



ASSOCIATION OF SERUM PROGESTERONE DETERMINED ON THE DAY OF FROZEN EMBRYO TRANSFER WITH THE PREGNANCY RATES IN INDIAN SETTINGS: A PROSPECTIVE COHORT STUDY

Obstetrics & Gynaecology

Sanjay Gupte Gupte Hospital, Pune, India

Sachin Jadhav* Gupte Hospital, Pune, India *Corresponding Author

Usha Subramaniam Gupte Hospital, Pune, India

Pallavi Tiple Gupte Hospital, Pune, India

ABSTRACT

Background and Aim: Indian literature determining the optimal progesterone levels for successful pregnancy outcomes through frozen embryo transfer (FET) cycles is scarce. Therefore, this study was conducted to correlate progesterone levels on the day of FET with clinical pregnancy outcomes in Indian women undergoing FET cycles. **Methods:** This was a prospective single-center study conducted at a commercial fertility clinic that included women undergoing FET. Women undergoing FET were prospectively enrolled and received standard luteal phase support. Based on progesterone levels, 110 women were categorized into either Group A (<10 ng/mL) or Group B (>10 ng/mL). The main outcomes were the correlation between progesterone levels and pregnancy outcome. Serum progesterone levels were measured on the day of the embryo transfer cycle. **Results:** A total of 38 women were enrolled in group A and 72 in group B. The mean (SD) age of women was 32.5 (3.5) years and the majority of women were from age group 31-40 years (70.9%). A total of 47 and 63 women had endometrial thickness <10 mm and >10 mm, respectively, on the day of FET. Post FET cycles, 43.6% of women were confirmed with clinical pregnancy (56.4%: negative for pregnancy). The women with progesterone <10 ng/mL in FET had statistically significantly lower pregnancy rate than those with progesterone >10 ng/mL ($P=0.001$). Endometrial thickness did not show any significant association with pregnancy outcome ($P=0.08$). **Conclusion:** This study highlights that in Indian women undergoing FET, serum progesterone level <10 ng/mL on the day of FET is negatively correlated with pregnancy outcomes.

KEYWORDS

Embryo Transfer, Endometrial Thickness, India, Pregnancy Rate, Progesterone

INTRODUCTION

The use of frozen embryo transfer (FET) during the assisted reproductive technology (ART) has benefited many women in terms of achieving clinical pregnancy and preventing in vitro fertilization (IVF)-related complications including ovarian hyper-stimulation syndrome and multiple pregnancies.^{1,2} The importance of mid-luteal progesterone levels in achieving successful pregnancies with the use of FET cycles has been demonstrated in multiple evidences. Both, the low as well as high levels of progesterone during mid-luteal phase have been shown to result in unsuccessful pregnancy outcomes.³⁻⁶ Appropriate endometrial maturation is a vital aspect in achieving a successful outcome from FET cycles and abnormal progesterone levels is a significant risk factor for disruption of the endometrial maturation ultimately impacting the implantation and early development of pregnancy.^{3,4}

In spite of multiple evidence available worldwide, the cut-off values of mid-luteal progesterone levels suggested in these evidences varies. Furthermore, Indian literature determining the optimal progesterone levels for successful pregnancy outcome through FET cycles is scarce. A recent study from India has suggested a mid-luteal serum progesterone levels <15 ng/mL are associated with negative pregnancy outcomes in both fresh and FET cycles.⁷ This prospective study was conducted to evaluate correlation between progesterone levels on the day of FET and pregnancy outcomes in Indian women undergoing FET cycles.

MATERIAL AND METHODS

This was a prospective cohort study that included patients undergoing FET at the Cradle IVF center, Pune, between February 1, 2019 and June 31, 2019.

Inclusion And Exclusion Criteria

Patient less than 40 years of age undergoing single FET cycle, with a triple layer endometrium >8 mm after hormonal replacement therapy and those in which the good quality blastocysts transferred on Day 5 of embryo through hormone replacement therapy (HRT) were included. Patients with history of recurrent implantation failure, uterine diseases (e.g. fibroids, polyps, previously diagnosed Müllerian abnormalities), bad embryo quality or presence of hydrosalpinx were excluded from study.

Study Procedure

All the enrolled patients underwent the Gonadotropin-releasing

hormone antagonist protocol with gonadotropins stimulation for IVF. The selection of doses of gonadotropins was done by IVF specialist on the basis of individual patient condition. The injectable beta human chorionic gonadotropin (β -hCG) was given as a trigger for follicular maturation to all the patients. Under general anesthesia, egg retrieval was done around 36 hours after the β -hCG injection, using transvaginal ultrasound guidance. All FET cycles were performed by HRT cycles. All patients underwent transfer of two blastocyst at 2 cleavage stage. At day 2, patients initiated with progynova 2mg three times a day. The first scan was done on day 10 or 11 followed by micronized vaginally progesterone 400 ng twice a day. FET was done on day 6 morning i.e. after completion of 5 day of progesterone.

Serum progesterone was tested with blood sample being collected at day time between 11 am and 1 pm. Serum β -hCG was done on day 14 and early pregnancy scan on day 21 of FET.

For FET, luteal support with progesterone was started when endometrial thickness was more than 8 mm. Luteal phase support was given with combination of vaginal micronized progesterone capsule 400 mg vaginally twice a day and oral administration of Dydrogesterone 10mg twice a day. No steroids were used. Adjuvant therapy that included low dose aspirin and arginitric sachet once a day along with folate and Vitamin B12 supplements was given to all patients. Serum progesterone measurement was done on day of embryo transfer by drawing 2 mL of blood in a plain vacutainer. The progesterone levels were analyzed from serum, after centrifugation, using the automated chemiluminescent microparticle immunoassay. Then, based on the serum progesterone levels women were categorized into two group: (Group A: progesterone <10 ng/mL and Group B: progesterone >10 ng/mL).

Pregnancy is measured by β -hCG testing after 2 weeks of embryo transfer. Pregnancy is considered positive if β -hCG is more than 25 IU. The presence of gestational sacs with cardiac activity was further evidence of clinical pregnancy.

Statistical Analysis

The data analysis was done using SPSS Version 20 (IBM SPSS Statistic Inc., Chicago, Illinois, USA). Descriptive statistics was used to present observation either in the form of number and percentages, or means and standard deviations (SD). A Chi-square test was used for comparison of qualitative data. A $P<0.05$ is considered statistically significant.

RESULTS

A total of 110 women undergoing embryo transfer were prospectively enrolled. The mean (SD) age of women was 32.5 (3.5) years and majority of women were from age group 31-40 years (70.9%). The mean (SD) anti-Müllerian hormone levels and progesterone levels before FET were 2.4 (2.4) ng/mL and 0.36 ng/mL, respectively.

These women received standard luteal phase support with progesterone in the form of progesterone soft gelatin capsules (400 mg, vaginally, twice daily) for 106 women, dydrogesterone tablets for three women and progesterone (200 mg) sustained release tablet for one woman. Out of 110, 38 women were enrolled in group A and 72 in group B. Total of 47 women had endometrial thickness <10 mm and 63 women had endometrial thickness >10 mm on the day of FET. Out of total 110 FET, majority women received frozen day 5 blastocyst (n=94; 85.5%) and remaining women received day 3 blastocyst transfer (n=10; 9%) or day 4 blastocyst transfer (n=5; 4.5%). Only one woman received expanded blastocyst of day 5. Post FET cycles, total of 43.6% of women were confirmed with clinical pregnancy while 56.4% were negative for pregnancy (Table 1).

Table – 1 Demographic And Baseline Clinical Characteristics

Patient characteristics	Total (n=110)
Age (years), mean (SD)	32.5 (3.5)
Age groups (years)	
20-30	30 (27.3)
31-40	78 (70.9)
>40	2 (1.8)
AMH [n=73]	2.4 (2.4)
Progesterone levels before FET	0.36 (0.2)
Progesterone levels on the day of FET	
<10 ng/mL	38 (34.5)
>10 ng/mL	72 (65.5)
Endometrial thickness	
<10 mm	47 (42.7)
>10 mm	63 (57.3)
Progesterone support	
Progesterone soft gelatin capsules (400 mg, vaginally)	106 (96.4)
Dydrogesterone tablet	3 (2.7)
Progesterone (200 mg) sustained release (BD)	1 (0.9)
Clinical outcome	
Pregnancy positive	48 (43.6)
Pregnancy negative	62 (56.4)
Data presented as n (%), unless otherwise specified. AMH, Anti-Müllerian Hormone; BD, twice daily; FET, frozen embryo transfer; SD, standard deviation.	

The women with progesterone levels <10 ng/mL in FET had statistically significant lower pregnancy rate (P=0.001). Endometrial thickness did not show any significant association with pregnancy outcome (P=0.08) (Table 2).

Table – 2 Correlation Of Progesterone Levels And Endometrial Thickness With Pregnancy Outcome

Parameter	Pregnancy positive	Pregnancy negative	P-value
Progesterone levels on the day of FET			0.001
<10 ng/mL	8 (21.1)	30 (78.9)	
>10 ng/mL	40 (55.6)	32 (44.4)	
Endometrial thickness			0.08
<10 mm	16 (34.0)	31 (66.0)	
>10 mm	32 (50.8)	31 (49.2)	
Data presented as n (%), unless otherwise specified. FET, frozen embryo transfer.			

DISCUSSION

For women undergoing embryo transfer, progesterone supplementation is commonly used to prepare endometrial tissue for successful implantation and pregnancy maintenance during the early pregnancy phase. However, the recommended duration of progesterone supplementation prior to and after embryo transfer varies significantly in different parts of the world. The paucity of Indian data concerning association between serum progesterone levels on the day of FET and pregnancy outcome has emphasized the need of clinical studies evaluating progesterone levels on the day of FET and its impact on reproductive outcomes. In view of this, the current study, was conducted to evaluate correlation between progesterone levels on the

day of FET and pregnancy outcomes in Indian women undergoing FET cycles.

The importance of serum progesterone supplementation for success with ART was also emphasized in several previous studies. Observations from a prospective randomized study by Sharma et al. highlighted that supplementation of progesterone for three days instead of four days before the transfer of cleavage stage (day 2) cryopreserved embryos resulted into significant improvement in the pregnancy and implantation rates.⁸ A retrospective cohort study from the USA demonstrated that clinically significant success rate of pregnancy outcome was associated with the transfer of a single frozen day 5 blastocyst compared to day 6 blastocyst (P=0.044).⁹ Similar results were obtained by , a systematic review and metanalysis showing higher successful reproductive outcomes in terms of clinical pregnancy and live birth rates with day 5 blastocyst transfer compared to day 6.¹⁰ Correspondingly, in the present study, majority of FET were performed using Day 5 blastocyst (85.5%) with the aim of achieving successful reproductive outcomes.

The key observation from the present study suggested that the cut-off levels of serum progesterone are 10 ng/mL in our settings and women with progesterone levels >10 ng/mL had significantly higher pregnancy rate than those with progesterone <10 ng/mL. However, a similar study including women of Indian origin undergoing fresh or FET cycles reported that the mid-luteal serum progesterone levels <15 ng/mL have a negative impact on pregnancy outcome in both fresh and FET cycles.⁷ The cut-off value for mid-luteal progesterone levels determined in this study are slightly higher than the present study. This may be attributable to the fact that reproductive system in every woman produces, absorbs and metabolizes different levels of progesterone and both these studies included relatively similar but small sample size. Therefore, these results need further validation by conducting clinical studies with large-scale cohort.

An old study from 2004 has indicated that women with progesterone levels below 15 ng/mL have decreased chance of positive pregnancy outcome or elevated risk of early pregnancy loss.¹¹ Another study has also reported corroborating results using cut-off value of 15 ng/mL.¹² A recent study by Alsbjerg et al. reported a relatively lower cut-off value of <11 ng/mL of serum progesterone with negative impact on ongoing pregnancy rates.¹³ Another study demonstrated that progesterone levels ≥20 ng/mL contributed to reduced live birth rate and elevated pregnancy loss rates.¹⁴ In the present study, we decided to use a lower cut-off value of serum progesterone (10 ng/mL). Furthermore, a recent retrospective study showed a different trend wherein progesterone levels ≤ 10 ng/mL on the day of FET did not improve pregnancy outcomes. However, progesterone levels < 5 ng/mL were linked to reduced rates of live birth.¹⁵ Thus, positive outcomes post FET cycles cannot be achieved if progesterone levels are below a certain threshold on the day of embryo transfer. In addition, it is beneficial in terms of improving pregnancy rates and live births if progesterone levels are measured one day prior to FET cycle.¹⁶

Further, the present study did not show any association between endometrial thickness cut-off value (<10 mm) and pregnancy outcomes suggesting endometrial thickness of <10 mm but above 8 mm may not have any negative impact on pregnancy positivity. This might be attributable to the inclusion criteria of women with endometrial thickness above 8 mm which has been suggested to be sufficient for achieving successful implantation of transferred embryo.¹⁷ On the other hand, another study including women with single blastocyst transfer reported no impact of both endometrial thickness and endometrial pattern on implantation and pregnancy outcomes.¹⁸

The most frequently investigated routes of progesterone administration for luteal support in women undergoing IVF are oral, intramuscular and vaginal. In the present study, all the women vaginally administered progesterone reported positive pregnancy rate among 55.6% of women with >10 ng/mL progesterone levels on the day of FET. A previous study compared the outcomes of IVF embryo transfer in women who used vaginal or intramuscular progesterone demonstrated that luteal support with vaginal progesterone produced similar treatment outcomes as intramuscular progesterone.¹² A literature base review article compared the different routes of administration of progesterone for luteal support in IVF. It was noted that progesterone administered vaginally has an advantage over

progesterone administered intramuscularly due to its ability to induce adequate secretory endometrial transformation.¹⁹

different routes of progesterone administration as luteal phase support in infertility treatments. Human reproduction update, 6(2), 139–148.

One of the strengths of the present study is the prospective study design; whereas most of the previous studies had a retrospective study design. Furthermore, the present study did not find a negative impact of high levels (>20 ng/mL) of progesterone in a small subset of patients. The small sample size and single-center study design of the present study has limited the applicability of these observations to a small cohort. Therefore, future prospective cohort studies involving larger sample size and population of different ethnicities and from multiple centers across India are required to validate these results. Additionally, comparison of impact of lowest as well as highest optimal progesterone levels on pregnancy outcomes in Indian women undergoing FET are necessary to better understand the relationship between progesterone levels and pregnancy rates.

CONCLUSIONS

Overall observations suggest that in Indian women undergoing FET, serum progesterone level <10 ng/mL on the day of FET have a negative impact on pregnancy outcome. In a small subset of patients, progesterone values >20 ng/mL did not impact pregnancy outcome negatively, but had good success rates. Determination of serum progesterone levels in the early luteal phase may enhance the pregnancy outcome in an ART cycle by providing individualized luteal phase support.

REFERENCES:

- [1] Givens, C. R., Markun, L. C., Ryan, I. P., Chenette, P. E., Herbert, C. M., & Schriock, E. D. (2009). Outcomes of natural cycles versus programmed cycles for 1677 frozen-thawed embryo transfers. *Reproductive biomedicine online*, 19(3), 380–384.
- [2] Zheng, Y., Li, Z., Xiong, M., Luo, T., Dong, X., Huang, B., Zhang, H., & Ai, J. (2013). Hormonal replacement treatment improves clinical pregnancy in frozen-thawed embryos transfer cycles: a retrospective cohort study. *American journal of translational research*, 6(1), 85–90.
- [3] Thomsen, L. H., Kesmodel, U. S., Erb, K., Bungum, L., Pedersen, D., Hauge, B., Elbak, H. O., Povlsen, B. B., Andersen, C. Y., & Humaidan, P. (2018). The impact of luteal serum progesterone levels on live birth rates—a prospective study of 602 IVF/ICSI cycles. *Human reproduction (Oxford, England)*, 33(8), 1506–1516.
- [4] Arce, J. C., Balen, A., Platteau, P., Pettersson, G., & Andersen, A. N. (2011). Mid-luteal progesterone concentrations are associated with live birth rates during ovulation induction. *Reproductive biomedicine online*, 22(5), 449–456.
- [5] Labarta, E., Mariani, G., Holtmann, N., Celada, P., Remohi, 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. *Human reproduction (Oxford, England)*, 32(12), 2437–2442.
- [6] Yovich, J. L., Conceicao, J. L., Stanger, J. D., Hinchliffe, P. M., & Keane, K. N. (2015). Mid-luteal serum progesterone concentrations govern implantation rates for cryopreserved embryo transfers conducted under hormone replacement. *Reproductive biomedicine online*, 31(2), 180–191.
- [7] Kaur, J., Naidu, P., Kumkum, R., & Mahajan, N. (2018). Impact of mid-luteal serum progesterone levels on pregnancy outcome in fresh and frozen embryo transfer cycles in women of Indian ethnicity. *The Onco Fertility Journal*, 1(1), 30–5.
- [8] Sharma, S., & Majumdar, A. (2016). Determining the Optimal Duration of Progesterone Supplementation prior to Transfer of Cryopreserved Embryos and Its Impact on Implantation and Pregnancy Rates: A Pilot Study. *International journal of reproductive medicine*, 2016, 7128485.
- [9] Hibray, C., Britt, M., Ball, G. D., Davis, L., & Halverson, R. P-1263. Comparison of clinical pregnancy rates with single blastocyst transfer when using frozen day 5 or day 6 embryos. *Fertility & Sterility* 2013. S511. Available from: [https://www.fertstert.org/article/S0015-0282\(13\)01000-5/pdf](https://www.fertstert.org/article/S0015-0282(13)01000-5/pdf)
- [10] Bourdon, M., Pocate-Cheriet, K., Finet de Bantel, A., Grzegorzczak-Martin, V., Amar Hoffer, A., Arbo, E., Poulain, M., & Santulli, P. (2019). Day 5 versus Day 6 blastocyst transfers: a systematic review and meta-analysis of clinical outcomes. *Human reproduction (Oxford, England)*, 34(10), 1948–1964.
- [11] Ellenbogen, A., Gidoni, Y., Michaeli, M., Rotfarb, N., & Ballas S. (2004). Mid-luteal serum progesterone and estradiol levels as predictors of pregnancy in IVF-ET cycles: May increasing the dosage of progesterone supplementation improve the outcome? *Fertility and Sterility*, 82, S205–6.
- [12] Mitwally, M. F., Diamond, M. P., & Abuzeid, M. (2010). Vaginal micronized progesterone versus intramuscular progesterone for luteal support in women undergoing in vitro fertilization-embryo transfer. *Fertility and sterility*, 93(2), 554–569.
- [13] Alsberg, B., Thomsen, L., Elbak, H. O., Laursen, R., Povlsen, B. B., Haahr, T., & Humaidan, P. (2018). Progesterone levels on pregnancy test day after hormone replacement therapy-cryopreserved embryo transfer cycles and related reproductive outcomes. *Reproductive biomedicine online*, 37(5), 641–647.
- [14] Kofinas, J. D., Blakemore, J., McCulloh, D. H., & 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. *Journal of assisted reproduction and genetics*, 32(9), 1395–1399.
- [15] Volovsky, M., Pakes, C., Rozen, G., & Polyakov, A. (2020). Do serum progesterone levels on day of embryo transfer influence pregnancy outcomes in artificial frozen-thaw cycles? *Journal of assisted reproduction and genetics*, 37(5), 1129–1135.
- [16] Alur-Gupta, S., Hopeman, M., Berger, D. S., Barnhart, K. T., Senapati, S., & Gracia, C. (2020). Measuring serum estradiol and progesterone one day prior to frozen embryo transfer improves live birth rates. *Fertility research and practice*, 6, 6.
- [17] El-Toukhy, T., Coomarasamy, A., Khairy, M., Sunkara, K., Seed, P., Khalaf, Y., & Braude, P. (2008). The relationship between endometrial thickness and outcome of medicated frozen embryo replacement cycles. *Fertility and sterility*, 89(4), 832–839.
- [18] Gingold, J. A., Lee, J. A., Rodriguez-Purata, J., Whitehouse, M. C., Sandler, B., Grunfeld, L., Mukherjee, T., & Copperman, A. B. (2015). Endometrial pattern, but not endometrial thickness, affects implantation rates in euploid embryo transfers. *Fertility and sterility*, 104(3), 620–8.e5.
- [19] Tavaniotou, A., Smits, J., Bourgain, C., & Devroey, P. (2000). Comparison between