

FACTORS PREDICTING SUCCESS IN OVARIAN STIMULATION

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Success after ovarian stimulation for In Vitro Fertilization (IVF) is depending on several factors. Some of them are inherent to the patient, as age, female and male infertility causes, or body mass index. On the other hand, other factors are generated during ovarian stimulation, and can affect the final outcome.

Ovarian stimulation performed for IVF provides a dramatic variation of the normal hormonal profile on the menstrual cycle. These variations can be summarized as:

- a) A 5-10 fold increase on Estradiol (E_2) serum levels in the follicular phase, due to multiple follicular development
- b) A subtle raise in serum progesterone (P) levels, before the human chorionic gonadotrophin (hCG) administration

Since more than one decade ago, our group has analyzed the role of high serum E_2 on embryo implantation. The first approach was reported in 1995, when significantly lower implantation and pregnancy rates were observed in women with high response, compared to those that showed a normal response (1). In that same study, it also was observed that in the oocyte donation model, implantation and pregnancy rates remained comparable in recipients independently of the response of the correspondent donor. Altogether, this data suggested that the negative impact of high serum E_2 on embryo implantation was due to a detrimental effect on the endometrium.

In a subsequent study, the endocrine milieu during the pre-implantation period was analyzed (2). It was seen that in high responders, there was a significantly higher $E_2:P$ ratio during days 4 to 6 after the oocyte retrieval, demonstrating that the hormonal variations caused during ovarian stimulation, lasted longer in these patients, evidencing an altered endocrine environment during the pre-implantation period in high responders.

To deeper analyze the effect of high E_2 levels on embryo implantation, Valbuena et al. developed an "in vitro" model to evaluate mice embryo adhesion to a monolayer endometrial epithelial cell culture (3). This model allowed analysing separately the effect of increasing doses of E_2 on the endometrium and on the embryo. The different experiments showed that increasing doses of E_2 in culture affected progressively embryo adhesion because of a negative impact on the endometrium, but also on the embryo. Moreover, E_2 affected embryo development in a dose dependent manner, and 100% of embryos were degenerated when E_2 doses were highly supraphysiological.

A subtle raise in serum P levels at the end of the follicular phase, before hCG administration can also affect embryo implantation. Although there is not a clear consensus to define a detrimental P level, most authors use a value between 1.0 and 1.5 ng/mL for cut-off level. This elevation has been shown to occur in a considerable number of IVF cycles, and for both kind of GnRH analogues used for preventing a premature LH surge. Thus, in GnRH agonist cycles, it has been described in 5 to 35% of cycles and in 20 to 38% of GnRH antagonist cycles.

To deeper analyse this event, our group designed a prospective cohort study with the objective of quantifying its incidence in GnRH antagonist cycles, and determine if this premature P raise had any adverse effect on cycle outcome in terms of implantation and pregnancy rates (4). Ninety four normogonadotropic patients aged 18-37 years old undergoing their first IVF/ICSI cycle were included. Eighty one cycles were completed, and all of them followed ovarian stimulation with recombinant FSH alone, and 0.25 mg/day of GnRH antagonist were introduced from stimulation day 6 in a fixed manner until the day of hCG administration.

Elevated P was defined as a serum P level ≥ 1.2 ng/mL on day of hCG administration, according to a previous retrospective study in which such value was identified as the best cut-off level. This circumstance was observed in 38.3% of the cases (31/81). The number of oocytes collected and number of embryos transferred were comparable between groups, but implantation (13.8 vs 32.0%) and pregnancy rates (25.8 vs 54.0%) were significantly lower in patients whose serum P was ≥ 1.2 ng/mL the day of hCG administration ($p < 0.01$). The Odds Ratio (and 95% confidence interval) of achieving pregnancy of these patients compared to those whose serum P level was < 1.2 ng/mL was 0.30 (0.11-0.79); ($p = 0.015$).

The capability of predicting pregnancy of serum E_2 , LH and P level on day of hCG were submitted to a Receiving Operating Characteristic (ROC) curve. Only P levels showed a statistically significant area under the curve (AUC) value (0.672; 0.555-0.789).

The question rising after this study was to determine whether the negative impact of high P on cycle outcome was due to a detrimental effect on the oocyte-embryo factor, or on the endometrium. To further analyse this point, we analysed the outcome of 289 oocyte recipients from donors that after undergoing two GnRH agonist long protocol cycles, had high P in one cycle ($n = 141$) but not in the other one ($n = 148$) (5). Results of this study showed that implantation and pregnancy rates were similar between both groups, with the only difference of a higher number of mature oocytes obtained from the donors who's P on day of hCG was ≥ 1.2 ng/mL. This finding suggests that the negative impact of high serum P observed in IVF-ET cycles could probably be due to adverse effects on the endometrial, rather than in the oocyte-embryo factor.

In conclusion, the analysis of the effects of serum E_2 and P levels achieved after ovarian stimulation allow defining the objectives that need to be determined when performing an IVF-ET cycle. This means to obtain enough mature and good quality oocytes to ensure a nice embryo cohort for a good quality embryo transfer, avoiding too high E_2 and P levels at the end of stimulation. The achievement of proper intervals of retrieved oocytes, and serum E_2 and P levels may optimize cycle outcome from the point of view of ovarian stimulation.

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