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Original Article

Progesterone/estradiol ratio <0.25 on the day of human chorionic gonadotropin administration is associated with adverse pregnancy outcomes in prolonged protocols for *in vitro* fertilization/ intracytoplasmic sperm injection

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ABSTRACT

Objective: It has been suggested that a progesterone/estradiol ratio (P/E2) ≥ 1.0 on the day of human chorionic gonadotropin (hCG) administration indicates premature luteinization and might be associated with an adverse pregnancy; however, a lower limit of this ratio has not been determined. We aimed to identify a lower limit of P/E2 that correlates significantly with an increase in adverse pregnancies in patients undergoing a prolonged *in vitro* fertilization/intracytoplasmic sperm injection therapy.

Materials and Methods: This retrospective analysis involved 7451 patients who received the first cycle of *in vitro* fertilization/intracytoplasmic sperm injection therapy treatment at the Reproductive and Genetic Hospital of Citic–Xiangya between January 2008 and April 2012. Patients were stratified into six groups according to their P/E2 on the day of hCG administration. Primary pregnancy outcomes, rates of implantation, clinical pregnancy, ongoing pregnancies, spontaneous abortions, and live births were recorded. The association between P/E2 on the day of hCG administration and primary pregnancy outcomes was assessed using logistic regression analysis.

Results: The rates of implantation (23.85–33.44%), clinical pregnancy (47.42–67.12%), ongoing pregnancy (40.83–61.48%), and live birth (34.40–57.65%) were significantly decreased in patients with a P/E2 < 0.25. These indicators were significantly associated with P/E2, but no significant correlation was observed between P/E2 and early spontaneous abortion rate.

Conclusion: P/E2 < 0.25 on the day of hCG administration was associated with adverse pregnancy outcomes in extended treatments of gonadotropin-releasing hormone agonist IVF/ICSI.

Introduction

It is believed that increased luteinizing hormone (LH) during the late follicular phase of the menstrual cycle causes increases in progesterone (P), and attempts have been made to use gonadotropin-releasing hormone as an agonist to prevent the rise in LH and subsequent premature luteinization. Administration of gonadotropin-releasing hormone agonist (GnRH-a) induces pituitary desensitization, and can effectively suppress the surge of early endogenous LH by 95–98% [1]; however, premature luteinization can still occur during controlled ovarian hyperstimulation, with an incidence rate of 13–71% [2–9]. Plasma P levels are associated with the level of follicle-secreted P, the number of mature follicles, and the level of estradiol (E2) on the day of human chorionic gonadotropin (hCG) administration; therefore, premature luteinization, defined as the average P value per follicle and referred to as ng/mL P × 1000/pg/mL E2 (P/E2) is suggested to be more appropriate for assessing pregnancy outcomes [1,10,11].

Previous studies have suggested that P/E2 ≥ 1.0 on the day of hCG administration correlates with unfavorable pregnancy outcomes [1,11,12]. The major cause is that supraphysiological levels of steroid hormones P and E2 (P/E2 ≥ 1.0) might have the potential to advance endometrial maturation, and elevated P might hasten the

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closure of the implantation. According to this theory, an insufficient P/E2 and serum P level might delay endometrial development, and day-3 embryos would be placed in an asynchronous endometrium with a subsequent failure of establishing an embryo–endometrium cross dialogue and failure of implantation. Although many studies have attempted to define the impact on pregnancy of higher P/E2 levels on the day of hCG administration, results have proved contradictory, large-sample studies are lacking, and no lower P/E2 cutoff value has been established that can predict adverse pregnancy outcomes. Thus, we performed a retrospective analysis of patients with normal ovarian reserve who received long-term *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatment and attempted to define the lowest P/E2 value that reliably predicts adverse clinical pregnancy outcomes.

Methods

Study participants

This retrospective analysis involved 7451 infertile women who received the first cycle of IVF/ICSI following an extended GnRH-a protocol at the Reproductive and Genetic Hospital of Citic–Xiangya between January 2008 and April 2013. Women who reported a normal sex life but were not pregnant 1.0 year after beginning sexual intercourse without contraception were defined as infertile.

Inclusion criteria

The inclusion criteria for this study were: (1) age <35 years; (2) regular menstrual cycles of 24–35 days; (3) baseline follicle-stimulating hormone (FSH) < 9.0 IU/L; (4) more than seven preantral follicles; and (5) endometrial thickness ≥8.0 mm on the day of hCG administration.

Exclusion criteria

The exclusion criteria were: (1) polycystic ovary syndrome; (2) uterine abnormalities (double uterus, bicornuate uterus, unicornuate uterus, and uterine mediastinum); (3) intrauterine adhesions, endometriosis, adenomyosis, hydrosalpinx, uterine fibroids (submucosal fibroids, nonmucosal fibroids >4.0 cm and/or endometrial pressure), thyroid dysfunction, or hyperprolactinemia; (4) P/E2 ≥ 1.0 on the day of hCG administration; (5) history of adverse pregnancy (including spontaneous abortion, stillbirth, and fetal malformation); or (6) chromosomal abnormalities or partners with chromosomal abnormalities.

Study groups

The women were divided into six groups according to their P/E2 on the day of hCG administration based on a recent study reporting that P/E2 ≥ 0.55 might adversely affect pregnancy outcome [13]. The groups were as follows: (1), $n = 800$ ($0.55 \leq$ P/E2 < 1.0); (2), $n = 640$ ($0.45 \leq$ P/E2 < 0.55); (3), $n = 1150$ ($0.35 \leq$ P/E2 < 0.45); (4), $n = 1655$ ($0.25 \leq$ P/E2 < 0.35); (5), $n = 1962$ ($0.15 \leq$ P/E2 < 0.25); and (6), $n = 1244$ (P/E2 < 0.15).

Controlled ovarian hyperstimulation

Controlled ovarian hyperstimulation [14] was performed on each patient using the following protocols. Patients were subcutaneously injected with 1.5 mg triptorelin acetate (Ipsen Biotech, Paris, France) during the midluteal phase of their menstrual cycle, 7.0 days after ovulation. From 3.0 days to 5.0 days after the first day of menstruation, the patient's serum estrogen, follicle stimulating hormone, LH, P, testosterone, and prolactin levels were measured, and follicular diameter and endometrial thickness were monitored using ultrasonography. Patients who met the established criteria for pituitary downregulation, E2 < 50 pg/mL, LH < 5.0 IU/L, follicular diameter < 10 mm, and endometrial thickness < 5.0 mm, received an intramuscular injection of recombinant human FSH (r-hFSH, Gonal-F, Merck-Serono) to induce ovulation. The r-hFSH dose was adjusted according to serum hormone levels and ultrasonography observations and ranged from 75 IU/d to 300 IU/d. When one or two follicles ≥18 mm in diameter were detected, patients were injected with 5000–10,000 IU hCG (Livzon Pharmaceutical Group Inc., Gaungong, China). Oocyte recovery was performed 34–36 hours later using a transvaginal ultrasound-guided technique, followed by a conventional IVF/ICSI procedure, and cleavage-stage embryos were transferred 72 hours later. The luteal phase was supported by intravaginal sustained-release P vaginal gel (Fleet Laboratories Ltd., Watford, Herts, UK) or 400 mg utrogestin (Besins-Iscovesco Pharmaceuticals, Paris, France) administered daily (200 mg in the morning and 200 mg in the evening) beginning on the day of embryo transfer with or without 60 mg intramuscular P in oil. The standard for the high risk of ovarian hyperstimulation syndrome leading to cancellation of the program in our hospital were: (1) E2 ≥ 7000 pg/mL on hCG day; (2) ≥ 28 oocytes retrieved; or (3) significant abdominal pain or other obvious discomfort.

Follow-up and observational indices

Blood β-hCG was measured 2.0 weeks after transplantation to detect pregnancy. Pregnant participants received ultrasound examination after 2.0 weeks, and pregnancy was confirmed if an intrauterine embryo was observed and a beating heart tube was detected. Ongoing pregnancy refers to cases in which the fetus survived for 70 days after transplantation. Early spontaneous abortion refers to miscarriage within 12 weeks. The live birth rate refers to the birth rate after transplantation.

Statistical analyses

Statistical analyses were performed using PASW 18.0 (SPSS Inc., Chicago, IL, USA). Numerical data are presented as the mean ± standard deviation. Non–normally-distributed data are presented as the median (interquartile range). Comparisons between multiple mean values were made using the Kruskal–Wallis one-way analysis of variance. Categorical data are presented as number (n) and percentage (%), and comparisons were made using the Chi-square test. Logistic regression was performed using the pregnancy outcomes as dependent variables and the baseline FSH, LH, E2, and P/E2 on the day of hCG administration as independent variables. Statistical significance was defined at $p < 0.05$.

Results

Patient characteristics

The 7451 women were divided into six groups according to their P/E2 on the day of hCG administration (Table 1). The average age of patients; duration of infertility; type of infertility; body mass index; levels of baseline FSH, LH, and E2; and the number of transferred embryos per patient did not differ significantly among the six patient groups, but there were significant statistical differences in the causes of ovulation dysfunction of infertility among the six patient groups (Table 1).

Table 1
Patient demographic and clinical characteristics.

	Group 1 (0.55 ≤ P/E2 < 1) (n = 800)	Group 2 (0.45 ≤ P/E2 < 0.55) (n = 640)	Group 3 (0.35 ≤ P/E2 < 0.45) (n = 1150)	Group 4 (0.25 ≤ P/E2 < 0.35) (n = 1655)	Group 5 (0.15 ≤ P/E2 < 0.25) (n = 1962)	Group 6 (P/E2 < 0.15) (n = 1244)	p
Age (y)	29.75 ± 3.35	29.62 ± 3.31	29.68 ± 3.37	29.70 ± 3.30	29.67 ± 3.33	29.72 ± 3.35	0.986
Duration of infertility (y)	4.36 ± 2.66	4.47 ± 2.9	4.7 ± 2.96	4.55 ± 2.84	4.67 ± 2.94	4.47 ± 2.8	0.127
Type of infertility							
Primary	49% (392/800)	52.18% (334/640)	49.3% (567/1150)	50.39% (834/1655)	50.3% (987/1962)	54.42% (677/1244)	0.095
Secondary	51% (408/800)	47.82% (306/640)	50.7% (583/1150)	49.61% (821/1655)	49.7% (975/1962)	45.58% (567/1244)	0.095
Cause of infertility							
Male factor	7.12% (57/800)	9.84% (63/640)	9.04% (104/1150)	7.73% (128/1655)	7.7% (151/1962)	8.36% (104/1244)	0.316
Tubal factor	85.62% (685/800)	82.65% (529/640)	82.96% (954/1150)	83.15% (1376/1655)	81.96% (1608/1962)	81.11% (1009/1244)	0.155
Ovulation dysfunction	4.75% (38/800)	6.25% (40/640)	5.83% (67/1150)	7.73% (128/1655)	8.92% (175/1962)	9.23% (115/1244)	<0.001
Unexplained	0.63% (5/800)	0.63% (4/640)	0.52% (6/1150)	0.36% (6/1655)	0.25% (5/1962)	0.65% (8/1244)	0.544
Male and female factors	1.88% (15/800)	0.63% (4/640)	1.65% (19/1150)	1.03% (17/1655)	1.17% (23/1962)	0.65% (8/1244)	0.065
Body mass index (kg/m^2)	21.36 ± 2.56	21.28 ± 2.54	21.33 ± 2.57	21.27 ± 3.27	21.23 ± 3.41	20.29 ± 5.25	0.266
Baseline FSH (mIU/mL)	4.3 ± 2.37	4.18 ± 2.38	4.32 ± 2.35	4.25 ± 2.37	4.31 ± 2.37	4.24 ± 2.35	0.736
Baseline LH (mIU/mL)	3.50 ± 1.60	3.43 ± 1.54	3.50 ± 1.59	3.48 ± 1.57	3.47 ± 1.59	3.52 ± 1.60	0.859
Baseline E2 (pg/mL)	20 (9.96, 36)	19.19 (9.49, 35)	19.13 (9.36, 36.7)	18.5 (8.76, 35.1)	18.7 (9.23, 35)	18.2 (8.86, 33.9)	0.297
Number of transferred embryos	2.04 ± 0.27	2.03 ± 0.25	2.04 ± 0.25	2.03 ± 0.25	2.04 ± 0.26	2.02 ± 0.24	0.595

Data are mean ± standard deviation, % (n/total), or odds ratio (95% confidence interval).
E2 = estradiol; FSH = follicle-stimulating hormone; LH = luteinizing hormone; P = progesterone.

Primary pregnancy outcome

Patients with a P/E2 between 0.35 and 0.45 (Group 3) achieved the highest implantation rate (33.44%), clinical pregnancy rate (67.12%), ongoing pregnancy rate (61.48%), and live birth rate (57.65%). These rates were slightly lower in patients with a P/E2 between 0.25 and 0.35 (Group 4), and significantly reduced in patients with a P/E2 < 0.25. Patients with a P/E2 between 0.35 and 0.45 (Group 3) had the lowest rate of early spontaneous abortion (4.2%), while >10% of patients with a P/E2 < 0.35 suffered early spontaneous abortions (Table 3).

Association analysis

The association among pregnancy outcome (rate of implantation, clinical pregnancy, ongoing pregnancy, spontaneous abortion, and live birth) and patient demographic and clinical characteristics (age; duration of infertility; baseline FSH, LH, and E2; and P/E2 on the day of hCG administration) was analyzed. The results suggested that P/E2 on the day of hCG administration was an independent predictor of implantation rate [odds ratio (OR) = 1.060, 95% confidence interval (CI) 1.003–1.119; p = 0.038]. A logistic regression analysis was performed to evaluate the association between pregnancy outcome and P/E2 on the day of hCG administration. We observed that the rates of implantation and clinical pregnancy were significantly decreased (p < 0.001) in patients with a P/E2 < 0.25 (Groups 5 and 6) when compared with that of patients with P/E2 between 0.55 and 1.0 (Group 1). OR of ongoing pregnancy and live birth were significantly reduced in patients with a P/E2 < 0.35 (Groups 4, 5, and 6, OR < 1.0, p < 0.001), and the early spontaneous abortion rate was significantly associated with P/E2 between 0.25 and 0.35 (Group 4, OR = 2.556, 95% CI 1.575–4.147, p < 0.001; Tables 3 and 4).

Table 2
Primary pregnancy outcome.

	Group 1 (0.55 ≤ P/E2 < 1) (n = 800)	Group 2 (0.45 ≤ P/E2 < 0.55) (n = 640)	Group 3 (0.35 ≤ P/E2 < 0.45) (n = 1150)	Group 4 (0.25 ≤ P/E2 < 0.35) (n = 1655)	Group 5 (0.15 ≤ P/E2 < 0.25) (n = 1962)	Group 6 (P/E2 < 0.15) (n = 1244)	p
Implantation rate	31.93%	33.06%	33.44%	30.79%	23.85%	27.95%	<0.001
Clinical pregnancy rate	65%	66.44%	67.12%	61.78%	47.42%	55.04%	<0.001
Ongoing pregnancy rate	57.16%	60.91%	61.48%	48.16%	40.83%	47.45%	<0.001
Early abortion rate	4.36%	5.8%	4.2%	10.45%	7.01%	7.93%	<0.001
Live birth rate	55.54%	57.05%	57.65%	41.70%	34.40%	38.71%	<0.001

E2 = estradiol; P = progesterone.

Table 3
Logistic regression of progesterone/estradiol ratio (P/E2) ratio with primary pregnancy outcomes (rate of implantation, clinical pregnancy, and long-term pregnancy).

Group	Implantation rate			Clinical pregnancy rate			Ongoing pregnancy rate		
	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
1	1			1			1		
2	1.086	(0.872, 1.352)	0.46	1.034	(0.827, 1.292)	0.77	1.135	(0.912, 1.412)	0.258
3	1.167	(0.964, 1.411)	0.112	1.094	(0.902, 1.329)	0.362	1.173	(0.97, 1.417)	0.099
4	0.945	(0.792, 1.128)	0.535	0.869	(0.726, 1.041)	0.127	0.68	(0.57, 0.811)	<0.001
5	0.508	(0.428, 0.603)	0.00	0.482	(0.404, 0.575)	0.00	0.501	(0.421, 0.596)	<0.001
6	0.566	(0.469, 0.682)	0.00	0.582	(0.48, 0.706)	0.00	0.593	(0.49, 0.718)	<0.001

CI = confidence interval; OR = odds ratio.

Table 4
Logistic regression of progesterone/estradiol ratio (P/E2) ratio with primary pregnancy outcomes (rates of spontaneous abortion and live birth).

Group	Early abortion rate			Live birth rate		
	OR	95% CI	*p*	OR	95% CI	*p*
1	1			1		
2	1.351	(0.736, 2.479)	0.332	1.265	(0.851, 1.88)	0.245
3	0.962	(0.546, 1.694)	0.893	1.037	(0.747, 1.438)	0.829
4	2.556	(1.575, 4.147)	<0.001	0.354	(0.265, 0.472)	<0.001
5	1.652	(0.990, 2.758)	0.055	0.45	(0.335, 0.605)	<0.001
6	1.889	(1.102, 3.239)	0.021	0.404	(0.295, 0.553)	<0.001

CI = confidence interval; OR = odds ratio.

Secondary pregnancy outcome

Patients with a P/E2 between 0.25 and 0.15 had the highest rate of high-quality embryos (86.69%) and LH levels (1.28, 0.93, 1.78 mIU/mL) on hCG day, and the highest dosage of micronized P (323.76 ± 157.30 mg/d). By contrast, patients with a P/E2 < 0.15 had the most oocytes retrieved (17.03 ± 6.54), highest cycle cancellation rate (22.75%), and the highest total gonadotropin dosage (1774.59 ± 3055.23; Table 5).

Discussion

Previous studies have predominantly focused on the upper limit of P/E2 on the day of hCG administration. In this study, we focused on patients with P/E2 < 1.0 on the day of hCG administration. Based on the reported predictive value of P/E2 > 0.55 [13], we assigned patients with 0.55 ≤ P/E2 < 1.0 into one group to use as a reference in logistic regression analysis, and subdivided patients into five additional groups according to P/E2 on the day of hCG administration. In our sample, P/E2 < 0.35 was correlated with adverse pregnancy outcomes and live birth rate, and patients with P/E2 < 0.25 had significantly decreased rates of implantation, clinical pregnancy, ongoing pregnancy, and live birth. We observed that the rate of implantation, clinical pregnancy, ongoing pregnancy, and live birth increased gradually with increasing P/E2. Logistic regression confirmed that a lower P/E2, as seen in Group 5 (0.15 ≤ P/E2 < 0.25) and Group 6 (P/E2 < 0.15), was negatively associated with the rates of implantation (OR = 0.508, 0.566; $p < 0.001$), clinical pregnancy (OR = 0.482, 0.582; $p < 0.001$), ongoing pregnancy (OR = 0.501, 0.593; $p < 0.001$), and live birth (OR = 0.45, 0.0, 404; $p < 0.001$), respectively.

Our data suggested that Group 5 had the most high-quality embryos, but the implantation rate was significantly lower. Superphysiological levels of steroids might not only affect endometrium morphology, but might also damage endometrial receptivity by modulating P/E2 [15,16]. We observed that P/E2 < 0.25 (lower P level and higher E2 level, $p < 0.001$, Table 5) was associated with an adverse pregnancy outcome, the reason of which might be related to endometrial receptivity. The source of P in the early follicular phase of the menstrual cycle is merely of adrenal origin; however, in the late follicular phase, P accumulates mainly from the growing follicles and on rare occasions, from the premature luteinization of the leading follicle. In our study, serum P was gradually decreased with an increase in E2 and more oocytes retrieved from the groups. This phenomenon might be related to P metabolism. P is further metabolized to androgens by theca cells under the trophic influence of LH (highest LH level on hCG day in Group 5). In theca cell, androgens are subsequently converted into estrogen through aromatization in the granulose cells. It is probable that the greater the LH drive to the theca cells, the more P catabolism into androgens, leaving fewer products throughout the general circulatory system [17]; however, the P levels in Group 6 did not increase as expected (with lower LH levels on hCG day); therefore, the theory of P metabolism remains unproven and more research is needed. By contrast, high levels of estrogen can change the endometrium physiological environment. Simon et al [18] demonstrated that E2 > 3000 pg/mL is detrimental to implantation and pregnancy, for which the possible explanation is that high concentrations of E2 induce nonsynchronized development of the endometrial gland matrix, and reduce endometrial secretions, which results in an environment that is not conducive to embryo implantation. Our data indicated E2 levels > 3000 pg/mL in Groups 5 and 6 (P/E2 < 0.25). High E2 concentrations on hCG day reduced endometrial blood flow, which might be the reason for the low pregnancy rate. Another theory suggests that the pinopodes used as the endometrial receptivity markers are positively associated with P levels, and that lower P might destroy endometrial receptivity by reducing pinopode expression [19,20].

Changes in hormone levels (lower P levels, see Table 5) during controlled ovarian hyperstimulation might cause insufficient

Table 5
Secondary pregnancy outcomes.

	Group 1 (0.55 ≤ P/E2 < 1) (*n* = 800)	Group 2 (0.45 ≤ P/E2 < 0.55) (*n* = 640)	Group 3 (0.35 ≤ P/E2 < 0.45) (*n* = 1150)	Group 4 (0.25 ≤ P/E2 < 0.35) (*n* = 1655)	Group 5 (0.15 ≤ P/E2 < 0.25) (*n* = 1962)	Group 6 (P/E2 < 0.15) (*n* = 1244)	*p*
No. of follicles (≥15 mm)	7.38 ± 4.17	7.73 ± 4.06	8.50 ± 4.22	9.14 ± 4.63	10.13 ± 5.14	11.98 ± 5.56	<0.001
Number of oocyte retrieved	10.76 ± 4.87	11.42 ± 4.93	12.48 ± 5.09	13.19 ± 5.29	14.71 ± 5.96	17.03 ± 6.54	<0.001
High quality embryo rate	67.52%	65.71%	66.49%	66.78%	86.69%	68.36%	<0.001
Cancellation rate	7.5% (60/800)	6.9% (44/640)	4.52% (52/1150)	6.4% (106/1655)	11.11% (218/1962)	22.75% (283/1244)	<0.001
The high risk of OHSS for cancellation	3.3% (2/60)	2.3% (1/44)	3.8% (2/52)	1.9% (2/106)	11.92% (26/218)	17.66% (50/283)	<0.001
E2 on hCG day (pg/mL)	1558.5 ± 570.8	1873.1 ± 608.3	2169.9 ± 683.2	2595.4 ± 871.8	3335.4 ± 1184.1	4328.9 ± 2223.2	<0.001
P on hCG day (ng/mL)	1.05 ± 0.37	0.93 ± 0.3	0.86 ± 0.26	0.77 ± 0.25	0.66 ± 0.23	0.46 ± 0.21	<0.001
LH on hCG day (mIU/mL)	1.18 (0.87, 1.69)	1.22 (0.89, 1.70)	1.24 (0.91, 1.80)	1.27 (0.92, 1.75)	1.28 (0.93, 1.78)	1.24 (0.86, 1.64)	0.005
Total gonadotropin dosage (IU)	2734.02 ± 5037.33	2570.24 ± 1586.33	2325.60 ± 987.32	2223.14 ± 1035.62	1981.73 ± 1038.85	1774.59 ± 3055.23	<0.001
Endometrial thickness (mm)	12.38 ± 6.26	12.26 ± 2.35	12.69 ± 6.42	12.33 ± 3.68	12.35 ± 4.09	12.37 ± 2.25	0.385
Luteal phase support							
Micronized P (mg/d)	297.98 ± 174.94	268.75 ± 188.55	292.03 ± 177.95	280.66 ± 183.26	323.76 ± 157.30	281.33 ± 183.02	0.003
P sustained-release vaginal gel (mg/d)	76.93 ± 31.72	76.78 ± 31.88	77.08 ± 31.56	75.75 ± 32.86	74.95 ± 33.59	75.22 ± 33.34	0.421
IM of P in oil (mg/d)	0	0	0	0	13.07 ± 24.93	15.63 ± 26.44	–

Data are mean ± standard deviation, % (*n*/total), or odds ratio (95% confidence interval).
E2 = estradiol; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin; IM = intramuscular; LH = luteinizing hormone; OHSS = ovarian hyperstimulation syndrome; P = progesterone.

development of the endometrium, and transplantation of cleavage-stage embryos on endometrium with unsynchronized development is likely to affect embryo–endometrium cross dialogue and implantation adversely; however, the results of our data must be confirmed by additional experimental studies.

Furthermore, Group 5 ($0.15 \leq P/E2 < 0.25$) had the highest number of high-quality embryos, which suggests that $P/E2 < 0.25$ did not reduce embryo quality. Low P levels might need more luteal phase support and lead to adverse outcomes, but our results suggested that insufficient endogenous P production cannot be rescued by exogenous supplementation (micronized P with/without intramuscular P in oil, Table 5), and this was supported by Ioannidis et al [21] At the same time, we found that there were significant statistical differences in ovulation dysfunction among the six patient groups ($p < 0.001$; Table 1). Ovulation dysfunction often means insufficient luteal function, not enough exogenous luteal phase support (Table 5), and a higher spontaneous abortion rate in IVF/ICSI (Table 2). Freezing–thawing embryo transfer with more exogenous luteal-phase support might be a choice in patients with $P/E2 < 0.25$. Patients with a $P/E2 < 0.15$ had the highest treatment cancellation rate (22.75%), potentially as a result of the high levels of estrogen in these patients after hCG administration, and the high risk for ovarian hyperstimulation syndrome (Table 5). Lai et al [22] found that $P/E2 > 1.2$ on the day of hCG administration did not have a negative impact on the clinical pregnancy rate in women with normal ovarian reserve treated at length with GnRH-a. In their study, the researchers used a receiver operating characteristic analysis to determine the most efficient cutoff value for the P/E2 associated with premature luteinization to discriminate between successful and unsuccessful IVF outcomes. However, their small study ($n = 139$) had low specificity (32.0%) and the area under the receiver operating characteristic curve was unsatisfactory (area under the curve 0.534, 95% CI, 0.456–0.613). To indicate that a parameter might have prognostic value, it must result in an area under the curve > 0.80 [23] in a large study.

Study limitations

In our study, we were limited by the fact that we designed our protocol on the results of previously published reports and did not conduct a prospective study; however, we identified significant differences in pregnancy outcomes in patients stratified by P/E2, and concluded that a $P/E2 < 0.25$ on the day of hCG administration correlates with adverse pregnancy outcomes in extended IVF/ICSI treatment in patients with normal ovarian response.

Conflicts of interest

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

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