>
<td>5%</td>
<td>~200,000</td>
<td>10,000</td>
</tr>
<tr>
<td>35-40</td>
<td>10%</td>
<td>~200,000</td>
<td>20,000</td>
</tr>
<tr>
<td>41-43</td>
<td>35%</td>
<td>~40,000</td>
<td>14,000</td>
</tr>
<tr>
<td>&gt; 43</td>
<td>60%</td>
<td>~10,000</td>
<td>6,000</td>
</tr>
<tr>
<td>Tot</td>
<td></td>
<td>500,000</td>
<td>~50,000</td>
</tr>
</tbody>
</table>
Life table (age ≤ 40 yrs)  
(Ongoing pregnancy rate)

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Oocyte quality in POR

The lower PR in POR is related to:
- the low number (limited possibility of performing embryo selection)
- a compromised viability of the oocyte itself
POR in old patients

- Low number of oocytes
- Poor implantation
- High frequency of aneuploidy

Implantation rate (as an index of oocyte quality) in women < 40 yrs

- March 2004 – April 2009
- Only 3 oocytes inseminated
- No embryo selection

An unique model to evaluate the degree of correlation between oocyte "QUANTITY AND QUALITY"
Implantation rate depending on the number of collected oocytes

- Insemination of 3 eggs
- Insemination of all eggs

Number of collected eggs

An unique model to evaluate the degree of correlation between oocyte "QUANTITY AND QUALITY"

Implantation rate depending on the number of collected oocytes

- Group A
- Group B

The oocyte number "per se" is not an index of oocyte quality.
A sub-optimal number of oocytes for insemination is crucial for in vitro embryo selection and transfer of most viable embryos.

- Oocytes of lower quality?
- Limited embryo selection?

A lower probability of term pregnancy in cycles with poor ovarian response compared seems to be more related to the reduced number of oocytes (limited possibility of performing embryo selection) than to their quality.

The persistent lower PR in young poor responders is related to the persistent low number of oocytes available or also to a compromise oocyte quality?
Life table (age ≤ 40 yrs)
(Ongoing pregnancy rate)

No morphological aspects are particularly relevant in oocytes generated from poor responder patients

Requirements for oocyte competence

Meiotic maturation
Cytoplasmatic maturation
Epigenetic maturation
FIRST POLAR BODY FISH ANALYSIS
INCIDENCE OF ANEUPLOIDY IN RELATION TO THE NUMBER OF RETRIEVED OOCYTES

<table>
<thead>
<tr>
<th>N. retrieved oocytes</th>
<th>Aneuploidy %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td></td>
</tr>
<tr>
<td>4-6</td>
<td></td>
</tr>
<tr>
<td>7-10</td>
<td></td>
</tr>
<tr>
<td>11-14</td>
<td></td>
</tr>
<tr>
<td>≥15</td>
<td></td>
</tr>
</tbody>
</table>

Techniques available to test oocyte competence in research projects, but no oocytes available from PORs for research.

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How to improve the probability of pregnancy in poor responders: systematic review and meta-analysis of stimulation protocols (Kyrou et al., Fert Steril 2009).

- Short vs long agonist protocol
- Agonist (long and short protocols) vs antagonist
- rFSH vs uFSH
- CC vs rFSH
- Addition of:
  - LH/HCG
  - GH or GHRF
  - Pyridostigmine
  - L-arginine
  - Testosterone
  - Letrozole
- Natural cycles

Interventions for poor responders to COH in IVF

COCHRANE review (Pandian et al 2010)

- Various Agonist protocols (flare-up, down-regulation, step protocol, daily vs depot, mini-dose long protocol)
- Antagonist protocol
- Gonadotrophins (urinary vs recombinant) alone
- GT with clomiphene

The search strategy identified 295 studies, but only 18 met the basic inclusion criteria. Of those, after further evaluation, only 10 were included for the final analysis.

LBR was considered the primary end point: only one trial reported LBR.

Authors' CONCLUSIONS

- With the exception of GH co-administration (six studies, five of which performed before 1996), none of the examined approaches appears to be beneficial.
- The management of poor responders still represents a challenge for the clinician, which is further complicated by the variations in the definition of poor ovarian response.
- Evaluation of the interventions proposed is usually performed in single, underpowered studies, which might not allow the detection of the true effect of an intervention.
- An internationally accepted definition is needed, which should be universally used in future trials so as to compare results and relevant interventions in in vitro fertilization.
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An internationally accepted definition is needed, which should be universally used in future trials so as to compare results and relevant interventions in in vitro fertilization.

Addition of LH to FSH in POR

Day 1

R-LH (75 IU)

FSH-HP

Day 6

Several trials

Addition of LH to FSH in POR

Day 6

No significant effect

New protocol for repeated (≥ 2 cycles) and young (≤ 40 yrs) poor ovarian responders

Down-regulation with daily low dose GnRH agonist, pre-treatment with rLH (150 IU/day for 4 days) before FSH stimulation (400 IU/day)

LH

FSH
Material and Methods

- Women < 40 years old who presented a poor ovarian response (cycles cancelled because of ≤3 growing follicles or retrieval of ≤3 oocytes) in at least two previous cycles using conventional stimulation in the first cycle and maximal stimulation in following cycles.

- Couples with severe male factors, altered karyotype, abnormal uterine cavity, and metabolic or autoimmune diseases excluded.

- New protocol tested in a prospective controlled study:
  - 400 IU of rFSH/day in down regulation protocol (half dose of daily Triptoreline) with LH pre-treatment (study group) and without LH pre-treatment (control group)
  - Study period: January 2004 - December 2008

Material: 43 patients with recurrent poor response in at least 2 previous cycles

<table>
<thead>
<tr>
<th>Previous cycles</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Started cycles</td>
<td>94</td>
</tr>
<tr>
<td>Cycles cancelled</td>
<td>34 (36%)</td>
</tr>
<tr>
<td>Eggs retrievals (mean eggs)</td>
<td>60 (2.1±1)</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>67%</td>
</tr>
<tr>
<td>Cleavage rate</td>
<td>89%</td>
</tr>
<tr>
<td>Grade 1 embryos</td>
<td>63%</td>
</tr>
<tr>
<td>Transferred cycles (mean embryos transferred)</td>
<td>56 (1.3±0.9)</td>
</tr>
<tr>
<td>Clinical pregnancies (PR/ cycle)</td>
<td>30(7%)</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>5.9% (4/72)</td>
</tr>
<tr>
<td>Miscarriages</td>
<td>3</td>
</tr>
<tr>
<td>Live birth rate/patient</td>
<td>0%</td>
</tr>
</tbody>
</table>

Study results

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>LH pre-treatment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>21</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>No. of cancelled cycles (%)</td>
<td>7 (33%)</td>
<td>5 (23%)</td>
<td>ns</td>
</tr>
<tr>
<td>No. of collected oocytes (mean)</td>
<td>2.5±1.2</td>
<td>3±1.6</td>
<td>ns</td>
</tr>
<tr>
<td>Range</td>
<td>0-7</td>
<td>1-15</td>
<td></td>
</tr>
<tr>
<td>No. of transferred cycles</td>
<td>13</td>
<td>27</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>No. of clinical pregnancies</td>
<td>1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Implantation rate</td>
<td>6 (50%)</td>
<td>29 (100%)</td>
<td>ns, 0.06</td>
</tr>
<tr>
<td>No. of early miscarriages</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Live birth rate/started cycle (%)</td>
<td>5% (1/20)</td>
<td>32% (7/22)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
Study conclusions

- The strategy proposed was never tested before in PORs.
- According to PORs ESHRE definition, homogeneous population of women was selected.
- The results are supporting that LH pre-treatment was able to offer a significantly higher LBR compared to control.
- The significant increase in LBR may be partially associated to the increased number of collected eggs. Although not significant, the increase from 2.4 to 3.5 oocytes/egg retrieval led to a higher number of transferred cycles.
- However, it is possible to speculate that LH pre-treatment also improved oocyte competence; in addition to a reduced cohort of follicles to be recruited by FSH, some poor responders may have border-line maturation defects at the early stages of oocyte development that could alter the imprinting and/or gene expression mechanisms.
- These defects, if not corrected when still reversible, could be amplified by the hormonal environment induced by FSH stimulation, leading to a drastic reduction in the generation of viable embryos. Exposure of small follicles to LH prior to FSH recruitment seems to adjust this process by improving the oocyte cytoplasmic maturation and, therefore, the future embryogenesis.

LH pre-treatment protocol in PORs (age ≤ 40 yrs) selected according to ESHRE definition (update to November 2011)

<table>
<thead>
<tr>
<th>Category</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>43</td>
</tr>
<tr>
<td>No. of cycles with POR</td>
<td>98</td>
</tr>
<tr>
<td>No. of cycles with LH pre-treatment</td>
<td>47</td>
</tr>
<tr>
<td>No. of cancelled cycles (%)</td>
<td>11 (23%)</td>
</tr>
<tr>
<td>No. of collected oocytes (mean)</td>
<td>3.5 ± 1.3</td>
</tr>
<tr>
<td>No. of transferred cycles (%) on started cycles</td>
<td>30</td>
</tr>
<tr>
<td>No. of clinical pregnancies</td>
<td>54</td>
</tr>
<tr>
<td>No. of early miscarriages</td>
<td>2</td>
</tr>
<tr>
<td>Ongoing PR / started cycle (%)</td>
<td>25%</td>
</tr>
</tbody>
</table>

Stimulation and oocyte quality in POR

- There is insufficient evidence to identify the use of any particular intervention to improve treatment outcomes in poor responders because of the heterogeneity in the definition of poor ovarian response. It is time to produce evidence-based medicine in the field for the management of these very difficult patients.
- The ESHRE definition of POR is simple and reproducible. If uniformly adapted as the “minimal” criteria needed to select patients for future trials, more homogeneous populations will be tested for any new protocols designed.
- No conclusive data are available on oocyte quality in PORs.
- Young PORs can still have reasonable prognosis of pregnancy for continuation of treatment.