

## Association of raised serum progesterone level with ovulation trigger and histology of endometrium in stimulated cycles

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This was a forthcoming study of those patients, who undergo *in-vitro* fertilization (IVF) and freeze-all embryo, who acquiesce for the study. The number of participated patients (n=350) in this study, underwent for IVF. The blood sample was collected from patients to evaluate the level of serum progesterone in vacuum vials on the day of ovulation trigger. After 36 hrs of ovulation trigger, ovum picked up was done. Quantitative methods were used to estimate the level of serum progesterone through the electrochemiluminescence immunoassay and correlation of serum progesterone with embryo transfer (ET) outcomes. Main outcome of this current study was to evaluate the value of mean serum progesterone level *i.e.* 0.868± 0.712 ng/ml and 0.88±0.723 ng/ml was found in case of pregnancy positive and negative respectively, at p=0.216 value. In antagonist (n=40) and agonist (n=310) cases, it was 8(20%) and 37(11.94%) PL occurrence was noted at p=0.143 respectively. An overall value of the premature lutenization (PL) occurrences was 13.63% and 15.25% observed in both positive and negative cases of pregnancy at p=0.216 respectively. This study concluded that 12.66% of PL occurrences were recorded in the case of IVF. Study results proved, there were no significant effect of PL on pregnancy outcomes.

**Keywords:** Serum progesterone. Premature lutenization. *in-vitro* fertilization. Human gonadotropins. Ovulation trigger.

### INTRODUCTION

In recent years, the number of infertility cases have increased and it has been recorded that only 1.33% of the issue of the live birth are coming in front through the usage of IVF and intracytoplasmic sperm injection (ICSI) reproductive technologies (Kamel, 2013).

The problem of Infertility arises due to the altered endometrial environment which is supported by reduced embryo implantation in the wall of the endometrial as observed in IVF cycles compare to natural cycles

reviewed by Haouzi *et al.* (2009). To overcome the problem of reduced implantation on endometrium wall, a regular assessment of endometrium function has to be done by elevating the serum progesterone on or before the day of ovulation trigger hence, it is defined as premature-lutenization (PL) (Al-Azemi *et al.*, 2012; Requena *et al.*, 2014). Other recommended steroid hormones in addition to progesterone, are gonadotrophins and estradiol are generally useful for IVF treatment and it may also affect on endometrial wall development. Previously reported Studies by Bosch *et al.*, 2010 and Papanikolaou *et al.*, 2009 suggested that the premature-lutenization phase adversely affect the pregnancy outcome, if the serum progesterone level is exceeded from 1.5 ng/ml on the day of ovulation trigger. This exceeded levels of peripheral progesterone

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during the late follicular phase, may lead to induce endometrial histological maturation and differential gene expression pattern (Labarta *et al.*, 2011) but do not have any negative effect on the egg development or embryo quality (Shapiro *et al.*, 2010). It affects the wall of endometrium therefore it is called as a window of implantation, which means there is no relation exist between the endometrium and developing embryo (Bourgain *et al.*, 2002). Still, several controversies exist between the association of PL and its effect on IVF outcome. Because earlier studies do not find out the significant result concerning with pregnancy rate determination during *in vitro* fertilization through the use of high or low level of progesterone on the day of ovulation stimulation (Allahbadia, 2014; Ochsenkuhn *et al.* 2012). To improve the pregnancy outcomes, some strategies/protocols were designed to promote ovulation induction through the Gonadotrophin and progestin-primed ovarian stimulation protocols for over age women (over 40 years) patients with poor ovarian response.

The objective of the current study was to investigate progesterone raised serum level favors both the phenomenon, the ovulation induction and histological changes in the wall of the endometrium.

## MATERIAL METHODS

The number of patients participated in this study were (n=350), underwent *in-vitro* embryo transfer (IVF-ET). Approx 12-month time duration was taken to accomplish this study. Ethical committee was set up to approve the study and written informed permission was obtained from participated patients.

### Study group

All the participated patients were undergone for *in vitro*-fertilization Department of Reproductive Medicine Center, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, 200233, China from July 2018 to August 2019.

### Selection criteria

All the 350 patients were undergoing for IVF-ET process were lie between the age group of 20-40 years during this study period. Diseased individuals are not part of this study.

## MATERIAL AND METHODS

Before IVF-ET, ovarian stimulation was done by using one of the two protocols, either long agonist or antagonist protocols. For the long agonist protocols accomplishment, intake of oral contraceptive drugs was started at the 5<sup>th</sup> day of menstruation cycle and gonadotropins agonist i.e. Leuprolide acetate dose of 1mg daily quantity was started from the 21<sup>st</sup> day of the menstruation cycle. The endometrial and ovarian changes were closely observed through the transvaginal ultrasonography technology. Estradiol and LH level evaluation was done by following same ultrasonography technology. Initially; ovarian stimulation was done by follitropin- $\alpha$  intake started from the 2<sup>nd</sup> of menstruation cycle (Corti *et al.*, 2013). In case of polycystic ovarian syndrome (PCOS), intake of recombinant LH dose was started at the end of follicular maturation phase. The dose of gonadotropins intake was started after completing the 6-doses of follitropin- $\alpha$  for normal ovarian reserve (NOR) and decrease ovarian reserve (DOR) cases. Each dosage of drugs was titrated that is based on the response of ovary follicles monitored by ultrasonography. In case of antagonist protocol, ovarian stimulation begins from the 2<sup>nd</sup> day of menstrual cycle followed by intake of gonadotropins antagonist injections. Cetrolinx 0.25 mg drug intake was recommended, when the follicle size reached to 15 mm or on the 5<sup>th</sup> day of stimulation. Minimum ovarian follicular size would be 18 mm or more, at this stage, ovulation triggering was done by using human chorionic gonadotropins (HCG)-250  $\mu$ g injection.

### Experimental procedure

First of all, a blood sample was collected from patients to evaluate the level of serum progesterone in vacuum vials containing separating gel before ovulation

trigger starts. After the 36hrs of ovulation trigger, ovum picked up was done. We processed the samples within 8 hrs of sample collection. Quantitative methods were used to estimate the level of serum progesterone through the Electro chemiluminescence immune-assay. For this purpose, Progesterone kit of 0.030-60 ng/ml range concentration was utilized. The required concentration of serum progesterone was more than the 1.5 ng/ml considered in this study for premature lutenization (PL). *In-vitro* fertilization was observed at 1<sup>st</sup> day of normal fertilization after insemination and blastomers stage. The transfer of embryos was done at 4-6 cell stage (Elgindy *et al.*, 2011) instead of avoiding exceptional cases of ovarian hyperstimulation syndrome (OHSS), where a failure of fertilization or underdevelopment of endometrium occasionally occurred.

Quantitative assay was performed to measure the serum HCG level (Mascarenhas *et al.*, 2015) by electrochemiluminescence immunoassay on ELISA reader after the 16<sup>th</sup> day of embryo transfer (ET). The  $\beta$ -value of HCG of  $\geq 50$  IU was considered as positive pregnancy in this study. Statistical data analysis was done to find out the correlation between the serum progesterone level on the day of HCG activation and *in-vitro* fertilization results.

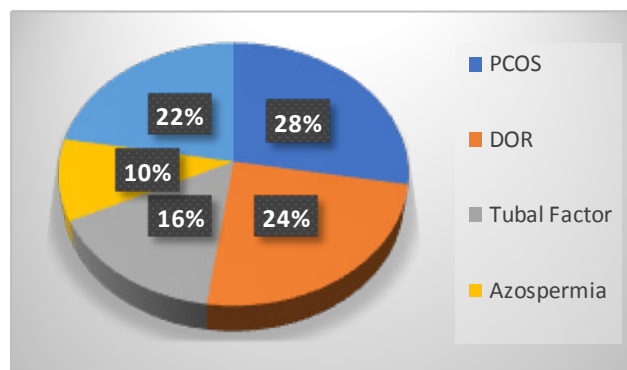
### Statistical analysis

All the statistical analyzes were performed using SPSSvers.16. A comparative study between groups was done using independent t-test and paired t-test. One-way ANNOVA analysis was used to analyze the different study group population of progesterone level secretion from ovarian reserve categories. In addition, protocol-wise analysis of the serum progesterone level was studied through the Cross-tabulation and Chi-square test. Test of significance of the data was done at  $p < 0.05$  level.

## RESULTS

Infertility factors were considered in this study such as PCOS (28%), DOR (22%), tubal factor (16%),

azoospermia (10%), and unexplained infertility (24%) defined in Figure 1.



**FIGURE 1** - Average age of female subjects was 30 years.

The total included study groups were  $n=350$  patients, of those 310 patients underwent the against protocol (88.57%) and remaining 40 patients (11.43%) follow the antagonist protocol as shown in Table I. The % of PL in studied group was 9.68 %, it was 9.67% and 11.66% found in the agonist protocol and an antagonist protocol respectively, at  $p=0.152$  as depicted in Table I. An observed value of % premature lutenization in diseased female patients were 13.33%, 8.97% and 17.58%, respectively, at  $p=0.219$  as shown in Table II. The result of pregnancy outcomes was positive in 31.42% and negative in 33.71%, embryo transfer (ET) was not done in 34.85% of the cycles because of the following reasons, such as (OHSS) risk, chances of fertilization is nil, follicle syndrome and poor endometrium response were high. The calculated value of the mean serum progesterone level was  $0.868 \pm 0.712$  ng/ml and  $0.88 \pm 0.723$  ng/ml found in pregnancy positive and pregnancy-negative cases. Statistical data analysis was performed and no significant outcomes were observed between studied groups at  $p=0.91$ . An overall value of the (%) PL occurrences was 13.63% and 15.25% observed in both positive and negative cases of pregnancy at  $p=0.216$  respectively, as depicted in Table III.

**TABLE I** - Level of serum progesterone in antagonist and agonist protocols and premature luteinization occurrence

Protocol	Antagonist (n=40)	Agonist (n=310)	P value
Progesterone (ng/ml)	0.93±0.632	0.93±0.678	0.996
PL*occurrence	8(20%)	37 (11.94%)	0.143

\*PL=Premature luteinization

**TABLE II** - Serum progesterone level and premature luteinization occurrence during polycystic ovarian syndrome, decreased ovarian reserve, and normal ovarian reserve

Study Groups	Serum progesterone level	P value	PLO occurrences	P value
PCOS (n= 90)	0.911±0.562	0.908	12 (13.33%)	0.219
DOR (n= 78)	0.87±0.545		7 (8.97%)	
Normal ovarian reserve (n= 182)	0.941±0.443		32 (17.58%)	
Study group (n= 350)	0.93±0.61			

**TABLE III** - Observed changes in *in-vitro* fertilization and embryo transfer, premature luteinization occurrence on the day of ovulation trigger

Categories	IVF outcome n (%)	Progesterone level (ng/ml)	P-value	Occurrences of PL	P value
Pregnancy positive	110(31.42%)	0.868±0.712	0.91	15(13.63%)	0.216
Pregnancy negative	118(33.71%)	0.88±0.723		18(15.25%)	
ET not done	122(34.85%)	0.90±0.376		17(13.93%)	

**TABLE IV** - Level of progesterone in patients with no Embryo transfer occurred

Reason behind not doing ET	N (%) of ET not done	Progesterone level (ng/ml)	P value
Fertilization failure	72(59%)	0.789±0.68	0.099
OHSS prevention	21(17.21%)	0.992±0.610	
Vacant follicular abnormality	11(9%)	1.089±1.09	
Poor grade embryos	9 (7.38 %)	0.534±0.233	
Thin endometrium	7 (5.74%)	0.999±0.613	
Hemoperitonium	2 (1.64%)	0.814	

## DISCUSSION

Physiological changes in endometrium wall resulted by ovarian steroid hormones. Ovarian hyperstimulation also occurred due to alteration in hormone level during *in-vitro* fertilization and it may affect the overall outcomes of IVF cycles. Earlier studies finding showed that the level of serum progesterone was found to be 0.84 ng/ml in a menstrual cycle at the mid-follicular stage (Allahbadia, 2014). In this study, the mean value of serum progesterone of participated study groups was  $0.93 \pm 0.61$  ng/ml, the rest of against was  $0.93 \pm 0.678$  ng/ml and  $0.93 \pm 0.632$  ng/ml of the antagonist protocol respectively, as shown in Table I. Similarly, reported in previous studies, the obtained mean value of serum progesterone was  $1.02 \pm 0.50$  ng/ml on the same day of HCG administration (Venetis *et al.*, 2015), its mean value figures were nearly analogous to obtain an average value of serum progesterone found in this study. The average value of the serum progesterone level was determined among different ovarian reserves categories but no significant differences were found, as depicted in Table II. Previously reported studies noticed that the serum progesterone level does not depend on abnormal factors during the day of ovulation trigger (Park *et al.*, 2015). PL phase was also occurred in both the case of agonist and antagonist protocols, PL varies from 5%-35%, and 20%–38% during agonist and antagonist cycles (Castillo *et al.*, 2015; Direito *et al.*, 2013). Reported PL occurrences were found to be 13.02% with further disturbed as 18.0% and 9.31% in case of agonist and antagonist sub-categories (Huang *et al.*, 2015). With reference of other reported literature found that there were no significant differences were reported in elevated progesterone level among the gonadotropins affected agonist and antagonist cycles i.e. 8.3% and 6.8% respectively at  $p=0.117$  (Venetis *et al.*, 2015; Ecochard *et al.*, 2013). Recently, studies on Indians about PL incidence among agonist and antagonist subgroups were found to be in 16% cases only at  $p=0.02$  level (Choudhary *et al.*, 2016). An observed value of PL occurrence was 13.33% in studied groups but no significant difference was found among agonist and antagonist subgroups. Earlier study by Houmard *et al.*, 2014 reported that the 11% PL occurrence was found if the level of serum progesterone is exceeded from 1.5 ng/ml concentrations. Some clinical

outcomes found while measuring the positive or the negative pregnancy rate, it was 13.63% positive, 15.25% negative and rest 13.93% (122 cases) were not undergone for embryo transfer (ET) as shown in Table IV. ET was not done in 122 cases because of incomplete fertilization (in 72 cases), OHSS risk (in 21 cases), vacant follicular abnormality (11 cases), poor grade embryo's (9 cases), reduced thickness of endometrial wall (7 cases) and rest of the 02 cases of hemoperitoneum shown in Table IV. The foremost limitation of this study was error prone cases, statistically it proves the chance of affecting the resulting outcomes of these cases were almost negligible, due to no correlation establish between these groups *i.e.* pregnancy positive, pregnancy negative and not transferred embryo cases, in terms of measuring the mean serum progesterone level and PL occurrence as depicted in Table III. Previously studied (Fatemi *et al.*, 2015) data set finds the pregnancy rate between studied groups with higher serum progesterone than required 1.5 ng/ml or less or equal to 1.5 ng/ml level, it was 41.03% and 37.04% observed at  $p=0.50$  (Venetis *et al.*, 2013; Huang *et al.*, 2015). From this study, our observation concludes the pregnancy rate was not affected by lowering or increasing the level of serum progesterone, supported by another study (Hajishafiha *et al.*, 2015; Xu *et al.*, 2012), suggested that the no significant results were obtained on the day of HCG injection at  $p=0.98$  value. One more study supports this study which states that egg development, egg quality and endometrium change were not affected by higher level (1.8 ng/ml) of serum progesterone but it affects the pregnancy distribution on the day of ovulation trigger (Leiva *et al.*, 2015) with the increment of up to 0.5 ng/ml concentration above this 0.5–3.0 ng/ml progesterone concentration, there were no significant changes observed in pregnancy distribution supported by many other previously reported studies. Therefore, no significant differences in serum progesterone levels were found between the positive and negative pregnancy groups.

## CONCLUSION

This study concludes that 12.66% of premature lutenization (PL) occurrences were recorded in the case of IVF. The PL occurrence, neither depending on different IVF protocols nor affects the pregnancy rate.



Study results showed that no significant effect of PL on pregnancy outcomes was found; hence embryo transfer phenomenon was not dependent on PL uncertainty.

## CONFLICT OF INTEREST

No conflict of interest

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