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# Serum Progesterone Level at the Day Prior to Frozen Thawed Embryo Transfer and Pregnancy Rate

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Cryopreservation's success rate varies depending on woman age, as low as 14.8% (if eggs were extracted from 40-year-old women), and as high as 31.5% with 25-year-old women. The goal of the research is to improve laboratory methods for freezing-thawing embryos, leading to elevated embryo survival rate. Yet, in hormonal replacement therapy frozen-thawed embryo transfer (HRT-FET) cycles, effective endometrial preparation before embryo transfer attracted less focus. The present research's aim is to see whether there's a link between blood progesterone levels and pregnancy rates the day before frozen-thawed embryo transfer.
**Methods:** This prospective observational research has investigated 120 patients of frozen-thawed embryo transfer cycle treatment, only 100 individuals subdivided to 2 groups for serum level of progesterone one day before frozen-thawed embryo transfer. The subjects visited both the Obstetrical and Gynecological Department of Tanta University as well as private centers between May 2020 and January 2021.
**Results:** We discovered no correlation between maternal age with pregnancy test results. Yet, our

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study discovered highly significant variation among both groups regarding endometrial thickness one day preceding embryo transfer, and regarding pregnancy rate ($p<0.05$).
**Conclusion:** The serum progesterone hormone preceding frozen embryo transfer has significant and direct relation and impact upon pregnancy rates. The present research detected low serum progesterone less than 10 nanograms/ml in the day before frozen-thawed embryo transfer in HRT-FET cycles significantly decreased probability of ongoing pregnancy post frozen-thawed embryo transfer.

*Keywords: Cryopreservation; progesterone; pregnancy rate; frozen embryo transfer; hormone replacement therapy cycles; hysterosalpingography.*

## 1. INTRODUCTION

Recent years have witnessed global rise in cryopreservation for frozen embryo transfer not just due to single embryo transfer policy, yet additionally as a result of increase in freeze-all policy throughout the board [1,2].

So, cryopreservation conducted for embryos and oocytes becomes important for practical application of further methods representing crucial implications for lengthy storage and transportation of embryos and it has several indications like severe ovarian hyperstimulation syndrome. As well as elevated progesterone in human chorionic gonadotropin HCG day, or being required for preimplantation genetic diagnosis (PGD) before the transfer. In addition, the existence of fluid in uterine cavity on day of embryo transfer [3,4].

As a result of these factors, number of frozen embryo transfers rose dramatically, leading to significant increase in embryo survival rates. Although there is some debate about frozen-thawed embryo transfer as opposed to fresh embryo transfer, consequences of frozen embryo transfer got significantly enhanced since 2010s due to cryopreservation technology breakthroughs [3].

Progesterone hormone is crucial for implantation, preparing endometrium for implantation in addition to controlling trophoblast invasion, as well as migration via genomic and non-genomic effects [5].

Both nuclear progesterone receptors PR-A and PR-B mediate progesterone's genomic effects; nevertheless, nuclear progesterone receptors are able to activate fast cytoplasmic processes [6].

Both genomic and extranuclear action of progesterone represent a must for adequate decidualization as well as implantation, which refers to the attachment of a fertilized egg or blastocyst to the endometrium. Membranes bound progesterone receptors were concerned in progesterone's rapid non-genomic actions.

By inhibiting the proliferative impact of estrogen and activating genes which enable endometrium to allow embryo attachment, progesterone helps to develop uterine receptivity. It also serves as negative regulator of trophoblast invasion via regulating matrix metalloproteinase (MMP) activity [7].

At pregnancy development, embryo needs implant in receptive endometrium between days 22 and 24, indicating that endometrial thickness should be measured using ultrasonography to identify a receptive endometrium during frozen-thawed embryo transfer. The endometrium thickness on the same day amid embryo transfer considers a cutoff point before transfer if below 7mm the cycle is canceled, the embryo is frozen till good artificial endometrial preparation [2].

There are many indications for cryopreservation such as the effective means to conserve the germ plasmas of enlarged species, fertility preservation, methods to reduce multiple pregnancies, and the large range of stocks available.

Cryopreservation of oocytes and embryos has become a critical step in the widespread use of other methods, with significant implications for long-term storage and transit of embryos [8].

Development of techniques for cryopreservation of embryos as IVF has become more widely available and the need for cryopreservation has become apparent, pressure for technical development has increased [9].

The enhancement and simplicity of cryopreservation techniques in use in embryo transfer programs has made significant progress.

Slow pace, programmed freezing, and vitrification of embryos have provided veterinarians, scientists, and producers with new options in herd reproduction [10].

Many studies have been carried to check the relation between the level of progesterone and the success of IVF-HRT treatment, the effect of progesterone administration, and its serum level elevation, and its effect on the pregnancy rate also studies [11].

The goal of this research is to see whether there's a link between blood progesterone levels and pregnancy rates one day that precedes frozen-thawed embryo transfer.

## 2. PATIENTS AND METHODS

The present prospective observational research was conducted at Obstetrical and Gynecological Department at Tanta University Hospital, in addition to a private center. A sum of 120 patients of IVF/ICSI cycle with frozen-thawed embryo transfer, occurred between May 2020 and January 2021, only 100 patients who continued, the other patients were canceled. The subjects were selected based on inclusion criteria where all the patients below 40 years old in addition to body mass index (BMI) less than 35kg/$m^2$ as well as have blastocysts caused by old IVF/ICSI cycle.

On the other hand, the exclusion criteria encompassed medical disorders like diabetes mellitus, renal disease, thyroid dysfunction, or cardiovascular diseases. Hormonal therapy more than 3 months before the study. And anatomical disorders or congenital malformation like the bicornate uterus or septate uterus.

All patients were investigated to detect serum progesterone level one day preceding frozen-thawed embryo transfer, based on serum progesterone level one day preceding frozen embryo transfer, we grouped patients to 2 groups: Group A which contained subjects with serum progesterone level at one day preceding frozen-thawed embryo transfer less than 10 nanograms/ml. and Group B for who had serum progesterone level at one day before frozen-thawed transfer more than 10 nanograms/ml.

The number reported as progesterone production via corpus luteum in normal cycle is closest to the 10 nanograms/ml criterion [12].

The method of the procedure started with exogenous artificial endometrial preparation through exogenous supplementation of estradiol valoperate at a dose of 6mg every day starting from 2$^{nd}$ day of natural menstrual cycle for about 12-14 days. After about 10 days from starting the exogenous administration of estrogen, vaginal ultrasound was done by Mindray DC 30 ultrasound for measuring the endometrial thickness, a longitudinal slice of endometrial, including the cervix, is shown in the best picture. If the endometrial thickness is more than 7mm we started the exogenous progesterone supplementation and if less than 7mm we continue the estradiol supplementation till the endometrial thickness exceeds 7 mm with repeated vaginal ultrasound after 2 days to determine the endometrial thickness. If endometrial thickness didn't get optimal thickness the cycles were canceled and excluded from the study there were 20 patients excluded from the study.

The exogenous progesterone administration started when the endometrial thickness was more than 7mm by a dose of 400mg vaginally twice per day, we transferred the frozen-thawed embryos after 5 days of starting progesterone treatment, continued progesterone supplementation till pregnancy test conducted if pregnancy test appeared positive continued till 12 weeks of pregnancy.

We measured the endometrial thickness and the serum progesterone level at one day preceding frozen-thawed embryo transfer after 1-3 hours of the morning dose using a commercially available automated electrochemiluminescent immunoassay (Cobas modular analytics E170; Roche diagnostic, Switzerland.

The thawed embryo transfer was done via full bladder by drinking about 1.5 liters of water before the process which assist in changing the uterus's tilt to make transfer simpler. In the transfer room, the patient was undressed up to the waist, a cover-up by warm sheet or blanket, sleep on exam table. The transfer was conducted with lithotomy position. We transfer 2 blastocyst grades A or B after 5 days of progesterone supplementation.

First placing prep Wallace catheter, the outer sheath which is thin, long, and under ultrasound guidance, a flexible catheter is softly guided through the cervix. The second process started when the prep catheter (Wallace embryo transfer catheter) was ready, the embryo was loaded into a smaller catheter (the inner sheath) which pass through the prep catheter till the right spot 1.5 to

2 cm away from the fundus of the uterus where embryo placed, then final step checked catheter tip under a high-powered microscope to confirm embryo transferring. Afterwards, when transferring process ends completely, we preferred the patients to take rest as possible as they can and continue on progesterone supplementation at the same dose of 400mg vaginally twice per day till do the pregnancy test.

### 2.1 Statistical Analysis of the Data

The IBM SPSS software programme version 20.0 was used to examine the data that was input into the computer. (IBM Corporation, Armonk, NY). Numbers and percentages were used to describe qualitative data. The Kolmogorov-Smirnov test has been performed to ensure that the distribution was normal. Range (minimum and maximum), mean, standard deviation, median, and interquartile range were used to represent quantitative data (IQR). The Chi-square test and Monte Carlo adjustment for chi-square were employed at over 20% of cells had the anticipated count. In addition to the Student t-test. The significance of acquired findings was determined at a 5% level of significance.

## 3. RESULTS

The current research discovered none correlation among both groups regarding age as well as Body Mass Index (BMI).

Table 3 compares other parameters like endometrial thickness which were measured 1 day prior to pregnancy rate and embryo transfer. It shows a high statistically significant variation among both groups.

Table 4 shows a high statistically significant variation among both groups based on pregnancy test results according to the p-value.

## 4. DISCUSSION

In disagreement with our findings, the study of Jiaying Lin et al. [16] is a retrospective analysis of 4958 women being treated for infertility at university tertiary center from January to December 2017 which compared pregnancy rate as well as neonatal outcomes among both groups at control age 30 years old. They discovered that live birth rates significantly dropped for elder mothers.

In another retrospective observational study of Yan, et al. [13] to examine the impact of mother age on in vitro fertilization as well as embryo transfer results (IVF-ET). Yan's study comprised total of 11830 IVF-ET cycles from 10,268 females. A total of 4 mother groups of various ages were compared. A group for whose aging between 21 and 30 years old (4,549 cycles), another for subjects ranging from 31 to 35 years old (4,424 cycles), a third for subjects aging between 36 and 40 years old (2,429 cycles), and last for whose above 40 years old (2,429 cycles) were age groups (428 cycles). In groups encompassing elder maternal age period, the mean beginning dosage of Gonadotropin (Gn) as well as mean total dose of Gn at every cycle showed substantially greater (P0.01), but the mean recovered oocyte number showed significantly less (P0.01) than in each of the lower groups. In groups with greater mother age periods, the biochemical pregnancy rate as well as clinical pregnancy rate showed substantially less (P0.01), whereas miscarriage rate showed significantly increased (P0.01). it discovered no variation at rate of two-pronuclear zygotes (2PN) or high-quality embryos among groups. The incidence of birth defects got similarly equivalent among groups of newborns. The pregnancy rate was 26.87 percent in group of subjects older than 40 years, while clinical pregnancy rate stood at 19.39 percent, and miscarriage rate post clinical pregnancy got 36.14 percent. Finally, individuals with a greater mother age showed inferior IVF results. Subjects ranging from 20 to 30 years old had the greatest IVF results among women of reproductive age. Patients above the age of 40 had poor IVF results and a significant incidence of miscarriage, indicating the need for preimplantation genetic screening (PGS).

In comparison with study of Padilla and Garcia. [14] between July 1, 1985, and June 30, 1988, 512 patients had 1,101 oocyte retrievals for in vitro fertilization (IVF). Eighty-four percent of retrieved oocytes were transferred. The average number of oocyte retrievals per patient got 2.2, while average number of embryo transfers (ET) got 1.8. The pregnancy rate per retrieval, ET, and individual reached 23 percent, 27 percent, as well as 49 percent, respectively. A sum of 77 spontaneous abortions (31%) took place, as well as five ectopic pregnancies (2%). A very significant negative linear connection among age and continuing pregnancy rate per ET was found in a linear regression study evaluating the impact of women's age on ongoing pregnancy rate per ET (P less than 0.005). The continuing pregnancy rate per ET for subjects below 30 years old reached 26%, in contrast with 9% with those 37 years and older (P less than

0.01). Individuals above the age of 40 had 50% miscarriage rate, in contrast with just 29% in patients under 40 years old. (P greater than 0.05). For patients having one to seven tries, the clinical pregnancy rate was 25 percent, 29 percent, 28 percent, 33 percent, 35 percent, 30 percent, and two out of five, respectively.

In another retrospective study P, Surat. [15] was conducted with 785 frozen embryo transfer cycles and 870 blastocysts were transplanted in a multi-center study. Excellent, good, average, and poor blastocysts were classified into five categories. The late embryos were usually collected on day 5, although in rare instances they were taken on day 6. Before evaluating the implantation potential, these embryos were immobilized and 3–7 cells got extracted at studies and genetic screening. The ladies were split into four age groups: one group for subjects at 35 years old, another for ones aging between 35 and 37 years old, and a third for whose ranging between 38 and 40 years old, and finally for those aging between 41 and 42 years old. When compared to good quality embryos, exceptional quality blastocysts were linked with a substantially greater live birth rate (79 percent vs 64 percent). The live birth rate for poor quality embryos was 28 percent, which is a significant difference. Similarly, as compared to medium and poor-quality embryos, excellent-quality embryos had a greater implantation rate as well as lower spontaneous abortion rate.

Surprisingly, research discovered no differences in implantation ability of euploid embryos or embryos with healthy number of chromosomes from subjects at various ages (35 years, 35 to 37 years, 38 to 40 years, and 41 to 42 years). Euploid embryos were similarly equivalent in form, structure, and development across age groups. Despite the fact that the older women had less euploid or embryos via healthy chromosomal numbers, detected no variation in euploid embryo's potential in terms of how they implanted, developed, and resulted in a live delivery.

In another study Lin et al. [16] carried on PCOS women (n = 1556) undergoing FET got categorized to groups upon weights. The normal weight subjects had a BMI of 18.5–24.9 kg/m2, overweight individuals had BMI of 25–29.9 kg/m2, as well as obese subjects had a BMI of 30 kg/m2. These groups got contrasted upon pregnancy and perinatal outcomes. As a consequence, pregnancy outcomes were comparable in normal-weight, overweight, as well as obese categories, with clinical pregnancy and miscarriage rates, continuing pregnancy rate, as well as live birth rate. The three categories had similar birth features in terms of infant gender, gestational age, birth weight, as well as length at delivery among singletons. After adjusting for poor neonatal outcomes, they discovered no significant variance among three categories in rates of decreased birth weight, very low birth weight, preterm birth, as well as very preterm birth. Furthermore, obstetric difficulties and the frequency of live-born abnormalities were similar across the three groups, with the exception that obese females more than normal-weight women to have given birth via caesarean section.

In another study of Megan et al. [17] with retrospective analysis for entire subjects having IVF between 1st January 2005 and 1st March 2006 at large private practice using single IVF laboratory. Standard procedures for adjusted ovarian hyperstimulation as well as embryology parameters were used on patients. The clinical pregnancy rate represented primary outcome measure. As much as 2,167 fresh, non-donor IVF cycles got examined, however just initial treatment cycle for every subject (n = 1273) was examined to reduce bias. Multiple regression models using BMI and age as basic factors, as well as a BMI x Age interaction, were used to analyze the data. BMI didn't seem to show significant impact on IVF result when evaluated as a main effect, however detected significant BMI x Age interaction. An increased BMI showed significant detrimental impact on fertility at earlier ages, but this effect faded as subject's age rose. With rising BMI and age, clinical pregnancy rates dropped. BMI has a substantial detrimental influence on fertility in younger IVF patients, but this effect fades when patients approach their mid-thirties. After the age of 36, BMI has little bearing on fertility.

In agreement with our study of Micah J. Hill, Steve Hong, John L. Frattarelli [18] which has prospectively reported if BMI indicates live birth rates in IVF subjects. That prospective research included 117 infertile subjects, using IVF success rates as the main end measure. The mean BMI of patients who had good outcomes and those who didn't vary. BMI and number of activated follicles had a significant positive association ($r = 0.19$, $P<.05$). They reported substantial negative association among BMI and gonadotropin ampules utilized ($r = 0.25$, $P<.01$) and BMI as well as days of stimulation ($r = 0.19$, $P<.05$). Women with a higher BMI generate more follicles, stimulate faster, as well as need fewer

gonadotropins amid IVF, according to these findings. BMI, on the other hand, had no discernible impact on pregnancy outcomes.

In agreement with our findings, the study of Annica Bergendal, Sophie Naffah, Christina Nagy [19]. The goal of this retrospective research was to compare the results of in vitro fertilization as well as embryo transfer for females with endometriosis to control group of females who were infertile due to tubal factors. At Huddinge University Hospital, 48 endometriosis patients completed 65 rounds of in vitro fertilization as well as embryo transfer. The tubal-factor infertility control group comprised of 98 cycles in 98 individuals. The stimulation, fertilization, embryo development, implantation, as well as pregnancy outcomes of these groups were studied retrospectively. The fertilization rate was substantially less in females with endometriosis, but cleavage, implantation, as well as pregnancy rates weren't different.

In another study by Paula Kuivasaari-Pirinen, Kaisa Raatikainen, Maritta Hippelainen, and Seppo Heinonen [20] .This retrospective cohort research compared the results of IVF singleton pregnancies in general Finnish population to those of spontaneous pregnancies. The study included 255 women who had given birth as a result of singleton IVF pregnancies. The mentioned causes of infertility were divided into six subgroups: anovulation (27 percent), endometriosis (19 percent), male factor (17 percent), tubal factor (15 percent), polycystic ovary syndrome (11 percent), as well as unexplained infertility (11 percent) (12 percent). There were 26,870 spontaneously conceived women in the reference group. Using logistic regression analysis, attuned odds ratios (AORs) for confounding factors like age and parity got calculated. Preterm birth was more common in women with endometriosis and anovulation (AOR 3.25, 95 percent CI 1.5–7.1 and AOR 2.1, 95 percent CI 1.0–4.2, respectively), though females in couples with male factor infertility got twofold risk of being admitted to neonatal intensive care (AOR 2.5, 95 percent CI 1.2–5.3). The results reported that infertility's factors got impact on obstetrics outcome, and pooling results could mask a few elevated risks amongst subgroups.

Agreeing with our findings, Richter's study, [21] was conducted to check if there's link among endometrial thickness as well as clinical result of IVF and ET. Endometrial thickness was shown to be higher in cycles that resulted in pregnancy than in cycles that didn't lead to pregnancy in this retrospective analysis of 1,294 infertile individuals (11.9 vs. 11.3 mm, respectively). Clinical pregnancy rates progressively elevated from 53% among subjects with lining of lower than 9 mm to 77% among individuals with lining of more than or equivalent to 16 mm. Age, embryo quality, in addition to endometrial thickness all had significant impacts on clinical pregnancy rates in addition to live-birth or continuing pregnancy rates, according to multiple logistic regression analysis. With increasing endometrial thickness, found marginally significant trend toward lower incidence of spontaneous pregnancy loss.

This was similar to the study of Eftekhar et al. [22] The results indicated that the pregnancy rate rose from 8 mm to 11 mm, then dropped, and that the pregnancy rate reached zero in endometrial thickness (Ent) > 14 mm. Between age groups, there were substantial variations in endometrial thickness as well as pregnancy outcomes. The pregnancy rate was greater (32%) in women aged 23–30, and Ent range was 6–12mm in this group. In addition, the results revealed positive relationship among Ent and AMH, estradiol levels, as well as quantity of COC and MII oocytes, as well as negative relationship among female age and Ent. Although certain clinical factors may influence Ent, the results showed that Ent during day of HCG injection is related with pregnancy outcomes in fresh IVF/ICSI cycles with diverse age groups. To get a definite conclusion, large research is required.

**Table 1. Distributing studied cases for progesterone level (n = 100)**

| Progesterone level | Total | | Group A (n=30) | | Group B n=70) | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| <10 | 30 | 30.0 | 30 | 100.0 | 0 | 0.0 |
| >10 | 70 | 70.0 | 0 | 0.0 | 70 | 100.0 |
| Min. – Max. | 4.60 – 18.0 | | 4.60 – 9.0 | | 10.60 – 18.0 | |
| Mean ± SD. | 12.23 ± 3.97 | | 6.89 ± 1.17 | | 14.52 ± 2.08 | |
| Median (IQR) | 13.05 (8.20 – 15.45) | | 6.70(6.10 – 8.10) | | 14.60(12.80 – 16.20) | |

*Comparing the two groups in regards to the different infertility factors showed none significant variation*

**Table 2. Comparing both studied groups based on infertility factor**

| Groups | Infertility factor | | | | | | | $\chi^2$ | $^{MC}p$ |
|---|---|---|---|---|---|---|---|---|---|
| | Endometriosis (n=1) | Male factor (n=24) | Mixed (n=18) | Ovulatory factor (n=18) | Tubal factor (n=23) | Unexplained (n=14) | Others (n=2) | | |
| | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | | |
| Group A | 0 (0.0%) | 5 (16.7%) | 3 (10.0%) | 9 (30.0%) | 8 (26.7%) | 5 (16.7%) | 0 (0.0%) | 7.083 | 0.285 |
| Group B | 1 (1.4%) | 19 (27.1%) | 15 (21.4%) | 9 (12.9%) | 15 (21.4%) | 9 (12.9%) | 2 (2.9%) | | |

**Table 3. Comparing both studied groups based on endometrial thickness**

| Groups | Endometrial thickness | | | | | $\chi^2$ | $^{MC}p$ |
|---|---|---|---|---|---|---|---|
| | 8 – 9 mm (n=14) | 9 – 10 mm (n=11) | 10 – 11 mm (n=34) | 11 – 12mm (n=36) | >12 mm (n=5) | | |
| | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | | |
| Group A | 10 (33.3%) | 7 (23.3%) | 5 (16.7%) | 6 (20.0%) | 2 (6.7%) | 22.966* | <0.001* |
| Group B | 4 (5.7%) | 4 (5.7%) | 29 (41.4%) | 30 (42.9%) | 3 (4.3%) | | |

**Table 4. Comparing both studied groups based on pregnancy test result**

| Groups | Pregnancy test result | | | | $\chi^2$ | p |
|---|---|---|---|---|---|---|
| | Negative (n=41) | | Positive (n=59) | | | |
| | No. | % | No. | % | | |
| **Group A** | 17 | 56.7 | 13 | 43.3 | 4.349* | 0.047* |
| **Group B** | 24 | 34.3 | 46 | 65.7 | | |

In another study of von Wolff M, Fäh M, Roumet M, Mitter V, Stute P, Griesinger G, and Kohl Schwartz A. [23] that retrospective, single-center research that looked at 105 women who had normal menstrual cycles and were having their first NC-IVF cycle with embryo transfer. Data was corrected for mother's age, cycle day of follicle aspiration, in addition to BMI to determine clinical pregnancy in addition to live birth rates.

The average age of participants reached 35.0 years [32.0; 37.0]. On day 14.0 [12.0; 15.0] of cycle, follicle aspiration was done. The overall clinical pregnancy rate reached 24.8 percent, with a 15.2 percent live birth rate per each single transfer. The pregnancy rate was 7.4% in females having endometrial thickness less than 7 mm (n = 27) and 30.8 percent in females having endometrial thickness more than 7 mm (n = 78) (OR 5.56, 1.22–25.36) (P = 0.03). The rates of live births weren't substantially diverse. Women with thin (about 8 mm) and thick (approximately >11 mm) endometria had decreased pregnancy rates, according to quadratic regression analysis. After adjusting for age, day of aspiration, BMI, and p-value after crude quadratic analysis reached 0.028, and after adjusting for age, day of aspiration, in addition BMI, it reached 0.039. The significance threshold for live birth rates wasn't met. Thin endometrium must be an independent unfavorable prognostic factor regarding obtaining pregnancy in females without ovarian stimulation, according to these findings.

In agreement with Labarta's study [3], our results are consistent with Labarta as this prospective cohort analysis of 244 patients who underwent ET following artificial endometrial preparation cycle with estradiol valerate as well as vaginal micronized progesterone (400 mg/12 h) in an oocyte donation cycle. The research was place from February 22nd to October 25th, 2016. (8 months). Subjects having their first/second oocyte donation cycle, aged 50, BMI 30 kg/m2, triple-layer endometrium >6.5 mm, and 1-2 good quality transferred blastocysts were included in the study. During ET day, serum P was determined and a 3D ultrasound of uterine cavity was done. Virtual organ computer-aided analysis (VOCALTM) system got used to assess endometrial volume. The main outcome was OPR after week 12 of pregnancy. Only 211 of the 244 patients that were recruited met all of inclusion/exclusion criteria. During embryo transfer day, the mean blood P level reached 12.7 5.4 ng/ml (Centiles 25, 9.2; 50, 11.8; 75,15.8). The OPRs were 32.7 percent in the first quartile, 49.1 percent in the second, 58.5 percent in the third, and 50.9 percent in the fourth. Q1 had a lower OPR than Q2-Q4: 32.7 percent vs 52.8 percent; P = 0.016; RR (95 percent CI): 0.62. (0.41-0.94). The endometrial volume reached 3.4 1.9 ml on average. Endometrial volume didn't connect with serum P on day post ET. OPR got significantly lower in females with serum P 9.2 ng/ml as opposed to 9.2 ng/ml (OR: 0.297; 95 percent CI: 0.113-0.779); P = 0.013, according to logistic regression analysis that controlled for all potential confounders. The ROC curve revealed that blood P levels one day of ET had a substantial predictive value for OPR, with an AUC (95 percent CI) = 0.59. (0.51-0.67).

In another study of Yovich et al. [6] This prior research looked at the significance of mid-luteal serum hormonal levels in cryopreserved embryo transfer cycles that included single-embryo transfer (SET) of 529 vitrified blastocysts and were performed under hormone replacement therapy (HRT) supervision. The specific HRT regimen resulted in a wide range of mid-luteal estradiol and progesterone concentrations. Although estradiol showed null effect on clinical pregnancy or live birth rates, this research discovered optimum progesterone range of 70 to 99 nmol/l (P 0.005). Progesterone concentrations under 50 nmol/l and over 99 nmol/l got linked to lower implantation rates. Although results showed no interaction among estradiol and progesterone levels, embryo quality grading had significant impact on outcomes (P0.001 and P0.002, respectively, for clinical pregnancy and live birth rates). Regardless of embryo grade, BMI, or woman's age, the progesterone impact was significant during vitrification or cryopreserved embryo transfer, according to multiple comparison studies. The findings support the idea that blood progesterone

concentrations in HRT-cryopreserved embryo transfer should be closely monitored.

In another recent study, Gaggiotti-Marre et al. [24] Progesterone (P) levels on day preceding to embryo transfer of euploid embryos represented influence on pregnancy consequences in artificial endometrial preparation for frozen embryo transfer (FET) cycles, according to that retrospective cohort study. In the period from January 2016 to June 2017, 244 FETs were analyzed in a private university clinic. Estradiol valerate as well as vaginal micronized progesterone were used to prepare the endometrium. The levels of serum P and estradiol were measured one day preceding embryo transfer. A multivariable analysis was used in examining relationship among serum P levels and pregnancy consequences, with confounding variables taken into account. The p-value was 11.3 5.1 ng/ml on average. The levels of progesterone got divided into four quartiles: Q1: 8.06 ng/ml, Q2: 8.07-10.64 ng/ml, Q3: 10.65-13.13 ng/ml, and Q4: > 13.13 ng/ml. In comparison to higher P quartile, patients in less P quartile showed significantly elevated miscarriage rate as well as significantly less live birth rate (LBR). A low serum P level one day before FET (10.64 ng/ml) is linked to less pregnancy as well as LBR post FET of euploid embryos.

Alsbjerg et al. [25] from January 2016 to December 2017, a cohort research got fulfilled on 244 HRT-FET cycles at Danish public center, in which all subjects received endometrial preparation with 6mg estradiol valoperate on cycle's 2nd day, preceded by vaginal progesterone. During the luteal phase, all subjects got their serum progesterone levels checked. Based on sensitivity analyses, best cut-off point was 35 nmol/l. They discovered no significant variation among both groups in terms of number of embryos transferred, embryo quality, age, or BMI. The measurements ranged from 0.3 to 110nmol/l. With p =0.04, unmodified OPR got significantly less in group with less than 35 nmol/l compared to other group with more than or equal to 35 nmol/l (38 percent versus 51 percent). In HRT-FET cycles, serum progesterone levels lower than 35 nmol/l reduce continuing pregnancy potential, according to that study.

Gaggiotti-Marre et al. [24] that new retrospective cohort research supports our results and was conducted to see whether progesterone (P) levels the day preceding embryo transfer of euploid embryos made effect on pregnancy consequences in artificial endometrial preparation for frozen embryo transfer (FET) cycles. In the period between January 2016 and June 2017, 244 FETs got evaluated in a private university clinic. Estradiol valerate as well as vaginal micronized progesterone were used to prepare the endometrium. The levels of serum P and estradiol were tested one day preceding embryo transfer. A multivariate analysis was used in examining association among serum P levels and pregnancy consequences, with confounding factors taken into account. The p-value reached 11.3 5.1 ng/ml on average. The levels of progesterone got divided into four quartiles: Q1: 8.06 ng/ml, Q2: 8.07-10.64 ng/ml, Q3: 10.65-13.13 ng/ml, and Q4: > 13.13 ng/ml. In comparison to higher P quartile, patients in lower P quartile had substantially greater miscarriage rate as well as significantly LBR rate. A low blood P level one day before FET (10.64 ng/ml) is linked to decreased pregnancy and LBR post FET of euploid embryos.

## 5. CONCLUSION

In HRT-FET cycles, low blood progesterone less than 10 nanograms/ml one day preceding frozen-thawed embryo transfer decreases possibility for continuing pregnancy post frozen-thawed embryo transfer.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

## CONSENT AND ETHICAL APPROVAL

All cases were subjected to presenting written informed consent that's accredited via Medical Ethical Committee at Tanta University Hospital. We used code number for each participant with the name and address in a special file to hide the patient's name.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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