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Do serum progesterone levels on the day prior to Day 5 Embryo transfer influence pregnancy outcome in artificial frozen-thaw embryo transfer cycle?

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Abstract
Background: Progesterone (PG) is an essential hormone in the process of implantation and pregnancy maintenance. Frozen-thawed embryo transfer (FET) is being performed worldwide. This study was designed to investigate whether serum progesterone levels on the day prior to day 5 frozen-thawed embryo transfer (FET) to hormonally prepared endometrium correlates with pregnancy outcomes.
Materials and Methods: A single center longitudinal observational study was conducted at an infertility center over a period of three months from October 2022 to December 2022. The study participants were the patients attending the infertility center who satisfied the inclusion and exclusion criteria. A total of 30 participants were included in this study. Serum progesterone levels were measured on the day of starting the progesterone (P0) and on day four (P4), the day prior to embryo transfer. Data analysis was conducted using SPSS version 21.
Results: The mean (SD) age in those who had positive pregnancy outcome was 34.22 (1.26) years and in those who had negative pregnancy outcomes was 39.92 (2.11) years and the difference was significant (P = 0.001). The mean (SD) serum progesterone levels in those who had positive pregnancy outcome at day 0 was found to be 0.61 (1.19) ng/ml and in those who had negative pregnancy outcomes was 0.28 (0.27) ng/ml but, the difference was not significant (P = 0.347). The mean (SD) serum progesterone levels in those who had positive pregnancy outcome at day 4 was found to be 10.84 (8.13) ng/ml and in those who had negative pregnancy outcomes was 8.13 (3.81) ng/ml and the difference was significant (P = 0.039).
Conclusion: Pregnancy loss increases with age and serum progesterone measurements at day 4 prior to embryo transfer could be used to assess pregnancy outcomes in frozen-thawed embryo transfer (FET) technique of infertility treatment. Monitoring of serum PG levels can be done for diagnosing potential luteal support defects before performing FET with artificial cycle (AC).

Keywords: Progesterone, frozen-thawed embryo transfer, pregnancy outcomes

Introduction
Progesterone (PG) is an essential hormone in the process of implantation and pregnancy maintenance. It plays important and crucial roles including luteal phase support, modulation of maternal immune response, suppression of inflammatory response, and reduction of uterine contractility and improvement of uteroplacental circulation [1].
Progesterone is a steroidal hormone produced primarily by the corpus luteum and the placenta. An investigation conducted by Csapo *et al.* [2] showed that lutectomy induced abortion. Progesterone is necessary to obtain a secretory phase transformation of the endometrium. During the luteal phase, it prepares the endometrium for pregnancy by stimulating proliferation in response to human chorionic gonadotropin (hCG) [3]. During conditions of insufficient progesterone, which is defined as a luteal phase defect, progesterone may not maintain normal secretory endometrium and may prevent normal embryo implantation and growth. A mid-luteal serum progesterone <10 ng/mL was established to predict a luteal phase defect [4].
Nowadays, frozen-thawed embryo transfer (FET) is being performed worldwide due to several factors, including implementation of vitrification with an extremely high survival rate, prevention of ovarian hyperstimulation syndrome and avoiding the negative effect of late follicular elevating progesterone on embryo implantation [5].
Hormonal replacement therapy (HRT) also known as artificial cycle (AC) is a commonly used protocol due to its flexibility and convenience in programming the embryo transfer day and excellent results [6].

In this method, oestrogen is administered from the beginning of the menstrual cycle to inhibit follicle stimulating hormone (FSH) inter cycle rise and follicular growth, resulting in the corpus luteum not being formed. Exogenous progesterone administration may begin 2, 3 or 5 days before the scheduled day of transfer depending on the stage of the frozen embryos.

There are various luteal phase support protocols in AC, utilizing different routes to administer PG (vaginal, intra-muscular, sub cutaneous, oral) and different dosages, with no consensus on the most efficient protocol. These micronized PG are exogenous, without endogenous production. According to pharmacodynamic studies, serum PG level is considered a reliable measure: vaginal PG rapidly reaches the systemic circulation, with a maximum concentration peak within 8 h and a steady state achieved within 24 hours. Given this, after 24 h of administration, PG level is supposed to be stable regardless of the interval between serum PG measurement and PG administration. Positive pregnancy outcome was defined as having serum beta hCG value of more than 100 IU/ml. The mean serum progesterone level was found to be 11.4 ng/ml on the fourth day by Cédrin-Durnerin I *et al*. [6] and 11.7 ng/ml on the fourth day by Commissaire M *et al*. [7].

According to some studies, negative pregnancy outcome is higher with AC endometrial preparation for FET than with other protocols, even though pregnancy rates are similar, leading to lower live birth rates. A potential inadequate luteal phase support, potentially due to a suboptimal exogenous PG substitution, could explain these findings. Extent data is scarce and contradictory on whether serum PG rate is related to ongoing pregnancy rate and on which serum PG level is required to optimize ongoing pregnancy rate after FET. An inadequate luteal phase due to poor adherence to treatment or lack of absorption of PG could impair ongoing pregnancy rate [7].

Therefore, this study was designed to investigate whether serum progesterone levels on the day prior to day 5 frozen-thawed embryo transfer (FET) to hormonally prepared endometrium correlates with pregnancy outcome and to compare day 4 serum progesterone levels among the patients who had a pregnancy after FET with AC endometrial preparation.

Materials and Methods

Study design

A single center longitudinal observational study conducted at an infertility center over a period of three months from October 2022 to December 2022.

Study Population

The study participants were the patients attending the infertility center during the study period. The study participants who satisfied the inclusion and exclusion criteria and those who gave consent to take part in the study were included. A total of 30 participants were included in this study.

The inclusion criteria for the study participants were,

- Patients undergoing Assisted Reproductive Technology (ART) with frozen embryo transfer from 18 to 45 years of age.
- Body mass index (BMI) from ≥18.5 kg/m^2 to 22.9 kg/m^2.
- Endometrial preparation with hormone replacement in frozen embryo transfer cycles with day 5 embryos.
- Endometrial thickness >= 7 mm, Flow zone >=2 on colour doppler as per Applebaum & Bilateral Uterine PI >1.1 and <3.

The exclusion criteria included the following,

- Oocyte donor cycles.
- Surrogacy.
- *In vitro* maturation of the oocytes.
- Preimplantation genetic testing.
- Abnormal uterus (fibroid types 0–3 according to the Internation Federation of Gynaecology and Obstetrics (FIGO) classification, endometrial polyp, adenomyosis, congenital malformation).
- Uterine fibroid types 4 and 5 (FIGO classification) >4 cm.
- History of recurrent implantation failure (>2 times of embryo transfer failure).
- Caesarean scar defect (isthmocoele).
- Endometrial thickness <7 mm on the day of embryo transfer.

Study Methodology

After completing the IVF profile, patients undergo ovarian stimulation, oocyte retrieval and embryo transfer. The remaining embryos are cryopreserved for future use in frozen embryo transfer cycle. In the FET cycle, patients come to the clinic on days 1–4 of their period to start endometrial preparation with estradiol valerate. Estradiol valerate is administered at a dosage of 6 -12 mg per day. Transvaginal Ultrasound and color doppler scans are performed every 3–7 days to monitor the development & blood flow of the endometrium. The dosage of estradiol may be increased up to 12 mg per day to maximize the growth of endometrium. When the endometrium reaches 7 mm or higher and the duration of endometrial preparation is somewhere between 12-18 days, vaginal & oral progesterone are started. Embryo transfer occurs on the sixth day of progesterone administration for day 5 embryos.

During the study period, 30 FET cycles were under study. In these, serum progesterone levels were measured on the day of starting the progesterone (P0) and on day four (P4), the day prior to embryo transfer. Serum progesterone levels were assessed in relation to pregnancy outcomes which included both biochemical and clinical pregnancies. A threshold of P0 <= 1.5 ng/ml was used to simulate the currently accepted levels for administering progesterone before embryo transfer in FET cycle and P4 values were observed and analyzed to see further prognosis.

Statistical analysis

Data collection sheet included patient's demographic information, clinical features, examination findings, and laboratory investigation. Statistical analysis was carried out using SPSS software version 21. Descriptive statistics included mean and standard deviation. Normality of the data was checked with Shapiro-Wilk's test and unpaired t test was used for normally distributed data and Mann Whitney U test was used for data in non-normal distribution. In all the statistical tests, P value of less than 0.05 was considered to be statistically significant.

Ethical issues

The study was approved by the institutional ethical committee (IEC) before data collection. Informed written consent was obtained from the study participants before administering questionnaire and performing clinical examination, laboratory investigations and interventions.

Results

The total sample size was 30. All the participants agreed to take part in this study and the response rate was 100%. Among 30 study participants, positive pregnancy outcome was observed in 18 (60%) and negative pregnancy outcomes were seen in 12 (40%).

Age distribution in study participants

The mean (SD) age of the study participants were 36.50 (3.27) years. The minimum and maximum ages were found to be 32 and 43 years respectively.

The mean (SD) age in those who had positive pregnancy outcome was 34.22 (1.26) years and in those who had negative pregnancy outcomes was 39.92 (2.11) years. It was found that the mean age was significantly higher in those who had negative pregnancy outcomes with a P value of 0.001 and is represented in figure 1.

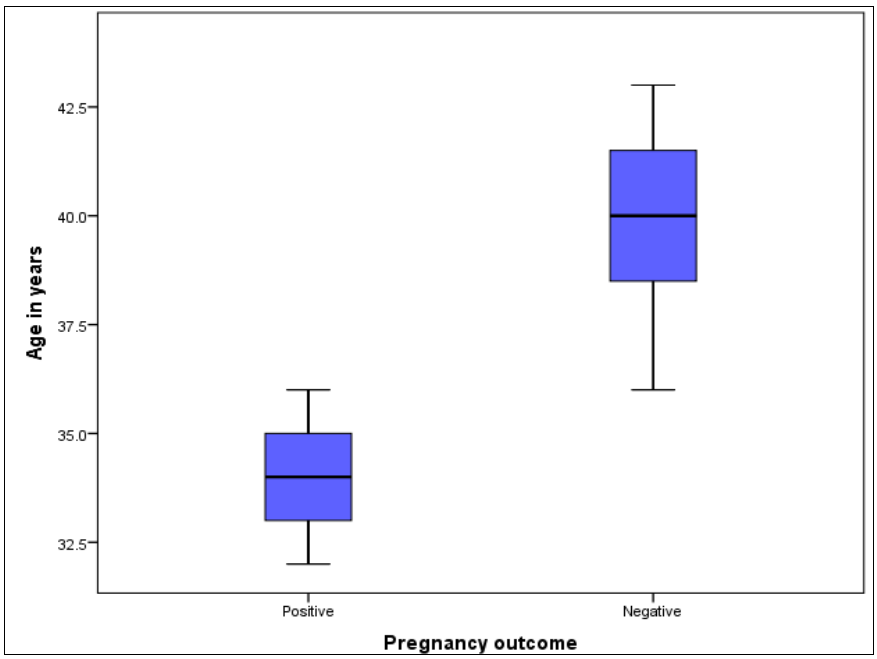

Fig 1: Age distribution among the study participants

Serum progesterone levels at baseline

Among 30 study participants at day 0, the mean (SD) level of serum progesterone levels were found to be 0.48 (0.29) ng/ml.

The mean (SD) serum progesterone levels in those who had positive pregnancy outcome at day 0 was found to be 0.61 (1.19) ng/ml and in those who had negative pregnancy outcomes was 0.28 (0.27) ng/ml. It was found that the mean progesterone levels at day 0 were higher in those who had positive pregnancy outcome than in those who had negative pregnancy outcome. But, the difference was not statistically significant with a P value of 0.347. The data is illustrated in figure 2.

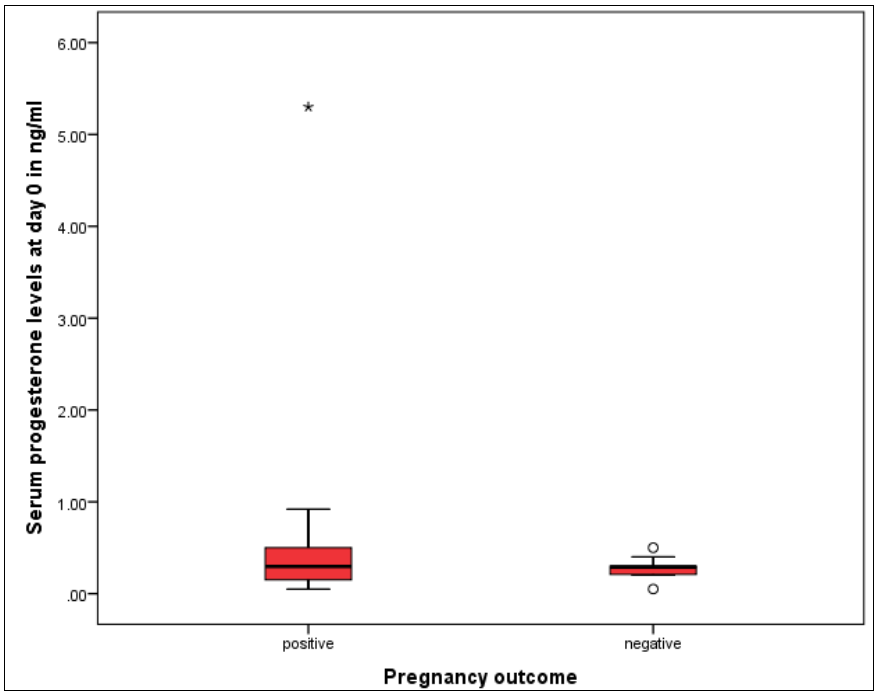

Fig 2: Serum progesterone levels in study participants at day 0

Serum progesterone levels at day 4

Among 30 study participants at day 4, the mean (SD) level of serum progesterone levels were found to be 9.76 (4.41) ng/ml.

The mean (SD) serum progesterone levels in those who had positive pregnancy outcome at day 4 was found to be 10.84 (8.13) ng/ml and in those who had negative pregnancy outcomes was 8.13 (3.81) ng/ml. It was found that the mean progesterone levels at day 4 were higher in those who had positive pregnancy outcome than in those who had negative pregnancy outcome and the difference was found to be statistically significant with a P value of 0.039 and is illustrated in figure 3.

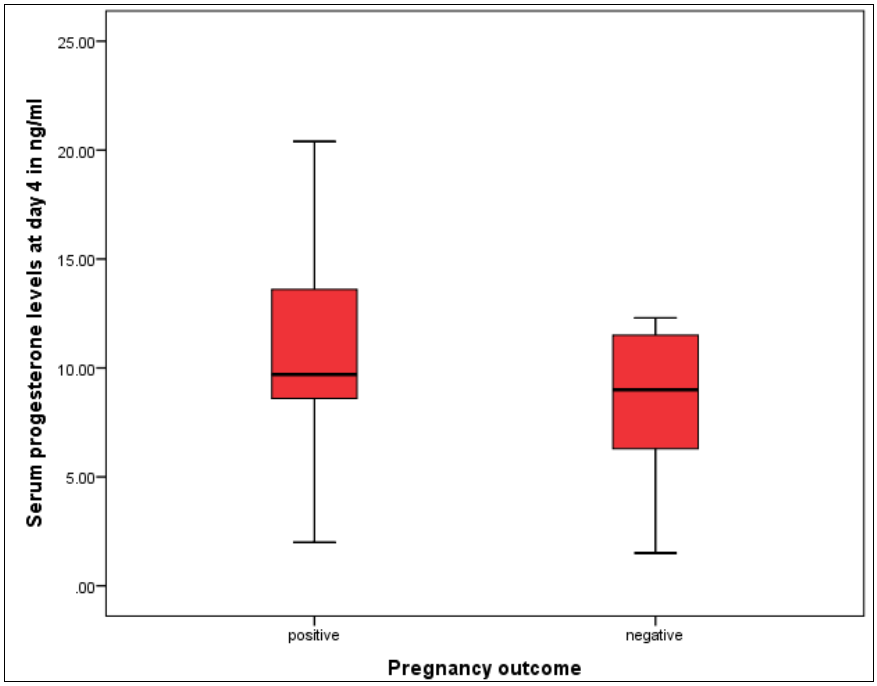

Fig 3: Serum progesterone levels in study participants at day 4

Discussion

A total of 30 participants took part in this study. Among 30 study participants, positive pregnancy outcome was observed in 18 (60%) and negative pregnancy outcomes were seen in 12 (40%). The mean (SD) age of the study participants were 36.50 (3.27) years. In study participants who had positive pregnancy outcome, the mean (SD) was 34.22 (1.26) years and in those who had negative pregnancy outcomes was 39.92 (2.11) years. It was found that the mean age was significantly higher in those who had negative pregnancy outcomes with a P value of 0.001. These findings highlight the fact that the chances of positive pregnancy outcome decrease with age.

We found that serum PG levels were lower in women with negative pregnancy results than those with ongoing pregnancy. In the AC protocol, all hormones are of exogenous origin. Serum PG levels are a direct and reliable reflection of what is absorbed by the patient; therefore, a low serum PG level might be related to an inadequate luteal phase support that could play a role in negative pregnancy outcome [8].

In the present study, it was found that the mean (SD) serum progesterone levels in those who had positive pregnancy outcome at day 0 was found to be 0.61 (1.19) ng/ml and in those who had negative pregnancy outcomes was 0.28 (0.27) ng/ml. The mean progesterone levels at day 0 were higher in those who had positive pregnancy outcome but, the difference was not statistically significant with a P value of 0.347. Commissaire M *et al*. in their study had also reported no significant difference in serum progesterone levels at day 0 [7]. This may have been caused by a fault involving the implantation linked to insufficient PG supplementation as previously suggested by the work of Devine *et al*. [9].

We found that at day 4, the mean (SD) serum progesterone levels in those who had positive pregnancy outcome was 10.84 (8.13) ng/ml and in those who had negative pregnancy outcomes was 8.13 (3.81) ng/ml. It was found that the mean progesterone levels at day 4 were higher in those who had positive pregnancy outcome than in those who had negative pregnancy outcome and the difference was found to be statistically significant with a P value of 0.039. These findings were similar to the results obtained by Commissaire M *et al*. [7]. Progesterone deficiency leads to implantation defect leading to pregnancy loss and this explains the lower serum progesterone levels in those having negative pregnancy outcomes. Our findings agree with the literature with mean PG levels of 8 ng/ml in case of negative pregnancy outcome and 12 ng/ml in case of ongoing pregnancy [9].

The main limitation of our study was its small sample size that reduced the statistical power of the results. The study was carried out in a single infertility center and included only biochemical and clinical pregnancy cases. This could reduce the validity of the results. Similar studies may be done on a large sample size in multiple centers to increase the validity of the results.

Conclusion

The results of the study highlight the fact that the Serum progesterone measurements at day 4, a day prior to the day of embryo transfer could be used to assess pregnancy outcomes in frozen-thawed embryo transfer (FET) technique of infertility treatment. Monitoring serum PG could help in diagnosing potential luteal support defects before performing FET with AC and this could also allow physicians to adapt PG supplementation in subsequent cycle. Large multicentric randomized controlled trials should be undertaken to assess

these preliminary results and generalize the study findings.

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