Original Article

Progesterone/Oestradiol ratio can Better Predict Intracytoplasmic Sperm Injection Outcomes than Absolute Progesterone Level

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Abstract

Background: Several parameters were proposed to predict the impact of premature luteinization on intracytoplasmic sperm injection (ICSI) outcomes such as isolated progesterone (P) level, progesterone to oocyte ratio, and progesterone/estradiol ratio (P/E2). **Aim:** The aim of this study is to compare the predictive value of P/E2 ratio and isolated P level on the ovulation triggering day for pregnancy outcomes in fresh GnRH antagonist ICSI cycles. **Settings and Design:** A retrospective cohort study conducted in a university-affiliated *in vitro* fertilization center between January 2017 and April 2019. **Methods:** The study included women who underwent their first- or second-ranked GnRH antagonist ICSI cycles with day-3 embryo transfer. P/E2 ratio was calculated as (P [ng/mL] × 1000)/E2 (pg/mL). Cutoff values of ≥1.5 ng/ml for high P (HP) and ≥0.55 for HP/E2 ratio were chosen based on the literature. **Statistical Analysis:** A receiver operating curve was performed to detect the predictability of serum P/E2 and P for the ongoing pregnancy rate. First, patients were divided according to either P level (low $P < 1.5$ ng/mL and HP ≥1.5 ng/mL) or P/E2 ratio (low P/E2 <0.55 and HP/E2 ≥ 0.55). Patients were further divided into four subgroups: (Group A: HP and HP/E2 ratio, Group B: low P and low P/E2 ratio, Group C: HP only, and Group D: HP/E2 only). A multivariate regression analysis models were used to account for the effect of the cycle confounders on the likelihood of pregnancy. **Results:** A total of 402 ICSI cycles were analyzed. The area under the curve was 0.67 and 0.59 for P/E2 and P, respectively. P/E2 showed a significant association with ongoing pregnancy (adjusted odds ratios [aOR]: 0.409, 95% confidence interval [CI] 0.222–0.753, $P = 0.004$) while HP revealed no significant predictive value (aOR: 0.542, 95% CI 0.284–1.036, $P = 0.064$) after the multivariate analysis. **Conclusions:** P elevation may not present as an independent predictor for cycle outcomes. P/E2 ratio has a better prognostic value than *P* alone in predicting pregnancy of GnRH antagonist cycles.

Keywords: *Infertility, intracytoplasmic sperm injection, pregnancy outcomes, premature luteinisation, progesterone, progesterone/estradiol ratio*

Introduction

In *in-vitro* fertilization (IVF) cycles, there has been a controversy about significance of the premature progesterone (P) rise during the late follicular phase,

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commonly known as premature luteinization (PL) and its impact on ART outcomes. PL is broadly defined as an elevation of serum $P \geq 1.5$ ng/ml in the follicular phase before the trigger administration for final oocyte maturation in controlled ovarian stimulation cycles (COS).[1,2]

In a meta-analysis of >60,000 IVF cycles, Venetis *et al*.[3] concluded that PL is associated with a decreased pregnancy probability in fresh embryo transfer (ET) cycles. This detrimental effect could be explained by accelerated endometrial maturation leading to a desynchronization between embryo growth and endometrial receptivity.[4,5] The success of frozen-thawed embryos originating from the cycles complicated by PL as well as the data of the oocyte donation cycles supports this judgment.[3] However, there is growing evidence regarding the effect of PL on embryo or oocyte quality.[6,7]

PL is not uncommon. Neither gonadotropin-releasing hormone agonist nor GnRH antagonist regimens could eliminate its risk. PL could be detected in all categories of patients undergoing COS, such as hyperresponders, normal responders, and poor responders.[8] The incidence of PL was linked to different factors such as daily follicle-stimulating (FSH) hormone dosage,[2] total gonadotrophin dose,[9] stimulation days,[10] number of retrieved oocytes, and peak estradiol level.[2]

To date, an effective prevention of PL is still lacking. In the literature, several measures were suggested to reduce the incidence of PL on IVF cycles: (1) addition of corticosteroids to COS cycles in patients with higher basal P,[11] (2) optimal timing of ovulation triggering,[12,13] (3) step-down stimulation approach with avoidance of enhanced ovarian stimulation toward the late follicular phase,[10] and recently (4) metformin.[14,15] However, further well-designed studies are needed to prove their success in the prevention of PL in IVF cycles.

Different indicators were suggested for diagnosing the PL such as absolute P level, progesterone/estradiol (P/E2) ratio, P/oocyte ratios or different P levels based on the ovarian response.[16] Many reports questioned the accuracy of absolute P level on the ovulation triggering day to predict the pregnancy outcomes. Instead, the use of P/E2 ratio to take into accounts the number of the developing follicles in COS cycles, was proposed.[17-19]

Cetinkaya *et al*.[20] reported that the *P* value on the late follicular phase is positively correlated with the number of mature follicles and peak estradiol levels. Moreover, the use of P/E2 can take into account the number of growing follicles during COS.[18,19] Progesterone elevation was linked to compromised pregnancy rates in poor responders yet not in high responders.[20] Hence, whether this adverse outcome is created by poor ovarian reserve or high P (HP) can be examined more precisely with the poor ovarian reserve group.[21] Progesterone/estradiol ratio was purposed to be a more useful predictor for PL in the regard of differentiating the source of P production either from numerous growing mature follicles or immature dysregulated ones.[17,22] However, some authors presented low sensitivity and positive predictive value for P/E2 and disputed its clinical application.[23,24] Aflatoonian *et al*.[25] revealed that neither P nor P/E2 has valid predictability for pregnancy and introduced the P to oocyte (P/oocyte) ratio to become a more efficient parameter for PL-induced adverse effects.

Our study aims to compare the predictive value of trigger day P/E2 and isolated progesterone (P) level on the ovulation triggering day for the pregnancy outcomes among GnRH antagonist cycles with day-3 ET.

Methods

Study type, setting, and duration

This was a retrospective, cohort study performed at in a single university-affiliated IVF center after obtaining Institutional Review Board approval. All patients included in the study consented to use their anonymized data for education/research purpose. Women who underwent their first or second intracytoplasmic sperm injection (ICSI) with GnRH antagonist and day-3 fresh ET between January 2017 and April 2019 were included. The study sample was determined according to the number of patients who met the eligibility criteria during the study's period and not on a previously calculated equation. Only levels of Anti-Müllerian hormone (AMH) ≥1 ng/ml and a basal FSH <10 mIU/mL were eligible for the study. IVF cycles involving uterine factor or surgically retrieved sperm were excluded.

Ovarian stimulation

Ovarian stimulation was started on either spontaneous or assigned day-2 after priming with low-dose oral contraceptive pills containing 0.03 mg of ethinyl estradiol and 0.075 mg gestodene (Gynera, Bayer Schering Pharma, Germany). Stimulation was started with 4–5 days of recombinant FSH (Gonal-F, MerkSerono Pharmaceutical, Egypt) followed by intramuscular menotropins injections (Menogon, Ferring, Germany). GnRH antagonist (InjCetrotide 0.25 mg SC daily, Merck-Serono, Germany) was added from the day when estradiol level reached ≥500 pg/mL, or the leading follicle was ≥14 mm. Gonadotropins dose was determined according to age, body mass index (BMI), ovarian reserve, and ovarian response history for patients undergoing the second ICSI trial. For

ovulation triggering either 10,000 IU human chorionic gonadotropin (Choriomon, IBSA Pharmaceutical, Egypt) or 250 μg of rHCG (Ovidrel; EMD Serono, Canada) were administered when ≥3 follicles reached a mean diameter of 17 mm. A transvaginal ultrasound-guided follicular aspiration was performed 34–36 h after the trigger.

Mature oocytes were fertilized by ICSI 6 h after the retrieval with the husband's sperm. Embryos that reached eight-cell stage on day 3 with <20% fragmentation are defined as good quality embryos.[26] Intramuscular P (Prontogest, IBSA Pharmaceutical, Egypt) at 25 mg twice daily was used for luteal phase support after oocyte retrieval until a pregnancy test. One or two best quality embryos were transferred on day-3 after egg retrieval according to the patients' age and embryo quality. A serum pregnancy check was done 14 days after ET.

Measurement of outcomes

The primary outcome was the ongoing pregnancy rate defined as the number of cases with pregnancy >12 weeks of gestation divided by the cycles initiated per 100. The secondary outcome was the implantation rate calculated as the number of gestational sacs observed, divided by the number of embryos transferred.

Hormone measurements

Serum P and E2 levels were measured on a triggering day and analyzed by the Mini-Vidas technique with a sensitivity of 0.2 ng/ml (range of measurement was 0.2–40 ng/ml). Progesterone/estradiol ratio was calculated as [(P (ng/mL) × 1000)/E2 (pg/mL)]. Our hormone measurements were usually performed between 8 and 10 am to limits the diurnal variation of hormones.[27] Coefficients of variations of hormonal measurements were <3% (internal laboratory data).

Grouping of patients

A receiver operating curve analysis was performed to detect the predictability of serum P and P/E2 for pregnancy outcomes [Figure 1]. Nevertheless, the area under the curve (AUC) was insufficient to obtain an efficient cutoff level. The AUC was 0.59 for serum P and 0.67 for P/E2. Thus, we chose cutoff values of 1.5 ng/ml for serum P and 0.55 for P/E2 based on a literature review.[21] First, patients were divided into two groups according to either *P* level (low *P* <1.5 ng/ml and HP ≥1.5 ng/ml) or P/E2 ratio (low P/E2 <0.55 and HP/E2 ≥ 0.55). Thereafter, patients were further divided into four subgroups [Group A: HP ≥1.5 ng/ml and HP/E2 ≥0.55, Group B: low *P* <1.5 ng/ml and low P/E2 <0.55, Group C: HP ≥1.5 ng/ml and low P/E2 <0.55 (HP only), and Group D: low *P* <1.5 ng/ml and HP/E2 >0.55 (HP/E2 only)].

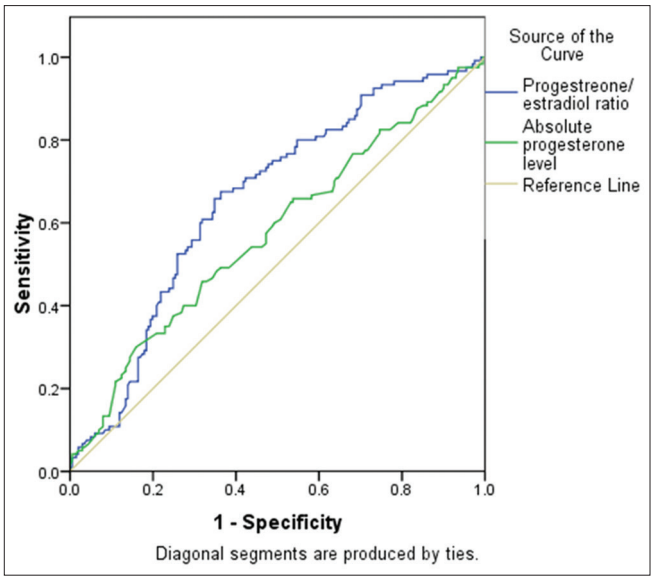

Figure 1: Receiver operating curve for the predictability of progesterone/estradiol ratio and progesterone for the ongoing pregnancy rate

Statistics

The collected data were entered into a Microsoft Access database and analyzed using the Statistical Package for the Social Sciences software (SPSS Inc., Chicago, Illinois, USA, version 21). Data are presented as mean ± standard deviation or frequencies and percentages. Patient's characteristics and cycle outcomes were compared between the groups of HP (≥1.5 ng/ml) and low P (<1.5 ng/ml) and between the groups of HP/E2 (≥0.55) and low P/E2 (<0.55) using the Student's *t*-test. Univariate analysis was used to study the association between HP and HP/E2 and pregnancy outcomes. Then, a multivariate binary logistic regression model was conducted to account for the cycle covariates. Patients were subdivided into four groups to investigate further whether the P/E2 ratio can add more information over P alone. The clinical characteristics and cycle data were analyzed using the ANOVA for the continuous variables and Chi-square for categorical ones. A two-sided $P < 0.05$ was considered to be statistically significant.

Results

A total of 512 fresh GnRH antagonist ICSI cycles was performed during the study period, of which 402 had day-3 ET and met the eligibility criteria of our study. The most frequent causes of infertility were male factor (32.6%), unexplained infertility (23.4%), anovulatory disorders (22.6%), tuboperitoneal factors (12.5%), and combined factors (8.7%). Primary infertility was encountered in 278 (69.2%) of cases and 331 (82.3%) patients had their first ICSI trial.

The baseline characteristics and stimulation cycle data of the study groups are presented in Table 1. The cohort with $P \geq 1.5$ ng/ml achieved comparable top quality embryos and implantation rate with those having $P < 1.5$ ng/ml. Nevertheless, ongoing pregnancy rate was lower when serum $P < 1.5$ ng/ml (24% vs. 37.7%, $P = 0.029$). On the other hand, the difference was more significant between patients with low P/E2 and HP/E2 in terms of the number of top quality embryos, implantation, and ongoing pregnancy rates (5.0 ± 2.8 vs. 3.3 ± 2.5, $P < 0.001$; 24.7% vs. 12.3%, $P < 0.001$; 42% vs. 16.6%, $P < 0.001$, respectively).

Table 2 summarizes the subgroup comparisons of P level and P/E2 ratio. As compared with low P and HP/E2 group (Group D), the group of HP and low P/E2 (Group C) yielded higher number of mature follicles, retrieved oocytes, mature oocytes, and embryos ($P < 0.01$) despite lower gonadotrophins dose used ($P = 0.023$). In addition, Group C had the highest peak estradiol level and number of good quality embryos among all groups.

The highest ongoing pregnancy rate was observed in Group B (low P and low P/E2), whereas the lowest one was in Group A (HP and HP/E2). Patients with HP/E2 and low P (Group D) had a lower pregnancy rate than those with low P and low P/E2 ratio (Group B) (21.3% vs. 42.2%, $P < 0.001$). On the contrary, pregnancy was not significantly different between the groups of HP and low P/E2 (Group C) and low P and low P/E2 (Group B) (34% vs. 42.2%, $P = 0.33$).

Table 2 also illustrates that Group C (HP and low P/E2) and Group B (low P and low P/E2) generated the highest number of good quality day-3 embryos with a nonsignificant difference in the pairwise comparison of them ($P > 0.05$). On the other hand, the elevation of P/E2 alone (Group D) led to a similar number of good embryos compared to the rise of both P and P/E2 ($P > 0.05$).

In unadjusted univariate analysis, both P and P/E2 showed a statistically significant effect on the ongoing pregnancy rate ($P = 0.031$, $P < 0.001$ for P and P/E2, respectively). The multivariate logistic regression analysis model demonstrated that HP did not have a significant association with pregnancy (adjusted odds ratios [aOR]: 0.542, 95% confidence interval [CI] 0.284–1.036, $P = 0.064$), yet P/E2 still has a significant inverse effect on pregnancy (aOR: 0.409, 95% CI 0.222–0.753, $P = 0.004$) [Table 3].

A correlation analysis was performed to further investigate the patient's profile and cycle parameters in relation to follicular P elevation [Table 4]. Taking all cycle confounders into account (age, BMI, AFC, AMH, number of mature follicles, total

Table 1: Baseline characteristics and cycle parameters

Characteristics	*P*			*P*/E2		
	High P (≥1.5) (*n*=75)	Low P (<1.5) (*n*=327)	*P*	High P/E2 ratio (≥0.55) (*n*=114)	Low P/E2 ratio (<0.55) (*n*=288)	*P*
Age (years)	31.3±4.3	30.8±4.3	0.434	31.9±4.9	30.5±4.0	0.002*
BMI (kg/m^2)	26.5±4.4	28.4±4.8	0.003*	26.7±4.1	28.6±4.9	0.001*
Duration of infertility (years)	6.7±3.3	6.4±3.0	0.581	6.4±3.7	6.5±2.9	0.851
AFC	16.2±9.9	14.8±8.5	0.235	12.6±7.9	16.0±9.0	<0.001*
AMH (ng/mL)	3.6±3.6	3.5±2.9	0.905	2.5±2.3	3.9±3.2	<0.001*
Basal FSH (IU/L)	6.2±2.4	6.2±2.7	0.963	7.4±3.2	5.7±2.3	0.001*
Total gonadotrophin dose	3384.7±1123.9	3126.8±1087.2	0.066	3719.7±1312.3	2959.3±916.4	<0.001*
Stimulation days	11.7±1.7	11.6±1.5	0.406	11.7±1.8	11.6±1.4	0.342
Endometrial thickness	9.6±1.7	9.6±1.7	0.808	8.9±1.9	9.8±1.5	<0.001*
Peak estradiol	3857.9±2564.1	2845.1±1685.87	<0.001*	1732.4±821.2	3549.3±1983.9	<0.001*
Follicles ≥15 mm	21.6±11.6	17.5±9.2	0.001*	14.2±9.5	19.8±9.4	<0.001*
Retrieved oocytes	18.3±9.9	14.5±7.4	<0.001*	12.0±8.2	16.5±7.7	<0.001*
Mature oocytes	13.6±7.5	11.0±5.7	<0.001*	9.1±6.4	12.5±5.8	<0.001*
Fertilized	9.8±5.4	8.3±4.7	0.020*	6.7±4.9	9.3±4.7	<0.001*
Number of good quality day-3 embryos	4.6±2.9	4.5±2.7	0.872	3.3±2.5	5.0±2.8	0.001*
Rate of top quality embryo formation	54.5±23.6	56.8±28.5	0.589	57.9±39.2	55.6±19.3	0.051
Transferred embryos	1.38±0.9	1.74±0.6	<0.001*	1.45±0.8	1.76±0.6	<0.001*
Implantation rate (%)	18/104 (17.3)	126/558 (22.6)	0.074	19/155 (12.3)	125/507 (24.7)	<0.001*
Ongoing pregnancy (%)	18/75 (24)	122/327 (37.3)	0.029*	19/114 (16.6)	121/288 (42.0)	<0.001*

Data are presented as mean±SD or number and %. *Statistically significant difference. P=Progesterone, P/E2=Progesterone/estradiol ratio, BMI=Body mass index, AFC=Antral follicle count, AMH=Anti-Müllerian Hormone, FSH=Follicle-stimulating hormone, SD=Standard deviation

Table 2: Subset analysis based on progesterone level and progesterone/estradiol ratio

Characteristics	Group A High P and high P/E2 (*n*=33)	Group B Low P and low P/E2 (*n*=251)	Group C High P and low P/E2 (*n*=38)	Group D Low P and high P/E2 (*n*=80)	*P*
Age (years)	32.3±4.5	30.5±4.0	30.3±3.8	31.8±5.1	0.05
BMI (kg/m^2)	26.6±3.9	28.9±4.9	26.5±4.9	26.8±4.2	0.001*
Duration of infertility (years)	6.7±3.7	6.4±2.9	6.9±2.7	6.2±3.7	0.751
AFC	13.6±8.8	15.7±8.8	19±10.3	12.1±7.3	<0.001*
AMH (ng/ml)	2.5±1.9	3.8±3.0	4.7±4.4	2.5±2.4	<0.001*
Basal FSH (IU/L)	6.6±2.3	5.7±2.2	5.8±2.4	7.7±3.4	<0.001*
Total gonadotrophin dose	3520.9±1163.9	2934.3±899.6	3167.6±1022.2	3764.1±1378.2	<0.001*
Stimulation days	11.7±1.7	11.6±1.4	11.7±1.7	11.7±1.8	0.950
Endometrial thickness (mm)	9.1±1.7	9.7±1.5	10.1±1.6	8.9±2.0	<0.001*
Peak estradiol	2312.9±1031.8	3270.1±1699.6	5441.4±2705.9	1522.3±613.9	<0.001*
Follicles ≥15 mm	17.7±12.5	19.1±9.2	25.6±9.6	12.3±7.0	<0.001*
Retrieved oocytes	15.0±10.8	15.8±7.3	21.5±8.1	10.7±6.2	<0.001*
Mature oocytes	11.1±7.8	11.9±5.5	16.2±6.5	8.3±5.4	<0.001*
Fertilized	8.1±5.7	9.0±4.6	11.5±4.5	6.1±4.3	<0.001*
Number of good quality day-3 embryos	3.3±2.7	4.9±2.7	5.9±2.7	3.5±2.4	<0.001*
Rate of top quality embryo formation	45.4±28.8	56.0±19.8	52.4±16.6	63.7±41.4	0.004*
Transferred embryos	1.3±0.9	1.8±0.5	1.4±0.9	1.5±0.8	<0.001*
Implantation rate (%)	3/43 (6.9)	110/450 (24.4)	13/53 (24.5)	17/114 (14.9)	<0.001*
Ongoing pregnancy (%)	3/33 (9.1)	106/250 (42.2)	13/38 (34.2)	17/80 (21.3)	<0.001*

Data are presented as mean±SD or number and %. *Statistical significant difference. Pairwise comparisons (when overall $P \leq 0.05$): BMI: A versus B=0.03, B versus C=0.023, B versus D=0.004, AFC: A versus C=0.037, B versus D=0 0.008, C and D=0.001, AMH: A versus C=0.006, B versus D=0.006, C versus D=0.001, Basal FSH: B versus D=0.001, C versus D=0.002, Gonadotrophins dose: A versus B=0.009, B versus D=0.001, C versus D=0.023, Endometrial thickness: B versus D=0.001, C versus D=0.013, Peak estradiol: A versus B=0.005, A versus C=0.001, B versus C=0.001, B versus D=0.001, C versus D=0.001, Follicles≥13 mm: A versus C=0.002, A versus D=0.027, B versus C=0.001, B versus D=0.001, C versus D=0.001, Retrieved oocytes: A versus C=0.001, B versus C=0.001, B versus D=0.001, C versus D=0.001, Mature oocytes: A versus C=0.001, B versus C=0.001, B versus D=0.001, C versus D=0.001, Fertilized: A versus C=0.013, B versus C=0.017, B versus D=0.001, Number of good quality day-3 embryos: A versus B=0.004, A versus C=0.001, B versus D=0.001, C versus D=0.001, Rate of top quality embryo formation: A versus D=0.003, Transferred embryos: A versus B<0.001, B versus D<0.001, B versus C<0.001, Implantation rate: A versus B<0.001, A versus C=0.020, B versus D=0.010, Ongoing pregnancy: A versus B<0.001, A versus C=0.012, B versus D=0.001. P=Progesterone, P/E2=Progesterone/estradiol ratio, BMI=Body mass index, AFC=Antral follicle count, AMH=Anti-Müllerian hormone, FSH=Follicle-stimulating hormone, SD=Standard deviation

Table 3: Association between ongoing pregnancy rate and serum progesterone or progesterone/estradiol ratio by multivariate logistic regression analysis

	Adjusted OR (95% CI)	*P*
P (ng/mL) (≥1.5 vs. <1.5)	0.542 (0.284-1.036)	0.064
P/E2 (≥0.55 vs. <0.55)	0.409 (0.222-0.753)	0.004*

*Statistically significant difference. The multivariate regression model included age, body mass index, antral follicle count, anti-Müllerian hormone, number of mature follicles, total gonadotrophins dose, number of oocyte retrieved, mature oocytes, number of good embryos obtained, and number of embryo transferred. P=Progesterone, P/E2=Progesterone/estradiol ratio, OR=Odds ratio, CI=Confidence interval

gonadotrophins dose, number of oocyte retrieved, mature oocytes, triggering-day P level, number of good embryos obtained, and number of embryo transferred), the multivariate logistic regression model did not reveal any effect for peak E2 level on ongoing pregnancy (aOR: 1.0, $P = 0.82$).

Discussion

Our study suggests that P elevation may not be an independent predictor for pregnancy outcome in GnRH antagonist cycle with day-3 ET. The study indicated that HP alone is not linked to adverse pregnancy outcomes; yet elevated P/E2 ratio on the ovulation triggering day is associated with a decrease in the ongoing pregnancy rate in the multivariate analysis.

Studying the value of adding P/E2 to serum P is of great importance to differentiate the P sources in different ovarian responders. In hyper-responder population, the HP levels might come from the cumulative production of a physiologic amount of P from the numerous growing follicles.[1] This should be differentiated from excess P secretion by a relatively low number of dysregulated follicles in patients with poor ovarian reserve.[28] The HP levels in patients with a poor ovarian reserve may originate from the intense stimulation with high-FSH

Table 4: Correlations between progesterone levels and demographics and cycle characteristics

	R	*P*
Baseline characteristics		
Age	0.047	0.34
BMI	−0.152	0.002*
AFC	0.003	0.951
AMH	0.042	0.405
FSH	0.029	0.562
LH	0.126	0.024*
Cycle stimulation parameters		
Peak estradiol level	0.259	<0.001*
Number of mature follicles	0.123	0.014*
retrieved oocytes	0.151	0.002*
Mature oocytes	0.129	0.009*
Number of embryos	0.014	0.782
Gonadotropins dose	0.128	0.010*
Stimulation days	0.098	0.051

*Statistically significant difference. BMI=Body mass index, AFC=Antral follicle count, AMH=Anti-Müllerian hormone, FSH=Follicle-stimulating hormone, LH=Luteinizing hormone

doses to overcome the defect encountered in their steroidogenic pathway.[29]

Elgindy[21] proposed that the clinical pregnancy rate is significantly higher in patients who had $P < 1.5$ ng/ml or P/E2 < 0.55 in comparison to those with $P \geq 1.5$ ng/ml and P/E2 ≥ 0.55, respectively, in long agonist protocol with cleavage ET. This study was agreed by our current data, yet in the antagonist protocol. Nevertheless, our adjusted multivariate analysis revealed that $P \geq 1.5$ ng/ml was no longer associated with lower ongoing pregnancy rate ($P = 0.064$) while HP/E2 showed a significant association ($P = 0.004$).

Arora *et al*.[30] performed a retrospective analysis for the predictability of HP and P/E2 on the GnRH antagonist cycle with day-5 blastocyst ET. HP did not experience any significant effect on implantation rate or clinical pregnancy rate (OR, 0.56; 95% CI, 0.25–1.25, $P = 0.16$) in contrast to the negative effect demonstrated by the P/E2 ratio (OR 0.58; 95% CI, 0.34–1.00, $P = 0.05$).

However, Lee *et al*.[31] suggested that the use of P/E2 is unfeasible in the clinical practice due to its low sensitivity and positive predictive value in GnRH agonist protocol. Golbasi *et al*.[32] demonstrated that P/E2 is not a significant predictive factor for the live birth rate after a retrospective analysis of 176 fresh ET of GnRH antagonist ICSI cycles with serum $P \geq 1.5$ ng/ml. However, the study did not define specific ET day (2nd, 3rd, and 5th days' embryos).

Although endometrial asynchrony is the widely accepted rationale behind the negative impact of PL on pregnancy outcomes,[4,5,33] recent reports demonstrated a link between PL and embryo quality.[6,22] Embryo utilization rate was significantly lower in patients with HP.[7] Similarly, PL was related to a lower percentage of top-quality blastocysts formation.[23] Our data demonstrated that PL based on P/E2 ≥0.55 and not on absolute P level is linked to a lower number of good quality day-3 embryos (5.0 ± 2.8 vs. 3.3 ± 2.5, $P = 0.001$, for high and low P/E2, respectively). Furthermore, the group of HP and HP/E2 had lower good embryos than that of HP only ($P < 0.001$). The same difference was observed in favor of low *P* and low P/E2 in comparison to HP/E2 only group ($P < 0.001$). Therefore, P/E2 ratio showed a better prognostic value for the increasingly-reported impact of PL on embryo quality.

Various risk factors were found to be linked to premature P elevation in the late follicular phase such as history of recurrent IVF failure and the patient's profile including age, ethnicity, and BMI.[24,25,34] The stimulation protocol, daily FSH dose, number of retrieved oocytes, peak estradiol level,[2] total dose of gonadotropins,[9] and stimulation days[10] were assumed to be contributory for the chance of P elevation. The correlation analysis in our study revealed a positive association between P elevation and peak estradiol level, stimulation days, mature follicles, retrieved oocytes, and mature oocytes. Progesterone elevation had a weak inverse correlation, yet significant with BMI ($R = -0.152$, $P = 0.002$).

Embryo cryopreservation with deferring ETs is a widely accepted rescue strategy overcoming the PL-induced endometrial asynchrony. Nevertheless, embryo freezing represents an extra burden on the IVF laboratory and can be complicated by embryo losses during thawing.[16] Therefore, identifying the accurate indicator and cutoff level for PL is important for the cost effective IVF management.

The association of peak estradiol (E2) level with pregnancy outcomes is conflicting. Some authors reported a positive correlation between peak E2 and number of oocytes retrieved, embryos allowed for transfer, adequate end thickens, and pregnancy outcomes.[35-37] A retrospective study conducted by Kara *et al*.[38] showed that the number of oocytes and clinical pregnancy are higher in patients with E2 ≥4000 pg/ml than those with E2 <4000 pg/ml. Peak E2 was not detrimental to the implantation rate, clinical pregnancy, or live birth rate.[39] Other reports failed to draw a conclusion about the association between the supraphysiologic E2 level and pregnancy outcomes.[40-42]

The main limitation of this study is its retrospective design. The study being conducted in a single IVF

center is another limitation on the size of data source. The difference regarding the number of embryos transferred in favor of the group with low P and low P/E2 can be initially considered a confounder in interpreting results. However, the multivariate regression analysis model accounted for the effect of various cycle covariates including the number of transferred embryos on ongoing pregnancy rate. The major strength of our study is the uniform stimulation protocol and day of ET. The pairwise comparison of all study subgroups added a depth to identify the value of adding P/E2 to the P level in criticizing P elevation in the late follicular phase. The study results are significant also for the ongoing widely used shift in the ART practice in favor of the antagonist protocol.

Conclusions

Combining P/E2 to the absolute P level can assist the clinical decision for predicting the PL impact on pregnancy and embryological outcomes. Progesterone/estradiol ratio may be of high prognostic value for cycle outcomes when compared to serum P level alone. Thus, our study does not support the routine use of deferred ET in IVF cycles in cases of HP level ≥1.5 ng/ml that is currently practiced in IVF centers worldwide. Considering the retrospective design of the current study, more robust data are needed to endorse such a conclusion.

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Conflicts of interest

There are no conflicts of interest.

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