High progesterone levels on the day of HCG administration do not affect the embryo quality and the reproductive outcomes of frozen embryo transfers

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Abstract

Objectives. The aim of this study was to evaluate the impact of premature progesterone rise on the day of human chorionic gonadotropin (HCG) administration on the outcome of in vitro fertilization (IVF) of frozen embryo transfer (FET) cycles using cleavage-stage embryos.

Methods. This was a retrospective, cohort study of 131 ovarian stimulation cycles followed by cleavage-stage frozen embryo transfers. The first group consisted of women undergoing FET due to premature luteinization during controlled ovarian stimulation (n = 56, P ≥ 1.2 ng/ml). The controls were represented by women undergoing FET not complicated by high progesterone levels at induction (n = 75, P < 1.2 ng/ml). For both groups, the progesterone was measured on the day of hCG administration and the fertilization rate, cleavage rate, implantation rate, clinical pregnancy rate, ongoing pregnancy rate and Top-Quality Embryos (TQE) rates were compared.

Results. The increase of progesterone in patients of the Group A had no significant effects on the number of oocytes retrieved or available for the insemination. The fertilization rate, cleavage rate and implantation rates, as well as the clinical pregnancy rate and ongoing pregnancy were very similar in both study groups. The analysis of TQE rates between the two groups indicated a roughly comparable result.

Conclusions. The results of this study showed that progesterone elevation on the day of HCG administration did not affect the outcomes of IVF with frozen embryos at cleavage stage. This study therefore confirms that for patients with high progesterone levels the right way to obtain a healthy pregnancy should be to delay the embryo transfer at a successive FET cycle, not associated with the ovarian stimulation.


Key words: Progesterone, frozen embryo transfer, cleavage-stage embryo, pregnancy rate

Introduction

Progesterone is a synthesis product of the steroid pathway deriving from the conversion of pregnenolone by the 3β-hydroxysteroid dehydrogenase in the adrenals and within granulose and theca cells of ovaries (1). As progesterone plays a crucial role in endometrial morphology and receptivity, it significantly affects the process of embryo implantation as well as the success rate of IVF treatments (2). For these reasons, during the last 20 years increasing attention has been directed to serum progesterone measurements during the ovarian stimulation.

In 1991 it was demonstrated for the first time that progesterone may rise during the last days of ovarian stimulation (3). This phenomenon, often referred as “premature luteinization”, is detectable in 12.3-46.7% of IVF outcomes, based on the threshold used (4). From then on, several studies have shown that early elevation of progesterone might negatively influence the pregnancy outcomes in women undergoing IVF and ET treatments (5-10). On the contrary, other publications have reached opposite conclusions (11-13). A recent large meta-analysis on more than 55000 fresh IVF cycles has concluded that progesterone elevation on the day of HCG supplementation, in women undergoing ovarian stimulation, is correlated with a significant decrease of pregnancy rate. Such negative effect can be observed starting from progesterone concentrations in the range of 0.8-1.1 ng/ml and seems to increase when it reaches 1.2 ng/ml or more (14).

The adverse effect of progesterone on pregnancy rates is hypothesized to be exerted through its action on the endometrium, by interfering with its receptivity. In more detail, the increase of progesterone likely constrains the endometrium to move on earlier, causing an asynchrony between the developing embryo and the endometrial receptivity (15). At present, the optimal strategy to counteract progesterone rise during ovarian stimulation should be to delay the embryo transfer. For example, oocytes can be fertilized and the obtained embryos can be frozen, to be transferred during a successive FET cycle, not associated with the ovarian stimulation (16,17). Indeed, it has been demonstrated that, in this case, the pregnancy rate is not compromised (18-21).

In contrast to the effects of progesterone rise on the endometrial receptivity (22,23), the results on whether the increased progesterone may influence the quality of embryos are still limited (7,24,25). In particular, to exclude the role of endometrium in the evaluation of progesterone rise on the reproductive outcomes, the aim of this study was to
evaluate the impact of premature progesterone rise measured on the day of hCG supplementation on the transfer cycles of frozen-thawed embryos at cleavage-stage.

Materials and Methods

Study design

This single-center retrospective cohort study was performed in the Momò Fertility Private Center for Reproductive Medicine Bisceglie, Italy, in the period between February 2013 and June 2016. After the local ethics committee approval and the written informed consent, a total of 131 patients with different progesterone levels undergoing on frozen FET cycles using cleavage-stage embryos, were included in the study.

Ovarian stimulation and progesterone measurements

Patients of both groups were down-regulated with a conventional GnRH antagonist and stimulated with a recombinant FSH preparation. During cycle monitoring, progesterone level on HCG trigger day was measured by a bioMérieux assay®, combining an enzyme immunoassay competition method with a final fluorescent detection (ELFA), performed on a VIDAS automatic platform®. The patients were divided into two groups based on the serum level of progesterone on the day of HCG administration. Group A (n = 56) included patients with a progesterone level ≥ 1.2 ng/ml. Group B (n = 75) consisted of those patients whose progesterone level was < 1.2 ng/ml. Inclusion criteria were women age less than 38 years and normal semen quality parameters according to the World Health Organization criteria. Exclusion criteria were poor responder according to the Bologna criteria, POFS, cases with previous index retrieval was included in this study. A total of 131 patients undergoing frozen program due to premature luteinization (5.44 ± 2.67 in the group A vs 5.6 ± 3.29 in the group B; 5.21 ± 2.46 in the group A vs 5.45 ± 3.03 in the group B, respectively).

Embryo culture

Briefly, semen was collected in sterile containers by masturbation after 3/4 days of sexual abstinence and then maintained at 37°C for 30 min. After liquefaction, samples were analyzed for sperm concentration, motility and morphology according to the World Health Organization criteria. Oocytes were collected at 36h post-hCG administration. Oocytes retrieval was performed through vaginal puncture under ultrasound guidance (LOGIQ S8, GE Healthcare®). Cumulus-oocyte complexes were exposed to Hyaluronidase solution (25 IU/ml) to remove by pipetting the corona radiata. Metaphase II oocytes were evaluated and select under a stereomicroscope (Nikon SMZ 1500). Only oocytes in metaphase II were injected.

The oocytes were incubated in LGGF medium (Fertilization Global) and injected 38/40 hours after HCG administration. In both groups, the ICSI procedure was performed on heated stage at 37 °C under an inverted microscope (Nikon eclipse TE 200) at 400X magnification. The Intracytoplasmic Semen Injection was performed by oil-hydraulic microinjection system (Nikon eclipse TE 200).

Normal fertilization was defined as zygotes with 2 pronuclei (2PN), then fertilized oocytes were continuously cultured in LGGG medium (Global) for 3 days.

Quality embryo was defined on the third day by evaluating blastomere number and cytoplasmic fragmentation. Day 3 embryos with 8 equal size and no cytoplasmic fragments were defined as Top Quality Embryos (TQE).

Embryo cryopreservation was performed on the third day of culture. Vitrification and thawing protocols applied were the Cryotop method (Kitazato BioPharma Co., LTD., Fuji city, Shizuola, Japan). FET was carried out with a maximum of 2 best-quality embryos per attempt.

Statistical analysis

Both groups were compared about fertilization, cleavage and implantation rate, clinical pregnancy rate and ongoing pregnancy rate and top embryo quality. Data are expressed as mean ± standard deviation for continuous variables, while percentages were used for categorical variables. Data analysis was performed by using Statistica version 8.0 (StatSoft Italia Srl®, Padova). Mean values were compared by Student’s t test. Percentages were compared by Chi-squared test (p value < .05) was considered significant using both statistical analysis. All patients were included once for the analysis.

Results

The patients were selected by searching our database for FET cycles performed between 2013 and 2016. Retrieval cycles involving the selection of embryos at cleavage-stage and subsequent FET were selected. Only the first FET after index retrieval was included in this study. A total of 131 patients were included in this study, divided into two groups: patients undergoing frozen program due to premature luteinization during controlled ovarian (Group A, P ≥1.2 ng/ml) were compared to women undergoing frozen cycles not complicated by high progesterone levels at induction (Group B, P < 1.2 ng/ml). The number of patients in the two groups was comparable.

As shown in Table 1, in both groups, the average age of participants was about 34 for women and 36.8 for men and the basal sperm concentrations were analogous.

The increase of progesterone had no significant effects on the number of oocytes retrieved or available for the insemination (5.44 ± 2.67 in the group A vs 5.6 ± 3.29 in the group B; 5.21 ± 2.46 in the group A vs 5.45 ± 3.03 in the group B, respectively).
The Embryo quality and the reproductive outcomes of frozen embryo transfers

Table 1. Patients characteristics and outcomes of ovarian stimulation in the two groups included in this study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A</th>
<th>Group B</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female age (years)</td>
<td>34.07 ±3.24</td>
<td>34.10 ±2.93</td>
<td>.93</td>
</tr>
<tr>
<td>Male age (years)</td>
<td>36.79 ±4.52</td>
<td>36.9 ±3.96</td>
<td>.80</td>
</tr>
</tbody>
</table>
| Basal sperm concentra-
| tion (x 10^6/ml)        | 34.9 (±24.6) | 36.01 (±25.81) | .67     |
| Retrieved MII oocytes (number) | 5.44 (±2.67) | 5.6 (±3.29) | .63     |
| Injected MII oocytes (number) | 5.21 (±2.46) | 5.45 (±3.03) | .42     |
| Note: Value are expressed as mean ± sd or percentage |

Table 2. Reproductive outcomes of FET cycles in the two groups included in this study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A</th>
<th>Group B</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilization rate</td>
<td>80.00 (±18.35)</td>
<td>78.87 (±18.87)</td>
<td>.56</td>
</tr>
<tr>
<td>Cleavage rate</td>
<td>91.5 (±13.69)</td>
<td>92.3 (±12.58)</td>
<td>.8</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>19.25 (±30.90)</td>
<td>23.57 (±32.52)</td>
<td>.19</td>
</tr>
<tr>
<td>Clinical pregnancy/ cycle (%)</td>
<td>18/56 (32.1%)</td>
<td>28/75 (38.7%)</td>
<td>.22</td>
</tr>
<tr>
<td>Ongoing pregnancy/ cycle (%)</td>
<td>15/56 (26.8%)</td>
<td>24/75 (32.0%)</td>
<td>.23</td>
</tr>
<tr>
<td>Note: Value are expressed as mean ± sd or percentage</td>
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Finally, the analysis of TQE rates between the two groups in Figure 1 evidenced a roughly comparable result, with a value of 32.5% in the group A respect to 35.2% in the group B.

Discussion

In autologous fresh IVF cycles, it is frequently observed a rise of progesterone levels caused by an increased steroidogenesis by multiple ovary follicles. This adverse effect has been shown to induce an accelerated endometrial maturation in the successive luteal phase. So far, many studies have showed that high progesterone levels might have negative effects on the endometrial receptivity and pregnancy rate (26,30) but the results on the impact of progesterone rise on the embryo quality are still limited (31,32). One of the most recent of these studies described the drift of embryo quality in patients who had P >2.0 ng/ml on day oh hCG trigger undergoing fresh cycles (33).

The present study demonstrated that the elevation of progesterone in the day of hCG administration did not affect the number of oocytes retrieved and available for fertilization, differently from other works in which the rise of progesterone was found to increase the number of oocytes retrieved (7,34). However, the comparable characteristics of patients and the standard and reproducible stimulation protocol followed in our center, support the fact that the egg number may not change between the two groups.

In the same way, the fertilization rate, cleavage rate and implantation rates were very close in both the study groups, thus suggesting that the rise of progesterone might affect not the egg quality and the relative embryo quality but rather the endometrial receptivity. In fact, the comparison of the TQE rates evidenced no difference between the two group of this work, in accordance with previous studies in which the rise of progesterone did not affect the number of chromosomally normal embryos available for the transfer in a successive FET cycle (35). The scientific development goes quickly forward, and many ethical issues arise concerning the future of the human species. Many questions are asked about the choice of healthy embryos, but not only, also regarding the genetic repairs of the serious genetic disorders of human genome and recent editing of the human embryo with CRISPR/Cas9, which can be a solution to genetic mutations, but at the same time could have implications on the development and on-going the human species evolution (36).

In conclusion, to achieve a successful implantation, an embryo with a good implantation potential should be present in the uterus during the so-called “window of endometrial receptivity”. Such implantation window is missed in patients with elevated progesterone, in which fresh transfer of embryos into an endometrium with an accelerated maturation might cause a high risk of implantation failures. In this case, FET in subsequent cycles allow to synchronize endometrial and embryo development. This study demonstrated that premature progesterone rise has no negative effects on oocytes and embryos in frozen IVF cycles and cleavage-stage FET. Even further experiments are needed to extend this work, it supports the idea that rather than annulling a cycle for patients with high progesterone levels, the “freeze-all” strategy (35) can be carried out to obtain a healthy pregnancy in a safe and ethical manner (36).

Declaration of interest

The authors report no declaration of interest.
Ethical approval

The study protocol has been sent to the local Ethics Committee for evaluation and written informed consent form signed at the inclusion in the study, was obtained from each patient. The study protocol conforms to the ethical guidelines of the ‘World Medical Association Declaration of Helsinki—Ethical Principles for Medical Research Involving Human Subjects’ adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013.

Informed consent

Informed consent was obtained from all the patients included in the study.

References

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