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COMMENTARY

The source and implications of progesterone rise during the follicular phase of assisted reproduction cycles

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Abstract Moderate elevations in serum progesterone concentrations are observed following the use of gonadotrophin-releasing hormone agonists during ovarian stimulation. The clinical significance of this phenomenon has been investigated, but findings have been inconclusive. This commentary proposes that progesterone concentrations are indeed important in endometrial advancement and oocyte/embryo development, which, may lead to asynchrony between endometrial and embryo development. Based on the two-cell, two-gonadotrophin model, this commentary proposes a hypothesis to describe how progesterone concentration increases during ovarian stimulation and three factors influencing this during ovarian stimulation are identified: the number of follicles, the FSH drive and the LH activity. It also suggests how differences in gonadotrophin preparations used for ovarian stimulation may have differential effects on progesterone synthesis. It remains to be tested whether routine measurement of late follicular progesterone concentrations may prove beneficial as suitable assay methods are now available. However, strategies that reduce follicular recruitment in high-responding women and gonadotrophins that contain LH activity may reduce the degree of progesterone elevation prior to luteinization.

KEYWORDS: granulosa cell, luteinizing hormone, ovarian stimulation, progesterone, theca cell

Introduction

Since the introduction of gonadotrophin-releasing hormone (GnRH) agonists to eliminate premature luteinization (Fleming et al., 1982), there has been debate regarding the origins and clinical significance of modestly elevated progesterone concentrations seen in the peripheral circulation during the late follicular phase of cycles of ovarian stimulation. Several publications have reported modestly raised concentrations of progesterone in these circumstances, with Ubaldi et al. (1996) showing an association between absolute progesterone concentrations at the end of the follicular phase and the area under the curve of FSH during stimulation. This suggests that the source is probably related to granulosa cell activity in growing follicles, which are not luteinized as the LH surge has been eliminated.

This commentary postulates how this phenomenon may arise and addresses the potential clinical significance of any such event. It contends that raised peripheral concentrations of progesterone in the late follicular phase are likely to influence endometrial development, whilst having no influence upon oocyte/embryo development. This results in a change in the normal synchrony between the endometrium and oocyte/embryo (Achache and Revel, 2006). When excessively disturbed, this asynchrony may reduce embryo implantation and pregnancy rates.

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The endometrium and steroid activity

There is abundant evidence supporting observations that endometrial development is advanced in most ovarian stimulation cycles. Of course, a principal candidate for the cause of this is protracted exposure to supra-normal oestradiol concentrations. There has been little attempt to link these endometrial changes to elevated follicular-phase progesterone values. Direct clinical significance (influence on pregnancy rates) of increased follicular progesterone values has been addressed, but conclusions are not unanimous. The only meta-analysis performed to date (Venetis et al., 2007), whilst not addressing analytical aspects of the phenomenon, found no relationship and dismissed the importance of raised follicular-phase progesterone concentration (RFPPC) on pregnancy outcomes. However, this conclusion may be flawed, on the basis of the scientific analyses and clinical observations below.

Explorations of the clinical significance of RFPPC during ovarian stimulation include evaluation of the influence upon endometrial development in the subsequent early-to-mid luteal phase (Bourgain and Devroey, 2003) and several large programmes have provided strong evidence that RFPPC is associated with impaired expected pregnancy rates (Andersen et al., 2006; Bosch et al., 2003).

This commentary considers the evidence that these changes are natural developments of non-luteinized follicular steroid biochemistry and extrapolate their potential down-stream consequences. Specifically, it is possible that RFPPC produced by ovarian stimulation-induced multiple follicle growth may contribute to changes in the endometrium, leading to embryo–endometrial asynchrony. This effect may be more significant when ovarian stimulation occurs without sufficient LH drive.

Follicular-phase profile in the normal cycle

FSH acts on granulosa cells, promoting cell division and steroid biosynthesis from cholesterol terminating at progesterone biosynthesis (Fleming, 2008). The rate-limiting step in intrafollicular steroid biosynthesis is the side-chain cleavage process converting cholesterol (27-carbon molecule) to the 21-carbon products pregnenolone and progesterone; granulosa cells are very active manufacturers of progesterone, while thecal cells also make significant amounts of progesterone. According to the two-cell, two-gonadotrophin theory of oestrogen biosynthesis (Moon et al., 1978) progesterone is further metabolized to androgens (19-carbon) by the thecal cells under the trophic influence of LH and this step can only take place in the thecal cell compartment. Androgens are subsequently converted to oestrogens (18-carbon) through aromatization back in the granulosa cells (**Figure 1**).

Progesterone produced by the granulosa cells under FSH drive must pass to the vascularized thecal cell compartment to be catabolized to androgens. However, from here, steroids gain access to the general circulation as well as being metabolized in the normal steroid cascade. It is probable that the greater the LH drive to the thecal cells, the more progesterone catabolism to androgens will take place, leaving less product to find its way into the general circulation. This can be summarized as follows: there are two sources of progesterone in the follicle, but only one step of further metabolism (catabolism of progesterone to androgens) which is driven by LH activity in thecal cells. A lack of LH drive to the thecal cells is therefore likely to leave more progesterone to find its way into the general circulation.

Analyses of steroid concentrations in veins of the periphery and those draining the active and contralateral ovaries reveal the potency of steroid metabolism in normally growing follicles (Coutts et al., 1981). In the mid-follicular phase, the concentrations of oestradiol in the periphery and in the contralateral vein (the side without the dominant follicle) range from 500 to 1000 pmol/l, as expected in the mid-follicular phase, while those in the vein draining the active ovary are $>$6000 pmol/l. To attain these values, the steroids must cross the basement membrane between granulosa cells and thecal cells twice. For progesterone, the equivalent concentrations showed peripheral and contralateral values within the expected range ($<$6 nmol/l), while the active ovary showed values $>$15 nmol/l. Detection of the active ovary values in the periphery would be indicative of ovulation and these results show that progesterone is a major secretory product of the growing follicle, of which significant quantities reach the general circulation.

Profiles during ovarian stimulation

It can be predicted that an ovary with a large number of growing follicles, stimulated by maintained high FSH concentrations by dint of daily FSH injections, will produce and secrete more progesterone into the ovarian vein than a single follicle in the normal mid-follicular phase, with declining FSH concentrations. In the event of ovarian stimulation-induced multiple-follicle growth, the progesterone output to the periphery will be magnified in accord with the number of follicles and the FSH drive. This is likely to impact upon progesterone concentrations in the periphery and may influence endometrial development. Thus, the three major components to the degree of progesterone secretion from the ovaries will be: (i) the number of follicles (or granulosa cells); (ii) the degree of trophic stimulus (FSH drive to granulosa cells); and (iii) the degree of LH drive to thecal cells, which will encourage conversion of progesterone to androgens and oestrogen.

Explorations of progesterone rise in ovarian stimulation

The aetiology for RFPPC during ovarian stimulation, although not directly confirmed, is likely to be subsequent to the observations discussed above. The influence of the three factors upon circulating progesterone concentrations in ovarian stimulation was addressed through a study (R Fleming, unpublished data) in which follicular-phase steroid concentrations were measured in cycles (n = 40) stimulated with purified urinary FSH (Metrodin HP; Serono, UK) and controlled with long GnRH-agonist down-regulation. Daily blood samples were taken through the late follicular phase (from stimulation day 6) to the day of human chorionic gonadotrophin (HCG) administration and assayed for oestradiol, progesterone, LH and FSH. The roles of the three criteria were examined by defining categories: (i) large follicular

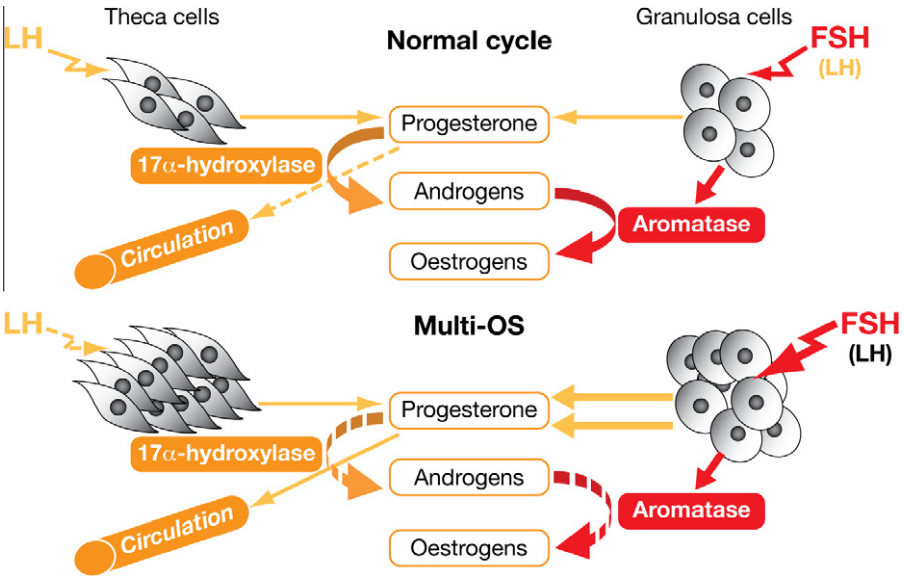

Figure 1 Steroidogenesis during normal follicular phase and following ovarian stimulation.

mass: at least five follicles with a diameter >17 mm on the day of HCG; (ii) high FSH: mean circulating value >12 IU/l; and (iii) low LH: mean value ≤0.7 IU/l. Progesterone concentrations were examined by reference to values seen in the normal cycle on the day of the LH surge, which are slightly higher than values of the preceding follicular phase.

In cases with a large follicular mass, mean circulating progesterone concentrations above the normal range were achieved 2.6 days before HCG administration. Those with a 'normal follicular mass' exceeded the normal range only 0.6 days prior to the day of HCG administration. Comparing high FSH and normal FSH revealed that mean circulating progesterone concentrations exceeded the normal range 1.7 and 0.9 days, respectively, prior to the day of HCG administration. In the normal FSH group, the concentration of LH was immaterial, with all values lying within the 'normal range'. In the high FSH group, when the LH was low, the circulating progesterone was significantly higher. The most striking group observation was when there was a combination of a large follicular mass, high FSH and low LH. Here, circulating concentrations of progesterone breached the 'normal range' on average 4 days prior to the day of HCG administration. By contrast, when LH concentrations were normal even with a large follicular mass and high FSH, elevated circulating progesterone concentrations occurred on average 2 days prior to the day of HCG administration. The RFPPC in many women during ovarian stimulation were equivalent to concentrations seen 2–3 days after the LH surge in a normal cycle. At the extreme of this effect, an asynchrony between endometrial and embryo development can be expected, as embryo development does not start until the luteinization signal.

Clinical implications of multiple follicular development in ovarian stimulation

It is important to establish whether there is evidence supporting the hypothesis that these changes in hormone concentrations are clinically significant and whether they can be predicted. One large, prospective study provides clear circumstantial evidence supporting a clinical consequence of this phenomenon. The MERiT study compared ongoing pregnancy rates in 731 young, normogonadotrophic women undergoing IVF after stimulation with highly purified human menopausal gonadotrophin (HP-HMG; Menopur; Ferring Pharmaceuticals A/S, Denmark; $n = 363$) or recombinant FSH (rFSH; follitropin alfa, Gonal-F; Serono, Switzerland; $n = 368$) following a long GnRH-agonist protocol (Andersen et al., 2006; Smitz et al., 2007). The HP-HMG formulation contains both FSH activity and HCG-driven LH activity, whereas rFSH contains only the FSH component. The critical value for defining 'elevated progesterone' concentration in this study was 4 nmol/l on the last day of stimulation. The serum progesterone concentrations were higher in rFSH-treated patients than in HP-HMG-treated patients, with the former showing a higher incidence of elevated circulating progesterone (23% versus 11%; $P < 0.001$: Andersen et al., 2006). In both treatment groups, elevated progesterone concentrations were associated with high ovarian responses (i.e. high oocyte yield), in parallel with the high follicular mass discussed above. Critically, patients showing higher progesterone values showed lower embryo implantation rate (HP-HMG, 24% versus 19%, not statistically significant; rFSH, 23% versus 11%, $P = 0.025$). These findings suggest that there may be a clinical consequence of a pre-HCG rise in progesterone. Furthermore, it appears that the effect may be seen at its most critical in those women considered to be 'ideal' (high-responding) patients, with a low body mass (associated with higher circulating FSH concentrations). According to the hypothesis, those cases showing raised progesterone on the last stimulation day may have breached 'normal' concentrations some days beforehand, which may be responsible for advancement of endometrial maturation, leading to asynchrony with embryo development and a detrimental effect on implantation.

As predicted by the hypothesis, the elevated progesterone concentration in the circulation is most pronounced in patients with large follicular response and stimulated with gonadotrophins that contain FSH activity alone. By contrast, the HCG-driven LH activity in HP-HMG stimulation may offset the rise in progesterone by stimulating thecal cell activity towards the catabolism of progesterone to androgens and, thereafter, metabolism to oestrogens in granulosa cells.

Bosch et al. (2010) have reported that high serum progesterone concentration on the day of HCG administration is a frequent event in cycles with both GnRH agonists and antagonists and, when observed, it is associated with a decreased pregnancy rate. The progesterone threshold concentration of 4 nmol/l in the MERiT study is equivalent to 1.26 ng/ml, which is similar to that used in the Bosch study (1.5 ng/ml, or 4.77 nmol/l), above which ongoing pregnancy rates fell. This evidence presents a strong case that elevations of progesterone derived from non-luteinized follicles, prior to HCG administration, can reduce pregnancy potential. This effect is most important in the young, high-responder patient (i.e. the 'ideal' patient) who is treated with GnRH agonists and with 'pure' FSH.

Discussion

It is postulated that all three factors examined — the number of follicles, the FSH drive and the LH activity — influence the concentration of progesterone in the circulation during the follicular phase of ovarian stimulation. The follicle number and FSH concentrations appear to have a positive association with raised progesterone output. The net effect in the more extreme examples is likely to lead to embryo/endometrial asynchrony, which may reduce the chance of implantation. By contrast, LH activity drives thecal cells to decrease progesterone concentrations in the circulation. The combination of scientific evaluations using a validated assay for progesterone and the circumstantial observations in the MERiT study — endometrial echogenicity and higher progesterone concentrations with FSH alone — supports this rationale (Andersen et al., 2006).

One further question to address is whether the measurement of progesterone in each cycle may help to improve implantation rates and, ultimately, pregnancy outcomes? In theory, yes; however, in the past poor reliability of assay methods at these low progesterone concentrations offered weak prospect of benefit (Fleming, 2008). More appropriate methods are now available (Coucke et al., 2007) and a prospective examination of appropriate strategic responses may prove beneficial. Although this concept appears to be relevant to a relatively modest proportion of the total patient cohort, it does indicate that responses to ovarian stimulation do impact upon outcome and that strategic approaches and drug choices reflecting the follicular demand for LH activity should be adapted to accommodate this.

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References

Achache, H., Revel, A., 2006. Endometrial receptivity markers, the journey to successful embryo implantation. Hum. Reprod. Update 12, 731—746.

Andersen, A.N., Devroey, P., Arce, J.C., 2006. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. Hum. Reprod. 21, 3217—3227.

Bosch, E., Valencia, I., Escudero, et al., 2003. Premature luteinization during gonadotropin-releasing hormone antagonist cycles and its relationship with in vitro fertilization outcome. Fertil. Steril. 80, 1444—1449.

Bosch, E., Labarta, E., Crespo, J., et al., 2010. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for in vitro fertilization: analysis of over 4000 cycles. Hum. Reprod., doi:10.1093/humrep/deq125.

Bourgain, C., Devroey, P., 2003. The endometrium in stimulated cycles for IVF. Hum. Reprod. Update 9, 515—522.

Coucke, W., Devleeschouwer, N., Libeer, J.C., et al., 2007. Accuracy and reproducibility of automated estradiol-17beta and progesterone assays using native serum samples: results obtained in the Belgian external assessment scheme. Hum. Reprod. 22, 3204—3209.

Coutts, J.R.T., Gaukroger, J.M., Kader, A.S., et al., 1981. Steroidogenesis by the Human Graafian Follicle: Functional Morphology of the Human Ovary. MTP Press, Lancaster, England, pp. 56—72.

Fleming, R., 2008. Progesterone elevation on the day of hCG: methodological issues. Hum. Reprod. Update 14, 391—392.

Fleming, R., Adam, A.H., Barlow, D.H., et al., 1982. A new systematic treatment for infertile women with abnormal hormone profiles. Br. J. Obstet. Gynaecol. 89, 80—83.

Moon, Y.S., Tsang, B.K., Simpson, C., et al., 1978. 17Beta-estradiol biosynthesis in cultured granulosa and thecal cells of human ovarian follicles: stimulation by follicle-stimulating hormone. J. Clin. Endocrinol. Metab. 47, 263—267.

Smitz, J., Andersen, A.N., Devroey, P., et al., 2007. Endocrine profile in serum and follicular fluid differs after ovarian stimulation with HP-hMG or recombinant FSH in IVF patients. Hum. Reprod. 22, 676—687.

Ubaldi, F., Smitz, J., Bourgain, C., et al., 1996. Premature luteinization in in vitro fertilization cycles using gonadotropin-releasing hormone agonist (GnRH-a) and recombinant follicle-stimulating hormone (FSH) and GnRH-a and urinary FSH. Fertil. Steril. 66, 275—280.

Venetis, C.A., Kolibianakis, E.M., Papanikolaou, E., et al., 2007. Is progesterone elevation on the day of human chorionic gonadotrophin administration associated with the probability of pregnancy in in vitro fertilization? A systematic review and meta-analysis. Hum. Reprod. Update 13, 343—355.

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