Controlled ovarian stimulation, progesterone, growing follicles, and progesterone assays

Hill et al. report in this issue of the journal (1) a new analysis of assisted reproductive technology (ART) outcome observed in women with elevated serum P on the day of hCG. Data based on 7,608 fresh ART cycles confirm the now widely published negative impact of pre-hCG P elevation on ART outcome. The originality of this article lies in the fact that Hill et al. queried whether the ratio of pre-hCG P over the number of oocytes (P:O) retrieved bore a possibly stronger predictive value for occurrence of pregnancy than serum P alone.

Hill et al. observed no added value of the P:O ratio over that provided by P (negative) and the number of oocytes (positive). Based on their findings, the authors conclude that it is the absolute P elevation that exerts a negative effect on endometrial receptivity independently of the number of oocytes retrieved. The authors imply therefore that whether a small number of follicles produce relatively large amounts of P, or the opposite, bears no practical consequences on ART outcome. Hence, the ovarian mechanism(s) responsible for the pre-hCG P elevation—likely different for high and poor responders—is (are) of no practical relevance.

The findings reported by Hill et al. are intriguing to those investigators—including us—who believed that pre-hCG P elevation bore a more ominous prediction when observed in poor as compared with high responders (2). Hill et al.'s findings are therefore provocative and prompted us to comment on the respective role of pre-hCG P, ovarian follicles, and P assays in controlled ovarian stimulation (COS) for ART. As described below, some of the discrepancies between the reported data on pre-hCG P elevation may find their root cause in P assay issues.

P ELEVATION IN ART

Routine pituitary suppression during COS starting when GnRH agonists (GnRH-a) became available initially led to an erroneous belief that pre-hCG P elevation was a worry of the past. This was soon proved untrue. Over 2 decades ago indeed, Schoolcraft et al. (3) and Fanchin et al. (4) and others observed pre-hCG elevations of P in some ART women despite endogenous gonadotropins being blocked by GnRH-a. In these early reports (3, 4), ART outcome with fresh ETs was decreased when P exceeded 0.9 ng/mL. Our threshold of 0.9 ng/mL was observed when using a radioimmunoassay (RIA) on extracted serum or plasma (3, 4), whereas studies reporting higher cutoff values used direct assay on unextracted serum and enzyme immunoassay.

Other reports confirmed the existence of pre-hCG P elevation in ART despite using GnRH-a but did not observe a decrease in ART outcome. Some investigators even reported higher pregnancy rates (PRs) in the case of pre-hCG P elevation.

More recently, however, large studies reviewed by Hill et al. (1) provided overwhelming evidence that late follicular phase P elevation alters ART in both agonist and antagonist cycles. These results were subsequently confirmed by others in systematic reviews and meta-analyses. Collectively, these later publications all reported lower PR after fresh but not cryopreserved ETs or donor egg ART (1).

P AND THE ENDOMETRIUM

In the menstrual cycle, P produced after ovulation induces transformations in the endometrial glands and stroma that collectively constitute the secretory changes of the endometrium. The early steps of these transformations—in endometrial glands—are actually initiated by the slight increase in P taking place in the late follicular phase.

In ART, however, the elevated E_2 levels induced by COS are known to cause some degree of resistance to the effects of P. In biopsies performed in oocyte donors, retarded decidualization was seen in high responders, also indicating that high E_2 levels in ART interfere with P effects. In light of these findings, it is possible that COS responses modulate the endometrial consequences brought by pre-hCG P elevations.

P AND DEVELOPING FOLLICLES

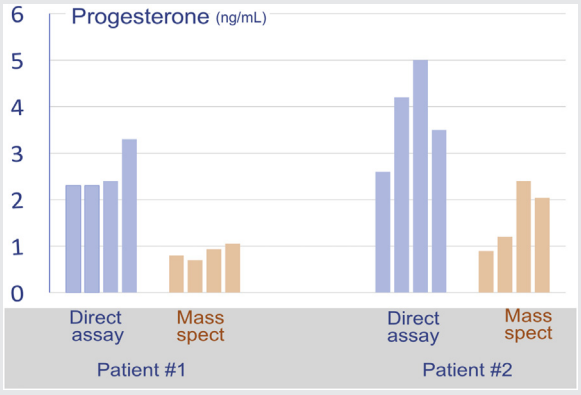
There is now a host of converging reports indicating that P elevation—pre- or postovulation—does not hamper oocyte quality or follicular growth. Overwhelming evidence of this is provided by publications that indicate that COS deliberately conducted in the luteal phase provides oocytes and embryos of similar quality and number as those obtained in the follicular phase. Likewise, so-called random-start COS protocols proposed in fertility preservation when time constraints preclude conventional follicular phase starts offer yields independent of when COS is started. Finally, in back-to-back or so-called duplex protocols we and others have shown that the first and second COSs have similar yields in spite of elevated serum P during COS number 2.

The fact that the follicular response to COS is independent of luteal P—demonstrated by the luteal phase, random-start, and duplex protocols—points at the endometrium as the primary target of the pre-hCG P elevation. This view—supported by Hill et al.'s findings—does not take into account two modulating factors: [1] as discussed above, the high E_2 levels of excessive COS responses may cause some degree of P resistance and thus mitigate the impact of pre-hCG elevations; and [2] as discussed below, P assay issues may differently affect high and poor responders to COS.

P ASSAYS

P assays have been tested and validated for measuring P produced by the luteinized granulosa cells forming the corpus luteum after exposure to the LH surge. Specificity, accuracy, and precision have been indeed defined—against values obtained by extraction-separation or mass spectrometry assays—in the presence of the pattern of P metabolites encountered in the luteal phase of the menstrual cycle.

FIGURE 1



P measurements for the last 4 days of COS in two high responder patients by direct assay and mass spectrometry (ng/mL). In both cases, hCG was withheld due to high risk of OHSS.

de Ziegler. Reflections. Fertil Steril 2016.

Measurements conducted in the late follicular phase therefore fall outside this tested envelope.

Today P is measured by direct assays managed by automated platforms. Four of the most commonly used assays compared against data obtained by mass spectrometry revealed that P data obtained in the low range—0.9–2.5 ng/mL—are of poor precision, accuracy, and reproducibility. Consonant with these findings, we made a seminal observation in two high responders to COS in whom hCG administration had been withheld because of the risk of ovarian hyperstimulation syndrome (OHSS) (5). As illustrated in Figure 1, these two women had 4 days of P elevation in excess of 0.9 ng/mL, after which—the ART cycle being cancelled—they had an endometrial biopsy. In both cases, the endometrial biopsy that was read by a pathologist experienced in reproductive histology showed no sign of secretory transformation. A secondary measurement of plasma P by mass spectrometry showed substantially lower P levels (Fig. 1). Taken together, these findings indicate that in these two very high responder patients, the 4-day pre-hCG P elevation detected by direct assay was not confirmed by mass spectrometry measurements and caused no endometrial changes. Hence, the direct measurements did not reflect true P elevations. Similar grossly erroneous findings have been made when using direct P assays for measuring P after oral administration. In this case, the grossly different metabolite profile encountered after oral administration and hepatic metabolism is the cause for the erroneous findings. Steroid measurements in follicular fluid conducted by one of us (D.R.M.) raised similar concerns (6). T measurement after serum extraction, separation on celite columns, and RIA resulted in levels that were a small fraction of those previously reported using direct measurements on serum (6). The T assay used was validated by finding an identical celite column profile of immunoreactive T and authentic radiolabeled T.

CONCLUSION

Hill et al. showed that the P:O ratio provides no added value for predicting ART outcome over that offered by P or the oocyte number alone (1). Hill et al.'s data indicate that with current assays, pre-hCG P elevation generally has a predictive value that is not dependent upon the number of oocytes retrieved. This does not exclude, however, that in certain circumstances—as with simplified assays currently used in most ART laboratories and therefore in most ART studies—pre-hCG P values may be erroneous in very high responders to COS, as in our two patients cancelled for OHSS risk.

Dominique de Ziegler, M.D.[a]
Renato Fanchin, M.D.[b]
David Meldrum, M.D.[c]

[a] Université Paris Descartes, Paris Sorbonne Cité—Assistance Publique Hôpitaux de Paris, CHU Cochin, Department of Obstetrics and Gynecology and Reproductive Medicine, Paris, France; [b] Center of Reproductive Medicine, Hôpital Foch, University Paris-Ouest, Suresnes, France; and [c] Reproductive Partners La Jolla and Division of Reproductive Endocrinology, University of California, San Diego, San Diego, California

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REFERENCES

1. Hill MJ, Healy MW, Richter KS, Widra E, Levens ED, DeCherney AH. Revisiting the progesterone to oocyte ratio. Fertil Steril 2017;107:671–6.
2. Fanchin R, Righini C, Olivennes F, Ferreira AL, de Ziegler D, Frydman R. Consequences of premature progesterone elevation on the outcome of in vitro fertilization: insights into a controversy. Fertil Steril 1997;68:799–805.
3. Schoolcraft W, Sinton E, Schlenker T, Huynh D, Hamilton F, Meldrum DR. Lower pregnancy rate with premature luteinization during pituitary suppression with leuprolide acetate. Fertil Steril 1991;55:563–6.
4. Fanchin R, de Ziegler D, Taieb J, Hazout A, Frydman R. Premature elevation of plasma progesterone alters pregnancy rates of in vitro fertilization and embryo transfer. Fertil Steril 1993;59:1090–4.
5. Romoscanu I, Stanczyk F, Bourgain C, Weintraub J, Paulson R, de Ziegler D. An elevation of plasma Progesterone (P4) during the last 4 days of controlled ovarian hyperstimulation (COH) fails to affect endometrial morphology, thus raising doubts about the veracity of P4 measurements in these circumstances. 21th Annual Meeting of ESHRE, Copenhagen, Denmark, June 20-22, 2005.
6. Cedars MI, Surey E, Hamilton F, Lapolt P, Meldrum DR. Leuprolide acetate lowers circulating bioactive luteinizing hormone and testosterone concentrations during ovarian stimulation for oocyte retrieval. Fertil Steril 1990;53:627–31.