Endocrine mechanisms and assay issues in premature progesterone elevation in assisted reproductive technology

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Progesterone elevation occurring in the late phases of controlled ovarian stimulation (COS) has been reported for over 25 years. Yet doubts remain regarding the mechanisms at play in this phenomenon and its net consequences on assisted reproductive technology outcome, which is known to occur in poor and good assisted reproductive technology responders. The pathophysiology of end-COS progesterone elevation encountered in gonadotropin-suppressed cycles is different from that prevailing at the time of, and just after, ovulation. The different divergence in practical consequences of end-COS progesterone elevation led to review the progesterone assays developed for measuring progesterone in the luteal phase of the menstrual cycle, but commonly used for measuring all forms of progesterone elevation. (Fertil Steril® 2018;109:571–6. ©2018 by American Society for Reproductive Medicine.)
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PROGESTERONE ELEVATION DESPITE GONADOTROPIN BLOCKADE

Premature luteinization, luteinizing hormone (LH) elevation causing progesterone production, was a lingering fear in assisted reproductive technology (ART) in the pre- gonadotropin-releasing hormone agonist (GnRH-a) era. This phenomenon, which happened in up to 15%-20% of ART cases, then called in vitro fertilization (IVF), ruined the affected cycles that needed to be cancelled. At first it was believed that the generalized use of GnRH-a, implemented in the mid-1980s, would fix the problem by blocking premature LH surges. However, in 1991, a seminal publication by Schoolcraft et al. (1) reported that cases of pre-human chorionic gonadotropin (hCG) progesterone elevation occurred in GnRH-a blocked ovarian stimulation cycles. It was soon established that this did not result from LH elevation that escaped GnRH blockade. Questions were raised as to the pathophysiology of premature progesterone elevation in GnRH-a and antagonist cycles and, to this date, no definitive answer has been generally agreed upon. The unknown mechanism of action also challenged the adequacy of progesterone assays for measuring progesterone elevations that did not result from a direct effect of LH on the growing follicles. Several queries were raised: Are the direct assays commonly used today sensitive enough for measuring the slight end-follicular phase progesterone elevation encountered in a fraction of controlled ovarian stimulation (COS) cycles? In a context where progesterone elevation does not result from an effect of LH on the growing pre-ovulatory follicle, what is the prevailing ratio of progesterone and progesterone metabolites in the whole process? Is this ratio similar to what is seen in the luteal phase of the menstrual cycle, which served for validating the progesterone assays currently used, or is it possibly different? Are such differences capable of challenging the specificity of direct progesterone assays due to interference with other steroids present at unknown levels?

Issues of end-COS phase progesterone elevation are encountered in low and high responders. In the former, a relatively large amount of progesterone

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emanates from a small number of follicles each producing relatively large amounts of progesterone. In the latter, on the contrary, the net progesterone elevation observed results from a relatively minute contribution of each follicle with a net effect multiplied by the number of follicles present. The net impact of end-COS progesterone elevation on ART outcome is complicated by the fact that the overall prognosis of these two populations, low and high responders, is inherently different.

In two cases of end-COS progesterone elevation occurring in strong hyper-responders, we made observations that led us to believe that in certain cases direct progesterone assays provide inappropriate readings (2). These two women had such excessive ovarian responses to a long GnRH-a COS protocol that it precluded triggering of ovulation with hCG. Both women, whose progesterone exceeded 2 ng/mL for at least 4 days, received no hCG and had an endometrial biopsy that failed to show any secretory changes, thus questioning the validity of the progesterone measurement (2). Actually, re-measurement of progesterone by mass spectrometry by one of us (F.Z.S.) showed much lower values, thereby confirming the data obtained by direct assay were inadequate in these two high responders (2). This observation together with the divergent conclusions, negative outcome or lack of effects, reported on the practical consequences of premature progesterone elevation led us to revisit this issue. In the following parts of this article we particularly focus on issues pertinent to the pathophysiology of progesterone elevation and its measurement by different available assays.

ENDOCRINE PATHOPHYSIOLOGY OF PROGESTERONE ELEVATION

Human ovarian steroidogenesis is regulated differently from many other species including many poly-ovulatory mammals. Whereas E2 is invariably a terminal product in most species, this is not the case with respect to sex steroids with progestogenic activity, in particular progesterone and 17-OH-progesterone. In women, however, progesterone and 17-OH-progesterone are terminal products alongside E2 in ovarian steroidogenesis, with nearly no conversion taking place before normal elimination pathways become active (3).

Human ovarian steroidogenesis involves conversion of cholesterol to pregnelonone, which takes place in the mitochondria and is normally considered the rate limiting step in the production of terminal products. Pregnelonone represents the branching point in human ovarian steroidogenesis, where the subsequent step may follow either the Δ4 or Δ5 pathway depending on where the first double bond between carbon atoms in the backbone is situated. If pregnelonone is further converted along the Δ5 pathway, the 17-hydroxylase/C_{17-20} lyase enzyme (i.e., CYP17) is active in catalyzing the production of 17-OH-pregnenolone, which may be further converted to DHEA by the same enzyme. Alternatively, pregnelonone may be converted by 3β-hydroxysteroid-dehydrogenase (3β-HSD) to progesterone representing the first product in the Δ4 pathway. The 3β-HSD exists in 2 versions, both of them being essentially unidirectional with no catalyzed conversion of products from the Δ4 pathway back to the Δ5 pathway. The CYP17 ortholog in women is able to convert progesterone to 17-OH progesterone via the Δ4 pathway, but unlike in other mammals the CYP17 enzyme in women catalyzes only to a very limited extent further conversion from 17-OH-progesterone to androstenedione via the Δ4 pathway. In practice, this implies that once either progesterone or 17-OH-progesterone has been produced it will not further convert into androgens and subsequently E2 in the ovary (3, 4).

The fact that progesterone and 17-OH-progesterone are very probably both terminal products in human ovarian steroidogenesis is illustrated by the measurement of intrafollicular concentrations of both steroids. In women's natural menstrual cycles the intrafollicular concentration of progesterone steadily increases as the follicular diameter increases and reaches very high concentrations that are more than 1000 times circulating levels in follicles that are close to ovulation (5). Also, 17-OH-progesterone reaches very high concentrations, around 20% of those of progesterone, so that the concentration of both progestogens exceeds levels seen in circulation by several orders of magnitude, suggesting that further conversion is limited (6).

In the human ovary both theca and granulosa cells possess the capacity to synthesize progesterone and 17-OH progesterone. The combined action of both cell types is required for synthesis of E2 since theca cells alone express CYP17 producing androgens, and granulosa cells alone express aromatase (i.e., CYP19).

To what extent theca or granulosa cells are the prominent cell type in synthesizing progesterone or 17-OH-progesterone in the follicular phase of the menstrual cycle is not yet clarified, and information is especially sparse in regard to ovarian stimulation where the levels of follicle-stimulating hormone (FSH) are supra-physiological and levels of LH often may be reduced. The exponential rise of progesterone in the follicular fluid from the time the follicle reaches a diameter of around 8-10 mm until ovulation suggests that a sizeable amount of the progesterone is produced by the granulosa cells (7).

However, granulosa cells from preovulatory follicles collected in vitro prior to the mid-cycle gonadotropin elevation from women in their natural menstrual cycles show a higher sensitivity towards LH stimulation than FSH in their ability to produce progesterone (8), which over a wide dose range was significantly higher with LH than with FSH. A randomized controlled study titrated the effect of LH-like activity (i.e., hCG) in which FSH stimulation was kept constant (9). Four groups of women received a standard agonist protocol with a fixed stimulation regimen employing FSH administration with the 4 groups receiving either 0, 50, 100 or 150 additional hCG injections daily (9). In all 4 groups, steady state levels of FSH around 12 IU/L and levels of LH around 2 IU/L were achieved after 6 days of stimulation. The steady state concentration of hCG was 0, 3, 6, and 11 IU/L in the 4 groups respectively, so basically the only difference between the groups was the level of hCG. Interestingly, levels of progesterone showed a dose-dependent positive association with the dose of hCG administered. Compared to the group with no hCG added, in the group receiving 150 IU hCG daily the concentration of all 3 steroids doubled on the day of final