Potential Benefit of Mild Stimulation Regimen on Embryo Quality in IVF-ICSI Cycles

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Abstract
Purpose: To assess the potential benefit of mild stimulation regimen on embryo quality in a specific subgroup of women who had previously experienced poor embryo quality after conventional regimen.

Methods: In this study performed in a single University Hospital, patients with at least 2 consecutive In Vitro Fertilisation (IVF) or Intra Cytoplasmic Sperm Injection (ICSI) attempts leading to poor embryo quality following conventional regimens were enrolled. The main purpose was to compare the absolute and relative number of good and top quality embryos following mild stimulation and conventional regimen.

Results: Mild stimulation regimen allowed the retrieval of a lower number of oocytes as compared to conventional protocol but the mean number and the percentage of good and top quality embryos were significantly increased in patients receiving the mild stimulation regimen.

Conclusion: In a specific subgroup of women with poor embryo quality following conventional IVF-ICSI protocols, a mild stimulation regimen seems to improve embryo quality.

Keywords: Mild stimulation; GnRH antagonist; Embryo quality; IVF-ICSI

Introduction
Ovarian stimulation is actually one of the major determining factors for a successful outcome of in vitro fertilisation (IVF) techniques. However, the optimal stimulation regimen is not firmly established. Indeed, it is still uncertain whether conventional regimen is the most effective approach for getting high quality embryos. Indeed, the potential negative impact of gonadotropin therapy has been reported in in-vivo animal models where gonadotropins negatively impact on embryo quality [1-4]. In addition, stimulation regimens using high doses of gonadotropin in unselected population may be associated with an increased rate of hyperstimulation syndrome (OHSS) and with a deleterious effect on endometrial receptivity [5,6]. Consequently, a more friendly ovarian stimulation using lower doses of gonadotropin for a shorter period of time has been advocated [7,8]. The “so-called” mild stimulation regimen [9] which consists in gonadotropin administration started from mid follicular phase after spontaneous follicular recruitment is actually close to the physiological events of the natural cycle. This mild strategy has been associated with a higher percentage of good quality embryos in relation with a lower rate of aneuploidy and mosaicism [10]. These data suggest that the strategy aiming at less interference with ovarian physiology could be recommended in a specific subgroup of young normo-ovulatory women who displayed unexplained poor embryo quality following conventional regimen.

Therefore, the purpose of this study was to assess the beneficial effect of mild stimulation regimen on embryo quality in a subgroup of patients who had previously experienced two attempts with poor embryo quality.

Materials and methods

Patients
This analysis was performed from data collected during the last 4 years in a single university ART centre. Patients who had previously undergone two IVF-ICSI cycles using conventional protocols and resulting in a low rate of good quality embryos were selected for this study. In this sub-group of patients, a mild stimulation protocol was proposed and then applied in order to assess whether embryo quality could be improved.

The inclusion criteria were as follows: women with regular, normal length and ovulatory cycles, aged < 43 years, BMI (Body Mass Index) between 19 and 35, two previous IVF – ICSI attempts using conventional GnRH analog protocol and resulting in a low (<20%) rate of good embryo quality.

The exclusion criteria were as follows: patients with no spontaneous follicular development, previous cycle cancellation for inadequate response to treatment, cycle without oocyte following ovarian pick-up, male indication related to non obstructive azoospermia.

Conventional protocols
GnRH agonist or antagonist protocols could be used in the 2 previous cycles.

GnRH long luteal agonist protocol consisted in luteal subcutaneous administration of Triptorelin (Decapeptyl™, Ipsen, Boulogne, France) for at least 12 days. When pituitary down regulation was achieved (serum estradiol (E2) level < 50 pg/mL, progesterone < 1ng/mL and no follicle > 10 mm), a daily administration of recombinant FSH (Follitropin alpha GonalF® Merck Serono Lyon, France; Follitropin beta Puregon® Schering Plough Neully, France) was started at a dose determined according to age, BMI and ovarian reserve. FSH dose

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adjustment was performed on day 6, 8 and 11 according to the ovarian response monitored by pelvic ultrasound and hormonal determinations. Ovulation was triggered by injection of human Chorionic Gonadotropin (r.hCG, Ovitrel® Merck Serono Lyon, France) performed when at least 3 follicles reached a diameter of 17 mm. Oocyte retrieval was performed 36h later and collected oocytes were inseminated according to IVF or ICSI procedures.


GnRH Antagonist protocol was prescribed in patients who previously received a pre-treatment with either oral contraceptive (Lévonorgestrel 0.15 mg / Ethinylestradiol 0.03 mg Minidril®) for 3 weeks or with natural estrogens (Micronized estradiol, Provames® 2 mg p.o) started on day 22 of the precedent cycle and maintained for at least 12 days. After a “wash out” period of respectively 5 and 3 days, a daily administration of r.FSH was started with a subsequent dose adjustment as previously reported for GnRH agonist protocol. GnRH antagonist (Cetrorelix Merck Serono Lyon, France; Ganirelix Schering Plough Neuilly, France) was administered in flexible way, when the largest follicle reached 14 mm or when serum E2 exceeded 500 pg/ml. Subsequent procedures were similar to those described for GnRH agonist protocols.

Ovarian mild stimulation regimen

A daily dose of r.FSH (100 to 200 IU) determined according to BMI, age and prior response to treatment was started on day 5 of a spontaneous cycle and was subsequently adjusted on day 8 according to the ovarian response. Administration of GnRH antagonist was introduced in a flexible way as described above. Subsequent procedures were similar to those described for conventional protocols.

Assessment of the embryo quality

Embryo quality was assessed on day 1, 2 and 3 following oocyte fertilization. Embryos were quoted according to the following criteria [11-15]:

“good quality”: < 20 % fragmentation rate and 3 – 5 cells on day 2 or 6 - 9 cells on day 3

“top quality”: < 20 % fragmentation rate and exactly 4 cells on day 2 or 8 cells on day 3.

Statistical analysis

In order to improve the statistic relevance we compared the data of the last conventional cycle to those of the mild stimulation. Non parametric tests were used for statistical comparison between the two treatment groups. For data obtained on embryo transfer at day 2, a Wilcoxon test for paired analysis was used whereas, for data obtained on embryo transfer at day 3, we used a Mann Whitney test suitable for unpaired analysis. P values < .05 were considered statistically significant. Results are expressed as mean +/- SEM.

Results

Among 2319 controlled ovarian stimulations performed in our centre for IVF-ICSI over 4 years, a retrospective analysis of our data allowed to identify 75 women who experienced 2 consecutive cycles with less than 20% of good quality embryos. Only 18 of them accepted to undergo a mild stimulation regimen.

The clinical characteristics of these patients are listed in Table 1: mean age: 34.2 ±1.25 yrs, BMI: 24.3 ± 0.82, average rank for IVF-ICSI attempt: 4 ± 0.24. Indications for ART were tubal infertility (9/18), pelvic endometriosis (3/18), male infertility (3/18), mixed factors (1/18) and unexplained infertility (2/18). As a low fertilization rate was previously observed in 6 couples, 9 patients underwent sperm microinjection following both conventional and mild regimens.

A paired comparison between the last conventional and the mild stimulation protocol showed that the total amount of r.FSH dose was significantly lower in patients treated with the mild protocol (1137 ± 63 versus 1967 ± 189 IU p <.01) but serum E2 values on the day of hCG day were not different (1307 ± 113 versus 1425 ± 184 pg/ml). In addition, serum LH and progesterone values at the time of hCG administration did not significantly differ.

As shown in Table 2, mild stimulation regimen was associated with a significantly lower number of retrieved oocytes but the numbers of metaphase II, fertilized oocytes and cleaved embryos were not significantly different. More importantly, the fertilization rate and the cleavage rate assessed by the percentage ofcleaved embryos per metaphase II oocyte were significantly higher in patients treated according to mild stimulation regimen as compared to conventional one.

Table 3 shows the number and the percentage of top or good quality embryos on day 2 and day 3 which were significantly higher after mild stimulation than after conventional regimen.

Discussion

This retrospective analysis shows that mild stimulation regimen may be associated with an improved embryo quality in a subgroup of women who had previously experienced 2 IVF-ICSI failures related to poor quality embryos following conventional IVF protocols.

Table 1: Patients’ characteristics: mean (SEM) or n (%).

<table>
<thead>
<tr>
<th></th>
<th>Age at (n-1) attempt (yrs)</th>
<th>Age at mild attempt (yrs)</th>
<th>Time between n-1 and mild attempt (month)</th>
<th>Rank of the mild attempt (n)</th>
<th>BMI (kg/m²)</th>
<th>Cause of infertility n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33.6 (1.3)</td>
<td>34.2 (1.2)</td>
<td>5.6 (0.8)</td>
<td>4.0 (0.2)</td>
<td>24.3 (0.8)</td>
<td>Tubal factor 9 (50%)</td>
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<td></td>
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<td>Severe endometriosis 3 (16.6%)</td>
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<td></td>
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<td>Male factor 3 (16.6%)</td>
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<td></td>
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<td>Mixed factors 1 (5.5%)</td>
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<td></td>
<td>Unexplained 2 (11.1%)</td>
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<td>IVF (n)</td>
<td>9 (50%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ICSI (n)</td>
<td>9 (50%)</td>
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<tr>
<td>Severe male factor</td>
<td>3 (33.3%)</td>
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<tr>
<td>Fertilization failure</td>
<td>6 (66.7%)</td>
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Table 2: Comparison of oocyte et embryo quantity between stimulation regimens.

<table>
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<tr>
<th></th>
<th>Retrieved oocytes (n)</th>
<th>Metaphase II oocytes (n)</th>
<th>Fertilized oocytes (n)</th>
<th>Cleaved embryos (n)</th>
<th>Fertilization rate (%)</th>
<th>Cleavage rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional stimulation</td>
<td>10.78 (1.05)</td>
<td>7.06 (0.91)</td>
<td>4.39 (0.70)</td>
<td>3.22 (0.56)</td>
<td>60.3 (7.15)</td>
<td>45.5 (6.05)</td>
</tr>
<tr>
<td>Mild stimulation</td>
<td>7.22 (0.71)</td>
<td>5.17 (0.61)</td>
<td>3.89 (0.49)</td>
<td>3.39 (0.42)</td>
<td>74.1 (6.26)</td>
<td>66.3 (5.8)</td>
</tr>
</tbody>
</table>

P Value < .01 for conventional vs. mild stimulation

P < .05 for conventional vs. mild stimulation
demonstrated the negative impact of ovarian stimulation on embryo addition, in vivo studies, performed in mice and hamsters, similarly was induced by high doses of FSH showed an increased rate of quality. The rationale behind the mild ovarian stimulation regimen is that a natural follicular recruitment could be associated with an improvement in oocyte and embryo quality.

In conclusion, while limiting the number of retrieved oocytes, a mild stimulation regimen may improve embryo quality in a subgroup of patients who experienced a low rate of high quality embryo in 2 previous consecutive cycles with conventional regimen. In our hand, the proportion of patients who fulfilled these criteria is actually low. This partly explains the limited number of women enrolled in this study. In addition, as mild stimulation was not universally advocated in this situation before the report [10], only a small number of patients were included in this protocol. Nevertheless, their clinical characteristics deserve further analysis. In our series, advanced age cannot explain the high incidence of poor quality embryos because, in the vast majority of patients, age was below 38 yrs. Similarly, a low ovarian reserve cannot account for the poor embryo quality as demonstrated by the range of retrieved oocytes following mild stimulation protocol. These data are in line with our previous report that, in young women without decreased ovarian reserve, cycle outcome remains fairly good even if the ovarian response to gonadotropins is lower than expected [23].

Regarding indications for IVF or ICSI, some of our patients underwent ICSI because of previous low fertilization rate following IVF. While our study was not focused on that issue, it is tempting to speculate that couples whose infertility is related to low fertilization rate could also benefit from mild stimulation regimens.

Therefore, our data suggest that a mild stimulation could be an interesting alternative to conventional regimen in a selected group of young women with normal ovarian reserve and whose the percentage of high quality embryo is repetitively low. This observation needs to be confirmed in additional trial with a larger number of enrolled patients.

In conclusion, while limiting the number of retrieved oocytes, a mild stimulation regimen may improve embryo quality in a subgroup of patients who had previously experienced IVF-ICSI failure due to a poor embryo quality.

References

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