Original Article

Pre-Ovulatory Hormones on Day of Human Chorionic Gonadotropin Trigger and Assisted Reproductive Technique Outcomes in Different Ovarian Response Groups

Subarna Mitra, Madhuri Patil[1], Milind Patil[1], Prasanta Kumar Nayak[2]

Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India, [1]Dr Patil's Fertility and Endoscopy Clinic, Bengaluru, Karnataka, India, [2]Department of Obstetrics and Gynecology, IMS SUM Hospital, Bhubaneswar, Odisha, India

Abstract

Background: Evidence regarding impact of pre-ovulatory hormone levels on assisted reproductive technique (ART) outcomes in different ovarian response groups is sparse. **Aims:** The objective of this study was to evaluate and compare the association between pre-ovulatory hormonal profile and ART outcomes in different ovarian responses. **Setting and Design:** This is a single-centre retrospective cohort study of 273 non-donor fresh ART cycles between January 2013 and June 2016. **Materials and Methods:** Data on clinical profile, basal and peak hormonal levels, characteristics of controlled ovarian stimulation and ART outcomes were collected. Progesterone elevation (PE) was defined as pre-ovulatory serum progesterone >1.5 ng/mL or progesterone to oestradiol ratio >1. The association between peak hormonal levels and ART outcomes in poor (≤4 oocytes retrieved), intermediate (5–13 oocytes retrieved) and high (≥14 oocytes retrieved) ovarian responders was analysed and compared. **Statistical Analysis:** Continuous and categorical variables were summarised as median (interquartile range) and percentages, respectively, and compared using Kruskal–Wallis H-test or Mann–Whitney U-test and Chi-square test or Fisher's exact test, respectively. **Results:** The incidence of PE, by both criteria and clinical pregnancy rates (35.7%, 36.8% and 18.6% in high, normal and poor responders, respectively; $P = 0.073$), was similar among the three response groups. Except fertilisation rates in normo-responders, PE did not influence ART outcomes in any response group. Furthermore, there were no differences between peak hormone concentrations or incidence of PE between those who conceived and those who did not. **Conclusion:** Pre-ovulatory sex steroid levels do not seem to be the primary determinant of ART outcomes in any ovarian response category; hence, decision to freeze all embryos in the event of PE should be tailored.

Keywords: *Assisted reproductive techniques, ovarian response, progesterone elevation*

Introduction

The impact of supra-physiological levels of oestradiol (E2) and progesterone (P4) in the late follicular phase on *in vitro* fertilisation/intra-cytoplasmic sperm injection (IVF/ICSI) outcomes remains contentious in reproductive medicine. Evidence reveals that the effect of progesterone elevation (PE) is stronger than that of E2, with lower pregnancy rate reported when serum P4 on the day of human chorionic gonadotropin (hCG) is above the threshold level,

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Address for correspondence: Dr. Subarna Mitra, Flat No. 703, Type-5 Quarters, AIIMS Housing Complex, Sijua, Bhubaneswar - 751 019, Odisha, India.
E-mail: subarnamit@gmail.com

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irrespective of the peak E2 concentration.[1] PE, a term preferred over premature luteinisation, is defined as unpredicted subtle rise in the serum P4 on the day of hCG administration without a concomitant increase in luteinising hormone (LH) levels. Several studies have shown a negative association of PE with clinical pregnancy rate (CPR) and live birth rate (LBR).[1-15] However, others have denied any such association.[16-20] These varied results can be attributed to the use of older P4 assays which lack analytical sensitivity, precision and accuracy at low hormone levels, different definitions and threshold values, diverse population and cycle characteristics and arbitrary cut-off levels employed in most of these studies.

PE can occur in high, normo and poor responders, and the cut-off value in each group is different. It would, therefore, be prudent to take into account not only the P4 levels but also the ovarian response and reserve when interpreting the cycles with raised P4.[21,22] Therefore, Younis *et al*. defined PE as a P4/E2 ratio of >1 and found it to be associated with low ovarian reserve as well as poor pregnancy outcomes.[21] This criterion could differentiate between the P4 secretion from dysmature follicles and physiologic secretion from multiple healthy mature follicles. There are limited studies exploring the relationship between hormonal profiles on the day of trigger and probability of pregnancy categorised by the quality of the ovarian response to controlled ovarian stimulation (COS), and most of them have observed higher P4 levels in high responders, probably owing to accumulation of normal amounts of hormones secreted by excess number of follicles.[10,23-26]

The aim of this study was to compare the hormonal levels on the day of trigger and assisted reproductive technique (ART) outcomes in different ovarian response groups. We also evaluated the association between hormonal profile on the day of trigger and ART outcomes in different ovarian response categories.

Materials and Methods

This is a retrospective, single-centre cohort study of patients undergoing ART. Ethical approval was not sought as it was a retrospective study conducted from January 2013 to June 2016, and ethical committee approval was not considered mandatory for retrospective studies at that time. Informed consent was obtained from all participants regarding the use of anonymised data from their records for educational and research purpose. Complete data of 273 non-donor fresh IVF/ICSI cycles during the study period were reviewed. Cycles with oocyte/embryo donation, frozen embryo transfer (FET) and intra-uterine insemination converted to IVF were excluded. Data from all patients were analysed retrospectively, irrespective of indication of ART and stimulation protocol in an attempt to mimic everyday clinical experience. The baseline characteristics of the women were evaluated. The baseline characteristics included were age, duration of infertility, body mass index (BMI), causes of infertility, baseline follicle-stimulating hormone (FSH), LH, E2 and P4 concentrations, anti-Mullerian hormone (AMH) and antral follicle count (AFC) on cycle day 2 or 3. The treatment protocol, type and doses of gonadotropins were individualised on a case-to-case basis according to the patient characteristics. Recombinant FSH, highly purified FSH and human menopausal gonadotropin were used alone or in combination for COS. The initial dose of gonadotropin was tailored for each patient according to age, basal FSH levels, AFC, BMI and previous response to ovarian stimulation. Dose adjustments were performed according to ovarian response, which was monitored by transvaginal scan (TVS) and E2 levels. Ovulation was triggered with recombinant hCG, or GnRH agonist in case of risk of ovarian hyper-stimulation syndrome (OHSS), when at least 2–3 leading follicles reached a mean diameter of 17 mm. Serum P4 and E2 levels were measured on the day of hCG administration by the chemiluminescent immunoassay using Access 2 Immunoassay system (Beckman Coulter) in the same laboratory where the basal hormonal parameters were estimated.

Oocyte pickup was done 35 h after trigger, and IVF or ICSI was performed depending on the indication, semen quality, the number of oocytes and previous fertilisation rates. Sperm preparation was done by the two-layer density gradient method. Fertilisation was defined as oocytes with two pronuclei (2PN) or polar bodies (syngamy) 16–20 h after insemination. Embryos were morphologically evaluated based on cell number, symmetry, granularity, type and percentage of fragmentation, presence of multinucleate blastomeres and degree of compaction. An embryo which is 4 cells on day 2, ≥8 cells on day 3, with equal-sized blastomeres and ≤20% fragmentation on day 3, and with no multinucleate cells is described as a top-quality embryo. Blastocyst grading was done based on degree of expansion, inner cell mass and trophectoderm. A blastocyst with grade 3AA and above was considered as a top-quality blastocyst.

Embryo transfer was performed either at the cleavage stage (day 2 or 3 of embryo development) or at the blastocyst stage (day 5 or 6 of embryo development), depending upon the number and quality of embryos. All surplus good-quality embryos were cryopreserved

for subsequent FET cycles. Luteal phase support was administered with intramuscular P4 100 mg along with either oestradiol valerate 2 mg BD or hCG 1500/2000 IU intramuscular every 3 days, depending upon the E2 levels on the day of hCG trigger. Serum β-hCG was estimated 10 or 12 days after day 5 or day 3 embryo transfer, respectively. Clinical pregnancy was confirmed 3 weeks after oocyte retrieval and foetal heart identified 1 week thereafter.

Biochemical pregnancy was defined as having a positive β-hCG but without any TVS evidence of gestational sac. Live clinical pregnancy was defined as report of foetal heart beat in TVS at 6 weeks. Implantation rate was defined as the number of gestational sacs on TVS divided by the total number of transferred embryos. An on-going pregnancy was defined as the pregnancy continuing beyond 20 weeks at the time of statistical analysis for this study.

We categorised ovarian responses into three arbitrary groups according to the number of oocytes retrieved – poor ovarian response (≤4 oocytes retrieved), normal ovarian response (5–13 oocytes retrieved) and high ovarian response (≥14 oocytes retrieved). We explored the relationship between hormonal levels on the day of hCG administration and the ART outcomes in different ovarian responders.

Statistical analysis

Statistical analysis was performed using the SPSS software package version 22 (IBM Corp., Armonk, NY, USA). We used Shapiro–Wilk test to evaluate the distribution of the quantitative parameters in the data. For descriptive statistics, median (interquartile range) and percentages were employed for continuous and for categorical variables, respectively. Kruskal–Wallis H-test or Mann–Whitney U-test was used for comparison of continuous variables and Chi-square test or Fisher's exact test for categorical variables, as appropriate. A $P < 0.05$ was considered statistically significant.

Assuming 20% CPR in the setting of PE based on a previous study by Bosch *et al.*,[4] an absolute precision of 5% and with the formula $4pq/d^2$, we estimated a sample size of 256.

Results

Data of 273 patients were available during the study period, achieving the final sample size of 273.

Table 1 depicts the baseline clinical and hormonal characteristics of the three groups of responders. Poor responders were older with lower AFC ($P < 0.001$) and had the highest BMI ($P = 0.01$). They also had higher basal serum FSH and lower serum AMH levels ($P < 0.001$). However, the basal serum P4 levels did not differ between the three groups.

Table 2 illustrates the characteristics and outcomes of COS cycles in the three groups. As evident, hyper-responders needed lowest dose of gonadotropins and produced highest number of follicles ≥14 mm compared to other two groups ($P < 0.001$). They also had higher levels of serum E2 ($P < 0.001$) and P4 ($P = 0.003$) on the day of trigger. Furthermore, number of oocytes retrieved, metaphase II (MII) oocytes, 2PN embryos, cleavage-stage embryos and blastocysts as well as proportion of top-quality blastocysts were higher ($P < 0.001$). Despite having favourable COS outcomes, it did not translate into higher CPR. More biochemical pregnancies were observed in poor responders. The incidence of PE was 23.21%, 14.37% and 9.3% (when defined as pre-ovulatory serum P4 >1.5 ng/ml) and 10.71%, 13.22% and 25.58% (when defined as pre-ovulatory serum P4:E2 >1) in hyper,

Table 1: Baseline clinical and hormonal characteristics in different ovarian response groups

Parameter	Overall	Hyper-responder	Normal responder	Poor responder	*P*
Number of cycles	273	56	174	43	
Age (years)	31 (5)	29 (5)	31 (5)	33 (6)	<0.001
BMI (kg/m^2)	25 (5.7)	24.3 (5.425)	25.1 (5.7)	26.4 (5.62)	0.01
1° infertility (%)	73.3	71.4	73.6	74.4	0.935
Duration of infertility (months)	36 (36)	36 (36)	36 (36)	48 (84)	0.102
AFC	11 (11)	23.5 (20.25)	9 (7)	7 (10)	<0.001
FSH (IU/L)	5.89 (2.93)	5.035 (2.1675)	5.88 (2.8575)	6.79 (3.85)	<0.001
LH (IU/L)	4.07 (2.645)	4.605 (2.505)	3.9 (2.89)	3.6 (1.93)	0.012
E2 (pg/mL)	39 (20.5)	42.5 (16)	37.5 (19)	42 (31)	0.024
P4 (ng/mL)	0.34 (0.475)	0.4 (0.535)	0.3 (0.445)	0.3 (0.43)	0.252
AMH (ng/mL)	2.5 (3.76)	5.3 (3.875)	2.1 (2.94)	1.06 (2.41)	<0.001

Continuous variables expressed as median (IQR) and proportion expressed as %. IQR: Interquartile range, BMI=Body mass index, AFC=Antral follicle count, FSH=Follicle-stimulating hormone, LH=Luteinising hormone, E2=Oestradiol, P4=Progesterone, AMH=Anti-Mullerian hormone

normal and poor responders, respectively, which was comparable between the three groups.

Table 3 demonstrates that the hormonal levels of sex steroids at trigger did not differ between conception and non-conception cycles. The incidence of PE by either definition was also similar in those who conceived compared to those who did not.

Tables 4 and 5 portray the comparison of ART outcomes between those with and without PE, in different ovarian response groups, based on two definitions of PE – serum P4 levels on the day of trigger >1.5 ng/mL or P4:E2 >1, respectively. Except for fertilisation rate in normo-responders, PE did not seem to affect any other outcomes irrespective of the ovarian response and the defining criteria.

When exploring the correlation between pre-ovulatory sex steroids and COS outcomes in different response groups, any impact of pre-ovulatory E2 and P4 was noted only in poor responders (not shown in table). There was a positive correlation between cleavage rate and pre-ovulatory serum E2 ($\rho = 0.388$, $P = 0.01$), negative correlation between serum P4:E2 ratio and cleavage rate ($\rho = -0.423$, $P = 0.005$) and negative correlation between blastulation rate and serum pre-ovulatory P4 ($\rho = -0.348$, $P = 0.022$).

Discussion

The main findings of this study were comparable CPR among different categories of ovarian response despite differences in pre-ovulatory hormone levels and COS characteristics. PE did not impact the ART outcomes in any response group. In addition, no difference in the pre-ovulatory sex steroid levels or incidence of PE was evident between those who conceived and those who did not.

Table 2: Controlled ovarian stimulation characteristics and assisted reproductive techniques outcomes in different ovarian response groups

Parameter	Overall	Hyper-responder	Normal responder	Poor responder	*P*
Total gonadotropin dose	2025 (1475)	1500 (928.125)	2100 (1368.75)	2700 (1725)	<0.001
Total days of COS	10 (3)	10 (3)	10 (3)	9 (3)	0.493
Number of follicles ≥14 mm at trigger	9 (7)	15 (6)	8 (5)	4 (4)	<0.001
Endometrial thickness at trigger	12 (2.05)	12.3 (1.8)	11.9 (2.025)	11.8 (2)	0.056
E2 at trigger	1964 (1728.5)	3171.5 (2290)	1919 (1375)	891 (999)	<0.001
P4 at trigger	0.7 (0.675)	0.9 (0.8575)	0.7 (0.6525)	0.56 (0.66)	0.003
P4:E2 at trigger	0.35 (0.405)	0.285 (0.2225)	0.35 (0.35)	0.6 (0.81)	<0.001
Number of oocytes retrieved	9 (8)	16 (4)	8 (4)	3 (2)	<0.001
Number of MII oocytes	8 (6)	15 (3.75)	7 (5)	3 (2)	<0.001
Number of 2PN embryos	5 (5)	12 (6)	5 (3)	2 (2)	<0.001
Number of cleavage stage embryos	5 (6)	12 (7)	5 (3)	2 (2)	<0.001
Number of blastocysts formed	2 (3)	5 (3)	2 (3)	0 (1)	<0.001
Top-quality D3 embryos (%)	67.7	69.4	66.5	67.4	0.448
Top-quality blastocysts (%)	84.3	93	79.5	69	<0.001
Fertilisation rate (%)	78.3	81.4	76.1	80	0.012
Cleavage rate (%)	97	96.5	97.1	99	0.402
Blastulation rate (%)	42.3	41.3	44	31.5	0.053
Implantation rate (%)	26.5	32.4	26.7	17	0.149
Biochemical pregnancy rate (%)	35.5	14.3	37.4	55.8	<0.001
Clinical pregnancy rate (%)	33.7	35.7	36.8	18.6	0.073
Miscarriage rate (%)	19.6	20	18.8	25	0.914

Continuous variables expressed as median (IQR) and proportion expressed as %. IQR: Interquartile range, COS=Controlled ovarian stimulation, E2=Oestradiol, P4=Progesterone, PN=Pronuclei, MII=Metaphase II

Table 3: Hormonal levels at trigger between conception and non-conception cycles

Parameter	Conception cycles (*n*=92)	Non-conception cycles (*n*=181)	*P*
E2 at trigger	2041.5 (1346.75)	1908 (1925.5)	0.968
P4 at trigger	0.6 (0.6)	0.77 (0.735)	0.165
P4:E2 at trigger	0.34 (0.3525)	0.36 (0.43)	0.187
Incidence of PE as per P4 >1.5 ng/mL	13.04	16.5	0.445
Incidence of PE as per P4:E2 >1	11.96	16.02	0.369

Continuous variables expressed as median (IQR) and proportion expressed as %. IQR: Interquartile range, E2=Oestradiol, P4=Progesterone, PE=Progesterone elevation

Table 4: Assisted reproductive techniques outcomes in different responders according to progesterone elevation defined as pre-ovulatory progesterone >1.5 ng/mL

	Hyper-responder (%)		*P*	Normal responder (%)		*P*	Poor responder (%)		*P*
	P4 >1.5 ng/mL	P4 ≤1.5 ng/mL		P4 >1.5 ng/mL	P4 ≤1.5 ng/mL		P4 >1.5 ng/mL	P4 ≤1.5 ng/mL	
Clinical pregnancy rate	30.77	37.21	0.671	28	38.26	0.325	25	17.95	0.73
Miscarriage rate	0	9.3	0.538	0	21.05	0.331	0	28.57	1.000
Fertilisation rate	79.3	83.36	0.35	69.42	77.34	0.014	83.33	79.63	0.761
Cleavage rate	96.11	96.67	0.721	99.3	96.74	0.091	100	98.84	0.732
Blastulation rate	46.82	39.42	0.087	45.07	43.85	0.787	30	31.71	0.913
Implantation rate	23.53	35.09	0.372	25.64	26.85	0.933	25	16.33	0.657
Biochemical pregnancy rate	7.7	16.28	0.438	32	38.26	0.55	50	56.41	0.806

Data expressed as %. P4=Progesterone

Table 5: Assisted reproductive techniques outcomes in different responders according to progesterone elevation defined as pre-ovulatory progesterone: oestradiol ratio >1

	Hyper-responder (%)		*P*	Normal responder (%)		*P*	Poor responder (%)		*P*
	P4:E2 >1	P4:E2 <1		P4:E2 >1	P4:E2 <1		P4:E2 >1	P4:E2 <1	
Clinical pregnancy rate	33.33	36	0.898	34.78	37.09	0.831	9.09	21.88	0.347
Miscarriage rate	0	22.22	1.000	12.5	19.64	0.628	0	28.57	1.000
Fertilisation rate	81.98	81.29	0.861	66.04	77.51	0.001	74.07	81.72	0.382
Cleavage rate	94.51	96.83	0.258	100	96.76	0.064	95	100	1.000
Blastulation rate	40.7	41.35	0.908	45.71	43.82	0.712	21.05	34.25	0.270
Implantation rate	28.57	32.86	0.819	27.03	26.64	0.960	11.11	18.18	0.607
Biochemical pregnancy rate	0	16	0.578	43.48	36.42	0.515	45.45	59.38	0.423

Data expressed as %. E2=Oestradiol, P4=Progesterone

Several studies have found pre-ovulatory P4 levels and incidence of PE to be positively associated with pre-ovulatory E2 levels, total gonadotropin dose, duration of COS and number of oocytes retrieved. This supports the hypothesis that increasing P4 levels is a simple mass effect due to excess number of follicles, and therefore, PE is more likely in hyper-responders.[1-7,9-11,13-19,23-25,27-32] We noted higher pre-ovulatory P4 levels in hyper-responders but not higher PE rates, probably because the mean values were lower than the chosen cut-off to define PE. In addition, we did not find higher CPR in hyper-responders despite better COS characteristics and hormonal profiles, in contrast to the findings of previous studies.[10,27,28] This could be due to the adverse effect of high pre-ovulatory sex steroid hormone concentrations on the endometrial receptivity.

Individual ovarian responsiveness should be considered when analysing the effect of PE on IVF outcomes.[14,26,28,29] We postulate that PE does not negatively influence ART outcomes, as evident from comparable IVF outcomes between PE and non-PE groups in each of the response category. This is in concordance with few reports[19,29] but in disagreement with most others.[1-15,23,25] Few have suggested higher cut-offs with increasing ovarian response, indicating that the threshold of 1.5 ng/mL cannot be applied to all patients.[10,23,25,26,28] However, Bosch *et al*. found that the threshold of 1.5 ng/mL is valid across all ranges of ovarian responses.[4] In fact, this cut-off of 1.5 ng/ml has been proposed based on the demonstration of a marked difference in endometrial gene expression profiles above and below this cut-off; hence, it is widely used for defining PE.[32,33] We, too, used this cut-off, but we could not demonstrate a negative effect of PE on ART outcomes, probably because other factors may be more important predictors of clinical pregnancy. Some have found deleterious effects of modest degree of PE on ART outcomes with relatively stable effect after a certain level.[9,10,30] However, some studies have found detrimental effect only at higher cut-offs, which is barely clinically significant in some of them.[5,18,24] Therefore, the use of such higher cut-offs in our study, especially in hyper-responders, is less likely to alter our results. The relationship between serum P4 and ART outcomes is linear according to some[3,8] but not so according to others.[4,6,9,13,28,32,34] However, in most studies, the predictive value is modest.[1,2,10] This further lends credence to our postulation that PE is not the predominant predictor of ART outcomes. We did not find a negative correlation between peak P4 levels and CPR. The lower mean peak levels P4 levels in our study which did not reach the threshold value of 1.5 ng/mL may exlain this lack of correlation.

PE does not seem to affect all patients equally. A differential effect depending upon ovarian response has been observed.[8,18,24,26,28] Specifically, in high responders, the availability of good-quality or faster developing embryos may negate any adverse effect of PE on endometrial receptivity.[21,27,35] Our study also affirms that PE is not associated with a decreased chance of pregnancy in high responders, similar to some reports,[14,17,18,24,28] but discordant to others.[4,10,23,25,26] The results of the meta-analysis by Venetis *et al*. in 2013 contend that a detrimental effect of PE is present already from 0.8 to 1.1 ng/mL in the general IVF population and in poor responders. However, in high responders, such a negative effect is exhibited only when the level of pre-ovulatory serum P4 reaches 1.9–3.0 ng/ml.[9] This indicates that an increased oocyte yield, a proxy indicator of quality of resulting embryos, might compensate for the detrimental effect of PE on the endometrium. Thus, it might be possible, at least in high responders, to offer freeze all to avoid the risk of OHSS and not for the reason of PE as they do not seem to affect CPR. Based on our findings of a lack of negative impact of PE on ART outcomes in hyper responders, we too second this strategy of freezing all embryos in hyper responders in the context of OHSS and not PE. Since the mean P4 concentrations in hyper-responders were much lower than the threshold suggested by the above meta-analysis, we could not demonstrate any detrimental influence on ART outcomes. In contrast, Arvis *et al*. concluded that, while in poor responders, PE may be ignored avoiding unnecessary cancellations or embryo freezing, in higher responders, the negative effect of PE is more pronounced mandating a wider application of freeze-all strategy.[34] Some studies do not support the moderating effect of ovarian response on the association between PE and ART outcomes and instead have observed negative influence regardless of ovarian response.[4,10-12,15,23,25]

PE has been demonstrated to be a dominant predictor of clinical pregnancy or live birth in multivariate analysis.[6,11,36] Despite having younger age and a better ovarian response with more follicles, oocytes and higher E2, the high P4 group still seems to fail to reach the best CPR, on-going pregnancy rates, LBR and cumulative LBR that one would expect this group to have.[4,15,16,31] The adverse impact could be on oocyte or embryo quality,[15,25,37] but most studies advocate that it is due to altered endometrial receptivity and hence favour freeze-all strategy in the event of PE.[1-6,9,13,23,30,32,33] Conversely, PE does not always halt implantation regardless of the threshold adopted. Preventing PE would theoretically increase on-going pregnancy rates by only 1%–2%.[24,27] It is estimated that monitoring P4 levels in 1000 cycles and intervening in 50–300 cycles with PE would potentially avoid 2–12 implantation failures by applying the freeze-all strategy.[35] These observations, including ours, suggest that probably, hormonal profile on the day of trigger does not play a major role in determining the probability of pregnancy. Clinical characteristics such as age of the patient, number of IVF attempts, duration of infertility and aetiology of infertility; hormonal parameters such as duration of PE, P4 levels in early follicular phase or on day of Ovum pick up, serial P4 measurements around hCG trigger rather than a single value, P4-to-follicle index and P4 to mature oocyte ratio as well as embryological factors such as total number of mature oocytes and embryos transferred, quality of embryos/blastocysts, early cleavage, blastulation and hatching and developmental stage of embryo at transfer may be more determinative of probability of pregnancy and/or live birth.[2,13,26,27,30,36] In addition, PE does not In addition, PE does not seem to affect all measures of ART universally or equally.[1,14,22,38] However, in our study, we found that normo-responders with P4:E2 > 1 had lower fertilization rates with comparable cleavage and blastulation rates compared to normo-responders with P4:E2 < 1. This hints at a possible, although trivial, adverse effect of PE on oocyte quality.

In the present study, fertilisation rate differed significantly between PE and non-PE groups only in normal responders, irrespective of the definition used, suggesting a potential negative effect of PE on oocyte quality, albeit not deleterious enough to affect CPR. Our findings are converse to others.[1-3,10,11,14,15,22,23,31,38] Interestingly, Peng *et al*. noted higher oocyte yield and higher fertilisation rate in PE group in polycystic ovary syndrome (PCOS) women using cut-off of pre-hCG P4 as 1.2 ng/mL although number of top-quality day 3 embryos, top-quality embryo rate, implantation rates and CPR were comparable. They proposed that PE is not an obstacle to successful IVF outcomes in PCOS patients.[17]

Reports on relationship between E2 levels at trigger and ART outcomes have been inconsistent, but majority have not found a detrimental effect and hence do not consider it to be significant and independent predictor of IVF success.[1,2,10] Our results corroborate this fact.

Using a single hormone level on trigger day to predict ART outcomes may be confounding because of the positive correlation of peak P4 with peak E2 and number of follicles and because PE is observed both in high and poor responders. Therefore, the role of P4:E2 ratio has been investigated with conflicting results.[10,21,22,38,39] Most have found poor-to-modest predictive accuracy of P4:E2 ratio, thereby limiting its clinical utility.[10,38,39] High P4:E2 does not affect oocyte or embryo quality as evident from comparable rates of fertilisation, cleavage,

blastulation and top-quality embryo in those with low and high P4:E2 in different studies.[10,22,38]

We did not find any adverse hormone profile on the day of trigger in non-conception cycles compared to conception cycles. This is in agreement to some studies[20,29] but contradictory to others.[36,38,39] Furthermore, we found comparable rates of PE in both conception and non-conception cycles, similar to the findings of Nagaraja *et al*.[20] This further reiterates that pre-ovulatory serum P4 levels do not play a significant role in determining pregnancy.

The reasons for the discordance between the findings of our study compared to other reports are varied. Any expected negative impact of higher pre-ovulatory P4 levels on endometrial receptivity was probably negated by the positive impact of higher number of oocytes and good-quality blastocysts in hyper-responders. Any negative influence of high P4 levels or PE does not seem to have a major clinical significance in any response category as ART outcomes were similar in those with and without PE in all types of responders. P4 levels at trigger as well as incidence of PE were comparable between those who conceived versus those who did not in all response categories, which further corroborates the hypothesis that PE does not always lead to implantation failure or non-attainment of clinical pregnancy. Several other characteristics of the individual patient and the ART cycle should be taken into consideration when estimating the probability of pregnancy. Finally, the differences in population characteristics, study design, stimulation protocols, cut-offs for PE and statistical methods employed in different studies can also explain the discrepancy of the results.

Unlike most previous studies that assessed the relationship between serum P4 level and ART outcome, we evaluated the outcomes in different ovarian responses.

Our study has some limitations. Ours is a single-centre retrospective study. COS protocols and the type of gonadotropin used were not uniform which may mask the possible effects on serum P4 concentration and the cycle outcome. A multivariate analysis taking into account all confounders would have provided more accurate estimate of the relationship between PE and pregnancy.

Conclusion

PE does not impact likelihood of pregnancy adversely in fresh ART cycles, regardless of ovarian response. Hormonal profile on the day of trigger or rates of PE is comparable between those who conceived and those who did not. Thus, pre-ovulatory sex steroid levels do not seem to be the primary determinant of ART outcomes in any ovarian response category. Management of patients with PE should be individualised, and the decision to freeze all embryos or proceed with fresh transfer should take into account various clinical and embryological parameters rather than based on single P4 measurement on the day of hCG administration.

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

Data of this study is with the corresponding author and will be made available on appropriate request.

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