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Original Article

Impact of progesterone (on hCG day)/oocyte ratio on pregnancy outcome in long agonist non donor fresh IVF/ICSI cycles

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ABSTRACT

Objective: To assess the role of progesterone (P) on [human chorionic gonadotropin (hCG) day]/oocyte ratio rather than a single cut-off value of serum P on hCG day to predict *in vitro* fertilization (IVF) outcomes.
Materials and Methods: A Retrospective, single center, cohort study in 687 infertile women undergoing fresh IVF/intracytoplasmic sperm injection (ICSI) treatment with long agonist protocol. The data was categorized into three groups according to serum P levels (Group A < 1.0, Group B: 1.0–1.5, Group C ≥ 1.5) and two groups on the basis of P/oocyte ratio (Group A ≤ 0.15; Group B > 0.15) determined using receiver operating characteristic (ROC). For comparing categorical data, χ^2/Fishers exact test was carried out as appropriate. ROC analysis was performed to determine cut-off value for P and P/oocyte, which may discriminate between pregnancy and nonpregnancy.
Results: The mean age of participants was 31.6 ± 3.7 years and overall pregnancy rate was 26.1%. Elevation of both serum P levels and P/oocyte ratio was found to significantly reduce the pregnancy potential in IVF without affecting fertilization and cleavage rates. The detrimental cut-off value for P and P/oocyte was found to be >1.0 ng/mL (sensitivity 56%; specificity 52%) and >0.15 (sensitivity 62%; specificity 61%) respectively. Pregnancy rate (35.3%) among the patients having ≤0.15 P/oocyte ratio was significantly higher ($p < 0.001$) compared with 18.8% observed among the patients having value >0.15.
Conclusion: P/oocyte ratio may be considered as a valuable tool to predict IVF outcomes when compared with serum P levels alone, but more evidence from randomized studies is required.

Introduction

The effect of elevated serum progesterone (P) on day of human chorionic gonadotropin (hCG) administration on *in vitro* fertilization (IVF) outcome is still a matter of debate. It has been shown in a large number of studies that elevated serum P has a deleterious effect on pregnancy outcome of IVF cycles [1–6], while other studies have denied such an association [7–9]. The mechanism of this elevation of serum P on hCG day is still not clear. However, a number of factors influencing the serum P concentration have been found. The number of follicles and follicular stimulating hormone (FSH) drive have been found to be positively associated with raised P levels [10]. It has also been reported that the level of P elevation varies with type of ovarian response [11].

Different cut-off values of serum P levels that would be considered as detrimental for pregnancy outcome in IVF have been proposed in a number of studies. Considering the relationship between serum P and follicle number, it would be better to use ratio of serum P on hCG day to the number of oocytes retrieved (P/oocyte ratio) rather than a single cut-off value of P to predict pregnancy outcome in IVF. The number of studies evaluating P/oocyte ratio as a tool for prediction of pregnancy outcome are very limited. Thus, we tried to investigate the relationship between serum P on day of hCG administration and P/oocyte ratio with the pregnancy outcome in gonadotropin releasing hormone (GnRH) long agonist protocol in nondonor fresh IVF/intracytoplasmic sperm injection (ICSI) cycles in this study.

Materials and methods

This was a single-center retrospective cohort study of patients undergoing IVF/ICSI treatment from January 2012 to July 2014 at the assisted reproductive technology center of the All India Institute of Medical Sciences, New Delhi. Complete data of 687 women

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who underwent nondonor fresh IVF/ICSI cycles with long agonist protocol was reviewed. Exclusion criteria were as follows: age > 40 years, antimullerian hormone < 1.0 ng/mL, and frozen embryo transfer (FET) cycles. The main outcome measure was the effect of serum P and P/oocyte ratio on pregnancy outcome.

Stimulation protocol

Patients underwent controlled ovarian hyperstimulation (COH) with use of a GnRH agonist long protocol. The pituitary down-regulation was achieved by subcutaneous injection of 1 mg of leuprolide acetate daily from the midluteal phase of the preceding cycle. Ovarian stimulation was done with 150–300 IU recombinant FSH follitropin alpha (Gonal F, Merck Serono, Darmstadt, Germany) subcutaneously and dose adjusted according to the response. Recombinant chorionic gonadotropin alpha (250 mg; Ovitrelle, Merck Biopharma, Darmstadt, Germany) was given to trigger ovulation when at least two to three leading follicles reached a mean diameter of 18 mm. Serum P and E_2 levels were measured on the day of hCG administration by the chemiluminescent immunoassay using the Access 2 immunoassay system (Beckman Coulter, California, United States). Endometrial thickness was also recorded on transvaginal ultrasound. Oocyte pickup was done 36 hours after hCG administration. Oocytes were cultured in G-IVF plus media (Vitrolife, Sweden) containing 10% of human serum albumin with gentamicin as an antibacterial agent and inseminated with motile sperm prepared by the two-layer Percoll gradient method. Fertilization was defined as oocytes with two pronuclei 16–20 hours after insemination. Embryos were transferred to G-IVF plus media and were classified by blastomere equalization and cytoplasmic fragment. Day 3 or Day 5 embryo transfer was done depending upon the number of embryos and excess good-quality embryos were cryopreserved for subsequent FET cycles. An ongoing pregnancy was defined as a pregnancy test done after 14 days of embryo transfer with a positive heartbeat by ultrasound at 6 weeks of gestation.

Three groups were made according to serum P levels: Group A < 1.0, Group B 1.0–1.5, and Group C ≥ 1.5.

Two groups were made according to P/oocyte ratio as determined by ROC analysis: Group A ≤ 0.15, and Group B > 0.15.

All statistical analysis was carried out using SPSS, IBM version 19 (Armonk, NY: IBM Corp). Data was expressed as mean, standard deviation or frequencies, and percentages. For comparing categorical data, χ^2/Fishers exact test was carried out as appropriate. ROC analysis was performed to determine cut-off value for P and P/oocyte at an approximately equivalent sensitivity and specificity, which may discriminate between pregnancy and nonpregnancy. A value $p < 0.05$ was considered to be statistically significant.

Results

A total of 687 ovarian stimulation cycles were included in the study. Out of these, six cycles needed to be cancelled, as no oocyte could be retrieved. Out of the remaining 681 cycles, 64.9% ($n = 442$) were conventional IVF cycles while 35.0% ($n = 239$) were ICSI cycles. Baseline characteristics such as age, body mass index, FSH, luteinizing hormone (follicular phase), antimullerian hormone, total antral follicle count, total gonadotropin dose, endometrial thickness, E_2, P on day of hCG, and clinical indications for IVF-Embryo transfer (ET) are as presented in Table 1.

The pregnancy outcomes including the number of oocytes retrieved, fertilization rate, cleavage rate, pregnancy rate, P/oocyte ratio, and number of transferred embryos for all of the women are shown in Table 2.

Table 1
Baseline characteristics of patients.

Characteristics	Overall
No. of patients (n)	687
Age (y)	31.6 ± 3.7
BMI (kg/m^2)	25.4 ± 4.0
FSH(follicular phase) (mIU/mL)	6.0 ± 1.9
LH (mIU/mL)	4.2 ± 2.4
AMH (ng/mL)	3.2 ± 1.6
Total AFC	5.9 ± 2.3
Total gonadotropin dose (IU)	3498 ± 1118
Endometrial thickness (on hCG day) (mm)	9.5 ± 1.7
E_2 on hCG day (pg/mL)	3655.5 ± 2391.5
P on hCG day (ng/mL)	1.27 ± 0.8
Clinical indications:	
Tubal factor (%)	42
Endometriosis (%)	9
PCOS (%)	3
DOR (%)	14
Male factor (%)	8
Unexplained (%)	12
Multiple factors (%)	11

AFC = antral follicle count; AMH = antimullerian hormone; BMI = body mass index; DOR = Diminished Ovarian Reserve; FSH = follicular stimulating hormone; hCG = human chorionic gonadotropin; LH = luteinizing hormone; P = progesterone; PCOS = Polycystic Ovarian Syndrome.

Table 2
Treatment outcome of *in vitro* fertilization/intracytoplasmic sperm injection cycles.

Characteristics	Overall ($n = 681$ cycles)
No. of mature oocytes retrieved	7.2 ± 3.9
P/oocyte ratio	0.24 ± 0.27
Cleavage rate (%)	88.7
No. of embryos transferred	2.8 ± 0.8
Fertilization rate (%)	86.5
Pregnancy rate (%)	26.1

P = progesterone.

We analyzed the correlation between the serum P levels and the fertilization rate, cleavage rate, and pregnancy rate among the three groups made according to serum P levels (Group A ≤ 1.0, Group B 1.0–1.5, Group C ≥ 1.5) as shown in Table 3. It was found that pregnancy rate had a significantly decreasing trend (χ^2 trend in proportion = 11.6; $p = 0.003$) with increasing levels of serum P with no effect on fertilization and cleavage rate.

To determine an optimal detrimental threshold of P levels, ROC analysis was used. The area under curve (AUC) 0.58 (95% confidence interval: 0.53–0.63) was found to be significant ($p = 0.002$). The cut-off value of serum P on hCG day was found to be 1.00 for sensitivity and specificity value of 56% and 52%, respectively (Figure 1).

As the adverse effect of serum P on pregnancy outcome varies according to the type of ovarian response, thus we calculated a cut-off value of P/oocyte ratio to be used as a predictor for IVF outcome rather than P levels alone.

Table 3
Effect of serum progesterone (P) levels on fertilization, cleavage, and pregnancy rate.

Characteristics	Group A ($n = 239$) P ≤ 1.0	Group B ($n = 266$) (P = 1–1.5)	Group C ($n = 176$) P ≥ 1.5	p
Fertilization rate (%)	84.3	84.8	83.8	NS
Cleavage rate (%)	89.8	89.4	88.3	NS
Pregnancy rate (%)	30.8	28.2	16.6	0.003

NS = not significant.

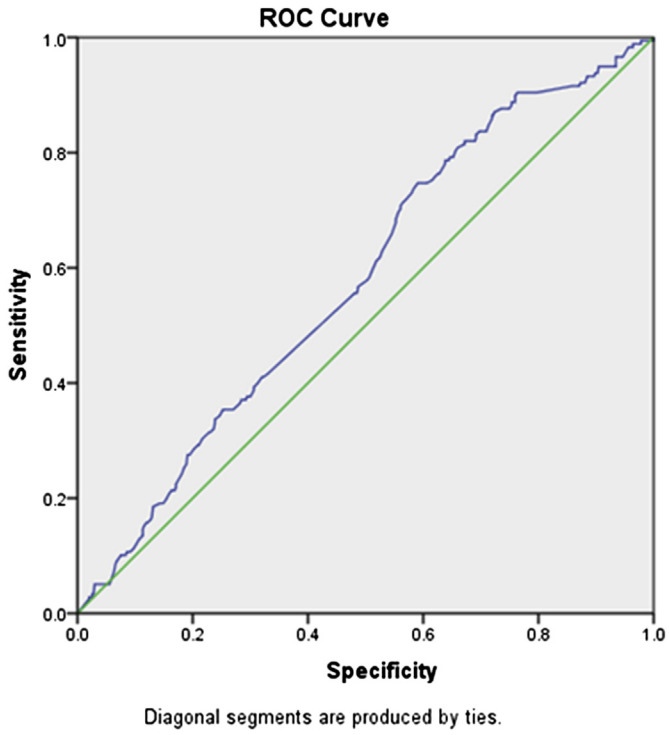

Figure 1. Receiver operating characteristic (ROC) curve for defining optimal detrimental cut-off value for progesterone (P) on human chorionic gonadotropin (hCG) day.

Using ROC, we determined a cut-off level of 0.15 for P/oocyte ratio (AUC: 0.58; $p < 0.001$) with sensitivity and specificity of 62% and 61%, respectively (Figure 2).

The fertilization, cleavage and pregnancy rate were compared in two groups made according to P/oocyte levels as shown in Table 4.

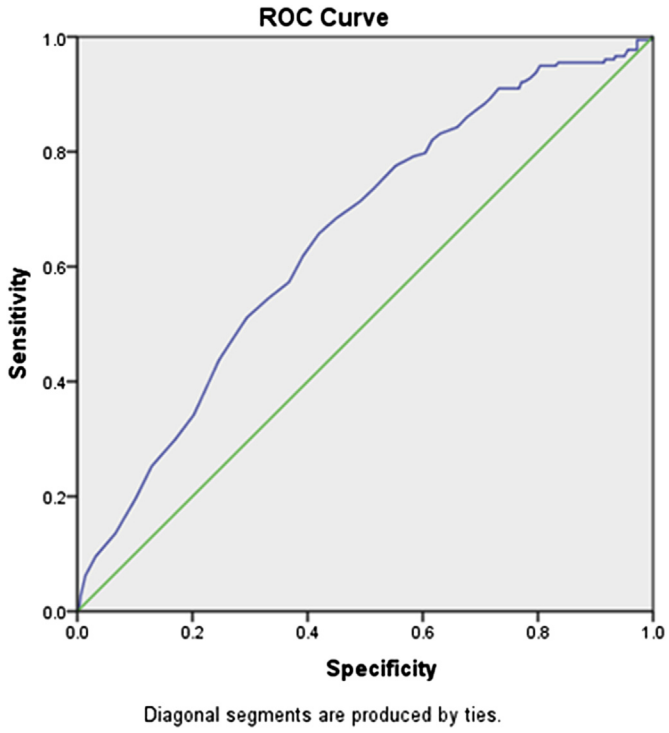

Figure 2. Receiver operating characteristic (ROC) curve for defining optimal detrimental cut-off value for progesterone (P)/oocyte ratio.

Table 4
Effect of progesterone (P)/oocyte ratio on fertilization, cleavage, and pregnancy rate.

Characteristics	Group A ($n = 303$) P/oocyte ≤ 0.15	Group B ($n = 378$) P/oocyte > 0.15	Chi-square (p)
Fertilization rate (%)	85.9	87.2	1.78 (0.182)
Cleavage rate (%)	89.5	87.7	3.15 (0.076)
Pregnancy rate (%)	35.3	18.8	23.8 (0.000)

Pregnancy rate (35.3%) among the patients having ≤0.15 P/oocyte ratio was significantly higher ($p < 0.001$) compared with 18.8% observed among the patients having value >0.15. However, fertilization and cleavage rates were not significantly different ($p > 0.05$) between the two categories.

Discussion

The number of follicles is considered to be one of the important factors that influences the concentration of serum P during COH [12]. Considering this fact, this study has suggested that not only the P levels, but also the ratio of serum P on hCG day to the number of oocytes retrieved can be used as a tool to prognosticate IVF outcome. It was found that both serum P levels and P/oocyte ratio have a negative association with pregnancy rate beyond a cut-off value of 1.00 ng/mL and 0.15 ng/mL, respectively, with no effect on fertilization and cleavage rate.

The issue of P elevation and its effect on pregnancy outcome in IVF is still unresolved. Despite the use of GnRH agonists and antagonists to prevent luteinizing hormone surge, serum P elevation at time of hCG administration has been reported in as high as 35% (5–35%) of stimulated cycles with GnRH agonists and 38% (20–38%) of cycles with GnRH antagonists [1,13]. The underlying mechanism of this P elevation and its effect on IVF outcome is still controversial.

Until now, numerous studies evaluating the role of P elevation on pregnancy outcome have been published. A recent meta-analysis and systematic review of over 60,000 IVF cycles by Venetis et al [14] showed that P elevation on day of hCG administration is associated with a significantly decreased probability of pregnancy in fresh IVF cycles using gonadotropins and GnRH analogues for ovarian stimulation. Different threshold values of P elevation, that would be considered as detrimental for occurrence of pregnancy, have been proposed in a number of studies, and vary from 0.9 ng/mL to 3 ng/mL [7,15]. The results of this study were also in agreement with it and found a detrimental cut-off value of >1.0 ng/mL for serum P elevation.

However, serum P levels alone may not be a good predictor for probability of pregnancy, as it is influenced by a number of other factors. Recently, the rise of serum P has been found to be related to multiple follicular development and increased ovarian steroidogenic activity occurring during COH [12,15,16]. The recent meta-analysis in 2013 also showed that the number of retrieved oocytes, E_2 levels on day of hCG, and total amount of FSH dose required for ovarian stimulation appeared to be increased significantly in women with P elevation when compared with those without P elevation [14].

The potential effect of P elevation and its negative effect on pregnancy outcome vary according to the type of ovarian response. In an analysis of more than 10,000 cycles carried out by Xu et al [11], different threshold values for serum P levels were determined among different ovarian responders: 1.5 ng/mL for poor responders, 1.75 ng/mL for intermediate responders, and 2.25 ng/mL for high responders. Therefore, P/oocyte ratio seems to be a better option to predict pregnancy outcome as compared with P levels alone.

The review of literature reveals a very limited number of studies evaluating the role of P/oocyte ratio to prognosticate the outcome of IVF treatment. In an earlier retrospective study carried out by Burns et al [17] in 114 IVF cycles, P/oocyte ratios were found to be inversely associated with clinical pregnancy ($p < 0.05$) and ongoing/delivered pregnancy ($p < 0.02$) for both the day of and the day after hCG. In a recent study by Aflatoonian et al [18], it was found that P per metaphase II oocyte was better than absolute P value and P/E_2 ratio for predicting the pregnancy and fertilization rate. A cut-off value of >0.32 for P/oocyte was considered to be related adversely with pregnancy rate. We used ROC analysis to identify optimal thresholds of P/oocyte to define these detrimental cut-offs and the value was found to be 0.15.

It has been proposed in a number of studies that the adverse effect of P elevation on pregnancy may be due to impairment of endometrial receptivity and not the oocyte/embryo quality [11,19,20]. The present study also supports this opinion as the fertilization and cleavage rate were not found to be affected with elevation of either P or P/oocyte ratio.

In conclusion, the present study shows that elevation of either P or P/oocyte ratio are inversely associated with probability of pregnancy. P/oocyte ratio may be considered as an important and better tool to predict IVF outcomes as compared with serum P levels alone. As this study is limited by its retrospective design, future randomized trials are required to evaluate the role of P/pocyte ratio as a valuable prognostic tool in IVF over P levels alone.

Conflicts of interest

The authors have no conflict of interest relevant to this article.

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References

[1] Wu Ze, Li Rong, Ma Yanping, Deng Bo, Zhang Xiaomei, Meng Yushi, 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.
[2] Al-Azemi M, Kyrou D, Kolibianakis EM, Humaidan P, Van Vaerenbergh I, Devroey P, et al. Elevated progesterone during ovarian stimulation for IVF. Reproductive Biomed Online 2012;24:381–8.
[3] Bosch E, Labarta E, Crespo J, Simón C, Remohí 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.
[4] Shulman A, Ghetler Y, Beyth Y, Ben-Nun I. The significance of an early (premature) rise of plasma P in in vitro fertilization cycles induced by a "long protocol" of gonadotropin releasing hormone analogue and human menopausal gonadotropins. J Assist Reprod Genet 1996;13:207–11.
[5] Fanchin R, de Ziegler D, Taieb J, Hazout A, Frydman R. Premature elevation of plasma P alters pregnancy rates of in vitro fertilization and embryo transfer. Fertil Steril 1993;59:1090–4.
[6] Ochsenkühn R, Arzberger A, von Schönfeldt V, Gallwas J, Rogenhofer N, Crispin A, et al. Subtle progesterone rise on the day of human chorionic gonadotropin administration is associated with lower live birth rates in women undergoing assisted reproductive technology: a retrospective study with 2,555 fresh embryo transfers. Fertil Steril 2012;98:347–54.
[7] Martinez F, Coroleu B, Clua E, Tur R, Buxaderas R, Parera N, et al. Serum P concentrations on the day of hCG administration cannot predict pregnancy in assisted reproduction cycles. Reprod Biomed Online 2004;8:183–90.
[8] Urman B, Alatas C, Aksoy S, Mercan R, Isiklar A, Balaban B. Elevated serum P level on the day of human chorionic gonadotropin administration does not adversely affect implantation rates after intracytoplasmic sperm injection and embryo transfer. Fertil Steril 1999;72:975–9.
[9] Andersen CY, Bungum L, Andersen AN, Humaidan P. Preovulatory progesterone concentration associates significantly to follicle number and LH concentration but not to pregnancy rate. Reprod Biomed Online 2011;23:187–95.
[10] 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.
[11] 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. 1321–1327.e1324.
[12] Fleming R, Jenkins J. The source and implications of P rise during the follicular phase of assisted reproduction cycles. Reprod Biomed Online 2010;21:446–9.
[13] Check JH, Chase JS, Nowroozi K, Dietterich CJ. Premature luteinization: treatment and incidence in natural cycles. Hum Reprod 1991;6:190–3.
[14] 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.
[15] Bosch E, Valencia W, 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.
[16] Harada T, Katagiri C, Takao N, Toda T, Mio Y, Terakawa N. Altering the timing of human chorionic gonadotropin injection according to serum P concentrations improves embryo quality in cycles with subtle P rise. Fertil Steril 1996;65:594–7.
[17] Burns WN, Witz CA, Klein NA, Silverberg KM, Schenken RS. Serum progesterone concentrations on the day after human chorionic gonadotropin administration and progesterone/oocyte ratios predict in-vitro fertilization/embryo transfer outcome. J Assist Reprod Genet 1994;11:17–23.
[18] Aflatoonian A, Davar R, Hojjat F. Elevated serum progesterone/MII oocyte ratio on the day of human chorionic gonadotropin administration can predict impaired endometrial receptivity. Iran J Reprod Med 2014;12:427–34.
[19] Bourgain C, Devroey P. The endometrium in stimulated cycles for IVF. Hum Reprod Update 2003;9:515–22.
[20] Huang R, Fang C, Xu S, Yi Y, Liang X. Premature progesterone rise negatively correlated with live birth rate in IVF cycles with GnRH agonist: an analysis of 2,566 cycles. Fertil Steril 2012;98:664–70.