



The role of progesterone/estradiol ratio in exploring the mechanism of late follicular progesterone elevation in low ovarian reserve women



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ABSTRACT

Late follicular progesterone (P) elevation continues to complicate a significant part of assisted reproductive technologies (ART) cycles, despite the ardent employment of gonadotropin releasing hormone (GnRH) analogues. In women with good ovarian reserve, late follicular P elevation is believed to be the result of the controlled ovarian stimulation (COS) itself, multiple follicular development and excessive ovarian steroidogenic activity. These mechanisms do not seem to be plausible in women with low ovarian reserve. In these women, excessive COS achieve a small number of pre-ovulatory follicles, which is not coupled to considerable ovarian steroidogenic activity. Therefore, other mechanisms should be pursued and explored.

Delicate paracrine and autocrine mechanisms within the oocyte-cumulus complex were suggested to preserve the integrity of the pre-ovulatory follicle, including inhibition of P increase and follicular luteinization. However, clinical studies to demonstrate the disruption of these mechanisms in cases with low ovarian reserve and ageing oocytes are still lacking. Late follicular progesterone/estradiol (P/E₂) ratio was introduced into clinical practice as a more appropriate way to analyze P rise in women undergoing COS to control for the E₂ increase. The current hypothesis claims that in a follicular environment were the mechanism that prevent premature luteinization is disrupted, independent to LH surge; P rise at the late follicular phase may relatively bypass normal E₂ production, at the pre-ovulatory stage of steroidogenesis, causing a rise in the P/E₂ ratio. Therefore, in women with low ovarian reserve and few pre-ovulatory follicles, undergoing conventional COS, a negative (reverse) correlation between number of maturing follicles and P/E₂ ratio may support the existence of such a mechanism as its disruption, while a no or a positive correlation may disapprove it.

Introduction

Elevated late follicular progesterone (P) continues to complicate a significant part of assisted reproductive technologies (ART) cycles, despite the persistent use of gonadotropin releasing hormone (GnRH) agonist and antagonists in this setting. An active discussion on the mechanisms underlying this phenomenon continues to be comprehensively dynamic. Furthermore, it is still controversial whether late follicular P elevation may adverse clinical outcome in ART as the literature continues to be divided between pros and cons. The cut-off level of P at the late follicular phase that may be detrimental for successful implantation and early pregnancy maintenance is still not settled [1–4].

It is believed today, that under the GnRH analogue regimens, elevated serum late follicular P concentration, in good ovarian reserve women, is related to controlled ovarian stimulation (COS) itself, multiple follicular development and increased ovarian steroidogenic

activity [4–9]. In these patients, the impact of elevated P on adverse ART outcome seems to be mainly on the endometrium and the window of implantation, leading to an asynchrony between the endometrium and the developing embryo [10,11]. Furthermore, in this group of women elevated P is advocated to have no negative effect on the oocyte or embryo quality [12,13].

Excessive ovarian stimulation does not necessarily cause the development of high number of pre-ovulatory follicles. Women with low ovarian reserve receiving high doses of gonadotropins achieve a small number of mature follicles, which does not lead to considerable ovarian steroidogenic activity. It is therefore not reasonable to impute late follicular P rise to an increased ovarian steroidogenic activity in these women. Other mechanisms should be searched for and explored [14]. The literature is still sparse concerning the mechanism of P elevation in women with low number of pre-ovulatory follicles in the ART setting. Whether P elevation in low ovarian reserve women is related only to

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the number of pre-ovulatory follicles or whether there are additional, mechanisms underlying this phenomenon is still to be explored.

The hypothesis

Delicate paracrine and autocrine mechanisms within the oocyte-cumulus complex were suggested to preserve the integrity of the pre-ovulatory follicle, including inhibition of P increase and follicular luteinization [15]. However, clinical studies to demonstrate the disruption of these “gate-keeper” mechanisms in cases with low ovarian reserve and ageing oocytes are lacking.

Serum progesterone/estradiol (P/E₂) ratio on the day of hCG administration was introduced as a more appropriate way to analyze P rise in women undergoing COS in the ART setting to control for E₂ increase [16,17]. The linear equation presented to calculate the P/E₂ ratio was $P \text{ (ng/mL)} \times 1000 / E_2 \text{ (pg/mL)}$. Over the years, several other groups have employed this measure to evaluate late follicular P level in the same setting [18–26].

In a follicular environment where the mechanism that prevent premature luteinization is disrupted, independent to LH surge, it may be speculated that P rise at the late follicular phase may relatively bypass normal E₂ production, at pre-ovulatory stage of steroidogenesis, causing a rise in the P/E₂ ratio. Therefore, in women with low ovarian reserve and few pre-ovulatory follicles, a negative (reverse) correlation between number of maturing follicles and P/E₂ ratio may support the existence of such a mechanism as its disruption, while a no or a positive correlation may disapprove it.

Evaluation of the hypothesis

Clinical studies

To the best of our knowledge, the topic of late follicular phase serum P concentration in women with low number of pre-ovulatory follicles under GnRH analogue treatment has not been previously targeted in a prospective clinical study. Indirect evidence from previous studies in the literature may support the concept of disrupted mechanisms related to the oocyte-follicle apparatus.

Elevated P level at the late follicular phase, was found to adversely affect pregnancy rate only in the weak responder group but not in the intermediate and strong ovarian response [27] suggesting a role for oocyte/follicle quality in this setting. Other investigators have found that elevated P at the late follicular phase significantly reduces top embryo quality and live birth rate [28–30] challenging previous observations that elevated P has no adverse effect on oocyte/embryo quality [12,13].

Furthermore, employing the long GnRH agonist treatment in the ART setting, follicular fluid P/E₂ ratio was found to be much higher in low responders as compared to controls, suggesting accelerated luteinization in these women [31]. Wu et al in a similar setting has demonstrated age-related functional decline in granulosa cell function, consistent with premature luteinization [25].

Basic research studies

The suggested hypothesis seems also to be backed-up by in-vitro studies, in human and animal models, targeting the intricate interaction between the oocyte and follicle. Sadeu et al have found that cigarette smoke condensate exposure inhibits follicle development and leads to premature luteinization in a mouse isolated follicle culture system that mimics murine folliculogenesis in vivo [32].

Experiments are accumulating to advocate that the oocyte itself play a major role in directing its own fate as well as the growth and differentiation of the follicle. This controlling capability has been shown to be accomplished with oocyte-secreted factors (OFS) by paracrine and autocrine mechanisms through the somatic cells within the follicle

(granulosa and cumulus cells). These factors, mainly growth-differentiation factor 9 and bone morphogenetic protein 15, activate signaling pathways in the somatic cells of the follicle at the pre-ovulatory phase to maintain its integrity including inhibition of P increase and follicular luteinization [15,33,34]. It is only reasonable to presume that in an environment where the quality of the oocyte is reduced, as in ovarian ageing cases, the OFS's will halt to act favorably leading to pre-ovulatory premature luteinization.

Testing the hypothesis

The suggested hypothesis could be prospectively tested in the ART setting focusing on women with low ovarian reserve. Women could be a priori selected, prior to COS initiation, based on the Bologna criteria or the POSEIDON criteria of poor ovarian response [35,36]. Alternatively, they could be selected, following conventional COS employing a gonadotropin-releasing analogue regimen to prevent premature LH surge. Retaining this approach, the selection is suggested to be performed on the day of HCG administration, to ensure a low number of developing pre-ovulatory follicles. Statistical correlation tests between serum E₂ level, serum P level, P/E₂ ratio and number of pre-ovulatory follicles on the day of hCG administration as well as number of oocytes and embryos achieved may be performed to look for significant positive or negative (reverse) correlations among these parameters. A negative (reverse) correlation between number of pre-ovulatory follicles and P/E₂ ratio may support the existence of a “gate-keeper” mechanism as its disruption. This oocyte-follicle mechanism acts to prevent early luteinization, independent to premature LH surge. Conversely, a no or a positive correlation between number of pre-ovulatory follicles and P/E₂ ratio may disapprove such a mechanism and support increased ovarian steroidogenic activity as the dominant mechanism of late follicular P elevation in all women undergoing COS.

Other approaches could be employed to test the existence of disrupted oocyte-follicle mechanisms. One of these approaches is to study the performance of the P/follicle ratio suggested by Shufaro et al. [37], originally conducted in a normal responder population. Finding a higher P/follicle ratio in low responder population as compared to controls may support the hypothesis further. In addition, evaluation of follicular fluid steroidogenic content, as have been previously suggested [31], could shed more light on testing the hypothesis. In all of these suggested studies, the role of FSH versus FSH and LH content of the COS employed is important to evaluate, to negate an inadequate LH dosing that may associated with a risk for a late follicular P elevation [7,38].

Consequences of the hypothesis and discussion

Current hypothesis if approved by clinical and experimental studies may pave the way for understanding the basic mechanisms of late follicular P elevation in low ovarian reserve women. It may also support the idea that the ageing oocyte is responsible for the fate of the maturing pre-ovulatory follicle. Furthermore, it could possibly provide ways to overcome this concern in low ovarian reserve women presenting with late follicular P during COS in the ART setting. Seeking to improve clinical outcome, one way to do that is to schedule oocyte retrieval at an earlier stage of the treatment cycle [25].

A key issue that should be taken into account, when engaged in such clinical studies, is to employ a serum P assay that is sensitive enough to subtle increase in P concentration as is the case in late follicular phase. Direct steroid immunoassays, mostly employed today to evaluate luteal P are convenient, simple, rapid, and relatively inexpensive and have a high throughput, however they lack specificity resulting in over-estimated values [39]. The accuracy of this method for quantifying low serum P concentrations has been recently questioned. Unconjugated P metabolites may cross-react with the P antiserum in a direct immunoassay and this may result in falsely elevated serum P level [37]. Conversely, the introduction of liquid chromatography/tandem mass

spectrometry assays in the past two decades has resulted in considerable improvements in the sensitivity, specificity, and automation of steroid hormone measurements [40]. This delicate issue of the P assay should be taken into consideration when planning a study focusing on late follicular P and P/E₂ ratio.

Furthermore, since DHEA supplementation, often prescribed for poor responders in the ART setting, may interfere with standard P immunoassays [41], such a supplementation ought to be excluded from women recruited to such a study.

Prospective, well-designed and targeted studies, employing late follicular P/E₂ ratio in women defined as poor ovarian response or with low number of pre-ovulatory follicles in the ART setting, may shed new light on the pathogenesis of elevated late follicular P in women with low ovarian reserve.

Statements of ethics

- The author declares he has no conflict of interest.
- The author declares he has not received any financial support for this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2019.02.047>.

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