Factors influencing serum progesterone level on triggering day in stimulated in vitro fertilization cycles

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Objective: Elevated serum progesterone (P) levels on triggering day have been known to affect the pregnancy rate of in vitro fertilization (IVF). This study aimed to identify the possible factors influencing serum P levels on triggering day in stimulated IVF cycles.

Methods: Three hundred and thirty consecutive fresh IVF cycles were included in the study. All cycles were first attempts and were performed in a single infertility center. The indications for IVF were male factor infertility (n = 114), ovulatory infertility (n = 84), endometriosis (n = 61), tubal infertility (n = 59), unexplained infertility (n = 41), and uterine factor infertility (n = 39). A luteal long protocol of a gonadotropin-releasing hormone (GnRH) agonist (n = 184) or a GnRH antagonist protocol (n = 146) was used for pituitary suppression. Ovarian sensitivity was defined as the serum estradiol level on triggering day per 500 IU of administered gonadotropins (OS[a]) or the retrieved oocyte number per 500 IU of administered gonadotropins (OS[b]).

Results: Univariate analysis revealed that the serum P level on triggering day was associated with the serum estradiol level on triggering day (r = 0.379, p < 0.001), the number of follicles ≥ 14 mm (r = 0.247, p < 0.001), the number of retrieved oocytes (r = 0.384, p < 0.001), and ovarian sensitivity (OS[a]: r = 0.245, p < 0.001; OS[b]: r = 0.170, p = 0.002). The woman's age, body mass index, antral follicle count, and basal serum follicle-stimulating hormone and estradiol levels were not associated with serum P level on triggering day. The serum P level on triggering day did not show significant variation depending on the type or cause of infertility, pituitary suppression protocol, or the type of gonadotropins used.

Conclusion: The serum P level on triggering day was closely related to the response to ovarian stimulation.

Keywords: Estradiol; in vitro fertilization; Ovarian stimulation; Progesterone

Introduction

Premature luteinizing hormone (LH) surges are a frequent cause of cycle cancellation in ovarian stimulation during in vitro fertilization (IVF) [1,2]. Even though the introduction of gonadotropin-releasing hormone (GnRH) analogues has dramatically decreased premature LH surges, there is still 5% to 30% chance of a subtle pre-ovulatory rise in the serum progesterone (P) during stimulated IVF cycles [3,4]. Controversy still exists about the association of clinical outcomes with elevated serum P levels at the time of ovulation triggering. Some have suggested that elevated serum P levels on triggering day are associated with negative clinical outcomes [1,4-7], while others have reported no effect on clinical pregnancy rates [8,9]. Nonetheless, a recent meta-analysis comprising 63 eligible studies (55,199 fresh IVF cycles) found that P elevation on the day of triggering is associated with a decreased probability of pregnancy [10]. The mechanism through which elevated serum P levels on trigger-
ing day lead to a lower pregnancy rate remains unclear, but early P elevation may have an adverse effect on endometrial receptivity instead of affecting the quality of the oocyte or embryo [11]. Poor endometrial receptivity may be explained by premature endometrial maturation, which leads to embryo-endometrium dyssynchrony [12].

A subtle P elevation on triggering day might be a simple mass effect due to an excess number of follicles, which all produce a certain amount of P during ovarian stimulation [13]. Thus, it has been suggested that P elevation may reflect the response of mature granulosa cells to high exogenous exposure to follicle stimulating hormone (FSH) [10]. In fact, it has recently been demonstrated that high serum estradiol levels on triggering day are associated with a higher serum P level on triggering day [9]. Thus, P elevation is more likely to occur in high responders. In the present study, we identified patient characteristics and responses to ovarian stimulation that influence serum P levels on triggering day in stimulated IVF cycles.

Methods

1. Study population

This retrospective study included 330 fresh IVF cycles performed from October 2005 to October 2013 at the Seoul National University Hospital. In order to minimize bias, only the first cycle was included. Patients with polycystic ovary syndrome were excluded. This study was approved by the Institutional Review Board of the Seoul National University Hospital (IRB No. 1502-076-679). The indications for IVF were male factor infertility (n = 114), ovulatory infertility (n = 84), endometriosis (n = 61), tubal infertility (n = 59), unexplained infertility (n = 41), and uterine factor infertility (n = 39). The body mass index, basal serum level of FSH and estradiol, and antral follicle count were recorded if they were measured within two months before starting the cycle.

2. Stimulation protocols and IVF procedure

Controlled ovarian stimulation was performed using recombinant FSH (Gonal-F, Serono, Geneva, Switzerland) with or without highly purified human menopausal gonadotropin (Merional, IBSA, Lugano, Switzerland) or recombinant LH (Luveris, Serono) using the luteal long protocol of a GnRH agonist (Decapeptyl 0.1 mg/day; Ferring, Malmo, Sweden) (n = 184) or the GnRH antagonist protocol (Cetrotide 0.25 mg/day; Serono) (n = 146). When two or more leading follicles reached a mean diameter ≥ 18 mm, 250 μg of recombinant human chorionic gonadotropin (hCG) (Ovidrel, Serono) was injected. The oocytes were retrieved 36 hours after the hCG injection. Ovarian sensitivity was defined as the serum estradiol level on triggering day per 500 IU of gonadotropins (OS[a]) or the retrieved oocyte number per 500 IU of gonadotropins (OS[b]). The oocytes were inseminated by the conventional method (n = 181) or by intracytoplasmic sperm injection (n = 149), depending on the quality of the sperm and oocyte. The embryos were transferred three or five days after the retrieval of the oocyte. Luteal phase support was performed using either a daily dose of 50 mg of P in oil (Progest, Samil, Seoul, Korea) or 8% P gel (Crinone, Serono), starting on the day of oocyte retrieval. Pregnancy was first assessed 14 days after oocyte retrieval by analyzing the serum β-hCG levels. In cases with a positive β-hCG result, transvaginal ultrasonography was performed to confirm the intrauterine pregnancy and to identify the number of gestational sacs and the fetal heart rate. Clinical pregnancy was defined as the presence of one or more gestational sacs.

3. Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences software (PASW ver. 18, SPSS Inc., Chicago, IL, USA). The data were analyzed using the Kruskal-Wallis test or the Mann-Whitney test as indicated. The correlation test was performed by the parametric Pearson’s test. Results were considered significant when the p-value was < 0.05.

Results

The distribution of serum P levels on triggering day is depicted in Figure 1. The mean serum P level was 0.56 ng/mL (standard deviation [SD], 0.45 ng/mL; range, 0.1–4.4 ng/mL). A serum P level on triggering day ≥ 1.5 ng/mL (considered an elevated serum P level according
to the standard criteria) was observed in 4.2% of the population of the study (14/330).

Table 1 shows simple linear correlations between the serum P level on triggering day and various numerical parameters reflecting the outcomes of ovarian stimulation. The parametric Pearson’s test revealed that the serum P level on triggering day was significantly associated with the number of follicles ≥ 14 mm \((r = 0.247, p < 0.001)\), the serum estradiol level on triggering day \((r = 0.379, p < 0.001)\), the number of retrieved oocytes \((r = 0.384, p < 0.001)\), and both metrics of ovarian sensitivity \((OS[a] r = 0.245, p < 0.001; OS[b] r = 0.170, p = 0.002)\). However, the woman’s age, body mass index, basal antral follicle count, and basal serum FSH and estradiol levels were not associated with serum P levels on triggering day.

Serum P levels on triggering day did not show significant differences depending on categorical parameters such as the type of infertility, the cause of infertility, the pituitary suppression protocol, and the type of gonadotropins (Table 2).

Among 330 IVF cycles, eight cycles were cancelled because transferable embryos could not be obtained. Among 322 transfer cycles, clinical pregnancy was identified in 81 cycles (clinical pregnancy rate: 25.1%), and live birth took place in 73 cycles (live birth rate: 22.7%). The serum P levels on triggering day were similar between the groups in which pregnancy did and did not occur (0.57±0.37 ng/mL vs. 0.56±0.48 ng/mL). Dividing the study population into two groups according to the serum P level on triggering day likewise resulted in no significant difference in the clinical pregnancy rate (25.2% among patients with p<1.0 ng/mL vs. 23.1% among patients with p≥1.0 ng/mL).

Discussion

Our study demonstrated that serum P levels on triggering day were closely associated with the patient’s response to ovarian stimulation. This finding is in agreement with a previous report, in which serum P elevation on triggering day was found to be closely related with a high estradiol level on triggering day, and thus was more frequent in high responders [9]. Another study has also shown that the serum estradiol level on triggering day and the number of oocytes were related to the occurrence of serum P elevation [10].

Table 1. Linear correlations between serum progesterone level on triggering day and clinically relevant numerical parameters

| Characteristic                          | Mean ± SD  | r-value | p-value |
|----------------------------------------|------------|---------|---------|
| Age (yr)                               | 35.4 ± 4.5 | −0.013  | NS      |
| Body mass index (kg/m²)                | 21.8 ± 3.3 | −0.039  | NS      |
| Basal serum FSH level (IU/mL)          | 6.6 ± 4.8  | −0.062  | NS      |
| Basal serum estradiol level (pg/mL)    | 28.2 ± 25.7| −0.031  | NS      |
| Antral follicle count                  | 11.9 ± 8.3 | 0.098   | NS      |
| Duration of stimulation (day)          | 9.3 ± 2.3  | 0.041   | NS      |
| Dosage of gonadotropins (IU)           | 2,373 ± 1,091| 0.017 | NS      |
| No. of follicles (≥ 14 mm) on triggering day | 5.4 ± 3.3 | 0.247   | <0.001  |
| Serum estradiol level on triggering day (pg/mL) | 1,516 ± 1,220| 0.379 | <0.001  |
| No. of oocytes retrieved               | 8.7 ± 6.8  | 0.384   | <0.001  |
| Ovarian sensitivity (a)                | 411 ± 430  | 0.245   | <0.001  |
| Ovarian sensitivity (b)                | 2.4 ± 2.5  | 0.170   | 0.002   |

SD, standard deviation; NS, not significant; FSH, follicle stimulating hormone; Ovarian sensitivity (a), serum estradiol level on triggering day per 500 IU of gonadotropins; Ovarian sensitivity (b), number of retrieved oocytes per 500 IU of gonadotropins.

Table 2. Serum progesterone levels on triggering day according to categorical parameters

| Parameter                        | n (%)        | Serum P level (ng/mL) | p-value   |
|----------------------------------|--------------|-----------------------|-----------|
| Type of infertility              |              |                       |           |
| Primary                          | 177 (53.6)   | 0.52 ± 0.37           | NS        |
| Secondary                        | 153 (46.4)   | 0.61 ± 0.52           |           |
| Main cause of Infertility        |              |                       |           |
| Male factor                      | 114 (28.6)   | 0.60 ± 0.63           | NS        |
| Ovulatory factor                 | 84 (21.1)    | 0.52 ± 0.31           |           |
| Endometriosis                    | 61 (15.3)    | 0.63 ± 0.39           |           |
| Tubal factor                     | 59 (14.8)    | 0.56 ± 0.41           |           |
| Unexplained                      | 41 (10.3)    | 0.44 ± 0.27           |           |
| Uterine factor                   | 39 (9.8)     | 0.59 ± 0.33           |           |
| GnRH analogue protocol           |              |                       |           |
| With GnRH agonist                | 184 (55.8)   | 0.56 ± 0.41           | NS        |
| With GnRH antagonist             | 146 (44.2)   | 0.57 ± 0.50           |           |
| Gonadotropin regimen             |              |                       |           |
| rFSH                             | 242 (73.3)   | 0.56 ± 0.46           | NS        |
| rFSH+hMG                         | 58 (17.6)    | 0.58 ± 0.44           |           |
| rFSH+LH                          | 30 (9.1)     | 0.60 ± 0.39           |           |

Values are presented as number (%) or mean ± standard deviation. P, progesterone; NS, not significant; GnRH, gonadotropin-releasing hormone; rFSH, recombinant follicle stimulating hormone; hMG, human menopausal gonadotropin; rLH, recombinant luteinizing hormone.
In fact, previous studies show marked variation in the incidence of serum P elevation (4.2%–23%). Discrepancies in the characteristics of patients and/or treatment protocols may cause this variability.

In conclusion, our study provides new insights into the mechanism of serum P elevation on triggering day. Therefore, individual ovarian responsiveness should be kept in mind when interpreting elevated serum P levels on triggering day.

**Conflict of interest**

No potential conflict of interest relevant to this article was reported.

**References**

1. Bosch E, Labarta E, Crespo J, Simon C, Remohi J, Jenkins J, et al. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for in vitro fertilization: analysis of over 4000 cycles. Hum Reprod 2010;25:2092-100.

2. Bosch E, Valencia I, Escudero E, Crespo J, Simon C, Remohi J, et al. Premature luteinization during gonadotropin-releasing hormone antagonist cycles and its relationship with in vitro fertilization outcome. Fertil Steril 2003;80:1444-9.

3. Elnashar AM. Progesterone rise on the day of HCG administration (premature luteinization) in IVF: an overdue update. J Assist Reprod Genet 2010;27:149-55.

4. Xu B, Li Z, Zhang H, Jin L, Li Y, Ai J, et al. Serum progesterone level effects on the outcome of in vitro fertilization in patients with different ovarian response: an analysis of more than 10,000 cycles. Fertil Steril 2012;97:1321-7.e1-4.

5. Wu Z, Li R, Ma Y, Deng B, Zhang X, Meng Y, et al. Effect of HCG-day serum progesterone and oestradiol concentrations on pregnancy outcomes in GnRH agonist cycles. Reprod Biomed Online 2012;24:511-20.

6. Santos-Ribeiro S, Polyzos NP, Haentjens P, Smitz J, Camus M, Tournaye H, et al. Live birth rates after IVF are reduced by both low and high progesterone levels on the day of human chorionic gonadotrophin administration. Hum Reprod 2014;29:1698-705.

7. Bu Z, Zhao F, Wang K, Guo Y, Su Y, Zhai J, et al. Serum progesterone elevation adversely affects cumulative live birth rate in different ovarian responders during in vitro fertilization and embryo transfer: a large retrospective study. PLoS One 2014;9:e100011.

8. Saleh HA, Omran MS, Draz M. Does subtle progesterone rise on the day of HCG affect pregnancy rate in long agonist ICSI cycles? J Assist Reprod Genet 2009;26:239-42.

9. Requena A, Cruz M, Bosch E, Meseguer M, Garcia-Velasco JA. High progesterone levels in women with high ovarian response...
do not affect clinical outcomes: a retrospective cohort study. Reprod Biol Endocrinol 2014;12:69.
10. Venetis CA, Kolibianakis EM, Bosdou JK, Tarlatzis BC. Progesterone elevation and probability of pregnancy after IVF: a systematic review and meta-analysis of over 60,000 cycles. Hum Reprod Update 2013;19:433-57.
11. Al-Azemi M, Kyrou D, Kolibianakis EM, Humaidan P, Van Vaerenbergh I, Devroey P, et al. Elevated progesterone during ovarian stimulation for IVF. Reprod Biomed Online 2012;24:381-8.
12. Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Hum Reprod Update 2006; 12:731-46.
13. Huang CC, Lien YR, Chen HF, Chen MJ, Shieh CJ, Yao YL, et al. The duration of pre-ovulatory serum progesterone elevation before hCG administration affects the outcome of IVF/ICSI cycles. Hum Reprod 2012;27:2036-45.
14. Filicori M, Cognigni GE, Pocognoli P, Tabarelli C, Spettoli D, Taraborrelli S, et al. Modulation of folliculogenesis and steroidogenesis in women by graded menotrophin administration. Hum Reprod 2002;17:2009-15.
15. Shufaro Y, Sapir O, Oron G, Ben Haroush A, Garor R, Pinkas H, et al. Progesterone-to-follicle index is better correlated with in vitro fertilization cycle outcome than blood progesterone level. Fertil Steril 2015;103:669-74.e3.
16. Papanikolaou EG, Pados G, Grimbizis G, Bili E, Kyriazi L, Polyzos NP, et al. GnRH-agonist versus GnRH-antagonist IVF cycles: is the reproductive outcome affected by the incidence of progesterone elevation on the day of HCG triggering? A randomized prospective study. Hum Reprod 2012;27:1822-8.
17. Andersen AN, Devroey P, Arce JC. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. Hum Reprod 2006;21:3217-27.