Serum Oestrogen and Progesterone Levels in the Early Luteal Phase as Predictors of Successful In-vitro Fertilisation Outcome: A Prospective Cohort Study

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ABSTRACT

Introduction: The serum levels of oestrogen and progesterone in the luteal phase of In-vitro Fertilisation (IVF) cycles using the long agonist protocol may play a role in predicting the outcome of the cycle.

Aim: To determine the levels of oestrogen and progesterone in the early luteal phase of IVF cycles and their association with the pregnancy rate.

Materials and Methods: A prospective cohort study was conducted in the IVF centre at Maulana Azad Medical College, Delhi, India, from September 2014 to August 2016. A total of 150 women undergoing IVF were recruited over a period of two years and underwent IVF using the standard long agonist protocol. Luteal phase support was provided according to the Institutional protocol. Blood samples were collected on the day of Ovum Pick Up (OPU) (day 0), the day of embryo transfer (day 3), the day of implantation (day 6), and the day of confirmation of

biochemical pregnancy (day 17) to estimate serum Estradiol (E2) and Progesterone (P) levels. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 16.0, and a p-value <0.05 was considered significant.

Results: The mean age of patients in the study was 31.17 ± 1.09 years, and the mean duration of infertility was 8.2 ± 4.2 years. Serum E2 (day 6, 1403.23 ± 376.67 vs 1258.98 ± 354.31 pg/mL; p=0.02) as well as P4 levels (day 6, 77.82 ± 21.82 vs 68.90 ± 22.17 pg/mL; p=0.01) were significantly higher on the day of implantation and day of confirmation of biochemical pregnancy (day 17, E2 541.80 ± 498.01 vs 289.34 ± 171.94 pg/mL, p<0.001; P4 70.61 ± 121.47 vs 36.17 ± 16.63 pg/mL, p<0.001) among those who conceived compared to those who did not conceive.

Conclusion: Serum E2 and P4 levels in the luteal phase may help guide luteal phase support and serve as predictors of a successful cycle outcome in women undergoing IVF using the long agonist protocol.

INTRODUCTION

With the increasing incidence of infertility worldwide, whether due to unexplained, tubal, male, or combined factors, IVF has become a significant option for infertile couples. Implantation is the major barrier to successful IVF outcomes, and the factors affecting implantation are poorly understood. The role of the functional corpus luteum in developing a receptive endometrium for successful implantation, as well as the optimal hormonal milieu to sustain pregnancy until the luteal-placental shift, has been well established. Therefore, early luteal phase hormone levels may predict the likelihood of conception as well as the pregnancy outcome [1].

The role of luteal phase E2 in predicting conception in Assisted Reproductive Techniques (ART) cycles remains controversial. Many studies have shown a significant difference in luteal phase E2 between conception and non conception cycles, suggesting a positive correlation between the early luteal increase in E2 and a positive pregnancy test [2-4]. However, other experts have denied this correlation as well [5]. Similarly, despite serum progesterone being the single best predictor of pregnancy outcome in spontaneous conceptions [6], its use in ART cycles is limited as iatrogenic progesterone administration in the luteal phase of the latter confounds the results. Although studies have shown that non conception cycles have low luteal phase P4 despite exogenous support [7], a study also highlighted the negative impact of an early luteal phase rise in progesterone on implantation [8]. The possible hypothesis is accelerated endometrial development causing desynchrony between endometrial and embryo development, which may lead to defective implantation.

The present study was thus designed to evaluate the role of both serum E2 and P4 in the luteal phase in a well-selected homogeneous population undergoing IVF with similar Controlled Ovarian Hyperstimulation (COH) protocols and similar luteal phase support. This may not only help reduce the anxiety of patients but also assist the clinician in counseling the couple and early pregnancy monitoring.

MATERIALS AND METHODS

A prospective cohort study conducted in the IVF and Reproductive Biology Centre, Department of Obstetrics and Gynaecology, Maulana Azad Medical College, and associated Lok Nayak Hospital from September 2014 to August 2016. A sample size of 150 patients was taken as a convenience sample. Ethical clearance was obtained from the Institutional Ethics Committee (IEC) (F.No./11/IEC/MAMC/2011/246). After screening 187 patients, 150 patients who met the inclusion and exclusion criteria were recruited.

Inclusion criteria: Women aged 20 to 39 years and those undergoing a long protocol of downregulation with Gonadotropin Releasing Hormone (GnRH) agonist and controlled ovarian hyperstimulation with gonadotropins were included in the study.

Exclusion criteria: Patients with a serum E2 level of more than 3000 pg/mL on the day of oocyte retrieval (due to the risk of ovarian hyperstimulation syndrome), decreased ovarian reserve (day 2 serum Follicle-stimulating Hormone (FSH) >10 mIU/mL}, severe endometriosis (stage greater than 2), severe male factor (less than 5 million per mL motile spermatozoa), and various endocrine disorders were excluded from the study.

Study Procedure

All the women enrolled in the study underwent controlled ovarian hyperstimulation using the "long" agonist protocol. Downregulation was done with an injection of leuprolide acetate 0.5 mg/day, subcutaneously, starting from day 21 of the cycle. Serum E2, P4, FSH, and LH levels were estimated on day 2 of the cycle. After successful downregulation, indicated by FSH <10 mIU/mL, LH <2 mIU/mL, E2 <50 pg/mL, and P4 <1 pg/mL, controlled stimulation was initiated using recombinant or urinary gonadotropins or Human Menopausal Gonadotropins (HMG). The starting dose was 225 IU/day of recombinant FSH. In women with markedly suppressed LH (<1 IU/L), HMG was used instead of FSH. Simultaneously, on day 2 of the cycle, the dose of injection leuprolide was reduced to 0.3 mg/day.

The stimulation cycle was monitored with serial measurements of serum estradiol and transvaginal ultrasound imaging. Most women required a total of 9-12 days of stimulation. The goal was to have atleast two follicles measuring 17-18 mm in mean diameter, ideally accompanied by a few others in the 14-16 mm range, and a serum E2 level consistent with the overall size and maturity of the cohort (approximately 200 pg/mL per follicle measuring 14 mm or greater). Endometrial development was also monitored during stimulation by measuring the endometrial thickness and grading. Once the targeted thresholds of stimulation were reached, Human Chorionic Gonadotropin (hCG) (5000-10000 IU) was administered subcutaneously to induce follicular maturation. Transvaginal ultrasound-guided oocyte retrieval was performed after 34-36 hours of hCG trigger under general anaesthesia.

Fertilisation was achieved by conventional microinsemination or Intracytoplasmic Sperm Injection (ICSI) in the case of male factor infertility. Embryo transfer was performed three days after oocyte retrieval using the transcervical ultrasound-guided Sydney IVF Embryo Transfer Catheter Set manufactured by Cook's Medical. Luteal phase support was given to patients according to the Institutional protocol, which included micronised progesterone, 400 mg, soft gel capsule, vaginally twice daily; micronised progesterone, 100 mg, intramuscular injection, alternate day; and highly purified hCG, 2000 IU, subcutaneous injection, alternate day, for a total of three doses.

The women were tested for conception using a Urine Pregnancy Test (UPT) and serum levels of beta hCG 14 days after embryo transfer. A positive UPT and/or serum beta hCG greater than 50 mIU per mL indicated successful implantation. Patients with a positive UPT were further followed-up for the detection of embryonic cardiac activity after two weeks. Blood samples (4 mL) were collected in plain vials on the day of oocyte retrieval (day 0), the day of embryo transfer (day three of oocyte retrieval), the day of implantation (three days after embryo transfer), and the day of confirmation of biochemical pregnancy (17 days after oocyte retrieval) in each patient. Serum levels of E2 and P4 were estimated by Radioimmunoassay (RIA).

STATISTICAL ANALYSIS

The statistical analysis was performed using SPSS version 14.0. The Mann-Whitney U test was used to compare E2 and progesterone levels between two groups. Receiver Operating Characteristic (ROC) curves were plotted and analysed. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The mean age of patients in the study was 31.17±1.09 years, and the mean duration of infertility was 8.2±4.2 years. The majority of the patients had primary infertility, specifically 112 (74.7%) out of 150. Regarding the aetiology of infertility, the majority of patients in the study had tubal factor 81 (54%), followed by unexplained infertility 40 (26.7%), male factor 22 (14.7%), and endometriosis 7 (4.7%). The overall pregnancy rate in the study was 58 (38.7%).

There was no significant difference in serum E2 and P4 levels on the day of OPU (day 0) and the day of Embryo Transfer (ET) (day 3) between pregnant and non pregnant patients, indicating the homogeneity of the cohort. However, on the day of implantation (day 6) and the day of confirmation of biochemical pregnancy (day 17), the levels of both E2 and P4 were significantly higher among women who conceived compared to those who did not conceive [Table/Fig-1].

	Pregnant (n=58)	Non pregnant (n=92)	p-value
E_2 level (pg/mL)			
Day 0	2120.57±538.48	1995.82±461.31	0.23
Day 3	1760.41±465.46	1606.17±405.27	0.06
Day 6	1403.23±376.67	1258.98±354.31	0.02
Day 17	541.80±498.01	289.34±171.94	<0.001
P_4 level (pg/mL)			
Day 0	40.55±30.60	30.15±18.95	0.13
Day 3	64.97±19.32	60.16±24.21	0.22
Day 6	77.82±21.82	68.90±22.17	0.01
Day 17	70.61±121.47	36.17±16.63	<0.001

[Table/Fig-1]: Comparison of serum E2 and P4 levels in pregnant and non pregnant women.

ROC curves were drawn as shown in [Table/Fig-2-5]. Using those curves, cut-off values for serum ethinylestradiol and progesterone on days 0, 3, 6, and 17 were determined, along with their sensitivity and specificity. A minimum of 60% sensitivity and 60% specificity for the cut-off value was considered significant for study purposes. Thus, a cut-off value of serum progesterone on the day of implantation was found: a serum level of progesterone greater than or equal to 76.5 pg/mL on the day of implantation predicted a positive pregnancy test, with a sensitivity of 60.3% and a specificity of 60.9%. Similarly, on day 17, serum E2 >320 pg/mL (sensitivity of 63.8% and specificity of 64.1%) and serum progesterone >42.60 pg/mL (sensitivity of 67.2% and specificity of 68.5%) were predictive of a successful cycle outcome [Table/Fig-6].
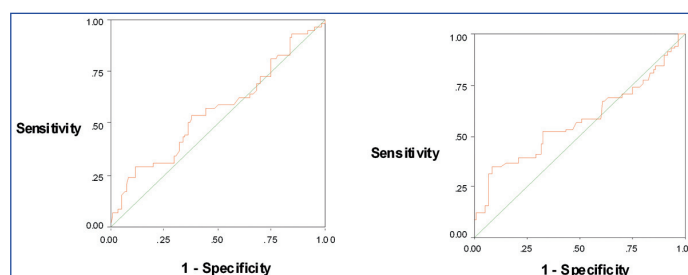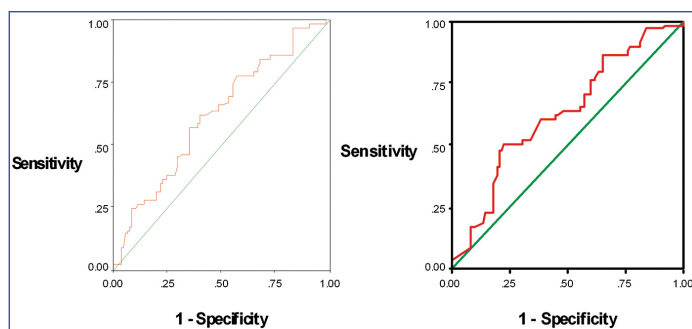

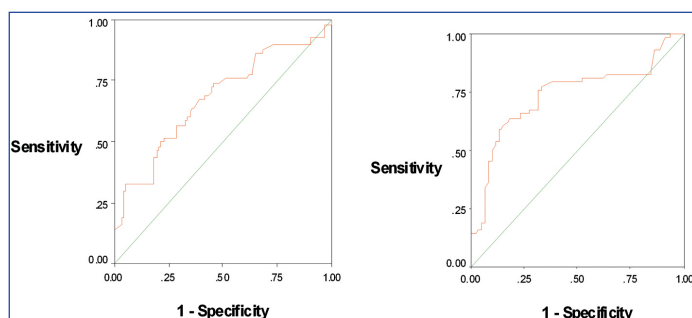[Table/Fig-2]: ROC curve for serum E2 (left) and P4 (right) level on day of oocyte retrieval.
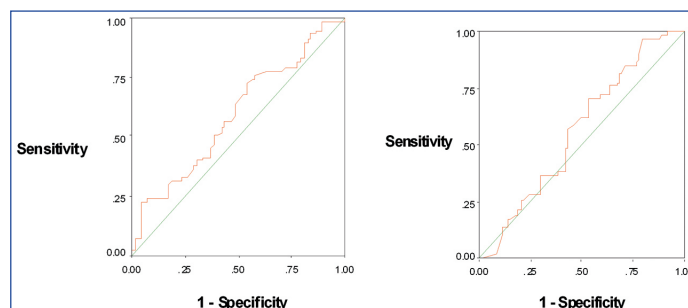
[Table/Fig-3]: ROC curve for serum E2 (left) and P4 (right) on day of embryo transfer.

DISCUSSION

In the present study, serum E2 and P4 levels were determined at different time points during the long agonist IVF cycle, and their association with cycle outcome was evaluated. A statistically significant difference was found between the levels of serum E2

[Table/Fig-4]:   ROC curve for serum E2 (left) and P4 (right) level on day of implantation.

[Table/Fig-5]:   ROC curve for serum E2 (left) and P4 (right) on 17th day after oocyte retrieval.

	Cut-off value (picograms/mL)	p-value	Sensitivity (%)	Specificity (%)
Serum ethinylestradiol				
Day 0	1972	0.23	53.4	55.4
Day 3	1647	0.06	56.9	53.3
Day 6	1266	0.02	58.6	59.8
Day 17	320	<0.001	63.8	64.1
Serum progesterone				
Day 0	28.25	0.13	53.4	53.3
Day 3	59	0.22	56.9	56.5
Day 6	76.50	0.01	60.3	60.9
Day 17	42.60	<0.001	67.2	68.5

[Table/Fig-6]:   Demonstration of cut-off values of hormonal parameters for conception.

in pregnant and non pregnant patients on both day 6 and day 17 post-OPU. Vicdan K and Zeki Isik A also evaluated luteal phase E2 levels on day 11 and day 13 post-OPU and concluded that an increase in levels was associated with a higher pregnancy rate (71.2% vs 18.2%) [2]. Ganesh A et al., reported significantly higher day 21 and day 28 E2 levels in conception cycles compared to non conception cycles [9]. Similarly, Sonntag B et al., reported significantly higher levels of E2 seven days after ET in conception cycles (3326±804 vs 1072±233 pmol/L, p=0.014) [3]. Additionally, Liu HC et al., found late luteal phase increases in E2 in all viable pregnancies [10]. However, Melnick AP et al., found that a day 28 E2 level <100 pg/mL did not preclude a successful outcome, with live birth rates of 15.4% (day 28 E2 < 50 pg/mL) and 41.2% (day 28 E2; 50-100 pg/mL), respectively [11]. Another study similarly failed to show any significant impact of either mid-luteal E2 level or a luteal phase E2 drop on pregnancy rates after IVF [12]. However, none of these studies have evaluated the hormonal profile as early as three days after ET [13,14].

In the present study, there was a significant difference in P4 levels between pregnant and non pregnant females on day six and day 17 post-OPU, with significantly higher levels in conception cycles. Sonntag B et al., also reported significantly higher P4 levels in conception cycles compared to non conception cycles as early as day seven post ET (244±68 vs 73±10 nmol/L, p=0.023) [3]. Ioannidis G et al., concluded that women with viable intrauterine pregnancies had significantly higher serum progesterone on day 14

post-oocyte retrieval compared to those with abnormal pregnancies or those who did not conceive [7]. Women who failed to conceive had consistently low serum progesterone levels despite exogenous supplementation. However, Vicdan K and Zeki Isik A reported that progesterone levels on day 11 post-oocyte retrieval were the same in conception and non conception cycles [2]. Another study by Kofinas JD et al., showed that P4 values >30 ng/mL on day 19 of the cycle had a clear detrimental effect on cycle outcome and suggested that maintaining levels between 10 and 20 ng/mL prior to implantation could maximise implantation and ongoing pregnancy rates, especially for frozen cycles [8]. Recently, a study by Brady PC et al., found that P4 values lower than 20 ng/mL on the day of ET were associated with lower rates of live birth and clinical pregnancy rates in donor recipient cycles [15]. To extrapolate the results to ART pregnancies universally, further multicentric studies including patients with different stimulation and embryo transfer protocols are needed.

Limitation(s)

The present study was a pilot study conducted over a limited period in women undergoing IVF using the long agonist protocol at a single centre. Additionally, in the present study, serum levels of ethinylestradiol and progesterone were only measured until the endpoint of biochemical pregnancy. Further studies are recommended to determine the cut-off levels for these hormones until clinical pregnancy is established.

CONCLUSION(S)

Implantation is the limiting factor in successful IVF outcomes, and the hormonal milieu plays a vital role in maintaining endometrial receptivity. Optimal hormonal levels are necessary to achieve and sustain endometrial receptivity. Estimating hormonal levels during the luteal phase can assist in guiding and titrating luteal phase support. Moreover, it can help alleviate couples' anxiety regarding cycle outcomes. Further large-scale studies are required to establish cut-off levels and/or the range of optimal levels for crucial hormones that directly impact endometrial receptivity and cycle outcomes.

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