Progesterone levels predict pregnancy outcome in individuals with fallopian tube associated infertility

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Research article

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Abstract

Background: To investigate the predictive value of human chorionic gonadotropin and progesterone levels on pregnancy outcomes in patients receiving in vitro fertilization due to simple fallopian tube factors.

METHODS: We retrospectively analyzed the clinical data of 854 cycles from the simple fallopian tube factor in vitro fertilization fresh embryo transfer. The clinical data of 854 cycles from January 2010 to December 2018 was divided into 7 groups according to the progesterone level on human chorionic gonadotropin day. Live birth rates and observe trends were calculated. The receiver operating characteristic curve was established to determine the optimal cutoff value for progesterone, which was used to further divide the data into 3 groups: Group 1 (progesterone \( \leq 1.0 \) ng/ml), Group 2 (1.0 ng/ml \( \leq \) progesterone \( \leq 1.25 \) ng/ml), and Group 3 (progesterone \( \geq 1.25\)ng/ml). We then compared the ovulation results and clinical outcomes between the 3 groups.

RESULTS There were no significant differences in age, infertility years, Gonadotropin dosage, Gonadotropin days, Luteinizing hormone level on human chorionic gonadotropin day, 2pronuclear fertilization rate, clinical pregnancy rate, live birth rates, full-term birth rate, and preterm birth rate among the three groups, but body mass index(P = 0.001), basal luteinizing hormone (P = 0.034), estrogen peak (P \( \leq 0.001 \)), number of oocytes obtained (P \( \leq 0.001 \)) were significantly different.

CONCLUSION The level of progesterone on human chorionic gonadotropin day does not affect the clinical pregnancy rate and live birth rates after in vitro fertilization. However, progesterone levels between 1.0-1.25ng/ml may lead to good clinical pregnancy outcomes.

Background

Progesterone(P) is known to play important physiological functions during the menstrual cycle and pregnancy[1]. In assisted reproductive treatment(ART), the use of P levels during the late follicular phase to predict pregnancy outcomes remains controversial. A recent study suggested that elevated P level on the day of human chorionic gonadotropin HCG administration negatively influence live birth rate and correlates with high rates of miscarriage. However, the detrimental impact of high P levels during pregnancy does not appear to be associated with endometrial receptivity [2]. A different study indicated that low P levels (\( \leq 0.5\)ng/ml) on the day of HCG administration are associated with low live birth rates[3]. Elevated P levels on the day of oocyte maturation have also been suggested to affect embryo quality while high P levels (>2.0 ng/ml) before oocyte maturation have been shown to be consistently impact the oocyte negatively [4]. It has also been proposed that premature luteinization does not seem to affect in vitro fertilization IVF outcome [5]. According to the controlled ovarian stimulation protocol, growth of multiple follicles may result in different levels of serum P in the late follicles[6]. Paulson and colleagues have suggested that when serum P exceeds a certain threshold of P levels , it triggers a various endometrial changes that result in the pre-transformation of the endometrium [7]. During the secretory phase, embryonic development rate gets out of sync with the endometrial phase, which negatively impacts the successful implantation. The serum P levels in the late follicular phase correlate with the number of follicles formed [6]. It has been suggested that ovarian reserve function decreases with rising serum P, and that too high or too low P level might lead to premature luteinization or follicle maturation [8]. The quality of the embryo is impaired, and the opening time of the endometrial implantation window is significantly shortened, which interferes with embryo implantation and development and causes reduced pregnancy rates [9]. Multiple studies have reported that in gonadotropin-releasing hormone (GnRH) downregulated cycles, premature increase in P levels on the day of HCG administration negatively correlates with IVF outcome. It should be noted that most of these studies included the patients with different caused of infertility and the transplant date and ovarian response degree were different. In addition, different P threshold values were used in different studies. These factors affect the reliability of the results and the capacity P levels as a predictor of...
pregnancy outcomes. To address these shortcomings, we carried out a retrospective analysis of the potential of P levels as a predictor of pregnancy outcomes in patients with infertility caused by simple fallopian tube defects. All participants in the study were normal ovarian responders and the embryos were transferred on the third day.

Methods

Study design

This retrospective study was conducted on a cohort of participants treated at the Center for Reproductive Medicine and Genetics at Shandong Provincial Hospital of Traditional Chinese Medicine, between January 2010 and November 2018. Of the cases seen during the study period, 854 met our inclusion criteria. Ethical approval for this study was provided by the ethics committee at Shandong Provincial Hospital of Traditional Chinese Medicine.

Patient enrollment criteria

Participating women were included only if they met the following criteria: a) they were undergoing assisted reproduction with fresh autologous embryo transferred on day 3 following oocyte retrieval, b) had infertility resulting from fallopian tube factors, c) they were normal ovarian responders (6–19 oocytes) [10] and d), had follicle-stimulating hormone (FSH) levels of <10IU/L on the second day of menstruation. Patients were excluded from the study if their reasons for IVF were linked to male sterility, ovarian, endometriosis, genetic, uterine or idiopathic factors. Those with hydrosalpinx were also excluded.

Controlled ovarian hyperstimulation protocols

Pituitary down-regulation was carried out by treatment with a GnRH agonist (Triptorelin®/Diphereline®) or antagonist (Ganirelix®/Cetrotide®). The choice of protocol and gonadotrophin dose were individualized to the patient’s clinical presentation and the clinician’s preference. The dosage of gonadotropins (Puregon®/Gonal®) used varied from 75 to 450IU/day of stimulation. When the dominant follicle reached ≥18mm in diameter, 10000IU of HCG(Ovidrel®) or 0.1mg of GnRH agonist plus 4000IU of HCG were added to induce final maturation and the ovum picked up 36 hours later post-treatment. Serum was collected from the participants before administration of HCG or the GnRH agonist. P levels in the serum were quantified using quantitative electro chemiluminescence immune assay “ECLIA” on an immunoassay analyzer (Beckman Coulter). Sperm was collected through masturbation prior to IVF and embryos transferred 3 days following oocyte retrieval. Embryo quality was determined in accordance with guidelines from the Society for Assisted Reproductive Technology and graded as good, fair, or poor. Luteal support was provided through vaginal P administration or intramuscular P injection. Serum HCG level was determined 14 days after fresh embryo transfer and beta values ≥50IU taken as positive indication of pregnancy. Clinically, pregnancy was defined by the presence of an intrauterine original heart beat on transvaginal ultrasound at 7 weeks of amenorrhea. Live births are defined as babies delivered after 28 weeks of gestation.

Data Collection

Data were retrieved from ART electronic medical records. The evaluated participant characteristics included age, body mass index (BMI), duration since infertility was diagnosed and basal serum FSH levels. Additional parameters that were analyzed included the protocols used for controlled ovarian hyperstimulation: total duration of Gonadotropin (Gn), dose of Gn, peak serum estradiol level, luteinizing hormone (LH) level, P levels on the day of HCG administration, number of oocytes obtained, 2 pronuclear (PN) fertility rate, total number of embryos, number of high quality embryos, number of transferred embryos on day 3, clinical pregnancy status, number of successful live births and the number of newborns.
The primary metric was the correlation between serum P levels on the day of ovulation induction during IVF and its effect on treatment outcome.

**Statistical analysis**

The data was analyzed and described in terms of mean±SD, frequencies or percentages. First, perform normality test and homogeneity of variance test. If the population distribution conforms to normality and the variance is satisfied, then the parameter test will be performed, then quantitative variables were compared using independent t-tests and variance analyses and categorical data were compared using χ2 tests. If not, the non-parametric test will be used. Pearson correlation coefficients were calculated to establish the relationship between P and clinical parameters. Receiver operating characteristic (ROC) analysis was conducted to search for the most efficient P cutoff values to discriminate between successful and unsuccessful IVF outcomes in women undergoing day 3 fresh embryo transplantation. The best cutoff values were set on the basis of an equivalent sensitivity and specificity and the highest value for area under the ROC curve (AUC). In each cohort, the univariate and multivariate analyses models were used to test the preferential effect of all independent on LBR. Statistical differences were considered to be significant when p = <0.05). Statistical analyses were done on SPSS statistical suite version 21 (IBM).

**Results**

A total of 854 cycles were included in this study. The average age of the participants was 31.99 years old (range 20-40 years). Patients were deemed infertile based on tubal factors only. The clinical features and cycle outcomes of the enrolled cycles are shown in Table 1.

First, we divide it into seven groups according to the level of progesterone on the day of HCG administration. Notably, P level below 1.25ng/ml were associated with high live birth rates but P level of 1.26ng/ml to 1.5ng/ml correlated with low live birth rates. P levels between 1.51ng/ml and 1.75ng/ml correlated with high live birth rates, but levels >1.75ng/ml tended to decrease live birth rates.

Correlation analysis for factors related to P level on the day of HCG administration (Table 2). Results show that P level was not significantly correlated with age (P=0.31); duration of infertility (P=0.35); basal FSH (P=0.79); total gonadotropin (P=0.89); duration of stimulation (P=0.49) and luteinizing hormone level on HCG day (P=0.25). In contrast, the P level on the day of HCG administration was significantly correlated with BMI (P<0.001); peak estradiol (P<0.001) and number of oocytes retrieved (P <0.001). P level was positively correlated with peak estradiol (Pearson Correlation Coefficients =0.261) and number of oocytes retrieved (Pearson Correlation Coefficients =0.158), but was negatively correlated with BMI (Pearson Correlation Coefficients =-0.160)

According to the ROC analysis (Figure 1), the optimal cut off value for P level was 1.0 ng/ml and the AUC was 0.506 (95%CI: 0.467-0.545). To further assess the effect of serum P on late follicular oocyte and clinical outcome, we divided the data sample in three groups: Group 1 (patients with P ≤1.0ng/ml), Group 2 (patients with P between 1.0ng/ml to 1.25ng/ml) and Group 3 (patients with P ≥1.25ng/ml) (Table 3). We concluded that patients’ BMI, basal FSH, peak estradiol and number of oocytes retrieved were different among these groups. Nonetheless, our analysis still showed that, patients with P level between 1.0ng/ml to 1.25ng/ml had higher clinical pregnancy rate and live birth rate, although not statistically significant (P=0.69,0.67;0.05). (Table 3)(Figure 2).

**Discussion**

In recent years, multiple studies have proposed the potential use of P levels on the day of HCG administration as a predictor of pregnancy outcomes [11]. Some studies have shown that high P levels during daily HCG administration may
adversely impact pregnancy outcomes. It has been reported that too high or too low P levels can adversely affect live birth rates. A 2013 meta-analysis based on 60,000 IVF cycles revealed that elevated daily P levels following HCG administration significantly lower pregnancy rate upon GnRH agonist and antagonist treatment in the fresh cycle [12]. It is speculated that raised follicular-phase P concentration produced by ovarian stimulation-induced multiple follicle growth may contribute to changes in the endometrium, leading to embryo–endometrial asynchrony [13]. A study by Yding and colleagues, analyzing 475 patients undergoing IVF assisted pregnancy, found that the daily P levels on HCG administration did not affect pregnancy rate, and that elevated P levels correlated with the number of oocytes obtained. To further investigate the effect of HCG P levels on pregnancy outcomes, we selected IVF cases for infertility caused by fallopian tube factors and performed embryo transfer on the third day after oocyte retrieval. Our study revealed that when P levels are below the range of 1.0-1.25ng/ml, the rate of live births increases with rising P levels indicating that a certain level of P is required for successful pregnancy. When P levels exceed this range, the LBR first drops. A follow up of the three groups revealed that the age, duration of infertility, Gn dosage, Gn days, daily HCG levels, daily LH level, 2PN fertilization rate, clinical pregnancy rate (CPR), LBR, full-term yield and preterm birth rate were not significantly different between the groups. While the BMI, basal LH levels, estrogen peak level and number of oocytes obtained were found to differ significantly between the groups. The numbers of oocytes obtained were highest in the high P group relative to the other two groups, suggesting that higher P levels may indicate the number of resulting oocytes. These observations are in agreement with previous reports showing that elevated P levels are significantly correlated with the number of oocytes retrieved, which is in turn associated with successful IVF outcomes [14]. The rates of 2PN fertilization and quality embryos were also observed to be higher in the group with elevated P levels, although the differences were not statistically significant. It has previously been reported that that post-HCG P levels are positively associated with the number of oocytes retrieved and this does not affect oocyte or embryo quality [16]. Other studies have focused on the effect of the P depending on the embryonic stage. It has been demonstrated that when P levels exceed 1.5 ng/ml, clinically defined pregnancy rates decrease. However, similar results were not seen following transfer at the blastocyst stage [15]. These observations suggest that controlling P levels in patients with low late follicular P levels may improve IVF outcomes [16]. To that end, limiting the total dose of FSH administered might be beneficial [17]. For example, previous randomized trials have reported that late follicular replacement of daily FSH with low-dose HCG achieves effects comparable to those of P receptor (PR) [18] [19] without the detrimental effects of late follicular P elevation [18]. In the current study, we observed that P levels ranging between 1.0-1.25ng/ml exhibit better clinical pregnancy and live birth rates. Additionally, we observed that HCG P levels negatively correlate with BMI and positively correlated with E2 and the number of oocytes. Together, these observations suggest that the BMI, in such clinical contexts may predict HCG P levels. Many published studies have relied on different P level thresholds. Typically, when these thresholds are surpassed, the embryos are collected for freezing. Indeed, no study has demonstrated any deleterious effects of P on frozen embryo transfer [20]. Overall, HCG daily P levels have limitations as predictors of IVF outcomes. There are reports suggesting that progesterone/oocyte ratio should be considered as a tool for the prediction of IVF outcomes in reference to serum P levels alone. However, more evidence from randomized studies is required to support this [21].

In this retrospective study, we analyzed the value of HCG daily P levels to predict the success of IVF in patients fallopian tube complications. P levels that fall within 1.0ng/ml to 1.25 ng/ml are associated with a higher rates of clinical pregnancy and live births in the 854 cycles we included. Although the observed differences are not statistically significant, we contend that the can be used as a clinical references.

**Conclusion**

Patients due to fallopian tube factors undergoing fresh autologous embryo transferred on day 3 following oocyte retrieval, their P level on HCG day does not affect the CPR and live birth rate after IVF. However, patients with P level...
between 1.0ng/ml to 1.25ng/ml had higher CPR and LBR, although not statistically significant (P=0.69,0.67,0.05). Therefore, determining P level on HCG day may have certain guiding significance for embryo transfer.

**Declarations**

**Ethical Approval and Consent to participate**

This retrospective study has been approved by Ethics Committee of Shandong University of Traditional Chinese Medicine. Because this is a retrospective study of this study, the patient was contacted by phone and given oral consent, and the ethics committee approved this procedure.

**Consent for publication**

All authors of this article agree to publish in your journal.

**Availability of data and material**

Some or all data, models, or code generated or used during the study are available in a repository or online in accordance with funder data retention policies.

**Competing interests**

All authors do not have any possible conflicts of interest.

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**Authors' contributions**

B WJ: Read and sort documents, collected data and write the manuscript;

Z N: audit data

All authors have read and approved the manuscript.

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**Abbreviations**

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Abbreviation | Full name
--- | ---
P | progesterone
HCG | human chorionic gonadotropin
IVF | in vitro fertilization
ROC | receiver operating characteristic
Gn | gonadotropin
LH | luteinizing hormone
PN | pronuclear
GnRH | gonadotropin-releasing hormone
ART | assisted reproduction technology
BMI | body mass index
FSH | follicle-stimulating hormone
LBR | live birth rate
CPR | clinical pregnancy rate

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**Tables**

Table1
Parameter

| Parameter                        | Value                  |
|----------------------------------|------------------------|
| No. of fresh cycles              | 854                    |
| Age (y)                          | 31.99±4.32             |
| BMI (kg/m^2)                     | 23.57±3.85             |
| Percentage of primary infertility (%) | 46.37 (396/854)        |
| Duration of infertility (y)      | 3.39±2.38              |
| Basal FSH (IU/L)                 | 6.80±1.61              |

Medication protocol

- GnRH agonist (%) 92.39 (789/854)
- GnRH antagonist (%) 7.61 (65/854)
- Total gonadotropin (IU) 2662±1087
- Duration of stimulation (days) 11.64±2.49
- Peak estradiol (pg/ml) 2,751±1,165
- luteinizing hormone on HCG day (IU/L) 1.8±2.16
- Progesterone on HCG day (ng/ml) 1.02±0.42

| No. of oocytes retrieved | 9.86±3.32 |
| 2PN fertilization rate (%) | 63.78±21.17 |
| Number of available embryos | 3.79±2.05 |
| Number of high quality embryos | 1.33±1.53 |
| Number of transferred embryos on day 3 | 1.98±0.39 |

Number of live birth babies

- 2 live birth babies 94
- 1 live birth baby 262
- No live birth baby 498

Table 2

| Parameter                                | Pearson Correlation Coefficients | P value |
|------------------------------------------|----------------------------------|---------|
| Age (y)                                  | 0.035                            | NS      |
| BMI (kg/m^2)                             | -0.160                           | 0.001** |
| Duration of infertility (y)              | -0.032                           | NS      |
| Basal FSH (IU/L)                         | -0.012                           | NS      |
| Total gonadotropin (IU)                  | 0.005                            | NS      |
| Duration of stimulation (days)           | -0.024                           | NS      |
| Peak estradiol (pg/ml)                   | 0.261                            | 0.001** |
| luteinizing hormone on HCG day (IU/L)    | 0.039                            | NS      |
| No. of oocytes retrieved                 | 0.158                            | 0.001** |

Table 3
| Patient demographics | Total | Group 1 | Group 2 | Group 3 | P-value |
|----------------------|-------|---------|---------|---------|---------|
| N                    | 854   | 440     | 162     | 252     |         |
| Age (y)              | 31.99±4.32 | 31.82±4.29 | 32.02±4.12 | 32.25±4.50 | NS      |
| BMI (kg/m²)          | 23.67±3.85 | 24.13±3.40 | 23.42±3.43 | 23.67±3.85 | 0.001**a |
| Duration of infertility (y) | 3.39±2.38 | 3.44±2.46 | 3.56±2.60 | 3.21±2.05 | NS      |
| Basal FSH (IU/L)     | 6.80±1.61 | 6.85±1.65 | 6.51±1.57 | 6.90±1.54 | 0.034*b  |

Treatment protocol and outcomes

| Duration of stimulation (days) | 11.64±2.49 | 11.70±2.61 | 11.57±2.48 | 11.58±2.26 | NS |
| Total gonadotropin (IU)        | 2662.23±1087.50 | 2668.16±1140.31 | 2629.49±1108.43 | 2672.92±977.73 | NS |
| Peak estradiol (pg/ml) on HCG day | 2751.16±1165.52 | 2527.78±1086.78 | 2872.97±1107.16 | 3062.86±1252.98 | 0.001**a |
| Luteinizing hormone (IU/L)     | 1.80±2.16 | 1.72±2.33 | 1.91±1.50 | 1.88±2.20 | NS      |
| No. of oocytes retrieved       | 9.86±3.32 | 9.45±3.30 | 9.70±3.15 | 10.66±3.34 | 0.001**c |

2PN fertilization rate (%)       
High quality embryo rate (%)     
Clinical pregnancy rate (%)      
Live birth rate (%)              
Premature rate (%)               
Full-term delivery (%)           

Figures
Figure 1

Receiver operation characteristic curve (ROC) showing the correlation between progesterone levels and live birth rate.

Figure 2

Comparison of clinical pregnancy rate, live birth rate, and full-term delivery rate among the three groups. Note: P1=0.690 among three groups for clinical pregnancy rate P2=0.673 among three groups for live birth rate P3=0.404 among three groups for full-term delivery rate