Original Article

The Enigma of Early Progesterone Rise: Is It Associated with the Type of Gonadotropin Used?

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Abstract

Background: It is debated whether the use of recombinant follicle-stimulating hormone (r-FSH) or human menopausal gonadotropin (HMG) is associated with progesterone (P) elevation in the late follicular phase. **Aims:** The aim is to determine whether the type of gonadotropin used for controlled ovarian stimulation (COS) in *in vitro* fertilization (IVF) is associated with P elevation in the late follicular phase using antagonist protocol. **Setting and Design:** This was a retrospective data analysis in the IVF unit. **Methods:** Data of women who underwent COS between January 2005 and December 2017. **Statistical Analysis:** Chi-square and unpaired Student's *t*-test in SPSS 20. **Results:** Data of 439 women was analyzed. Mean age was 31.45 ± 4.6 years. HMG was used in 193 (44%), r-FSH in 232 (52.8%), and a combination of both in 14 (3.2%) women. Proportion of women with elevated P was significantly higher in r-FSH group as compared to HMG group (19/232 [8.2%] vs. 6/193 [3.1%]; $P = 0.027$). Mean P levels were significantly higher in r-FSH group (0.75 ng/ml vs. 0.59 ng/ml; $P = 0.049$). Mean estradiol (E2) levels at trigger were significantly higher in women with elevated *P* as compared to normal *P* (2893.4 ± 2091.8 pg/ml vs. 1668.3 ± 1508.6 pg/ml respectively; $P < 0.000$). Fresh embryo transfers performed in 18/27 women with elevated P resulted in pregnancy in three (16.7%) women. Two had biochemical pregnancies and one was lost to follow-up. **Conclusions:** Use of r-FSH and E2 levels at trigger are associated with elevated P levels in the late follicular phase. Fresh embryo transfers performed in spite of elevated P levels were associated with low pregnancy rates and unfavorable outcomes.

Keywords: *Early progesterone rise, gonadotropin, human menopausal gonadotropin, recombinant follicle-stimulating hormone*

Introduction

The menstrual cycle involves a complex interplay of various hormones and autocrine and paracrine factors. Under the influence of follicle-stimulating hormone (FSH), selection of the dominant follicle is seen between day 5 and day 7 of the cycle. Estradiol (E2) derived from the dominant follicle steadily increases. The rising E2 level exerts a negative feedback on FSH levels and a positive feedback on the luteinizing hormone (LH) levels. Once a certain E2 threshold level is reached, the LH surge is initiated. The LH surge leads to luteinization of the granulosa cells, progesterone (P) production, and synthesis of prostaglandins in the follicle. This, together with the activity of proteolytic enzyme, leads to digestion and rupture of the follicle, that is, ovulation.

In vitro fertilization (IVF) necessitates the use of controlled ovarian stimulation (COS) for multifollicular development as against the unifollicular development of natural cycles. Relatively high doses of gonadotropins are administered to keep the gonadotropin levels above a certain threshold to support multifollicular growth.[1]

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These multiple follicles produce supraphysiological E2 levels with subsequent activation of the LH surge.

Gonadotropin-releasing hormone analogs (GnRHa) are used to prevent a premature LH surge. Prevention of LH surge using GnRHa is of utmost importance as it improves oocyte yield and number of embryos, allowing better selection, and consequently, better pregnancy rates.[2]

Whether clinical decisions have an impact on the early rise in progesterone levels is a question which warrants attention. Multiple factors influence the rise in the serum P concentration in the late follicular phase. The primary factor is the type of gonadotropin used for COS, namely, human menopausal gonadotropin (HMG) or recombinant FSH (r-FSH). The initial management decision pivots around the choice of gonadotropin to be used, namely, HMG or r-FSH. HMG is a urinary gonadotropin and is much cheaper than r-FSH. On the other hand, the bioactivity of r-FSH is accurate and predictable with no batch-to-batch variation. HMG, being a urinary derivative, may show inconsistent bioactivity of FSH between batches. HMG contains FSH and LH bioactivity in the ratio of 1:1, while r-FSH contains FSH alone. In the antagonist cycles with the endogenous LH suppressed, pharmacological LH administration would appear to be an apparent causative factor for P elevation. It would, therefore, seem logical that women who receive HMG for COS should have a higher incidence of premature P elevation. It has been documented by Filicori *et al*. that high levels of *P* at the end of stimulation and prior to human chorionic gonadotropin (hCG) administration are related to the FSH activity and not to LH activity.[3] Several other studies have shown that P concentrations in the late follicular phase were higher in women receiving r-FSH than those receiving HMG.[4-6] This demonstrates that the relationship between LH and P elevation is a complex one. The complexity is further reinforced by the LH threshold and ceiling concept. According to this concept, a minimum level of LH is required (threshold) for follicular growth and maturation, while beyond a certain level (ceiling), LH proves detrimental to the follicles.

The present study was aimed at determining whether the type of gonadotropin used for COS is associated with the incidence of P elevation in the late follicular phase before the administration of the trigger for oocyte maturation. Such an association could provide additional information for selection of appropriate gonadotropin for COS. In addition, we assessed the association, if any, between E2 levels and P elevation. We also examined pregnancy outcomes in patients in whom fresh embryo transfers were carried out in spite of elevated P levels to help guide patient selection for freeze-all strategy.

Methods

The study was approved by the Institutional Ethics Committee. Data were retrieved from the hospital's electronic medical record system. Analysis was carried out of data of all women who underwent COS for IVF with antagonist protocol in our superspecialty hospital between January 2005 and December 2017. Data of patients with all protocols other than the antagonist protocol were excluded. Variables of interest were age of patient, type of gonadotropin used, E2 and progesterone levels, and number of fresh embryo transfers in the elevated P group and their outcomes. Data were entered into SPSS (version 20) for Windows package (SPSS Science, Chicago, IL, USA) and analyzed. Categorical data have been expressed as frequencies (e.g., use of different types of gonadotropins), while continuous data such as age, E2, and P levels at trigger have been expressed as mean, range, and standard deviation. For examining associations such as between categorized *P* values at trigger and gonadotropin used, Chi-square test was employed. Unpaired Student's *t*-test was used to test the statistical significance of difference in the means of two independent groups (such as mean E2 levels between normal and elevated P groups). $P < 0.05$ was considered statistically significant.

Results

Data of 439 women were analyzed. The mean age of women in our sample was 31.45 ± 4.6 years (range was 19–43 years). The most frequently used gonadotropin was r-FSH (232, 52.8%), followed by use of HMG in 193 (44%) women and a combination of both was used in 14 (3.2%) women [Figure 1]. As per standard hospital policy, HMG and r-FSH used belonged to the same manufacturer, respectively.

As can be seen from Figure 2, P levels at trigger were high (≥1.5 ng/ml) in 19/232 (8.2%) women on r-FSH as compared to 6/193 (3.1%) women on HMG. This

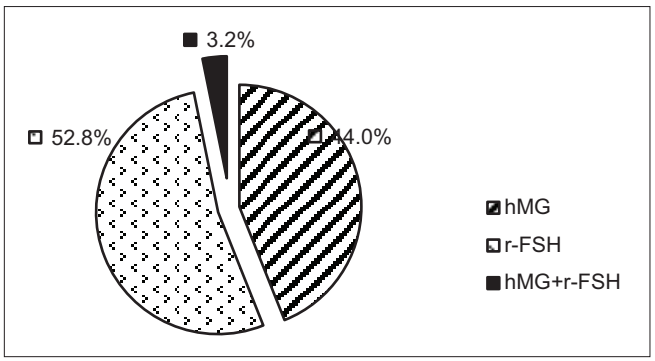

Figure 1: Gonadotropins used

difference was statistically significant ($P = 0.027$). When mean P levels at trigger were examined according to gonadotropin used, it was observed that mean P level in the r-FSH group was higher (0.75 ng/dl, range 0.09 ng/dl to 13.7 ng/dl) as compared to the HMG group (0.59 ng/dl, range 0.03 ng/dl to 3.2 ng/dl). This difference was also significant ($P = 0.049$). Our data revealed that 2/14 women who received a combination of r-FSH and HMG also had elevated P levels. These have not been included in the comparative analysis.

Mean ages of women in the r-FSH and HMG groups were 31.4 years and 31.5 years, respectively ($P = 0.8$, not significant). Similarly, there was no significant difference in the mean ages of women in the elevated and normal P level groups (31.2 years vs. 31.4 years respectively, $P = 0.8$).

Mean E2 level at trigger in patients with elevated P was 2893.44 pg/ml (range 344 pg/ml to 9680 pg/ml) and that in patients with normal P was 1668.26 pg/ml (range 57 pg/ml to 16,132 pg/ml). This difference was highly statistically significant ($P < 0.000$) [Figure 3]. When assessed according to the gonadotropin used, it was found that mean E2 level in women on r-FSH (1840.12 pg/ml, range 57 pg/ml to 16,132 pg/ml) was higher than that in women on HMG (1647.53 pg/ml, range 148 pg/ml to 11,300 pg/ml). This was, however, not found to be statistically significant ($P = 0.225$).

Fresh embryo transfers were performed in 18 out of 27 (66.6%) women with P levels ≥1.5 ng/ml. All transfers were carried out on day three. Fifteen patients had Grade I embryos, two patients had Grade II, and one had Grade III embryos. Pregnancy test was negative in 15 (83.3%) women. Out of the 3 (16.7%) women in whom beta-HCG was positive, two had biochemical pregnancies and one was lost to follow-up.

Out of 412 women with P levels <1.5 ng/ml, 10 women were treated using a combination of HMG and r-FSH, all embryos were frozen in 35 women, while 30 women were lost to follow-up. Hence, data of 337 women with P levels <1.5 ng/ml in whom fresh embryo transfers were performed is presented to determine the pregnancy rates when either HMG or r-FSH was used [Table 1]. Pregnancy test was positive in 106 (31.5%) women.

There was no statistically significant difference in the pregnancy rates when either HMG or r-FSH was used (31.4% vs. 31.5%, respectively, $P = 0.536$).

Discussion

Elevated progesterone concentration in the late follicular phase is defined as serum progesterone (P) levels above 1.5 ng/ml.[7] The rationale for this cutoff is that the endometrial gene profile expression is markedly different

Table 1: Pregnancy outcomes in women with normal P levels according to hormone used

Hormone used	Pregnancy outcome		Total, *n* (%)
	Positive, *n* (%)	Negative, *n* (%)	
HMG	48 (31.4)	105 (68.6)	153 (100)
r-FSH	58 (31.5)	126 (68.5)	184 (100)
Total	106 (31.5)	231 (68.5)	337 (100)

HMG=Human menopausal gonadotropin, r-FSH=Recombinant follicle-stimulating hormone

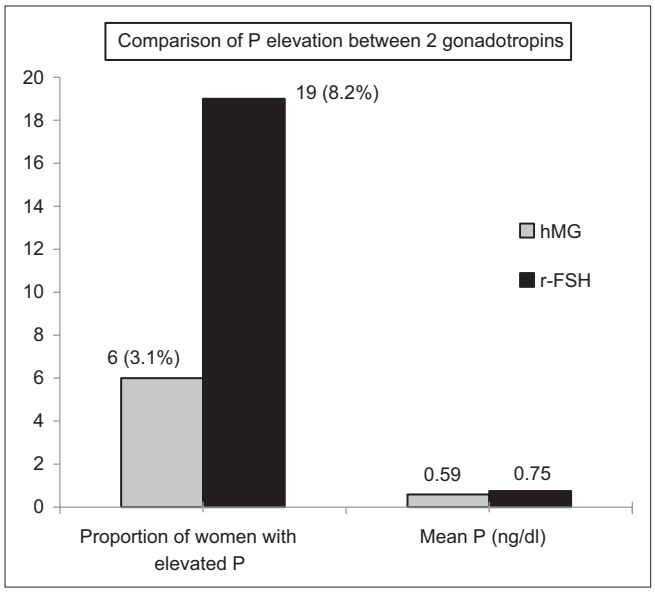

Figure 2: Comparison of proportion of women having P elevation ($P > 1.5$ ng/ml) (between human menopausal gonadotropin and recombinant follicle-stimulating hormone)

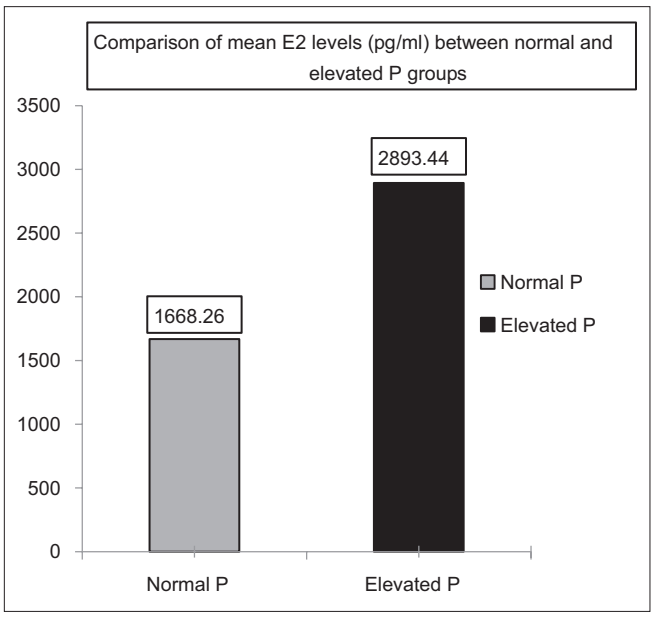

Figure 3: Comparison of mean estradiol levels at trigger between patients with normal ($n = 398$) and elevated P ($n = 25$)

above and below the level of 1.5 ng/ml.[7,8] This rise in progesterone occurs in spite of the LH surge being suppressed by the GnRH antagonist. This is different from premature luteinization, where the progesterone rise follows the LH surge.

In a large randomized controlled trial by Anderson, Devroey, *et al.* for the MERIT group, P concentrations were found to be significantly higher in the r-FSH group.[9] Similar finding was observed by Bosch *et al*. in their analysis of over 4000 cycles and by Werner *et al*. in their study comprising 10,280 first IVF cycles.[6,10] The results of our study also show that P elevation in the late follicular phase was significantly higher in the r-FSH group as compared to the HMG group. Fleming and Jenkins, in their commentary, have identified that the type of gonadotropin used for ovarian stimulation impacts progesterone production. They have further suggested that LH is responsible for increased progesterone catabolism in the theca cells by the 17 α-hydroxylase enzyme. This reduces the amount of progesterone entering the general circulation.[4] Andersen and Ezcurra have conducted an elaborate review on steroidogenesis and have discussed the potential implication of CYP17 on COS. CYP17 expression, seen only in theca cells, is enhanced by LH. This drives the pregnenolone metabolism via the D5 pathway. This, in turn, depletes the pregnenolone substrate for the enzyme 3-beta hydroxy-steroid-dehydrogenase (3β-HSD) which converts pregnenolone to progesterone.[11] The lack of LH in r-FSH may allow more and more pregnenolone to be available for conversion to progesterone. The P elevation in the late follicular stage in the r-FSH group observed in our study can be explained by these enzymatic reactions. In addition, changes in paracrine regulation may be responsible for the difference in P levels in HMG and r-FSH stimulated cycles. FSH-stimulated granulosa cells produce paracrine factors which stimulate production of insulin-like growth factor-1 and progesterone. LH inhibits intrafollicular transforming growth factor beta production which is known to stimulate the enzyme 3 β-HSD, which in turn metabolizes pregnenolone to progesterone. Thus, LH leads to suppression of conversion of progesterone to androgens via this paracrine action.[5]

The significantly higher mean E2 levels found in our study in the elevated P group may be due to the higher number of follicles recruited. Kyrou *et al*. and Hill *et al*. have found similar significant association between higher E2 level and P elevation.[12,13] Papanikolaou *et al*. have compared the outcomes of embryo transfers on days 3 and 5 with respect to elevated P levels. In this study, they have found that mean E2 levels were significantly higher in the group of patients with elevated P levels.[14] Although not significantly different, we also found lower E2 levels in the HMG group as compared to the r-FSH group. This can be explained by the two-cell two gonadotropin hypothesis by Moon *et al*.[15] LH initiates production of androgens through its action on the 17 α-hydroxylase enzyme present only in the theca cells. The androgens are then transported to the granulosa cells where they undergo aromatization to E2. Thus, in the presence of LH, the release of P in the general circulation is reduced.

Elevated P levels in the late follicular phase affect the outcome of IVF cycles. A systematic review and meta-analysis of over 60,000 cycles found that P elevation on the day of triggering is associated with decreased probability of pregnancy.[16] Several studies[9,10,17] have established evidence that elevated P concentration in the late follicular phase is associated with impaired pregnancy rates. Raised follicular phase progesterone concentration caused by ovarian stimulation may contribute to changes in the endometrium leading to embryo–endometrial asynchrony, thus affecting implantation and pregnancy rates.[4] According to Bosch, the negative impact of elevated serum P levels at the end of stimulation should not be underestimated. He reinforces the need for monitoring serum P levels during ovarian stimulation. In our study, fresh embryo transfers performed in 18/27 women with P levels ≥1.5 ng/ml yielded very poor pregnancy outcomes. Only three women had a positive beta-hCG 2 weeks after the transfer. Ultimately, two of the three women ended up having biochemical pregnancies and the third woman was lost to follow-up. Thus, there was not a single clinical pregnancy.

Conclusions

Elevated P levels at trigger are associated with the use of r-FSH for COS in IVF cycles using antagonist protocol. Furthermore, elevated P levels are associated with elevated E2 levels at trigger. Fresh embryo transfers performed in spite of elevated P levels are associated with very low pregnancy rates and unfavorable outcomes. Our results support the need for meticulous monitoring of P levels during COS cycles. We feel that this practice could better inform selection of patients for either fresh embryo transfers or freezing of all embryos.

Limitations

This study was a retrospective data analysis. Hence, we acknowledge that we could not maintain homogeneity regarding the brands of the drugs used. Furthermore, variability in the indications for IVF and ovarian reserve of patients could have influenced the results. Larger

data about fresh embryo transfers and corresponding pregnancy rates would have allowed generalization of results.

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Conflicts of interest

There are no conflicts of interest.

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