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Original Article

Increasing elevated progesterone levels on the day of human chorionic gonadotropin administration in an in vitro fertilization cycle agonist protocol had different predicted thresholds of pregnancy outcomes in different age groups: analysis of 5,566 cycles

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Abstract: The aim of this study was to investigate the relationships among the ages of patients, serum progesterone (P) levels on the day of human chorionic gonadotropin administration (D_{HCG}), and clinical pregnancy rates in a gonadotropin-releasing hormone (GnRH)-agonist long protocol. A total of 5,566 patients followed at the reproductive medicine center of a tertiary care public hospital who were undergoing in vitro fertilization/intracytoplasmic sperm injection treatment with a GnRH agonist long protocol that proceeded to embryo transfer (ET) were studied. Retrospective analysis was performed and clinical pregnancy rates were calculated. With increasing serum P levels on D_{HCG}, clinical pregnancy rates declined. The cutoff values of serum P on D_{HCG} in patients aged ≤30 years old, 31-34 years old, and 35-37 years old were 4.5 nmol/L, 2.5 nmol/L, and 5.5 nmol/L, respectively. Logistic regression analysis showed that age and serum P on D_{HCG} were risk factors that would affect the clinical pregnancy rate. Multiple linear regression analysis showed that in the overall sample group, the serum P levels on D_{HCG} were positively correlated with age, total follicle-stimulating hormone (FSH) level, estradiol (E2) level on D_{HCG}, and number of ova obtained; however, the serum P levels on D_{HCG} showed no correlation with different age groups. Increasing the elevated serum P level on D_{HCG} could decrease the clinical pregnancy rate, while different age groups had different cutoff values. Age was not only an important factor affecting the clinical pregnancy rate, but also affected the serum P level on D_{HCG}.

Keywords: HCG, progesterone, pregnancy rate, in vitro fertilization, GnRH-agonist

Introduction

During the process of controlled ovarian hyperstimulation (COH) in pituitary gonadotropin releasing hormone (GnRH) agonist downregulation cycles, 2-35% of the cycles demonstrate early onset of serum progesterone (P) level elevation on the day of human chorionic gonadotropin administration (D_{HCG}) [1, 2]; this phenomenon is called premature luteinization (PL), or premature progesterone rise (PPR).

As early as 1990, some researchers began to focus on the effect of PPR on D_{HCG} in the in vitro fertilization (IVF) cycle with regard to pregnancy outcomes [3]. It was reported that in the late follicular stage of the COH cycle, the serum P level had no effect on the clinical outcome, and could not be used to predict the success of clinical pregnancy [4, 5]. However, some other evidence [6, 7] suggested that the earlier elevation of P on D_{HCG} in the in vitro fertilization (IVF) cycle was negatively correlated with pregnancy outcomes. Urman et al. [8] studied 911 intracytoplasmic sperm injection (ICSI) patients, using a receiver operating characteristic (ROC) curve to select 0.9 ng/mL as the cutoff serum P level

on D_{HCG}, and found that an elevated P level on D_{HCG} had no effect on the implantation rate. Bosch et al. [9] used the Mantel-Haenszel test to study 4,000 patients undergoing IVF/ICSI, and found that when the serum P level on D_{HCG} was above 1.5 ng/mL, the ongoing pregnancy rate was significantly reduced, and irrelevant to the applied ovulation induction protocol. Recently, Huang et al. [10] reported that the cutoff values of serum P level on D_{HCG} in long-protocol and short-protocol groups were different (1.2 ng/mL and 2.0 ng/mL, respectively), and when the serum P level on D_{HCG} was higher than the cutoff value, the birth rate was significantly decreased. Previous research was inconclusive about the effects of PPR on D_{HCG} for IVF-ET pregnancy outcomes, and the cutoff values for PPR still lack uniform standards. Meanwhile, because of the differences in statistical methods in defining PPR cutoff, it is necessary to reevaluate the relationships of PPR on D_{HCG} and IVF pregnancy outcomes during the COH process.

It is well known that age is an important factor affecting pregnancy rates. With increasing age, ovarian reserve function diminishes, and the quantity and quality of ova also decrease; therefore, pregnancy rates correspondingly decrease, which should be extremely obvious in patients over 35-40 years old [11, 12]. We hypothesized that PPR on D_{HCG} in different age groups had different cutoff values in the IVF cycle, which would play an important role in predicting pregnancy outcomes. Therefore, this study retrospectively analyzed 5,566 cycles in the IVF long protocol, and divided the patients into four age groups to assess the impact and predictive value of PPR on D_{HCG} on the clinical pregnancy rate, aiming to identify relationships among age, elevated serum P levels on D_{HCG}, and pregnancy outcomes.

Methods

Subjects

This was a non-intervention retrospective study performed at a single reproductive medical center; 5,566 patients who underwent IVF/ICSI treatment in the Third Affiliated Hospital of Guangzhou Medical University from January 2011 to December 2012 were analyzed. All patients were in the first treatment cycle of a luteal-phase GnRH-agonist downregulation long protocol, and fresh embryos were transferred in this cycle; the number of embryos transferred was 2-3, and patients with only one embryo transferred were excluded. Because this was a retrospective study, no additional intervention was performed, and there was no need to obtain permission for the study from the ethics committee. The center was supervised by the Chinese National Health Planning Commission and was authorized to perform assisted reproductive technology treatments.

Controlled ovarian stimulation protocol

In the luteal-phase GnRH-agonist long protocol, the patient received a single intramuscular injection of 1.0-1.875 mg triptorelin depot (Ipsen, France) for pituitary downregulation in the mid-luteal phase of the previous menstrual cycle (6-7 days after ovulation), or approximately 16 days after orally administered short-acting contraceptives. Fourteen days after triptorelin injection, when the patient met the criteria for pituitary downregulation (vaginal ultrasound showing maximum follicular diameter <10 mm, serum follicle-stimulating hormone [FSH] <5.0 mIU/mL, luteinizing hormone [LH] <5.0 mIU/mL, and estradiol [E2] <183 pmol/L), she was given recombinant FSH (rFSH: Puregon, USA, or Gonal-F, Serono, Switzerland) to promote folliculogenesis; the initial dose was based on the patient's age, number of antral follicles, baseline FH, and anti-Mullerian hormone (AMH) levels, and the subsequent dosage administered was adjusted by monitoring follicular development by vaginal ultrasound, serum E2, P, and LH levels. When the diameters of two or more dominant follicles were over 18 mm, 10,000 IU of hCG (Profasi; Serono, Switzerland) was administered via intramuscular injection, and the ova were sampled 34-36 h later.

Embryo culture, transfer

Patients were conventionally inseminated by IVF/ICSI; 12-18 h later, pronuclei were assessed to confirm fertilization (Day 1), as well as embryonic development 24 h (Day 2) and/or 48 h later (Day 3). Based on the embryo prokaryotic score, growth rate, and morphological parameters, such as the number of blastomeres, size, shape, symmetry, and debris, embryonic quality was divided into 4 grade levels: level I blastomeres had uniform size, regular shape, and 0-5% debris; level II blastomeres showed slight-

Table 1. Baseline characteristics of the IVF/ICSI-ET population

Parameter	Overall n=5566	Age 1 n=2513	Age 2 n=1846	Age 3 n=702	Age 4 n=505
Age (years)	31.29±4.22	27.60±2.07	32.33±1.11	35.85±0.82	39.48±1.47
Type of infertility [n (%)]					
Primary infertility	2779 (49.93)	1458 (58.02)	901 (48.81)	259 (36.89)	161 (31.88)
Secondary infertility	2787 (50.07)	1055 (41.98)	945 (51.19)	443 (63.11)	344 (68.12)
Infertility cause [n (%)]					
Tubal factor	3232 (58.07)	1420 (56.51)	1104 (59.80)	408 (58.12)	300 (59.41)
Endometriosis	107 (1.92)	33 (1.31)	46 (2.49)	16 (2.28)	12 (2.37)
ovulation disorders	255 (4.58)	138 (5.49)	83 (4.50)	24 (3.42)	10 (1.98)
Male factor	890 (15.99)	454 (18.07)	263 (14.25)	90 (12.82)	83 (16.43)
Female + male infertility	1069 (19.21)	465 (18.50)	344 (18.63)	162 (23.08)	98 (19.41)
Unexplained infertility	13 (0.23)	3 (0.12)	6 (0.33)	2 (0.28)	2 (0.40)
Procedure [n (%)]					
IVF	4474 (80.38)	1962 (78.07)	1513 (82)	589 (83.90)	410 (81.19)
ICSI	956 (17.18)	491 (19.54)	294 (15.9)	90 (12.82)	81 (16.04)
IVF/ICSI	136 (2.44)	60 (2.39)	39 (2.1)	23 (3.28)	14 (2.77)

ly nonuniform size, slightly irregular shape, and 10-20% debris; level III blastomeres showed significantly uneven size, significantly irregular shape, and 21-50% debris; level IV showed severely uneven cell size, with a serious granular cytoplasm phenomenon, and >50% debris [13, 14]. An embryo scored as level I or Ii (normal fertilization, with 2 pronuclei) on Day 1, Day 2, and Day 3, with 4-5 blastomeres on Day 2, or 7-9 blastomeres on Day 3, was of high-quality. According to the specifications for assisted reproductive technologies issued by the Chinese National Health Planning Commission, no more than three embryos could be transferred on Day 2 or 3 after ovum sampling. The patient was intramuscularly administered 40 mg of progesterone (Shanghai General Pharmaceutical, China) on the day of embryo transplantation for luteal support.

Hormone assays

Venous blood was sampled at 8-10 AM on D_{HCG} for serum P and E2 levels by electrochemiluminescence assay (Abbott Laboratories, USA). The intra- and inter-lot coefficients of variation of the detection reagents were less than 10% and 15%, respectively. The minimum sensitivity values for P, LH, and E2 were 0 nmol/L, 0.56 IU/L, and 0 pmol/L, respectively, with measurement ranges of 0-1,233 nmol/L, 0.56-89.08 IU/L, and 0-1,890 pmol/L. The levels were measured using the blood sample as drawn; if the concentration exceeded the measurement range, the sample was diluted by 10 times for detection [Serum P unit conversion (nmol/L)/3.18 = ng/mL. serum E2 unit conversion (pmol/L)/3.67 = pg/mL].

Outcome measures

Fourteen days after embryo transplantation, serum β-HCG was measured. When the serum β-HCG exceeded 25 IU/L, pregnancy was confirmed; 30 days after the transplantation, vaginal ultrasound was performed, and a case with a gestational sac and beating heart tube was identified as a clinical pregnancy.

Statistical analysis

All patients were divided into four groups by age. Group 1 was ≤30 years old; group 2, 31-34 years old; group 3, 35-37 years old; and group 4, ≥38 years old. Each age group was then divided into six subgroups according to the serum P levels on D_{HCG}: <1.5, 1.5-2.49, 2.5-3.49, 3.5-4.49, 4.5-5.49, and ≥5.5 nmol/L. The Mantel-Haenszel test [9] was used to calculate the odds ratio [OR] and 95% confidence interval [CI] of the clinical pregnancy rate for these six subgroups, in order to compare each subgroup with the previous subgroup, and to analyze changing trends between the serum P levels on D_{HCG} and clinical pregnancy rates; this yielded the cutoff values for serum P in differ-

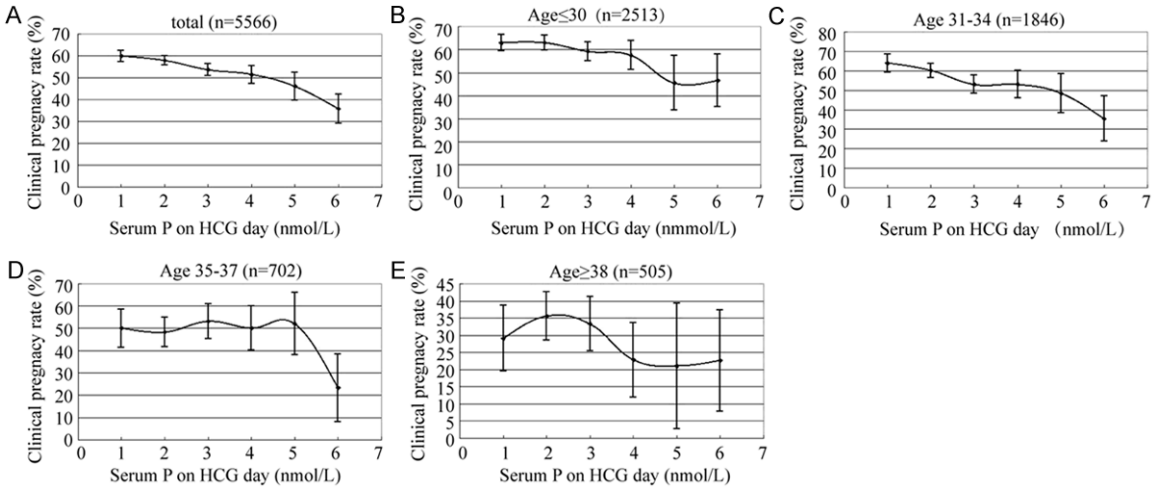

Figure 1. Correlations of P levels and clinical pregnancy rates in different age groups. A. Overall sample group; B. Correlations of P levels and clinical pregnancy rates in Group 1; C. correlations of P levels and clinical pregnancy rates in Group 2; D. Correlations of P levels and clinical pregnancy rates in Group 3; E. Correlations of P levels and clinical pregnancy rates in Group 4.

ent age groups that would affect clinical pregnancy rates. Logistic regression and advanced logistic regression variable selection methods were used to assess the 18 relevant factors that might potentially affect clinical pregnancy rates, including age, cause of infertility, primary/secondary infertility, assisted reproduction protocol (IVF, ICSI, and IVF/ICSI), baseline E2, baseline FSH, baseline LH, infertility duration, total Gn, total days of Gn, E2 on D_{HCG}, LH on D_{HCG}, P on D_{HCG}, endometrial thickness, ova obtained, embryonic age when transferred, number of embryos transferred, and number of high-quality embryos transferred. Multivariate stepwise regression analysis was further performed to analyze whether age, baseline FSH, baseline E2, total Gn, total days of Gn, E2 on D_{HCG}, LH on D_{HCG}, and ova obtained were correlated with the elevated serum P on D_{HCG}.

SPSS 16.0 software was used for the analysis, and the data were expressed as mean ± standard deviation (SD). Classification data were compared using the chi-square test, and continuous variables were compared using the two independent samples t-test or analysis of variance, with $P<0.05$ considered as statistically significant.

Results

Patient characteristics

The basic information for all the patients is shown in **Table 1**. A total of 5,566 patients were enrolled, among whom 2,513 were in group 1, 1,846 in group 2, 702 in group 3, and 4,505 in group 4. The average age was 31.29 years old (20-45 years old). The causes of infertility included tubal factors (58.07%), endometriosis (1.92%), ovulation disorders (4.58%), male factors (15.99%), male + female factors (19.21%), and unexplained infertility (0.23%).

Effect of serum P levels on clinical pregnancy rates in different age groups

Figure 1 shows the changing trend of serum P levels and clinical pregnancy rates among the overall samples; with increasing serum P levels on D_{HCG}, clinical pregnancy rates were decreased; the same declining trend appeared in the four age groups. In the overall samples, when P≥2.5 nmol/L, the clinical pregnancy rate was reduced much more significantly, indicating the cutoff value of serum P was 2.5 nmol/L (**Figure 1A**); the cutoff values of serum P in groups 1, 2, and 3 were 4.5 nmol/L, 2.5 nmol/L, and 5.5 nmol/L, respectively, (**Figure 1B-D**), while there was no corresponding cutoff value of serum P level for the clinical pregnancy rate in group 4 (**Figure 1E**).

Table 2 shows the OR (95% CI) for clinical pregnancy rates for each serum P level compared with the preceding P subgroup in the overall group and in the four different age groups. The difference of relative change in OR between intervals confirmed the nonlinear relationship

Table 2. Clinical pregnancy rates according to serum P levels

P level (nmol/l)	Overall		Age 1		Age 2		Age 3		Age 4	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
<1.5										
1.5-2.49	0.919 (0.798-1.058)	0.238	1.004 (0.818-1.232)	0.968	0.849 (0.659-1.094)	0.206	0.932 (0.608-1.428)	0.747	1.348 (0.772-2.356)	0.294
2.5-3.49	0.776 (0.665-0.906)	0.001	0.855 (0.681-1.074)	0.178	0.637 (0.484-0.838)	0.001	1.132 (0.716-1.789)	0.597	1.220 (0.678-2.194)	0.507
3.5-4.49	0.708 (0.582-0.861)	0.001	0.797 (0.590-1.078)	0.142	0.637 (0.450-0.901)	0.011	1.000 (0.594-1.684)	1.000	0.721 (0.332-1.564)	0.407
4.5-5.49	0.573 (0.433-0.758)	0.000	0.492 (0.298-0.812)	0.006	0.527 (0.336-0.825)	0.005	1.083 (0.566-2.075)	0.809	0.651 (0.197-2.154)	0.482
≥5.5	0.371 (0.272-0.506)	0.000	0.512 (0.512-0.316)	0.007	0.307 (0.178-0.530)	0.000	0.304 (0.122-0.757)	0.011	0.712 (0.272-1.862)	0.488

Table 3. Logistic regression analyses on the predictors of clinical pregnancy

Variable	Overall		Age 1		Age 2		Age 3		Age 4	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Age (y)	0.946 (0.931-0.962)	0.000	-	-	0.880 (0.808-0.958)	0.003	-	-	0.975 (0.687-0.919)	0.001
E2 level on D_{HCG} (pmol/L)	1.00002 (1.000007-1.000032)	0.002	-	-	-	-	-	-	-	-
P level on D_{HCG} (nmol/L)	0.844 (0.808-0.882)	0.000	0.859 (0.807-0.915)	0.000	0.838 (0.781-0.899)	0.000	-	-	0.843 (0.733-0.970)	0.017
LH level on D_{HCG} (IU/L)	-	-	-		-	-	1.457 (1.136-1.870)	0.003	-	-
Endometrial thickness (mm)	1.127 (1.094-1.160)	0.000	1.154 (1.103-1.205)	0.000	1.146 (1.089-1.205)	0.000	1.093 (1.014-1.177)	0.020	-	-
No. of embryos transferred	0.719 (0.567-0.911)	0.006	-	-	-	-	-	-	2.308 (1.336-3.990)	0.002
No. of high-quality embryos transferred	1.506 (1.396-1.624)	0.000	1.612 (1.427-1.822)	0.000	1.492 (1.307-1.702)	0.000	1.472 (1.222-1.774)	0.000	1.324 (1.087-1.614)	0.005

Table 4. Comparison of risk factors that would impact the clinical pregnancy rates between the < cutoff value and > cutoff value subgroups in different age groups

Variable	Overall		Age 1		Age 2		Age 3	
	P<2.5 nmol/l	P≥2.5 nmol/l	P<4.5 nmol/l	P≥4.5 nmo/l	P<2.5 nmol/l	P≥2.5 nmol/l	P<5.5 nmol/l	P≥5.5 nmol/l
Age (y)[a]	30.96±4.17	31.72±4.25**	-	-	32.31±1.12	32.37±1.10	-	-
P level on D_{HCG} (nmol/L)[a]	1.61±0.50	3.70±1.27**	2.16±0.93	5.85±1.39**	1.63±0.49	3.70±1.22**	2.59±1.16	6.79±1.57**
E2 level on D_{HCG} (pmol/L)[a]	9984.32±4524.80	12809.95±5133.84**	-	-	-	-	-	-
LH level on D_{HCG} (IU/L)[a]	-	-	-	-	-	-	0.83±0.70	0.84±1.16
Endometrial thickness (mm)[a]	10.62±1.92	10.56±1.92	10.66±1.88	10.66±1.82	10.69±1.90	10.58±1.90	10.50±2.05	10.45±2.18
No. of embryos transferred[b]	2.07±0.25	2.11±0.31**	-	-	-	-	-	-
No. of high-quality embryos transferred[b]	1.55±0.75	1.61±0.76**	1.61±0.66	1.62±0.63	1.51±0.72	1.55±0.71	1.55±0.83	1.67±0.85
Clinical pregnancy rate (%)[c]	58.7	51.3**	61.6	47.4**	61.5	51.2**	50.4	24.2*

Note: ** and *, statistical significance between the < cutoff value and > cutoff value subgroups; **P<0.001, *P<0.05. a: Two-independent samples t-test; b: Kruskal-Wallis equality-of-populations rank test (chi-square); c: One-sided Fisher exact chi-square test.

Table 5. Comparison of clinical pregnancy-related factors in different serum P subgroups in the 4 age groups

P level (nmol/L)	P level on DhCG (nmol/L)[d]	Age[d]	No. Of embryos transferred[e]	No. of high-quality embryos transferred	Clinical pregnancy rate (%)[f]
<1.5	1.02±0.28	39.65±1.60	2.65±0.48	1.51±1.07	25.3%
1.5-2.49	1.99±0.28	39.53±1.43	2.66±0.47	1.53±1.10	34.9%
2.5-3.49	2.94±0.28	39.41±1.40	2.79±0.41	1.77±1.12	36.2%
3.5-4.49	3.89±0.29	39.30±1.63	2.78±0.43	1.70±1.11	25%
4.5-5.49	4.84±0.30	39.78±1.69	2.89±0.32	2.07±1.04	18.5%
≥5.5	7.44±1.83	39.10±1.19	2.81±0.40	1.42±1.26	22.6%
P	0.000	0.34	0.02	0.07	0.15

Note: d: Analysis of variance; e: Dunnett 'T3 test; f: Chi-square test.

between clinical pregnancy rates and serum P level intervals. Meanwhile, statistically significant differences were observed between the 1.5-2.45 nmol/L and 2.5-3.45 nmol/L intervals (*P*=0.001) in the overall study group, the 3.5-4.49 nmol/L and 4.5-5.49 nmol/L intervals (*P*=0.006) in group 1, the 1.5-2.45 nmol/L and 2.5-3.45 nmol/L intervals (*P*=0.001) in group 2, and the 4.5-5.49 nmol/L and ≥5.5 nmol/L intervals (*P*=0.011) in group 3. However, the difference was not statistically significant for any interval in group 4 alone.

These data suggested that serum P concentration of 4.5 nmol/L, 2.5 nmol/L, and 5.5 nmol/L might represent the critical threshold levels to define PPR, at which there was a negative impact of P on the clinical pregnancy rates in groups 1, 2, and 3, respectively. Therefore, patients aged ≤30 years with serum P levels <4.5 nmol/L, patients aged 31-34 years with serum P levels <2.5 nmol/L, and patients aged 35-37 years with serum P levels <5.5 nmol/L had a higher likelihood of achieving clinical pregnancy than patients aged ≤30 years with serum P levels ≥4.5 nmol/L, patients aged 31-34 years with serum P levels ≥2.5 nmol/L, and patients aged 35-37 years with serum P levels ≥5.5 nmol/L.

Risk factors that might affect clinical pregnancy rates

Considering the complex factors that might affect clinical pregnancy rates, we used logistic regression analysis and found that these indicators in the overall sample group and the four age groups were slightly different (**Table 3**). Age and serum P level on D_{HCG} were risk factors that affected the clinical pregnancy rates; *P*=0.002 for E2 on D_{HCG} in the overall sample group, with OR 1.00002 and 95% CI 1.000007-1.000032; *P*-values in other age groups were greater than 0.05.

To further confirm the reasonableness of assessing cutoff values for serum P in different age groups, and to explore the reasons for the absence of a cutoff value for serum P in group 4, we divided patients into < cutoff value and > cutoff value subgroups to compare risk factors that would affect clinical pregnancy rates. The results showed (**Tables 4**, **5**) that in the overall sample group, the clinical pregnancy rate of the < cutoff value subgroup (P<2.5 nmol/L) was 58.7%, significantly higher than the > cutoff value subgroup (P≥2.5 nmol/L, 51.3%), and that age, E2 on D_{HCG}, P on D_{HCG}, number of embryos transferred, and number of high-quality embryos transferred showed significant differences (*P*<0.05). In groups 1, 2, and 3, the clinical pregnancy rates of the < cutoff value subgroups (the P<4.5 nmol/L group, the P<2.5 nmol/L group, and the P<5.5 nmol/L group) were 61.6%, 61.5%, and 50.4%, respectively, while those of the > cutoff value group (the P≥4.5 nmol/L group, the P≥2.5 nmol/L group, and the P≥5.5 nmol group) were 47.4%, 51.2%, and 24.2%, respectively; the values for the former were all significantly higher than for the latter. In addition, the serum P levels on D_{HCG} of the former groups were significantly lower than the latter groups, while LH on D_{HCG}, endometrial thickness, and number of high-quality embryos transferred within three age groups showed no statistical significance. Between the six serum P subgroups of group 4, the clinical pregnancy rates were similar (25.3%, 34.9%, 36.2%, 25%, 18.5%, and 22.6%, *P*=0.15), while the serum P

Table 6. Multivariate analysis of factors related to progesterone elevation

Variable	Overall			Age 1			Age 2			Age 3			Age 4		
	Regression coefficient	Standard error	P	Regression coefficient	Standard error	P	Regression coefficient	Standard error	P	Regression coefficient	Standard error	P	Regression coefficient	Standard error	P
Age (y)	0.041	0.005	0.006	-	-	-	-	-	-	-	-	-	-	-	-
Basic FSH level (IU/L)	-0.036	0.011	0.005	-	-	-	-	-	-	-	-	-	-	-	-
Duration of stimulation	-0.113	0.01	1.98e-12	-0.134	0.014	0.000	-0.156	0.018	0.000	-0.155	0.033	0.000			
Total dose of rFSH administered (IU)	0.309	2.72e-05	8.96e-57	0.256	4.46e-05	0.000	0.330	4.3e-05	0.000	0.315	7.19e-05	0.000	0.214	6.48e-05	0.000
E2 level on D_{HCG} (pmol/L)	0.302	4.09e-06	3.08e-90	0.286	6.51e-06	0.000	0.352	7.85e-06	0.000	0.419	1.27e-05	0.000	0.224	9.32e-06	0.000
No. of oocytes retrieved	0.171	0.0036	1.43e-29	0.131	0.005	0.000	0.154	0.006	0.000	0.115	0.012	0.01	0.343	0.013	0.000

levels on D_{HCG} and number of embryos transferred showed statistically significant intergroup differences ($P<0.001$, $P=0.02$) (**Table 5**).

Multivariate analysis of factors related to progesterone elevation

Using a stepwise method, multiple linear regression models were developed to analyze the relationships between the serum P level on D_{HCG} and other clinical variables; the results showed that in the overall sample group, the serum P level on D_{HCG} was positively correlated with age, total FSH, E2, and embryos obtained, while the serum P level on D_{HCG} was not correlated with the different age groups (**Table 6**).

Discussion

The effects of PL on clinical pregnancy outcomes in COH have been controversial and have shown no consistent or conclusive results among different studies. Younis et al. [15] considered these deviations to be the result of differences in study methodologies and designs, including different COH protocols, indicators used for assessing outcomes, statistical methods, and cutoff values used for defining PPR. Most previous studies often used one absolute serum *P* value or ROC curve as the diagnostic threshold for PPR, which ranged from 0.9 to 2.0 ng/mL [16-19]. We thought this was inappropriate because our data showed that the serum P levels on D_{HCG} were nonlinearly related to clinical pregnancy rates, consistent with the report by Bosch et al [9]. In order to correct defects in the above methods, the trend analysis method used by Bosch was introduced into our study, and further analysis was performed according to different age stratifications. We found that the serum P levels on D_{HCG} were negatively correlated with clinical pregnancy rates. With increasing P levels, the clinical pregnancy rates were decreased. The four age groups showed the same trend, but the cutoff values of serum P of the overall sample group and the other age groups were different: overall sample group, 2.5 nmol/L (0.79 ng/mL); group 1, 4.5 nmol/L (1.42 ng/mL); group 2, 2.5 nmol/L (0.79 ng/mL); and group 3, 5.5 nmol/L (1.73 ng/mL), respectively. However, a cutoff value of serum P in group 4 was not identifiable, in contrast with the reports of Huang et al. [10] (the cutoff value of serum P in a long protocol was 1.2 ng/mL) and Bosch et al. [9] (1.5 ng/mL). There are several possible reasons. First, the indicators selected to assess the outcomes were different. Huang used the birth rate while Bosch used the continuing pregnancy rate as the outcome evaluation indicator of pregnancy. In contrast, we used the clinical pregnancy rate as the outcome evaluation indicator because the miscarriage rate within IVF cycles was as high as 10-20% [20-22], and more than 50% of the aborted embryos were caused by embryonic chromosome abnormalities [23, 24] instead of high serum P on D_{HCG}; therefore, we believed that it would be more reasonable to use the clinical pregnancy rate as the evaluation indicator for the serum P and the corresponding pregnancy outcomes. Second, there was a difference in hormone detection methods. The first two studies used radioimmunoassay and microparticle enzyme immunoassay, while we used the electrochemiluminescence method. Third, there were differences in patient ages. The average ages in the first two studies were 31.1 and 35.3 years old, respectively, while that in our study was 31.29 years old. Age is an important factor affecting the pregnancy rate; with increasing age, ovarian reserve function diminishes, the quantity and quality of ova decline, and the pregnancy rate also decreases, especially in older patients [11, 12, 25]. Therefore, we speculated that in the IVF cycle, different age segments would have different PPR cutoff values on D_{HCG}. Our findings confirmed our hypothesis, and PPR could not be defined solely by the cutoff value of serum P for the overall sample group. Meanwhile, we performed logistic regression analysis and found that the age and serum P on D_{HCG} were risk factors affecting clinical pregnancy rates; in addition, although the E2 value on D_{HCG} was also an indicator affecting the clinical pregnancy rate in the overall sample group ($P=0.002$), the OR and 95% CI were only 1.00002 and 1.000007-1.000032, respectively; thus, when patients were placed into four age subgroups for statistical analysis, the effects of E2 level on D_{HCG} on the clinical pregnancy rate did not appear, indicating that assessment by age stratification was necessary.

Curiously, group 4 exhibited no clear cutoff value for serum P. To identify the reason, we set the cutoff value for serum P as the cutoff point and divided this group into < cutoff value and > cutoff value subgroups, aiming to compare the

risk factors that would affect the clinical pregnancy rate between these two subgroups. The results showed that in the overall sample group, the declining clinical pregnancy rate in patients with P≥2.5 nmol/L was not simply caused by serum P>2.5 nmol/L; the differences in age, E2 on D_{HCG}, number of embryos transferred, and number of high-quality embryos transferred also have some effect. In the < cutoff value subgroups of groups 1, 2, and 3 (the P<4.5 nmol/L group, the P<2.5 nmol/L group, and the P<5.5 nmol group), the clinical pregnancy rates were 61.6%, 61.5%, and 50.4%, respectively, which was significantly higher than the > cutoff value subgroups (the P≥4.5 nmol/L group, the P≥2.5 nmol/L group, and the P≥5.5 nmol group; 47.4%, 51.2%, and 24.2%, respectively). The intragroup comparisons of LH on D_{HCG}, endometrial thickness, and number of high-quality embryos transferred showed no difference, suggesting that the effects of LH on D_{HCG}, endometrial thickness, and number of high-quality embryos transferred on the clinical pregnancy rates were identical between the < cutoff value and > cutoff value subgroups, and the difference in the clinical pregnancy rates was mainly caused by the difference of serum P; thus, it was meaningful to set serum P at 4.5 nmol/L, 2.5 nmol/L and 5.5 nmol/L as the cutoff values in groups 1, 2, and 3, respectively. The reason for the absence of a cutoff value of serum P on D_{HCG} in group 4 might be due to the differences in the number of embryos transferred. These results further confirmed the reasonableness of evaluating serum P cutoff values by different age segments.

Previous studies proposed factors leading to PPR on D_{HCG} in the IVF cycle, including: 1) multiple follicular development [16, 26]; 2) excessive use of FSH [9, 27]; 3) PL [28, 29]; 4) poor ovarian response [15]; and 5) super physiologic E2 level [9]. In order to explore PPR-generated endocrine mechanisms, different research methods have been introduced. Some researchers divided patients into PL and non-PL groups and compared the differences for multiple clinical variables between the two groups [2, 16, 30] to identify the causes of PPR. This method was simple, but did not reflect the interactions among these variables. Bosch et al. [9] used logistic regression for multivariate analysis of all possible factors related to increasing serum P; however, because the serum P level was a continuous variable, the statistical method used in this study might exaggerate or reduce the effects of some variables. The regression model of serum P and other clinical variables established by the stepwise method used in our multiple linear regression model revealed that the excessive dose of FSH, high E2 level on D_{HCG}, and more ova obtained were the reasons for increasing serum P level on D_{HCG}, consistent with the reports of Bosch et al. [9] and Adonakis et al [31]; no other reason was found for a correlation with the elevated LH level in other studies [29, 30]. In addition, our results found for the first time that the serum P level on D_{HCG} was positively correlated with age, suggesting that the higher the age, the greater the use of FSH, the higher the E2 on D_{HCG}, the more the ova obtained, and the higher the probability of occurrence of PPR. This might be because the functions of ovular peripheral particles decrease with increasing age, manifesting as reduced proliferation ability, increased apoptosis, downregulation of FSH receptor (FSHR) and aromatase (CYP19A1), reduced response of FSH [32-34], upregulation of LH receptor (LHCGR), P450scc (CYP11A1) and progesterone receptor (PGR), and increased sensitivity of LH and P [35-37]. After performing age stratification, the analysis subsequently revealed that the correlations between the serum P levels on D_{HCG} and age disappeared. Therefore, age was an interactive factor with dual roles, which not only affected the serum P level on D_{HCG}, but also the clinical pregnancy rate; therefore, performing age stratification to assess the effect of PPR on D_{HCG} on the clinical pregnancy rate was correct.

There were two main explanations for the effect of PPR on D_{HCG} on clinical pregnancy outcomes. First, the high serum P level might cause endometrial histological changes to occur earlier, so that endometrial development was not synchronic with embryonic development [38]. Second, the increasing serum P would theoretically cause PL of ovarian follicles, thus affecting the qualities of the oocytes and embryos. Some researchers [39, 40] compared the clinical pregnancy rates between patients with elevated and normal serum P levels in fresh ET and freeze-thaw ET cycles, and found that the clinical pregnancy rate in the fresh ET cycle was very low, while that in the freeze-thaw cycle was not affected; this suggested that the increasing

serum P might not have affected the qualities of the oocytes and embryos, but instead reduced endometrial receptivity. This speculation was confirmed in the ovum-donation cycle [1] and the luteal pro-ovulation phase [41, 42], and also at the gene level [42, 43]. Melo et al. [1] found that the clinical pregnancy rate in the recipients in the ovum-donation cycle was not affected by the donors' high serum P levels on D_{HCG}. Kuang et al. [41] and Moffat et al. [42] also reported similar pregnancy outcomes with the ova obtained in the luteal and follicular pro-ovulation phase. Van Vaerenberg et al. [43] and Labarta et al. [44] both found that when serum P was >4.77 nmol/L, the gene sequence of the endometrium was significantly different from that seen with a normal P level, and this difference would continue until the transfer. Therefore, in order to prevent the harmful effects of high serum P level on D_{HCG}, cancellation of transfer in the fresh cycle and the use of frozen-thawed embryos were recommended [10]. Therefore, in the GnRH-agonist long protocol, patients aged ≤30 years old with serum P on D_{HCG}≥4.5 nmol/L, those 31-34 years old with serum P on D_{HCG}≥2.5 nmol/L, or those 35-37 years old with serum P on D_{HCG}≥5.5 nmol/L should be considered for transfer using frozen-thawed embryos.

This study proposed the concept of age stratification for the first time to assess the effects of high serum P level on D_{HCG} on the clinical pregnancy rate. Age was not only an important factor affecting the clinical pregnancy rate but also affected the serum P level on D_{HCG}; therefore, performing age stratification was necessary and meaningful. Meanwhile, the large sample size (n=5,566) in the current study also ensured the validity of these findings. However, this study had some limitations. This was a retrospective analysis. Moreover, no limitation was set on the number of embryos transferred in group 4, so no cutoff value of serum P was found in this group.

In summary, this study performed age stratification of 5,566 IVF/ICSI patients for the first time and assessed the effects of elevated serum P levels on D_{HCG} on clinical pregnancy rates: the high serum P level on D_{HCG} reduced the clinical pregnancy rate for IVF/ICSI, and different ages had different cutoff values. Age was not only an important factor affecting the clinical pregnancy rate, but also affected the serum P level on D_{HCG}.

Disclosure of conflict of interest

None.

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