Hosseini *et al.*
*Middle East Fertility Society Journal* (2023) 28:25
https://doi.org/10.1186/s43043-023-00151-9

Middle East Fertility Society Journal

RESEARCH

Open Access

# Effect of different levels of serum progesterone on day of frozen ET on clinical outcome

Sedigheh Hosseini[1], Saghar Salehpour[2], Parisa Taherzadeh Borujeni[2] and Nazanin Hajizadeh[2*]

## Abstract

**Background** Progesterone (P4) plays a critical role in a natural menstrual cycle. It is an essential hormone to have successful embryo implantation into the endometrium.

**Objective** In the current study, we compared the clinical outcomes of patients with different levels of P4 on embryo transfer day and aimed to determine the optimum threshold for P4 in the luteal phase.

**Material and methods** We performed a prospective cohort study on 100 patients who were referred to the Taleqani Infertility Treatment Institute to undergo frozen embryo transfer (FET). Endometrial preparation was programmed using an estrogen (E2) and progesterone supplement. FET was carried out 3 days after administration of the first dosage of progesterone. We measured serum progesterone levels on embryo transfer day. Clinical pregnancy was the main investigated outcome. We used a parametric receiver operating characteristic curve (ROC) to determine the best-cut points of P4 on embryo transfer day.

**Results** Overall, the average endometrial thickness was 7.9 ± 0.2 mm. We observed no association between endometrial thickness and clinical pregnancy (*P* value = 0.719). The mean number of the transferred embryos in all investigated cycles was 1.5 ± 0.5, and 80.0% of the transferred embryos were high quality (high and medium level). The average progesterone level in cycles with clinical pregnancy was 17.2 ± 3.3 ng/mL. It was only 13.4 ± 9.4 in patients without clinical pregnancy. The observed difference was statistically significant (*P* value = 0.032). The estimated AUC for the drawn ROC curve was 0.71 indicating a high predictive value.

**Conclusion** The current study demonstrated that high and low serum progesterone (P4) levels on embryo transfer day were associated with reduced clinical outcomes following blastocyst transfer during IVF/ICSI. However, more studies with large sizes are required in this regard.

**Keywords** Serum progesterone, Frozen embryo transfer, Luteal phase, Clinical pregnancy

## Background

Progesterone (P4) plays a critical role in a natural menstrual cycle. It is an essential hormone to have successful embryo implantation into the endometrium [1]. During the recent decade, GnRH agonist has been known as the protocol of assisted reproductive technology (ART). The use of GnRH analogs (agonist or antagonist) in ART (fresh cycles) may need luteal phase support. In hormonal FET cycles, LPS

*Correspondence:
Nazanin Hajizadeh
hajizadeh.nazanin94@yahoo.com
[1] Department of Obstetrics and GynecologyInfertility FellowshipSchool of Medicine, Ayatollah Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Obstetrics and Gynecology, School of Medicine, Men's Health and Reproductive Health Research Center, Ayatollah Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

is crucial. GnRH agonist inhibits the secretion of LH and disturbs the generation of endogenous progesterone (P4). Consequently, it will decrease implantation rate and pregnancy [2]. Therefore, in such cases, progesterone (P4) supplementation is recommended to make them capable of initiating secretory endometrium and maintaining pregnancy. Several studies provided strong evidence regarding the positive effects of progesterone supplementation on the outcome of FET [3, 4]. According to previously published studies, P4 luteal support increases the clinical outcomes of FET. Patients who received P4 luteal support had higher biochemical and clinical pregnancy with an increased chance of living birth. Moreover, they had a lower miscarriage [3, 4].

Even though the adequate level of P4 on the day of the embryo has an undeniable effect on clinical outcomes of IVF, there are growing pieces of evidence regarding the non-linear association between serum P4 and such outcomes [5]. There is an optimal window for P4 values in luteal phases that is in controversy with the traditional belief that higher values of P4 will lead to better clinical outcomes in IVF. If the P4 level is above 20 ng/Ml, this may decrease the pregnancy rate. It might be due to premature luteinization and its subsequent impairment in receptivity of the endometrium [5, 6]. As already mentioned, there are few studies regarding the role of P4 in luteal phases on clinical outcomes of IVF, and it is still regarded as a controversial issue. In the current study, we compared the clinical outcomes of patients with different levels of P4 on embryo transfer day and aimed to determine the optimum threshold for P4 in the luteal phase.

## Material and methods

We performed a prospective study on 100 IVF/ICSI cycles between February 2020 and February 2022 at Taleqani Infertility Treatment Institute. The inclusion criteria were: infertile women aged range of 18–40 years, candidacy for first or second freez embryo transfer. Exclusion criteria were cycles with missing data, patients with a preimplantation genetic diagnosis, Age over 40, ovarian hyperstimulation syndrome, history of implantation failure, history of recurrent miscarriage, and participation in sperm or oocyte donation program.

All the patients received hormone replacement therapy for endometrial preparation with pituitary down-regulation with a GnRH agonist. An ultrasound scan and $E_2$ measurement were carried out on days 2–3 of the spontaneous menstrual cycle to confirm pituitary desensitization. The endometrial preparation was started using 6 mg estradiol valerate daily if the endometrial thickness was less than 5 mm and serum estradiol level < 50 pg/ml. After 10–12 days of estradiol administration and if ultrasound showed an endometrial thickness (≥7 mm, with a triple-line pattern), E2 was continued at the same dosage and then a vaginal progesterone suppository (Cyclogest® 400 mg, Actover, Iran) was administered twice daily. Meanwhile, starting on the fourth day, 25 mg of progesterone (Aburaihan Pharmaceutical Co., Tehran, Iran). The estradiol dosage was increased to 8 mg/day to achieve the appropriate endometrial thickness. E2 and progesterone therapy was continued until a pregnancy test was performed, and in cases of a positive pregnancy, hormone therapy continued until week 10 of gestation. Finally, according to the women's age, up to two frozen embryos were thawed and transferred at the cleavage stage on the 3rd day of progesterone treatment. The quality of each embryo was determined based on the criteria described by Racowsky et al. [7]. The degree of fragmentation and the extent of symmetry besides blastomere numbers were considered to score embryo morphology. We measured serum progesterone level (P4) on embryo transfer day, then all serum progesterone samples were transferred to a laboratory and assayed using Electrochemiluminescence immunoassay. We expressed serum progesterone level as nanograms per milliliter. Clinical pregnancy was our main outcome. It was defined as ultrasound confirmation regarding at least one intrauterine gestational sac.

### Statistical analysis

Descriptive analyses were carried out using mean and standard deviation for continuous variables. We also used proportion and a 95% confidence interval for dichotomous variables. Patients were categorized into two groups according to their outcomes and then compared all baseline variables between these groups using independent *T*-test and chi-square analysis. We also used parametric receiver operating characteristic (ROC) analysis with two cut points to determine the best cut points. Statistical analysis was performed using the Stata statistical software package (Stata Corp. 2014. Stata Statistical Software: Release 14.1, College Station, TX: Stata Corp. LP). All *P* values of < 0.05 were considered significant.

## Results

In Table 1, we summarized the characteristics of 100 cycles meeting inclusion criteria. Study participants were categorized into two different groups according to the status of clinical pregnancy. The mean age of cycles that led to successful clinical pregnancy was 33.4 ± 4.7 years, while it was 32.9 ± 9.4 years in cycles without clinical pregnancy (*P* value = 0.560). Overall, the average endometrial thickness was 7.9 ± 0.2 mm and no association was found with

clinical pregnancy (*P* value = 0.719). The mean number of the transferred embryos in all investigated cycles was 1.5 ± 0.5, and 22.0% of the transferred embryos were high quality (grade A). According to Table 1, the average progesterone level in cycles with clinical pregnancy was 17.2 ± 3.3, whereas, it was only 13.4 ± 9.4 in patients with negative pregnancy, and the observed difference was statistically significant (*P* value = 0.032) (Table 1).

We draw a parametric ROC curve to determine the best cut point of serum progesterone level on embryo transfer day. Accordingly, the best cut-off was at 13.7 ng/Ml. However, we showed that the proportion of successful clinical pregnancy would decrease to 9.0% when progesterone values exceeded 20 ng/mL(CI:2.2, 30.5) (Table 2). The estimated AUC was 0.71. The proportion of clinical pregnancy in the first category of serum progesterone level was 10.0%. It drastically increased in cycle with progesterone level between 13.8 and 20 ng/mL and reached a peak in this quartile (92.8%, CI 75.0, 98.2). However, in the last quartile when the progesterone level value was higher than 20 ng/mL the proportion of clinical pregnancy declined to 9.0% that was the lowest reported percent of clinical pregnancy (Table 2).

## Discussion

Serum progesterone level is a contributing factor for successful implantation and is associated with beneficial effects in the ART and management of patients with recurrent spontaneous miscarriage of unknown cause [1]. There are several reports regarding the efficacy of progesterone support in the luteal phase. However, it seems that the extremely high level of P4 may reduce the implantation rate, clinical pregnancy, and live births [5]. The importance of progesterone support in the luteal phase has increased over the past decades as there has been a growing pattern in the number of women seeking GnRH agonist treatment [8, 9]. In the current study, we aimed to evaluate the effect of different levels of P4 on the day of ET on clinical outcomes of women with IVF/ICSI to determine the optimum window of the luteal phase. The overall proportion of clinical pregnancy as the main outcome of the study was 33%. However, we spotted a statistically significant association between serum P4 level on the day of ET and clinical pregnancy. The highest proportion of clinical pregnancy was 92.0% that was observed when P4 was between 13.7 and 20 ng/mL. However, in the values lower or higher than this window proportion of clinical pregnancy was less than 10%. Kofinas et al. have reported the same pattern in association between P4 level on embryo day and clinical outcomes of IVF/ICSI. They have argued that higher values of P4 might be associated with an earlier "implantation window". According to Kofinas et all, in the presence of higher values of P4, endometrial development is accelerated and consequently will lead to the impaired coordinated development of uterine and embryo. In such circumstances, poor implantation is expected because while the uterus is receptive and ready for implantation, the embryo is still developing [5]. Our findings support such a hypothesis as we illustrated the best clinical outcome when P4 values on embryo day were between 13.7 and 20 ng/mL. Yovich et al. have also supported our results and showed extremely low and high values of P4 on embryo day were associated with lower clinical pregnancy and live birth [10]. However, the upper threshold reported by Yovich was around 30 ng/mL which was much higher than the reported value in the current study. Alyasin et al. have also reported the same findings. According to their analysis, P4 levels higher than 32.0 ng/mL were associated with decreased live births [11]. All previous studies reported lower clinical outcomes in extremely high values of serum P4 values. However, there is wide heterogeneity regarding the best cut-off point.

We also reported a low clinical pregnancy rate in extremely low values of P4 on embryo transfer day.

**Table 1** Cycle and study participant's baseline characteristics

| Variable | With clinical pregnancy (*n* = 33) | Without clinical pregnancy (*n* = 67) | *P* value |
|---|---|---|---|
| Age, mean (SD) | 33.4 (4.7) | 32.9 (4.0) | 0.560 |
| Progesterone level, mean (SD) | **17.2 (3.3)** | **13.4 (9.4)** | **0.032** |
| Endo meter thickness | 7.9 (0.1) | 7.9 (0.2) | 0.719 |
| Quality of embryo | | | |
| High quality | 8 (25.8%) | 14 (21.2%) | 0.875 |
| Medium | 19 (61.2%) | 41 (62.1%) | 0.556 |
| Low quality | 6 (19.8%) | 9 (13.4%) | 0.822 |
| N of transferred embryos, mean (SD) | 1.4 (0.5) | 1.5 (0.5) | 0.575 |

*p* values < 0.05 was statistically significant

**Table 2** Clinical pregnancy rate by different categories of serum P4 level of embryo transfer day in women who underwent IVF/ICSI

| Progesterone level | Clinical pregnancy(*n*:33) | 95% CI | *P* value |
|---|---|---|---|
| 0–13.7 | 3.0% (*n*:1) | 4.1, 22.1 | |
| 13.8–20 | 92.8% (*n*:30) | 75.0, 98.2 | |
| > 20 | 6.0% (*n*: 2) | 2.2, 30.5 | |
| Overall | 33.0% | 24.3, 42.9 | *< 0.001 |

* Statistically significant difference; $p < 0.05$

The clinical pregnancy rate in patients with serum P4 level < 13.8 was 10%. Several studies have reported similar findings. Labarta et al. have pointed to 9.2 ng/mL as the cut point of serum P4 on the day of ET was associated with lower clinical outcomes [12]. In other studies, serum P4 values lower than 10 and 10.64 have demonstrated an association with lower clinical pregnancy and live birth that is lower than the reported value in the current study [13, 14]. However, in one study performed by Boynukalin et al. was shown that P4 values < 13.6 ng/mL were associated with a reduced likelihood of ongoing pregnancy and these findings were similar to our study [15]. Delay in the endometrium development and receptivity due to a low progesterone level is the main pathway to justify the lower clinical outcome of IVF/ICSI in cycles with a lower level of serum P4 [10].

The current study is one of the first attempts to determine the best cut point of serum progesterone level on embryo transfer day. However, our findings must be interpreted in the context of our limitations. The small sample size was one of the main limitations of the current study. Moreover, we did not address whether the investigated embryos were euploid or not.

In conclusion, the current study demonstrated that either high or low serum P4 levels on embryo transfer day were associated with reduced clinical outcomes following cleavage embryo transfer during IVF/ICSI. There is wide heterogeneity regarding higher and lower thresholds of serum P4 on embryo day. Monitoring and measuring P4 levels could increase the success rate during FET cycles. However, further studies are required.

#### Abbreviations

FET Freez embryo transfer
IVF In vitro fertilization
p4 Progesterone

#### Acknowledgements

The authors hereby express their appreciation toward the laboratory technicians of the IVF clinic of Taleghani Hospital and the Preventative Gynecology Research Center of Shahid Beheshti University of Medical Sciences for their support.

#### Authors' contributions

Sedighe Hosseini participated in the design of the study and performed the statistical analysis. Saghar Salehpour performed the statistical analysis, participated in its design and coordination, and helped to draft the manuscript. Dr. Parisa Taherzade participated in the statistical analysis and Nazanin Hajizadeh performed the statistical analysis and collected the data. All authors read and approved the final manuscript.

#### Funding

This study was supported by Preventative Gynecology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

#### Availability of data and materials

All data and materials are available.

### Declarations

#### Ethics approval and consent to participate

All of the women were fully explained about the process of laparoscopy and stem cell injection into both ovaries and complications. They all signed the informed consent form. The study protocol was approved by the Ethics Committee of Shahid Beheshti Medical University (Code: IR.Sbmu.. RETECH.1401.637).

#### Consent for publication

Not applicable.

#### Competing interests

All authors declare that they have no competing interests.

Received: 14 February 2023 Accepted: 27 September 2023
Published online: 16 October 2023

#### References

1. Daya S (2009) Luteal support: progestogens for pregnancy protection. Maturitas 65(Suppl 1):S29–34. PubMed PMID: 19913373. Epub 2009/11/17. eng.
2. Veleva Z, Orava M, Nuojua-Huttunen S, Tapanainen JS, Martikainen H (2013) Factors affecting the outcome of frozen–thawed embryo transfer. Hum Reprod 28(9):2425–2431
3. Bjuresten K, Landgren B-M, Hovatta O, Stavreus-Evers A (2011) Luteal phase progesterone increases live birth rate after frozen embryo transfer. Fertil Steril 95(2):534–537
4. Kim CH, Lee YJ, Lee KH, Kwon SK, Kim SH, Chae HD et al (2014) The effect of luteal phase progesterone supplementation on natural frozen-thawed embryo transfer cycles. Obstet Gynecol Sci 57(4):291–296
5. Kofinas JD, Blakemore J, McCulloh DH, Grifo J (2015) Serum progesterone levels greater than 20 ng/dl on day of embryo transfer are associated with lower live birth and higher pregnancy loss rates. J Assist Reprod Genet 32(9):1395–1399
6. Niemann H, Sacher B, Elsaesser F (1985) Pregnancy rates relative to recipient plasma progesterone levels on the day of nonsurgical transfer of frozen/thawed bovine embryos. Theriogenology 23(4):631–639
7. Racowsky C, Combelles CM, Nureddin A, Pan Y, Finn A, Miles L et al (2003) Day 3 and day 5 morphological predictors of embryo viability. Reprod Biomed Online 6(3):323–331
8. Penzias AS (2002) Luteal phase support. Fertil Steril 77(2):318–323
9. Shapiro DB, Pappadakis JA, Ellsworth NM, Hait HI, Nagy ZP (2014) Progesterone replacement with vaginal gel versus im injection: cycle and pregnancy outcomes in IVF patients receiving vitrified blastocysts. Human Reprod (Oxford, England) 29(8):1706
10. Yovich JL, Conceicao JL, Stanger JD, Hinchliffe PM, Keane KN (2015) Mid-luteal serum progesterone concentrations govern implantation rates for

cryopreserved embryo transfers conducted under hormone replacement. Reprod Biomed Online 31(2):180–191
11. Alyasin A, Agha-Hosseini M, Kabirinasab M, Saeidi H, Nashtaei MS (2021) Serum progesterone levels greater than 32.5 ng/ml on the day of embryo transfer are associated with lower live birth rate after artificial endometrial preparation: a prospective study. Reprod Biol Endocrinol 19(1):1–9
12. Labarta E, Mariani G, Holtmann N, Celada P, Remohí J, Bosch E (2017) Low serum progesterone on the day of embryo transfer is associated with a diminished ongoing pregnancy rate in oocyte donation cycles after artificial endometrial preparation: a prospective study. Hum Reprod 32(12):2437–2442
13. Cédrin-Durnerin I, Isnard T, Mahdjoub S, Sonigo C, Seroka A, Comtet M et al (2019) Serum progesterone concentration and live birth rate in frozen–thawed embryo transfers with hormonally prepared endometrium. Reprod Biomed Online 38(3):472–480
14. Gaggiotti-Marre S, Martinez F, Coll L, Garcia S, Álvarez M, Parriego M et al (2019) Low serum progesterone the day prior to frozen embryo transfer of euploid embryos is associated with significant reduction in live birth rates. Gynecol Endocrinol 35(5):439–442
15. Boynukalin FK, Gultomruk M, Turgut E, Demir B, Findikli N, Serdarogullari M et al (2019) Measuring the serum progesterone level on the day of transfer can be an additional tool to maximize ongoing pregnancies in single euploid frozen blastocyst transfers. Reprod Biol Endocrinol 17(1):1–8

## Publisher's Note