The relationship of premature progesterone rise with serum estradiol levels and number of follicles in GnRH antagonist/recombinant FSH-stimulated cycles

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ABSTRACT

Objective(s): To investigate the relationship between premature progesterone (P) rise and serum estradiol (E2) levels and the number of follicles in GnRH antagonist/rec-FSH stimulated cycles.

Study design: Two hundred and seven patients treated by IVF/ICSI at the Centre for Reproductive Medicine of the Dutch-Speaking Brussels Free University were included in this observational study. They received 200 IU/day rec-FSH from day 2 of the cycle and daily GnRH antagonist starting on day 6 of stimulation. The criteria for hCG administration included the presence of ≥3 follicles of ≥17 mm diameter. Serum P, E2 and LH were determined on the day of hCG administration. The outcome measure was to identify a threshold of E2 and number of follicles on the day of hCG administration which would define a progesterone rise on the day of hCG administration (cut-off value of 1.5 ng/ml).

Result(s): Patients with a P >1.5 ng/ml had significantly higher concentrations of E2 and increased number of follicles on the day of hCG administration compared to those with P ≤ 1.5 ng/ml. However, patients with a P > 1.5 ng/ml the day of hCG showed lower pregnancy rates than those with P < 1.5 ng/ml (17.8 vs. 32.7%, respectively; p < 0.05). A ROC curve was employed in order to estimate a cut-off for E2 on day of hCG >1790.5 pg/ml and more than 9.5 follicles of ≥11 mm in diameter for progesterone rise over 1.5 ng/ml.

Conclusion(s): A significant impact is shown on progesterone rise by E2 and number of follicles on the day of hCG administration in GnRH antagonist/rec-FSH-stimulated cycles. With this knowledge, an upcoming progesterone rise during follicular phase can be anticipated and prevented.

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1. Introduction

Serum progesterone (P) concentrations are low (<1.5 ng/ml) during the normal early follicular phase of ovulatory cycles, but tend to increase gradually 12–24 h before the onset of the LH surge [1]. The stimulation protocols in IVF treatment induce supraphysiological serum concentrations of estradiol (E2) during the follicular phase. Despite the administration of GnRH analogues, however, moderate elevation in serum progesterone concentrations can be observed during the late follicular phase, which could influence endometrial development [2].

Many researchers have incorrectly described the progesterone rise during the late follicular phase as “premature luteinization” [3–6]. This process is defined as a rise in serum P levels above 1.5 ng/ml towards the end of the follicular phase [7]. No association has been reported between progesterone elevation and fertilization rates or oocyte/embryo quality [8,9], which might indicate absence of a detrimental effect of elevated progesterone on the day of hCG administration on oocyte quality, as previously suggested [3–5,10–13]. However, a negative impact of elevated progesterone levels during the follicular phase on pregnancy rates has been described [3,14]. This may affect endometrial receptivity and embryo implantation.

Although progesterone rise has historically been associated with LH in the context of premature LH surges, more recent studies assign the elevated progesterone to FSH and estradiol exposure during the follicular phase [3,4,6]. Changes in paracrine regulation could explain an increase in progesterone production in the late follicular phase [15]. The pathologic mechanism is probably the high response of the ovary to the stimulation, which results in an excess number of follicles and proliferating granulosa cells. The high follicular P levels could lead to an advancement of the endometrium, disturbing the dialogue between embryo and endometrium and resulting in implantation failure [16,17].

Several studies [18,19] failed to demonstrate any negative impact of increased serum P levels during the follicular phase on pregnancy rates. Recent studies, however, did confirm significant
Changes in endometrial gene expression and reduced ongoing pregnancy in patients with elevated serum P during the late follicular phase [7,14,16,20].

Changes in hormone concentrations carry significant biological messages. Understanding and proving this theory could give rise to better approaches to treatment and risk assessment. Therefore, the objective of this study was to investigate the relationship between premature P rise and serum E2 levels and the number of follicles in GnRH antagonist/rec-FSH stimulated cycles.

2. Materials and methods

Two hundred and seven patients treated by IVF/ICSI at the Centre for Reproductive Medicine of the Dutch-Speaking Brussels Free University, were included in this observational study, between October 2007 and December 2008. Patients could enter the study only once. Inclusion criteria were age ≤39 years, body mass index (BMI) between 18 and 29 kg/m², presence of both ovaries and basal levels of estradiol (≤80 pg/ml) and progesterone (≤1.6 ng/ml) on day 1 of the cycle. Exclusion criteria were the presence of endometriosis stage ≥3 (American Fertility Society), polycystic ovarian syndrome (Rotterdam criteria), the need for preimplantation genetic diagnosis and azoospermia sperm extraction.

Recombinant FSH (rec-FSH) (Puregona; Merck Sharpe Dome) was started on day 2 of the menstrual cycle at a dose of 200 IU/day in all patients. The dose of rec-FSH remained fixed during stimulation until day 10 of the cycle. If it was necessary to increase the dose of rec-FSH after 10 days of stimulation, or to decrease the dose of rec-FSH due to a risk of ovarian hyperstimulation syndrome (OHSS) patients were excluded from the study. GnRH antagonist (Orgalutran; Merck Sharpe Dome) was started on day 6 of rec-FSH stimulation.

Triggering of final oocyte maturation was performed by using 10,000 IU hCG (Pregnyl; Merck Sharpe Dome) as soon as at least three follicles ≥17 mm were present on ultrasound (US) scan. Oocyte retrieval was carried out 36 h after hCG administration by transvaginal ultrasound-guided puncture of follicles.

Hormonal assessment was performed at the initiation of stimulation, on day 6 of rec-FSH stimulation, on day 8 and on the day of hCG administration. Additional blood samples were taken as necessary between antagonist initiation and hCG administration. Serum LH, FSH, E2, P and hCG were measured by means of the automated Elecsys immunoanaolzer (Roche Diagnostics, Mannheim, Germany). Intra-assay and interassay coefficients of variation (CVs) were <3% and <4% for LH, <3% and <6% for FSH, <5 and <10% for E2, <3 and 5% for P and <5 and <7% for hCG, respectively.

US scan was performed on day 6 of stimulation and thereafter as necessary in order to ensure that hCG would be injected on the first day that the patient had ≥3 follicles of ≥17 mm.

The objective of the study was to identify a threshold of E2 and number of follicles on the day of hCG administration which would define a progesterone rise on day hCG (cut-off value of 1.5 ng/ml; [14]).

The data are expressed as mean (SD). All continuous variables followed normal distribution as assessed with the Kolmogorov–Smirnov test. A logistic regression model was used to assess the relationship between E2 and number of follicles with high progesterone on day hCG (>1.5 ng/ml). Maximum accuracy calculated via the ROC curve was then employed in order to estimate the best cut-off for E2 and number of follicles on day hCG when progesterone is ≥1.5 ng/ml. JMP 8.0 (SAS Inst., Cary NC) was used for data analysis.

Baseline characteristics and stimulation data of the participants are shown in Table 1. Forty-five women (21.7%) had serum progesterone levels >1.5 ng/ml on the day of hCG administration.

Patients with a P >1.5 ng/ml had significantly higher concentrations of E2, and increased number of follicles on the day of hCG administration compared to those with P ≤1.5 ng/ml. However, patients with a P >1.5 ng/ml on the day of hCG showed lower pregnancy rates than those with P <1.5 ng/ml (17.8 vs. 32.7%, respectively; p <0.05) [Table 2]. In contrast, P levels higher than 1.5 ng/ml on the day of hCG, there was no difference in pregnancy rates between women with P levels above any cut-off value and women with P levels below this cut-off value.

A logistic regression model was performed to assess the relationship between E2 and number of follicles on hCG day with progesterone rise (over 1.5). The number of follicles on the day of hCG administration predicted serum progesterone levels >1.5 ng/ml on that day (odds ratio (OR) 1.090, 95% CI 1.026–1.159, p = 0.005) whereas serum E2 levels on the day of hCG administration were not predictive (OR 1.000, 95% CI 0.999–1.000, p = NS). The Nagelkerke R² of the model was 0.277.

The area under the ROC curve for prediction, from serum E2 levels on the day of hCG administration, of a serum progesterone level >1.5 ng/ml on that day was 0.600 (95% confidence interval (CI) 0.505–0.695, p <0.05), with a cut-off value of ≥1790.5 pg/ml for serum E2 levels giving a sensitivity, specificity, positive and negative predictive value of 57.8, 61.1, 29.2 and 83.9%, respectively, for predicting serum progesterone levels >1.5 ng/ml. The area under the ROC curve for prediction, from number of follicles on the day of hCG administration, of a serum progesterone level >1.5 ng/ml on that day was 0.590 (95% CI 0.501–0.679, p <0.05), with a cut-off value of ≥9.5 follicles giving a sensitivity, specificity, positive and negative predictive value of 64.4, 51.2, 26.8 and 83.8%, respectively, for predicting serum progesterone levels >1.5 ng/ml (Fig. 1).

A serum progesterone concentration >1.5 ng/ml had sensitivity, specificity, positive and negative predictive value for predicting an unsuccessful attempt at pregnancy induction of 34.2, 80.3, 80.6 and 33.8%, respectively.

As expected, serum P levels on day 6 and 8 correlated with serum P levels on the day of hCG administration (r = 0.321 and r = 0.486, respectively; p <0.001 for both correlations). In contrast, serum P levels on day 1 did not correlate with serum P levels on the day of hCG administration (r = 0.112, p = 0.111).

Table 1

Baseline characteristics and stimulation data of the study population.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>n = 207</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.9 ± 3.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 ± 3.3</td>
</tr>
<tr>
<td>No of trials</td>
<td>1.1 ± 0.5</td>
</tr>
<tr>
<td>Basal FSH (IU/l)</td>
<td>7.8 ± 2.6</td>
</tr>
<tr>
<td>Cause of infertility (%)</td>
<td>Male 132 (63.8)</td>
</tr>
<tr>
<td></td>
<td>IC50 168 (81.2)</td>
</tr>
<tr>
<td>Stimulation</td>
<td>Duration of stimulation (days)</td>
</tr>
<tr>
<td></td>
<td>Total FSH administered (IU)</td>
</tr>
<tr>
<td></td>
<td>Progesterone on day of hCG (ng/ml)</td>
</tr>
</tbody>
</table>

Values are means ± (standard deviation).
4. Comments

The results of the present study demonstrated that there is a relation between a premature progesterone rise during the late follicular phase and E2 and the number of follicles on hCG day in GnRH antagonist/rec-FSH stimulated cycles.

Despite the use of GnRH analogues, subtle increases in serum progesterone levels beyond a defined threshold value have been observed at the end of the follicular phase during ovarian stimulation. Although the frequency of elevated serum progesterone levels varies, incidences as high as 35% of stimulated cycles in women treated with GnRH agonists [18,19] and 38% of cycles in women treated with GnRH antagonists [3,6] have been reported. In a large retrospective analysis of over 4000 cycles, however, the incidence of progesterone rise on the day of hCG administration above 1.5 ng/ml, was estimated to be 8.4% in agonist and antagonist cycles [14].

Recent publications confirmed that a premature progesterone rise during the follicular phase is associated with lower pregnancy and implantation rates [14,16,21].

Despite the overwhelming evidence for the crucial role of progesterone in ovulation, its origin and the regulation of its secretion throughout the follicular phase of the cycle remains poorly understood. Some publications [2,15] hypothesized that the premature progesterone rise is related to a lack of LH activity in the drugs used for ovarian stimulation. However, the source of progesterone present during the follicular phase is not only the ovary but also the adrenal gland.

- Table 2

<table>
<thead>
<tr>
<th></th>
<th>Women with serum P &gt; 1.5 ng/ml on the day of hCG administration (n = 45)</th>
<th>Women with serum P ≤ 1.5 ng/ml on the day of hCG administration (n = 162)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum E2 levels on the day of hCG administration (pg/ml)</td>
<td>2303.2 ± 1383.3</td>
<td>1881.1 ± 1175.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Number of follicles on the day of hCG administration</td>
<td>12.6 ± 5.5</td>
<td>11.1 ± 5.9</td>
<td></td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>17.8</td>
<td>32.7</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Area under the receiver-operating characteristic (ROC) curve for serum E2 and number of follicles measurement on the day of hCG administration.

In the ovary, FSH acts on granulosa cells promoting cell proliferation and steroid biosynthesis from cholesterol leading to progesterone biosynthesis. Granulosa cells are very active manufacturers of progesterone. Theca cells also make significant amounts of progesterone. According to the two-cell, two-gonadotropin theory of estron biosynthesis [22], progesterone is then metabolized to androgens (mainly androstenedione) by only theca cells under the influence of LH. Androgens are subsequently converted to estrogens through aromatization back in the granulosa cells. This step is limited by the amount of precursor available, which in turn depends on LH levels.

Recently, it was suggested that once the progesterone has been produced by either the theca or granulosa cells it cannot be converted into androgens and subsequent estradiol. If anything it might be converted into 17-hydroxyprogesterone, which also possesses progestogenic activity [23]. This conception is in disagreement with the theory that granulosa cell-derived progesterone can be converted into androgens in the theca cell compartment [2,14].

In summary, there are two sources of progesterone in the follicle, but only one step of further metabolism (conversion of progesterone to androgens), which is driven by LH activity in theca cells. Therefore, if progesterone production (by active granulosa cells) exceeds the limit of LH available, it is likely to leave more progesterone to find its way into the general circulation. Therefore, the origin of late follicular phase progesterone has been assumed to be a lack of LH in the late follicular phase of stimulated cycles [15]. On the other hand, the ovary is not the only source of progesterone production during the follicular phase [22]. The dominant source of progesterone in the follicular phase is the adrenal gland under the control of ACTH. Fanchin et al. [24] observed that during ovarian stimulation with HMG for IVF, peak concentrations of plasma progesterone, testosterone and androstenedione occurred during the early morning, coincidental with the circadian elevation in ACTH and adrenal function. Casson et al. [25] showed that adrenal DHEA-S secretion was augmented by ovarian hyperstimulation. This implies that ovarian hyperstimulation may induce adrenal steroid synthesis. In rats, estrogen administration causes an elevation in ACTH, which induces adrenal progesterone secretion by impairment of the glucocorticoid receptor-mediated negative feedback on CRF and ACTH secretion [26]. This subtle increase in serum progesterone stimulates secretion of LH. Whether similar or alternative interactions between the hypothalamic–pituitary–ovarian and the hypothalamic–pituitary–adrenal axes exist in human is as yet unknown. The increase in estradiol concentration in stimulated cycles does increase ACTH concentrations, which stimulate progesterone secretion from the adrenals [22].

Although premature progesterone rise is an important issue for implantation failure in IVF, this is the first study to propose a predictive model in order to anticipate this phenomenon. Since premature progesterone rise is related to estradiol levels and the number of follicles [15], with this study we have demonstrated that in rec-FSH/GnRH antagonist-stimulated cycles, a serum E2 on day of hCG of ≥ 1790.5 pg/ml and more than 9.5 follicles of ≥11 mm in diameter have a significant effect on progesterone rise.
over 1.5 ng/ml on the day of hCG administration for final oocyte maturation. It becomes evident from the current analysis that even relatively low levels of serum E₂ (1.790.5 ng/l) and number of follicles (9.5), could lead to a premature progesterone rise.

Premature progesterone rise in stimulated IVF cycles is a frequent phenomenon, which is associated with lower pregnancy and implantation rates. With this knowledge, an upcoming progesterone rise during the follicular phase of GnRH antagonist/rec-FSH stimulated cycles can be anticipated and prevented. Prevention could take two different approaches. A shift towards milder ovarian stimulation for IVF could be one of the possible methods. However, further sufficiently powered prospective studies applying mild treatment regimens for IVF are required to demonstrate their efficiency.

On the other hand, very limited data are available regarding the timing of the trigger for final oocyte maturation in rec-FSH/GnRH antagonist-stimulated IVF cycles. It has to be mentioned that the diameter of 17 mm in this study was arbitrary. Kolibianakis et al. [27] demonstrated clearly that prolongation of the follicular phase by delaying hCG administration decreases significantly the ongoing pregnancy rates, but the cutoff level for triggering final oocyte maturation was taken from a follicle size of 17 mm. Recently, Kyrou et al. [28] showed that earlier administration of hCG (as soon as there are three or more follicles of size >16 mm) which was expected to result in lower progesterone levels on the day of hCG administration, is not associated with the probability of pregnancy in cycles stimulated with rec-FSH and GnRH antagonists. Therefore future studies should focus on whether triggering at a smaller size of follicles (<17 mm) in high responder patients would jeopardize the outcome by having too many immature oocytes.

A further possible approach, however, once the progesterone level breached the level compatible with successful outcome, might be freezing of all embryos and embryo transfer in a natural cycle. This approach is supported by Melo et al. [8] who concluded that progesterone rise does not appear to have a negative impact on the ongoing pregnancy rate in an oocyte-donation programme.

In conclusion, these data demonstrate a significant effect on progesterone rise by E₂ and number of follicles in GnRH antagonist/ rec-FSH stimulated cycles. Both of them have a predictive value on premature progesterone rise.

References