Confronting the Hidden Face of Progesterone During the Follicular Phase

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Submitted June 28, 2002; accepted July 24, 2002

Sporadic cases of late follicular phase progesterone elevation have been described in ART cycles despite routine blockage of endogenous gonadotropins with GnRH agonists, the mechanism of which has been eluding our scrutiny. In this issue of JARG, Levran et al. report the first cases of adult-onset mild form of congenital adrenal hyperplasia (CAH) linked to a deficit in 17-hydroxylase (17-OH ase). Contrary to CAH due to deficit 21-OH ase, these not only affect the adrenals but also the FSH driven production of E2 and androgen by the ovary thereby leading to progesterone “*accumulation*” during the follicular phase. Levran’s findings offer new hypotheses for explaining the cases late follicular progesterone elevation particularly, when encountered in COH cycles associated with poor E2 elevation. And more importantly, Levran’s findings offer new possible options for minimizing their consequences on ART outcome.

KEY WORDS: Progesterone; CAH; Follicular phase; COH.

It has been a long prevailing dogma that the only clinically meaningful production of progesterone by the ovary occurs in the corpus luteum formed after ovulation. According to this classical view, progesterone’s sole role is to trigger a wealth of changes in the estrogen primed uterus that are indispensable for embryo implantation and pregnancy development. At the endometrium level, progesterone opposes the proliferative properties of estrogen, triggering tissue differentiation through sets of secretory changes in glands and stroma. In the menstrual cycle, these culminate in the predecidual transformation of the endometrial stroma, starting on Day 26 (1). In case of pregnancy, we observe the formation of a near syncytium stage with confluence of stromal cells, or

“*Arias Stella*” phenomenon (1). At the myometrium level, progesterone promptly induces a state of relative utero-quiescence already established by the 4th day after ovulation (2). In case of pregnancy, the inhibition of contractility induced by progesterone resists the extraordinary degree of uterine stretching necessary for the development of the fetus.

Understandably, this vision of progesterone portrayed as a luteal phase hormone led to measuring plasma progesterone levels only during the second half of the menstrual cycle when it serves as a marker of ovulation. But because progesterone production is pulsatile, plasma levels are highly fluctuating, which ruined all hopes of correlating actual plasma values with luteal phase quality.

In this issue of the Journal, a publication by Levran et al. (3) stresses that progesterone elevation can in certain conditions also occur during the follicular phase with devastating consequences on fecundity. The mechanism at play in Levran’s report, a partial form of enzymatic deficiency in steroids’ synthesis, is one of several that can result in progesterone

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elevation during the follicular phase. Too often, the possibility that progesterone elevation occurs during the follicular phase has been ignored, thus leaving an uncharted number of undiagnosed cases and the affected women deprived of possible treatments. Levran's publication should be a wake up call arising our awareness of possible progesterone elevations during the follicular phase. It is time that we confront the "hidden face" of progesterone, the too often ignored facet of this hormone that in certain circumstances strikes during the follicular phase.

In controlled ovarian hyperstimulation (COH) commonly induced for IVF, we have long recognized that preovulatory increases in progesterone sometimes occur, with a negative impact on outcome. Originally, these were believed all to fall in the realm of premature luteinization, with the developing follicles being transformed by rising LH levels. The advent of GnRH-agonists (GnRH-a) that were soon routinely used in IVF, provided an easy and effective cure for all premature LH elevations. Yet to our and other's surprise, if GnRH-a eradicated premature LH rises, those of progesterone persisted with seemingly ominous consequences on IVF outcome, at least in some reports (4–7). The possibility that progesterone elevation reflects an escape from the blocking properties of GnRH-a on gonadotropins was soon dismissed, leaving us at a loss for understanding the fine mechanism(s) of this phenomenon. Yet, this represented the first demonstration of nonluteal/nonluteinization elevation of progesterone, with a possibly devastating toll on fecundity.

The debate on the true consequences of late follicular phase elevation of progesterone rapidly heated up with an equal number of papers scoring in favor of negative (4–7) and positive (or no) consequences (8–12) on IVF outcome. The mechanism by which late follicular phase elevation of progesterone could alter IVF results was less controversial, however. All who saw lower pregnancy rates when late follicular phase progesterone exceeded the fatidic cutoff value of 0.9 ng/mL admitted that it resulted from an effect on the endometrium while the embryos remained unaffected (13). In support of this latter theory contending that premature exposure of oocytes to progesterone or progestin is harmless, we observed a proper follicular development in a woman who mistakenly kept taking the oral contraceptive pill during her entire COH. The oocytes fertilized well and the cryopreserved embryos transferred at a later stage gave

rise to a pregnancy that developed uneventfully (de Ziegler D, Devroey P, Van Steirteghem A, unpublished observation).

Ultimately, the dust of the dispute over the clinical significance of late follicular phase elevations of progesterone settled when Fanchin *et al.* (14) showed that the consequences on IVF outcome depend on the quality of the ovarian response. While late follicular phase elevations of progesterone had catastrophic consequences on low responders, it left the high responders unaffected.

As stated earlier, less is known about the nature of the mechanism(s) responsible for the late follicular increase in plasma progesterone seen in some COH cycles. We now know, however, that exogenous LH (and hCG) contained in hMG preparations is not the culprit we had "naturally" designated at first. In a prospective trial, Adonakis *et al.* (15) randomly allocated women undergoing COH with pure FSH to receive either hMG or pure FSH after their follicles reached >14 mm (approximately, on Day 8). The group receiving hMG had higher E2 levels but similar late follicular phase increments in progesterone than women continuing on pure FSH. Further supporting the lack of role of LH in the late follicular phase increase in progesterone is the work of Filicori *et al.* (16). These authors prospectively compared the hormonal profile of COH induced with FSH or FSH supplemented with 50 IU of hCG. Here again, late follicular phase profiles of plasma progesterone were similar in patients receiving FSH or FSH supplemented with hCG. In an even more daring deviation from common COH protocols, the same authors switched the ovarian stimulant from FSH to small amounts of hCG (50–200 IU/day) when follicles exceeded 14 mm in diameter (17). The rationale justifying this innovation is that follicles acquire LH receptors when their size is >14 mm. In a trial conducted in regularly ovulating infertility patients, the progesterone and androgen profiles were again similar in women who stayed on FSH or switched to mini hCG doses during the last 3 days of COH (16). Taken together, these findings enable us to proffer with confidence that it is not LH but rather the ovarian stimulation itself (induced by FSH) that is responsible for the late follicular increase in progesterone.

In women displaying a hefty response to COH, the late follicular increase in progesterone is often seen as resulting from a mere multiplication by the large number of follicles present of a normal increase in progesterone production occurring in the latter stages

of follicular maturation. But when this takes place in women whose ovarian responses to COH are weak, a “*per follicle*” increase in progesterone production is seen. The nature of this latter phenomenon is more obscure and taken by some as an indirect sign of ovarian aging (14). The new emerging possibility of switching the COH stimulus from FSH to LH/hCG may tell us whether the late follicular phase increase in progesterone emanates from large (>14mm) or smaller follicles (<10mm), the latter regressing on mini hCG doses.

Still a different etiology for the follicular phase increases in progesterone is the host of enzymatic defects that hinder cortisol production and indirectly result in progesterone elevation by the adrenal and/or the ovary (depending on the enzyme affected). Globally, these genetically determined enzymatic defects are known as congenital adrenal hyperplasia or CAH. The most common is the deficit in 21-hydroxylase resulting from a single mutation in the *CYP21* gene. In homozygous individuals, this induces an excessive production of androgens and a lack of mineralocorticoids. The clinical picture is female pseudohermaphroditism or premature sexual development in male infants and often, a severe salt wasting syndrome (18). The full-blown picture of the disease is most often diagnosed at birth or soon after. Adult-onset or “*nonclassic*” and milder forms have also been recognized and now enter in the differential diagnosis of all cases of severe hirsutism (19). When not diagnosed through signs of masculinization, these disorders may result in infertility with persistent “*throughout*” elevation of plasma progesterone, including during the follicular phase (18). Affected women undergoing IVF typically show repeated implantation failures, with puzzlingly poor endometrium on ultrasound scans. These cases usually remain undiagnosed until plasma progesterone is measured during the follicular phase.

Until today’s report by Levran *et al.* (3), it was believed that some degree of hirsutism and/or acne was always part of the mild or nonclassic forms of CAH because “*adult onset*” forms of CAH were described only for the deficiencies in 21-hydroxylase. The originality of Levran’s publication is to reveal that mild nonclassic forms of 17-hydroxylase deficiencies also exist with suboptimal production of E2 and no increase (rather, a decrease) in androgen production. This finding should upstage our vigilance for the possibility of progesterone elevation during the follicular phase, even in women showing no sign of masculinization.

The incidence of heterozygous carriers for *CYP21* gene mutations, by far the most frequent form of CAH, observed after direct genetic testing of 600 newborns in New Zealand is 2.8% (20). This finding exceeds estimates derived from the frequency of classic CAHs observed in newborn screening, 1.3%. The issue of clinical findings in female heterozygous carriers is not totally settled yet. While the prevailing concept is still that their fecundity is unaffected, the debate remains open. On the one hand, mothers of children with classic CAH (thus, heterozygous carriers) are no more likely to show signs of androgen excess than do age-, sex-, and BMI-matched controls (21). On the other hand, children referred to an endocrine clinic for either premature pubarche or hirsutism showed a higher prevalence of heterozygous *CYP21* mutations than did asymptomatic controls (22).

How many mild-nonclassic cases of the various forms of CAH remain undiagnosed? We can keep asking ourselves the question but will not know the answer until we are more diligent at unveiling the “*hidden face*” of progesterone by looking for elevated levels during the follicular phase. If mild forms of enzymatic alterations in the machinery of sex and adrenal steroid synthesis are ignored, infertility will likely persist in the affected patients in whom common treatments are bound to fail. Does this imply that we should measure progesterone during the follicular phase in all our infertility patients? Probably not. How frequent are the mild/adult-onset forms of COH? While rare, they are nonetheless likely to concentrate in the implantation failure population of our IVF and ART practices. Can mild-nonclassic forms of CAH hide amongst common cases of late follicular increases in progesterone? Probably, yes. And this may even be more likely, now that nonclassic forms of 17-hydroxylase deficiency have also been recognized, which affect progesterone synthesis in both the adrenals and ovaries. How many of these cases are ignored? We do not know. But considering that specific treatments exist for women affected by COH, one is sure one too many.

Yes, we should be more aware that mild-nonclassic forms of CAH may hide amongst our IVF failures. We ought to measure progesterone in the follicular phase each time we become suspicious. Such hunches may come from an abnormal aspect of the endometrium on ultrasound or other features like possibly, the lack of copious mucus in the cervix in the late follicular phase of natural or COH cycles. We should also rule out follicular phase elevations of progesterone when confronted with repeated implantation failures.

Future work should determine whether suppressing the adrenal component of progesterone with timely use of dexametasone may be of value in women whose late follicular progesterone is excessively elevated, even in the absence of enzymatic disorders.

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