Original Article

Correlation between Serum Progesterone Level on the Day of Ovulation Trigger During *In vitro* Fertilization and Its Effect on Treatment Outcome

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Abstract

Background: Premature luteinization (PL) is defined as a premature rise in serum progesterone concentration on or before the day of ovulation trigger with human chorionic gonadotropin. The incidence of PL varies between 5% and 30% during *in vitro* fertilization and embryo transfer (IVF-ET). **Materials and Methods:** The prospective observational study comprising 380 patients undergoing IVF-ET. Blood samples were collected for serum progesterone level estimation on the day of ovulation trigger. Ovum pickup was done 36 h later and serum progesterone levels were correlated with IVF-ET outcome. **Study Outcome:** To correlate serum progesterone level on the day of ovulation trigger during IVF and its effect on treatment outcome. **Results:** Mean serum progesterone level in the positive pregnancy group and negative pregnancy group was 0.892 ± 0.752 ng/ml and 0.91 ± 0.688 ng/ml, respectively (P = 0.961). The overall incidence of PL was 12.8% with 12.7% and 13.6% in the agonist and antagonist protocol respectively (P = 0.9001). PL incidence was 13.5% and 13.4% in positive pregnancy and negative pregnancy group (P = 0.223). **Conclusion:** PL has been associated with 12.8% of the IVF cycles. There was no statistically significant difference observed in the incidence of PL between different IVF stimulation protocols. PL does not seem to affect IVF outcome.

Keywords: *Decreased ovarian reserve, human chorionic gonadotropin, in vitro fertilization, polycystic ovarian syndrome, premature luteinization*

Introduction

Progesterone is the primary hormone during the luteal phase of the menstrual cycle which is vital for the transformation of the endometrium for embryo implantation. In a natural conception cycle, there are many physiological events such as fertilization, embryogenesis, and endometrial changes in a synchronous manner. The blastocyst reaching the uterine cavity is exposed to perfect endometrial conditions required for implantation.

Premature luteinization (PL) is defined as a premature rise in serum progesterone level on or before the day of ovulation trigger.[1] Studies by Bosch *et al*. and Papanikolaou *et al*. have shown the negative effect of PL for pregnancy outcome when the progesterone level was >1.5 ng/ml on the day of ovulation trigger.[2,3] PL may have an adverse effect on endometrial receptivity, poor endometrial receptivity may be explained by premature endometrial maturation which may lead to asynchrony between the embryo and endometrium.[4,5] PL affects the endometrial gene expressions,[6,7] and it is known to occur with increased number and size of follicles, a higher dose of gonadotropin and poor ovarian response with increased luteinizing hormone (LH) sensitivity. PL is also associated with increased activity of follicular stimulating hormone stimulated, granulosa

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cells, and LH stimulated theca cells.[8] In the previous studies, the incidence of PL is found to be between 5% and 30% during stimulated *in vitro* fertilization (IVF) cycles.[9,10]

Data on the association between elevated serum progesterone level during ovulation trigger and its effect on IVF outcome have been inconsistent. Many studies including a meta-analysis have shown that PL is associated with a decreased probability of pregnancy during IVF.[11-13] On the contrary, some previous researchers did not find any significant differences in pregnancy rates during IVF with high- or low-progesterone levels on the day of ovulation trigger.[14] Two recent studies have shown that serum progesterone level on ovulation trigger day did not affect clinical pregnancy rate.[15,16] Controversy about the association of PL and its effect on IVF outcome in different studies inspired us to conduct this research at our center.

Materials and Methods

Study design

Prospective observational study comprising 380 patients undergoing IVF embryo transfer (IVF-ET), meeting inclusion and exclusion criteria were recruited into the study over a period of 6 months. The institutional ethical committee approved the study and written informed consent was obtained from the participants.

Study population

All willing patients who underwent IVF cycle at the Reproductive Medicine Center from September 2016 to March 2017.

Inclusion criteria

All patients undergoing IVF-ET with age ranging between 22 and 40 years during study period.

Exclusion criteria

1. Patients with disorders of hypothalamic–pituitary–adrenal axis
2. Patients with hydrosalpinx
3. Patients with thin endometrium or any endometrial pathology.

Methodology

Controlled ovarian hyperstimulation (COH) for IVF-ET was done by one of the two IVF stimulation protocol, i.e., long agonist or antagonist protocol. In the long agonist protocol, oral contraceptives were started on 5th day of menstruation and GnRH agonist, leuprolide acetate 1 mg daily (Luprorin®-4 mg/4 ml vial, Intas Pharmaceuticals, India) was started from day 21 of the cycle. The downregulation was confirmed by transvaginal ultrasonographic evaluation of the ovaries and endometrium. Estradiol and LH estimation were done only in cases where ultrasound findings were inconclusive.

Ovarian stimulation was started from the 2nd day of menstruation in a downregulated cycle with recombinant follitropin alpha (Gonal F®, Merck Serono, Italy). Recombinant LH (Luveris®, Merck Serono, Switzerland) was added toward end for follicular maturation in polycystic ovarian syndrome (PCOS) cases. In cases of normal ovarian reserve and decrease ovarian reserve (DOR) cases, highly purified human menopausal gonadotropin (Menodac® 150 IU, Bayer Zydus Pharma, India) was added after six doses of recombinant follitropin alpha. Dosages were titrated as per ovarian response with ultrasound follicular monitoring. In antagonist protocol, stimulation was started from the 2nd day of menstruation and GnRH antagonist injection cetrorelix 0.25 mg (Cetrolix®, Intas Pharmaceuticals, India) was added when the leading follicle reached 15 mm or on day 5 of stimulation whichever was earlier. Ovulation trigger was given with injection recombinant human chorionic gonadotropin (HCG) 250 μgm (Ovitrelle®, Merck Serono, Italy) when minimum two follicles reached 18 mm or more in size. Blood sample was collected for serum progesterone level estimation in vacutainer containing separating gel before ovulation trigger and ovum pickup was done 36 h after ovulation trigger. Collected samples were processed within 8 h of sample collection. Serum progesterone was estimated by quantitative electro chemiluminescence immune assay "ECLIA" on immunoassay analyzer with proper quality control and calibration methods. The measurement range for progesterone kit used was 0.030–60.00 ng/ml. A serum progesterone level >1.5 ng/ml was considered as PL in our study. IVF or Intracytoplasmic sperm injection was performed depending on seminal parameters and embryos were evaluated at 2PN stage and 4 cell stage. ET was done at 4–6 cell stage except in cases that were likely to develop ovarian hyperstimulation syndrome (OHSS), fertilization failure, or in cases of unsuitable endometrium. Luteal phase support was started in all patients from the day of ovum pickup. Serum HCG level was determined by quantitative electrochemiluminescence immunoassay "ECLIA" on immunoassay analyzer on day 16 post-ET, value of beta HCG ≥50 IU was taken as positive pregnancy. Data so obtained were statistically processed to determine the association between serum progesterone value on day of HCG trigger and IVF outcome.

Study outcome

Primary outcome

To correlate serum progesterone level on the day of ovulation trigger during IVF and its effect on treatment outcome.

Secondary outcome

The secondary outcomes were as follows:

- To study the incidence of PL during IVF-ET at our center
- The study was proposed as a prospective observational study on all willing patients during the study period September 2016 to March 2017.

Statistical analysis

The study descriptive was analyzed with SPSS version 22 (IBM Corp., Armonk, NY, USA). The study group comparisons were made using independent *t*-test and paired *t*-test. Cross-tabulation and Chi-square test was done for studied protocol-wise analysis of serum progesterone level. One-way analysis of variance was done to compare the progesterone level in the different ovarian reserve categories among the study population. Statistical significance was assessed at $P < 0.05$.

Results

PCOS (25%), DOR (22%), tubal factor (14%), azoospermia (9%), and unexplained infertility (20%) were the common causes of infertility [Figure 1]. Mean age of our female patients was 29.34 years. The study group contained 380 patients, of whom 314 patients had the long agonist protocol (82.6%) and 66 patients (17.4%) the antagonist protocol [Table 1].

Incidence of PL in our study group was 12.8%, it was 12.7% and 13.6% in the long protocol and antagonist protocol, respectively ($P = 0.157$) [Table 1]. Incidence of PL in women with PCOS, DOR, and normal ovarian reserve were 14.5%, 10.3%, and 13.1%, respectively ($P = 0.199$) [Table 2].

Pregnancy was positive in 31.1% and negative in 33.2%, ET was not done in 35.8% of the cycles for reasons such as high risk for OHSS, fertilization failure, empty follicular syndrome, and poor endometrium. Mean serum progesterone level was 0.892 ± 0.752 ng/ml and 0.91 ± 0.688 ng/ml in the pregnancy-positive and pregnancy-negative group, respectively. There was no statistical difference in the progesterone value between the two groups ($P = 0.961$). Incidence of PL was 13.5% and 13.4% in pregnancy-positive and pregnancy-negative groups, respectively ($P = 0.223$) [Table 3].

Discussion

Physiological variations of ovarian steroid hormones regulate the cyclic changes of the endometrium. COH during IVF is associated with supraphysiological hormonal levels and alteration in the hormonal milieu is likely to affect the clinical outcome of an IVF cycle.

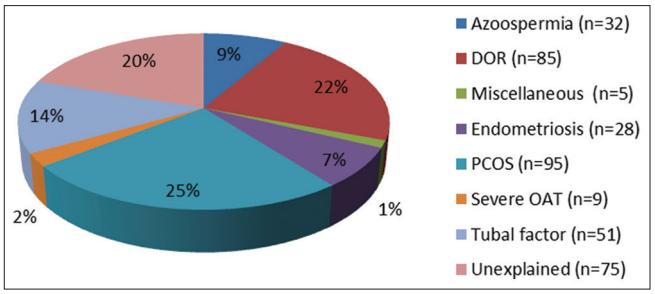

Figure 1: Etiological distribution of the study group

Table 1: Serum progesterone levels in different *in vitro* fertilization protocols and incidence of premature luteinization

Protocol	Antagonist (*n*=66)	Agonist (*n*=314)	*P*
Serum progesterone (ng/ml)	0.91±0.714	0.91±0.721	0.998
Incidence of PL	9 (13.6% of total antagonist cycle)	40 (12.7% total agonist cycle)	0.157

PL=Premature luteinization

Serum progesterone level in a natural menstrual cycle during the mid-follicular phase was found to be 0.84 ng/ml.[4] Mean serum progesterone in our study group was 0.91 ± 0.71 ng/ml, it was 0.91 ± 0.721 and 0.91 ± 0.714 ng/ml in the agonist protocol and antagonist protocol, respectively [Table 1]. Average serum progesterone level as per available literature was 1.02 ± 0.50 ng/ml on the day of HCG administration,[17] which is almost similar to the mean progesterone level of our study.

Mean serum progesterone levels were studied in different categories of ovarian reserves, and there was no statistically significant difference in the serum progesterone levels among these groups [Table 2]. Similar findings were also noticed in a study which states that serum progesterone levels on ovulation trigger day do not depend upon etiological parameters.[16]

PL is seen in both agonist and antagonist cycles, in agonist cycles it varies between 5% and 35% and 20%–38% in antagonist cycles.[18] It has been observed in a study that overall incidence of PL to be 13.02% with a further distribution as 18.00% in agonist and 9.31% in antagonist subgroups.[19] On the contrary, there is a study which has found no significant difference in the incidence of elevated progesterone between GnRH agonist and antagonist cycles 8.3 versus 6.8%, respectively ($P = 0.117$).[17] As per the latest Indian study, PL (>1.5 ng/ml) was noticed in 16% cases in the agonist group and 6% in the antagonist group (p-0.002).[12] The overall incidence of PL in our study was 12.8% without any significant difference between agonist and antagonist protocol. A study by Houmard *et al*. showed that the PL

incidence was found to be 11% when progesterone level cutoff was >1.5 ng/ml.[20]

In our study, clinical pregnancy rate was positive in 31.1%, negative in 33.2%, and 35.8% (136 cases) did not undergo ET. ET was not done in these cases due to various reasons such as fertilization failure (77 cases), to prevent OHSS (28 cases), empty follicular syndrome (15 cases), poor grade embryos (08 cases), thin endometrium (07 cases), and one case due to hemoperitoneum [Table 4]. This can be considered as a limitation of our study but as per the statistical analysis, the chance of these cases affecting the resulting outcome is almost negligible, as there is no significant difference in mean serum progesterone level and incidence of PL between positive pregnancy, negative pregnancy, and cases that did not undergo ET [Table 3].

It has been observed that the clinical pregnancy rates between patients with serum progesterone level ≤1.5 ng/ml versus >1.5 ng/ml to be 37.04% and 41.03%, respectively, with $P = 0.50$.[19] This study suggests that difference in progesterone level does not have significant effect on pregnancy rates which is further supported by another study which also states that there was no significant difference in pregnancy rate between groups with normal or high progesterone level on the day of HCG administration ($P = 0.98$).[15] Our study supports the above findings as the mean serum progesterone level in the pregnancy-positive group and negative pregnancy group was almost the same without any significant difference in the incidence of PL between the two groups. There is one more study to confirm the above findings which states that when progesterone levels were higher than 1.8 ng/ml, and it had no detrimental effect on oocyte quality or endometrial receptivity.[21]

Pregnancy distribution was also assessed at varying levels of serum progesterone on the day of ovulation trigger at an increment value of 0.5 ng/ml [Figure 2]. There was no significant change in serum progesterone levels

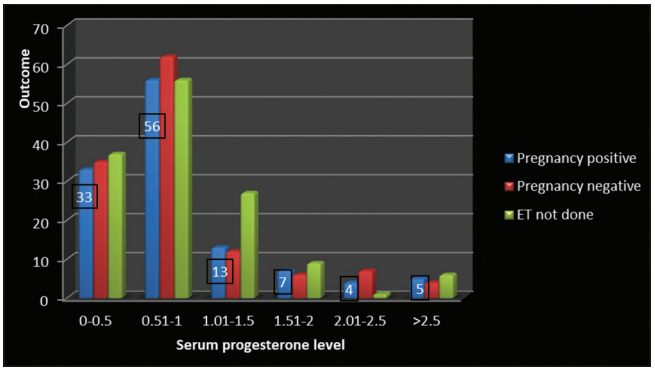

Figure 2: Pregnancy distribution at different serum progesterone levels

Table 2: Serum progesterone level and incidence of premature luteinization between polycystic ovarian syndrome, decreased ovarian reserve, and normal ovarian reserve

Group	Serum progesterone level (ng/ml)	*P*	Incidence of PL	*P*
PCOS (*n*=96)	0.914±0.552	0.903	14 (14.5% of total PCOS)	0.199
DOR (*n*=87)	0.88±0.824		9 (10.3% of DOR)	
Normal ovarian reserve (*n*=197)	0.921±0.743		26 (13.1% of normal ovarian reserve)	
Study group (*n*=380)	0.91±0.71			

PCOS=Polycystic ovarian syndrome, DOR=Decreased ovarian reserve, PL=Premature luteinization

Table 3: Outcome of the *in vitro* fertilization and embryo transfer, incidence of premature luteinization, and progesterone level on the day of ovulation trigger

Category	Outcome of IVF, *n* (%)	Progesterone level (ng/ml)	*P*	Incidence of PL	*P*
Pregnancy positive	118 (31.1)	0.892±0.752	0.961	16 (13.5% of pregnancy positive)	0.223
Pregnancy negative	126 (33.2)	0.91±0.688		17 (13.4% of pregnancy negative)	
Embryo transfer not done	136 (35.8)	0.922±0.722		16 (11.7% of ET not done)	

PL=Premature luteinization, IVF=*In vitro* fertilization, ET=Embryo transfer

Table 4: Serum progesterone level in embryo transfer not done cases

Reasons for not doing ET	ET not done cases, *n* (%)	Serum progesterone level (ng/ml), (mean±SD)	*P*
Fertilization failure	77 (57)	0.802±0.61	0.104
To prevent OHSS	28 (20)	1.120±0.605	
Empty follicular syndrome	15 (11)	1.222±1.33	
Poor grade embyos	8 (6)	0.619±0.303	
Thin endometrium	7 (5)	1.160±0.564	
Hemoperitonium	1 (1)	0.853	

OHSS=Ovarian hyperstimulation syndrome, SD=Standard deviation, ET=Embryo transfer

between pregnancy-positive and pregnancy-negative groups. Difference in pregnancy distribution was also not significant between different ranges of progesterone levels such as 0–0.5, 0.51–1.0 and 1.01–1.5 ng/ml, 1.51–2.0, 2.01–2.5, and >2.5 ng/ml. Our findings are further supported by the observations made by many studies which showed pregnancy distribution at similar range of progesterone level and the changes in the pregnancy distribution were not statistically significant at different progesterone levels.[15,19,20]

Conclusion

In our study, the overall incidence of PL was 12.8% of the IVF cycles. PL incidence did not differ with different IVF stimulation protocols nor did it affect pregnancy rates. Our study also suggests that the effect of PL on pregnancy outcome is not clinically significant; therefore, a decision to defer ET should not be based on apprehension of PL.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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