Original Article

Comparative Study of Serum Progesterone Levels at the Time of Human Chorionic Gonadotropin Trigger and Ovum PickUp in Predicting Outcome in Fresh *in vitro* Fertilization Cycles

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Abstract

Background: Studies have shown that premature rise of progesterone in controlled ovarian stimulation (COS) at the time of human chorionic gonadotropin (hCG) trigger is negatively associated with in vitro fertilization (IVF) outcome in fresh IVF cycles. Some authors have failed to demonstrate this. One large single centre retrospective cohort study has compared the pre and post hCG progesterone and observed that the ratio of the rise in progesterone could be a positive predictor. There is paucity of literature on this aspect. **Aims and Objectives:** To compare the serum progesterone at hCG trigger and ovum pick-up (OPU) with IVF outcome by estimating the respective paired hormone levels. **Material and Methods:** Serum progesterone levels at hCG trigger and OPU are compared retrospectively in 301 fresh IVF cycles with IVF outcome by long protocols with GnRH agonists for two years. Parametric and nonparametric testing of null hypothesis is performed. *P* value <0.05 is taken as significant. **Results:** There is no predictive association of IVF outcome with either progesterone levels. The ratio of rise in progesterone is strongly positively associated with IVF outcome ($P < 0.001$). However, after adjusting for confounders and modifiers the retrieved number of oocytes are positively associated with IVF outcome ($P = 0.044$). **Conclusions:** The ratio of rise in progesterone is significantly associated with number of oocytes retrieved which in turn is associated with successful IVF outcome in fresh cycles. Ratio of rise in progesterone seems to be therefore an indirect parameter for predicting successful IVF outcome in fresh cycles.

Keywords: *Fresh in vitro fertilization cycles, in vitro fertilization outcome, serum progesterone at human chorionic gonadotropin trigger, serum progesterone at ovum pick up*

Introduction

In clinical practice, the ovulation trigger of mid-cycle luteinizing hormone (LH) surge is usually achieved by human chorionic gonadotropin (hCG) due to its molecular similarity, binding to LH receptors, and longer half-life.[1] hCG trigger induces luteinization of mature follicle and formation of corpus luteum.[2] The primary role of progesterone produced by corpus luteum is the preparation of estrogen primed endometrium for implantation of an embryo and its maintenance. There of till the initial weeks of pregnancy.[1] Progesterone also plays a role in the expression of genes needed for implantation at the level of the endometrium.[3,4] It is seen in controlled ovarian stimulation (COS) protocols that there is premature release of progesterone in the late follicular phase due to simultaneous stimulation of large number of follicles resulting in asynchronous endometrium and implantation failure in fresh cycles.[1,4] Several studies have observed that this

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premature progesterone rise in gonadotropin-releasing hormone (GnRH) downregulated cycles on the day of hCG trigger is negatively associated with *in vitro* fertilization (IVF) outcome.[5-8] Other investigators failed to support this view. Few other studies evaluated the paired samples of serum progesterone levels at the time of hCG trigger and post-hCG administration to determine which one correlated better with IVF outcome. So far, only three studies are available.[9-11] There is a paucity of literature on this front and hence the present study.

Aim of the study

The aim is to compare serum progesterone levels at hCG trigger and ovum pick up (OPU) in predicting successful IVF outcome in fresh IVF cycles.

Objectives of the study

To estimate and evaluate the correlation of serum progesterone levels at hCG trigger and OPU with successful IVF outcome.

To evaluate which among the above two is a better predictor for a successful IVF outcome.

Subjects and Methods

A single-center retrospective cohort study is conducted on 306 fresh IVF cycles of normozoospermic semen samples and COS by long protocol with GnRH agonists followed by hCG trigger from 2016 to 2018.

All couples underwent complete evaluation including detailed history, physical examination, blood counts, urinalysis, hormone profile, blood sugar, blood group, coagulation profile, viral markers, serological tests for syphilis, hormone profile, Mantoux test, erythrocyte sedimentation rate, chest X-ray, hysterosalpingogram, ultrasonography of pelvis, ovulation studies, evaluation of ovarian reserve, diagnostic hystero-laparoscopy, and semen analysis (at least twice) as per the WHO manual 2010. All female partners received preconceptional folic acid supplementation 5 mg twice daily and tablet aspirin 75 mg once daily.

Inclusion criteria

Cycles with the retrieval of at least three mature oocytes and fresh semen preparations recovering morphologically normal sperms of a concentration ≥10 million/ml are included in the study.

Exclusion criteria

Cycles with poor/nonresponse to COS and fresh semen preparations recovering morphologically normal sperms of a concentration <10 million/ml are excluded from the study.

Additional inclusion and exclusion criteria with reasons are listed in Box 1.

COS is done by individualizing the starting dose of gonadotrophins based on age, basal follicle-stimulating hormone (FSH) levels, body mass index, and response during earlier stimulated cycles. Depending on ovarian response by ultrasound assessment, dose adjustments of gonadotrophins are carried out after at least 5 days of stimulation. When the follicular size is at 18–20 mm, 250 mcg of recombinant hCG and 5000 IU urinary hCG are administered for final maturation of the oocyte. OPU is carried out after 36 h of hCG trigger.

Insemination is performed using droplet method with oil overlay within 1 h of OPU/removing sperms from the final swim up, and embryos are cultured in sequential culture media (vitrolife).

The assessment of fertilization is made on day 1, i.e., 18–20 h postinsemination at 2-pronuclei stage. Denudation is carried out on day 1 at the time of assessment of fertilization. Day 2 embryos with 2–4 cells and day 3 embryos with 6–8 cells, and <20% fragmentation is taken as good-quality embryos (Grades 1 and 2), embryo transfer (ET) is performed at the cleavage stage (day 3) of the embryo development. One to three in number of embryos is transferred depending on the yield and quality of embryos.

Post-OPU and post-ET luteal phase support are given by micronized progesterone injections 100 mg once daily, and tablet dydrogesterone 10 mg twice a day from the day of OPU until the 12th week of gestation, if pregnancy is tested positive.

Outcome measures

Positive or negative pregnancy as tested by serum beta hCG (≥100 mIU/ml) on day 17 of ET is taken as the primary outcome variable. Number of mature oocytes retrieved is taken as an intermediate outcome/secondary outcome.

Progesterone measurements

Paired serum samples are collected from female partners at hCG trigger and at OPU. The samples underwent radioimmunoassay (RIA) using Beckman Coulter RIA kit for measuring serum progesterone level. The analytical sensitivity was 0.11 nmol/L intra- and inter-assay precision, expressed as coefficients of variation were 6.7% and 17.3%, respectively. The tests are done after taking ethical committee approval along with written and informed consent of patients. Staff at assisted reproductive technology center are blinded to progesterone levels. Staff at endocrine laboratory measuring the progesterone values are blinded to IVF outcome. Serum progesterone levels and IVF outcome are analyzed using retrospectively by the third group of investigators.

Statistical analysis

Statistical analysis is performed using the IBM SPSS statistics 20 (SPSS Inc., Armonk, New York, USA). Proportions are compared using the Chi-square test and means using independent samples *t*-test and analysis of variance. Binary logistics regression performed for adjusting the effect of confounders, modifiers, and moderators. We have considered parametric tests because of the large sample size, different spread/dispersion of compared groups, and to give better statistical power to study if there is a true significance effect that exits. We have also performed simultaneously non parametric tests to test the Null Hypothesis since many of the variables were following non normal distribution pattern. Value of $P < 0.05$ is considered as statistically significant.

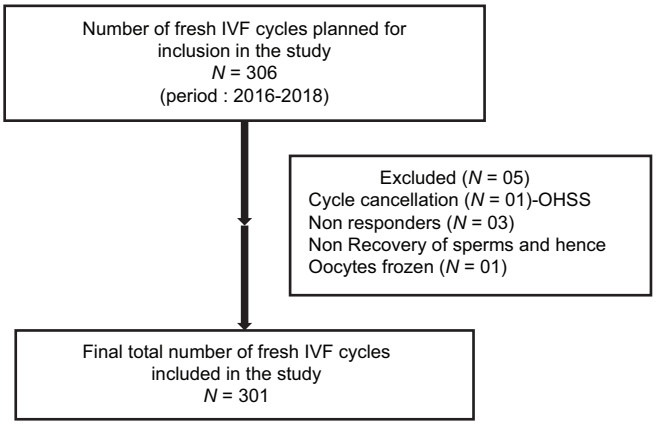
Results

Three-hundred and one fresh IVF cycles of 306 [Flow Chart] are analyzed with variable parameters-age, duration of infertility, serum progesterone at hCG trigger and OPU, the ratio of rise in serum progesterone, number of retrieved mature fertilizable oocytes, and IVF outcome.

Baseline characteristics – Table 1 depicts the baseline characteristics of the independent variables of our study. Overall IVF outcome – 154 (51%) of 301 are tested positive for pregnancy, and 147 (49%) are tested negative.

Summary of bivariate analysis by parametric tests [Table 2]

Table 2 shows the relationship of each independent variable with IVF outcome, *P* value and statistical significance. In the present study, it is seen that serum progesterone levels at hCG trigger, and OPU independently have no significant association with IVF outcome. However, the ratio of rise in progesterone is



Flow Chart: OHSS= Ovarian hyperstimulation stimulation syndrome

Box 1: Additional Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria (reasons for exclusion in parenthesis)
Fresh IVF cycles	Frozen embryo transfers (study relates to effects on fresh cycles)
Female partners with normal day 2 FSH, LH, prolactin levels, euthyroid status, and optimal levels of AMH	Difficult embryo transfers (outcome modifier)
Unexplained infertility	Infertility due to uterine and endometrial factors (outcome modifier)
Infertility due to absent or damaged fallopian tubes	Poor ovarian reserve (non/poor responders hence outcome modifier)
Endometriosis	PCOS (no hCG trigger and "freeze all embryos" policy)
Fresh semen samples collected by masturbation	Cases of ovarian hyperstimulation (cycle cancellation or deferment of embryo transfers)
Normozoospermic (WHO 2010) male partners	All male factors infertility: Azoospermia, retrograde ejaculation, oligasthenoteratospermia, immunological infertility (may be a confounding factor)
	All other forms of collection of semen or extraction of sperms other than masturbation (potential confounder)
	Cryopreserved semen samples (only fresh samples were taken)
	Presence of medical illness in either or both partners (potential confounders)
	Total fertilization failure in previous cycles (potential confounder)

IVF=*In vitro* fertilization, hCG=Human chorionic gonadotropin, FSH=Follicle-stimulating hormone, LH=Luteinizing hormone, AMH=Anti-Müllerian hormone, PCOS=Polycystic ovary syndrome

Table 1: Baseline characteristics

	Age	Duration of infertility	Serum progesterone (nmol/ml) at the time of hCG	Serum progesterone (nmol/ml) at the time of OPU	Ratio of rise in serum progesterone	Number of oocytes retrieved
Mean	28.9435	5.6312	5.0088	14.7593	7.286	10.9568
SD	3.65515	0.79176	13.62911	15.26560	8.15856	6.91145
Median	28.00	6.0	2.000	9.0	4.00	10.00
Range	22.00	4.0	163.70	78.70	50.00	45.00

SD=Standard deviation, OPU=Ovum pick up, hCG=Human chorionic gonadotropin

Table 2: Summary of bivariate analysis (parametric and nonparametric)

Independent variable	Dependent variable		*P* (parametric, nonparametric)	Significance
	IVF outcome	Number of mature oocytes		
Age groups	IVF outcome		0.003, 0.018	Significant
Duration of infertility	IVF outcome		0.976, 0.846	Not significant
Progesterone at hCG trigger	IVF outcome		0.821, 0.105	Not significant
Progesterone at hCG trigger		Number of mature oocytes	0.051, <0.001	Not significant/significant
Progesterone at OPU	IVF outcome		0.334, 0.155	Not significant
Progesterone at OPU		Number of mature oocytes	0.038, <0.001	Significant
Ratio of rise in serum progesterone	IVF outcome		<0.001, 0.914	Significant/non significant
Ratio of rise in serum progesterone		Number of mature oocytes	<0.001.<0.001	Significant
Number of oocytes retrieved	IVF outcome		0.022, 0.005	Significant

IVF=*In vitro* fertilization, OPU=Ovum pick up, hCG=Human chorionic gonadotropin

Table 3: Binary logistic regression analysis (parametric tests)

Independent variable	Dependent variable	*P*	Significance
Age group	IVF outcome	0.237	Not significant
Duration of infertility	IVF outcome	0.938	Not significant
Ratio of rise of progesterone	IVF outcome	0.959	Not significant
Number of oocytes	IVF outcome	0.044	Significant

IVF=*In vitro* fertilization

strongly associated with positive IVF outcome. It is also seen that the serum progesterone levels at hCG trigger have no significant association with a number of mature oocytes retrieved. However, serum progesterone at OPU, the ratio of rise in progesterone is positively associated with the number of mature oocytes retrieved which in turn have a significant positive association with pregnancy.

Summary of bivariate analysis by non parametric tests [Table 2]

We have also performed nonparametric tests taking into consideration that the median of the many of the groups actually represented the measure of central tendency [Table 2]. These tests included independent median test, Mann–Whitney U-test, Kolmogorov–Smirnov test, Kruskal–Wallis test, and Jonckheere–Terpstra test. All these tests show significant (<0.05) positive association of number of oocytes retrieved with positive IVF outcome. However, there is no direct association with either stand-alone progesterone values at hCG trigger, at OPU or the ratio of rise of these paired samples with IVF outcome. However, related samples Wilcoxon signed-rank test additionally show significant association of stand-alone progesterone value at hCG, at OPU, and their ratio of rise to the number of oocytes retrieved which, in turn, is associated with positive IVF outcome.

Binary logistic regression

The primary outcome is further analyzed using binary logistic regression with age groups, duration of infertility, the ratio of rise in serum progesterone, and number of oocytes retrieved [Table 3]. The number of oocytes after adjusting for age groups, duration of infertility, and the ratio of rise in progesterone is significantly positively associated with the pregnancy ($P = 0.044$).

Discussion

Bivariate analysis [Table 2] of two primary independent variables of our study progesterone levels at the time of hCG trigger and at OPU are not found to be associated with IVF outcome. Premature rise of progesterone before hCG trigger is shown to have a negative association with the pregnancy in fresh IVF cycles.[5-8] The incidence of rise in serum progesterone on or before the day of hCG trigger is 35% (5%–35%) in GnRH agonist cycles and 38% (9%–38%) in GnRH antagonist cycles.[12-16] Serum progesterone rise on the day of hCG has been a matter of debate with studies showing contrary evidence. Some studies have shown no correlation with the IVF outcome.[5] Venetis CA *et al* are of the opinion that this apparent lack of association of premature rise of serum progesterone at the time of hCG trigger with IVF outcome could be due to failure to adjust for confounders and modifiers by multivariate regression.[17] Endometrial receptivity is related the hormonal milieu during ET. High concentrations of estradiol during COS result in premature elevation of progesterone, asynchronous endometrium, and implantation failure. This negative effect of progesterone elevation is limited to the endometrium. There is no effect on oocyte maturation or fertilization as corroborated by donor-recipient IVF cycles and in frozen embryo cycles. Vitrification of embryos can be a useful alternative approach when progesterone levels exceed a suggested threshold (1.5 ng/ml).[5]

While stand-alone serum progesterone levels do not show significant association with successful IVF outcome, the ratio of rise in serum progesterone in the paired samples is significantly strongly associated with positive IVF outcome [Table 2]. However, it is also to be noted

that analysis by Mann–Whitney U-test does not show significant association [$P = 0.912$; Table 2]. Nevertheless, this is an important observation of our study. The aim of the study is to make a comparative analysis of serum progesterone at hCG trigger and at OPU to find out how they are associated with IVF outcome and therefore serve as predictors of successful IVF outcome in fresh IVF cycles. The ratio of rise in progesterone has emerged as a predictor instead. However, the significance is lost after adjusting for number of oocytes, age, and duration of infertility [Table 3]. Moreover, analysis by non parametric tests does not show the significance. Zhu *et al*.[9] in their single-center retrospective analysis of the first fresh IVF cycles of a large cohort of women aged 24–43 years among other findings, conclude that the ratio of change in post-hCG progesterone could be used as another indirect parameter to estimate the pregnancy rate and live birth rate. The primary outcome studied was the effect of this change in post-hCG progesterone levels with number and quality of oocytes and quality of embryos. The implantation rates and pregnancy rates were also studied. The investigators further stratified the ratio of rise to seven subgroups and found that the subgroup of patients with higher ratio of rise in progesterone has higher chances of retrieval of good number of oocytes therefore indirectly live birth rates. Besides, the above-quoted study there are only two more studies of small group of patients.[10,11] Our study seems to be the only study to estimate the post-hCG serum progesterone on the day of OPU.

Elevated serum progesterone levels at hCG trigger show no significant ($P = 0.051$) positive association with number of oocytes recovered by parametric tests although non parametric test shows a significant association (P=<0.001) [Table 2]. However, progesterone levels at OPU and ratio of rise are found to be significantly associated with number of oocytes retrieved by both parametric and non parametric tests ($P = 0.038$ and < 0.001) [Table 2]. Number of oocytes, in turn, is positively associated with positive IVF outcome both by bivariate analysis ($P = 0.022$) [Table 2] and by binary logistic regression ($P = 0.044$) [Table 3].

Zhu *et al*.[9] observed that post-hCG progesterone levels were positively associated with the number of oocytes retrieved, but did not affect oocyte or embryo quality. The effect of premature progesterone rise on IVF outcome may be entirely different depending on strong or weak response to COS. Low pregnancy rates were reported when the ovarian response was weak.[18] We need to consider ovarian reserve, oocyte, and embryo quality which could alter the IVF outcome vis-a-vis serum progesterone rise at hCG.[19,20] Several studies have also shown that strong premature progesterone rise at hCG trigger is linked to the quantum of ovarian response to stimulation.[5,15,21-24]

Ovarian response may be having a moderating influence on the premature rise of progesterone and the pregnancy rates. Pooled data published by Griesinger *et al*.[24] from six clinical trials suggested that premature rise of progesterone at hCG does not compromise pregnancy rates in high responders. However, Bosch *et al*.[15] and Xu *et al*.[25] showed deleterious effects of progesterone elevation at hCG to ongoing pregnancy rates regardless of the magnitude of ovarian response. Large multivariate analysis of 3296 fresh IVF cycles after adjusting for several confounders have shown that there is no evidence to support a moderating effect of ovarian response on the association of progesterone elevation at hCG trigger with live birth rates. However, the authors were of the opinion that there could still be a Type II error.[17]

Conclusion

Stand-alone serum progesterone levels either at hCG trigger or at OPU are not associated with IVF outcome. Serum progesterone at OPU and the ratio of rise of progesterone are significantly associated with number of oocytes retrieved which in turn is associated with successful IVF outcome in fresh cycles and therefore seem to be indirect parameters for predicting successful IVF outcome. This is in agreement with another large retrospective cohort study. Apart from our study and the large cohort mentioned, there are only two small group of studies comparing the paired samples of serum progesterone pre- and post-hCG trigger. Ours is unique study in the sense, we are the only investigators who analyzed post-hCG values at the time of OPU.

Limitations of the study

1. Single-center study
2. Retrospective study
3. Secondary outcomes such as implantation rate, miscarriage rate, and live birth are not included in the study
4. Limited number of independent variables are included in the binary logistic regression to find out adjusted association/relationship
5. Multivariate regression analysis is not carried out.

Therefore, we recommend multicentric prospective studies including all possible confounders, modifiers, and moderators such as COS by GnRH protocols (agonist and antagonist), basal FSH, E2 and progesterone levels, total dose of gonadotrophins administered, number of mature oocytes retrieved, number and quality and number of embryos transferred.

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Conflicts of interest

There are no conflicts of interest.

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