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Original Research Article

Serum progesterone level as a predictor of pregnancy in frozen embryo transfer

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ABSTRACT

Background: Aim of the study was to know the effect of serum progesterone (P4) level on pregnancy rate in frozen embryo transfer. The retrospective observational study was conducted in Institute of Reproductive Medicine, Madras Medical Mission Hospital, Chennai.
Methods: 126 patients underwent frozen embryo transfer with hormone replacement treatment using oral estradiol valerate and intramuscular progesterone beyond 7 mm of endometrial thickness were started on IM micronized progesterone 100 mg once a day till p+4. Morula transfer was done with at least one good-quality embryo. Serum progesterone level was measured one day before embryo transfer at our institute by chemiluminescence immunoassay. Adequate luteal support was given.
Results: The receiver operating curve (ROC) cut off showed serum progesterone level of 20.6 ng/ml had a sensitivity of 71.7% and specificity of 56.5%. Overall pregnancy rate was 50.7%. Live birth rate was significantly more in P4 >20.6 ng/ml.
Conclusions: In conclusion, the results of our study conclude that Progesterone level before embryo transfer day is an important prognostic factor for pregnancy outcome. According to this study, serum progesterone level >20.6 ng/ml one day before embryo transfer can be a good predictor of pregnancy in morula transfer. Further studies on a large scale on morula transfer are needed to modulate individualized treatment for patients for a successful pregnancy outcome.

Keywords: Morula transfer, Serum progesterone, Pregnancy rates, Livebirth rate

INTRODUCTION

In recent years, progesterone has become crucial support for frozen embryo transfer (FET). Many studies have shown that progesterone support can improve live birth rate during and after FET.[1] Some studies recommend individualized luteal support and that serum progesterone levels should be monitored during embryo transfer.[2] Programmed artificial FET cycles are different regarding the luteal phase because the endogenous corpus luteum is absent.[3] Benefits of programmed cycle are less monitoring for patients and ease of scheduling transfers.

The adequate level of P4 on the day of embryo transfer has an undeniable effect on the clinical outcomes of *in vitro* fertilization (IVF). There is an optimal window for P4 values in luteal phases, which is in controversy with the traditional belief that higher levels of P4 will lead to better clinical outcomes. Several studies have provided strong evidence regarding the positive effects of progesterone supplementation on clinical pregnancy outcomes.[4]

Due to the lack of typical morphological markers, morula transfer on day 4 has been understudied in humans. However, it has recently been demonstrated that the

densification process at the morula stage involves multiple self-correcting mechanisms critical in determining embryo quality, establishing the first cell lineage, and the entire development process, which means that morula transfer can be a feasible transfer strategy.[5]

Compared to single blast transfer, transferring two embryos can enhance the success rate of IVF.[5] Also, blastocyst culture is more complicated than morula culture, which requires strict technical support. On the other hand, the culture of morula and cleavage embryos is relatively simple and requires less support.[6] Some clinics prefer morula transfer due to technical constraints. In this study, we have studied the progesterone cut-off value for successful morula transfer and pregnancy outcomes.

METHODS

This retrospective study was conducted in the Institute of Reproductive medicine, The Madras Medical Mission Hospital. The institutional scientific and ethical committee approval was obtained. 126 patients underwent morula transfer in the Institute of Reproductive Medicine between January 2021 and December 2023 were included in the study. Maternal age between 21 and 45 were included in the study. Cell stage transfer, blast transfer, natural cycle, downregulated cycle, and the presence of any uterine factors were excluded from the study.

Patients who underwent frozen embryo transfer with hormone replacement treatment using oral estradiol valerate maximum of 10-12 mg and beyond 7 mm of endometrial thickness were started on IM micronized progesterone 100 mg once a day till p+4. Morula transfers were done with a maximum of two embryos. Serum progesterone levels were measured one day before embryo transfer by chemiluminescence immunoassay followed by adequate luteal support. A beta-human chorionic gonadotropin (B-hCG) tests were performed 16 days after embryo transfer.

The progesterone value was calculated using a receiver operating curve (ROC curve) for the statistical analysis. The coordinates of the ROC curve infer maximum sensitivity and specificity for the cut-off progesterone value positive for pregnancy, which was derived using Number Cruncher Statistical Systems (NCSS) statistical software.

RESULTS

In this study, a total of 126 FET cycles with artificial endometrial preparation with HRT were taken for analysis. The progesterone value on the day of the morula transfer was analyzed using NCSS statistical software. The Receiver operating curve cut-off for serum P4 value was 20.6n g/ml. Serum progesterone level cut-off of 20.6 ng/ml had a sensitivity of 71.7% and specificity of 56.5%, stating the study has a 95% confidence interval considered to be predictive of positive pregnancy.

Comparing the baseline characteristics (Table 1) between both the groups, there was no statistical difference in age (p value 0.398), body mass index (BMI) (p value=0.377), duration of infertility (p value=0.266), days of HRT (p value=0.259) and endometrial thickness (p value=0.945). The mean progesterone value in P4 >20.6 ng/ml was 26.87±3.4, and in P4 <20.6 ng/ml was 18.59±1.7.

Table 1: Demographic data of the study population.

Variables	P4 ≤20.6 ng/ml (n=50)	P4 >20.6 ng/ml (n=76)	P value
Age (in years)	32.61±3.4	31.74±2.9	0.398
BMI	26.42±4.12	25.61±4.4	0.377
Duration of infertility	6.4±3.2	5.9±3.4	0.266
Days of HRT	16.1±4.3	18.5±3.5	0.259
Endometrial thickness	9.64±1.9	9.69±1.5	0.945

The overall pregnancy rate in the 126 FET cycle was 50.7% (64/126). The progesterone concentration was divided into two groups. Group I measures P4 value less than 20.6 ng/ml. Group II measures a P4 value of more than 20.6 ng/ml as shown in Table 2.

The pregnancy rate in P4 more than 20.6 ng/ml showed a better pregnancy rate of 52.63% (40/76) compared to the pregnancy rate in P4 less than 20.6 ng/ml of 48% (24/50). The live birth rate in P4 more than 20.6 ng/ml was higher at 37.1% compared to the progesterone value of less than 20.6 ng/ml (28%). In this study, the pregnancy rate and LBR were statistically significant between both groups (p value=0.005) and (p value=0.001) (Table 2).

The biochemical pregnancy rate in P4 more than 20.6 ng/ml was 2.63% compared to P4 less than 20.6 ng/ml with 6% with no significance (p value=0.48). Ongoing pregnancy in P4 more than 20.6 ng/ml was 1.31% compared to a P4 value of less than 20.6 ng/ml with 6%. Both groups did not differ much between biochemical and ongoing pregnancies (Table 2).

The miscarriage rate in P4 more than 20.6 ng/ml was 14.47% compared to 6% in P4 less than 20.6 ng/ml. This could be due to the increased number of FET cycles in group II (60.3%) compared to group I (39.6%). The miscarriage rate for women above 30 years of age is around 10-15%. This study showed no statistical difference in age between both groups (Table 2).

In this study, one ectopic in P4 less than 20.6 ng/ml was managed medically. There was 1 DCDA twin pregnancy in each group, delivered at term with no complications as shown in Table 2.

This study concludes that patients with a progestrone value greater than 20.6 ng/ml had a better pregnancy rate and live

birth rate than those with a progestrone value less than 20.6 ng/ml. Although the miscarriage rate was increased in group I, better success rates can be achieved in morula transfer by individualising patients and treating them with appropriate management.

Table 2: Pregnancy outcomes between both groups.

Outcomes	P4 ≤20.6 ng/ml (n=50)	P4 >20.6 ng/ml (n=76)	P value
Pregnancy rate	24 (48%)	40 (52.63%)	0.005 (significant)
Biochemical pregnancy	3 (6%)	2 (2.63%)	0.48
Ongoing pregnancy	3 (6%)	1 (1.31%)	0.99
Miscarriage	3 (6%)	11 (14.47%)	0.005 (significant)
Live birth rate	14 (28%)	26 (37.14%)	0.0001 (significant)
Twins pregnancy	1 (2%)	1 (0.13%)	0.99
Ectopic	1 (2%)	0	

DISCUSSION

Although many studies have been made on serum progesterone in cleavage and blast transfer, this study was performed in morula transfer. Due to advancements in the technical aspects of the culture and skilled embryologists, morula transfer is now widely accepted for better pregnancy outcomes.[7]

This study analyzed the association between serum progesterone levels one day before embryo transfer and clinical outcomes in FET patients with morula transfer. The principal finding is that serum progesterone concentration less than 20.6 ng/ml had a significantly lower live birth rate than those with more than 20.6 ng/ml. In a study by Brady et al, he concluded that serum progesterone value less than 20 ng/ml had a lower live birth rate in the FET cycle ($p<0.001$).[1,8] In his study, he stated that despite additional progesterone supplementation after ET, it was insufficient to rescue pregnancy rates.

Only one retrospective study conducted by Kofinas et al with artificial endometrial preparation with HRT followed by IM progesterone of 50-75 mg with single euploid blastocyst transfer showed progesterone level on ET day less than 20 having better ongoing pregnancy rate (65% versus 49%) which is contradictory to our study.[9] The same group in 2017 studied trends of serum progesterone after a day of the transfer to predict the live birth rate and found no significant difference in embryo transfer day progesterone level and showed that >10% fall in serum progesterone from day of transfer associated with poor pregnancy outcomes (63% versus 26%).

Labarta et al conducted a prospective study of 211 donor oocytes cycles with artificial endometrial preparation transfer after micronized vaginal progesterone 400 mg twice daily.[10] The mean serum P on the day of embryo transfer was 12.7±5.4 ng/ml. Ongoing pregnancy/live birth rate was significantly higher in P4 >11 ng/ml compared with low P4 (55.6% versus 35%).

Cédrin-Durnerin et al studied serum progesterone and live birth in retrospective analysis in hormonally prepared endometrium with transdermal estradiol combined with 600 mg vaginal micronized progesterone.[11] Mean serum embryo transfer day progesterone was 11.4 ng/ml. Serum progesterone <10 ng/ml was observed in 37% of cycles and was associated with significantly lower pregnancy (34% versus 48%, p=0.04) and live birth rates (17% versus 31%, p=0.01).

Gaggiotti-Marre et al studied retrospective levels of serum progesterone on ET day with a live birth in hormonally prepared endometrium with estradiol valerate and vaginal micronized progesterone 600 mg with single euploid blastocyst transfer.[12] A low serum P level (10.64 ng/ml) one day before FET is associated with lower pregnancy and LBR following FET of euploid embryos (47.5% versus 62.3%).

Artificial reproductive technologies have rapidly evolved over the past few decades in order to improve pregnancy outcomes of infertile couples, including great advances in the cryopreservation process.[13] The impact of serum progesterone in FET cycles is widely studied, with evidence suggesting that luteal phase P supplementation improves live birth rates.[14] Many attempts have been made to find whether there is an optimal serum progesterone value around the time of embryo transfer in the luteal phase.[15]

One of the study's main limitations was the small sample size. Moreover, we did not address whether the embryos were euploid or not. Studies on different populations or the administration of different routes of progesterone will require further validation.

Evidence-based prospective and retrospective studies using exogenous progesterone administration, irrespective of embryo stage, support the negative impact of low serum progesterone levels on pregnancy outcomes.

CONCLUSION

In conclusion, the results of our study conclude that Progesterone level before embryo transfer day is an important prognostic factor for pregnancy outcome. According to this study, serum progesterone level >20.6 ng/ml one day before embryo transfer can be a good predictor of pregnancy in morula transfer. Further studies on a large scale on morula transfer are needed to modulate individualized treatment for patients for a successful pregnancy outcome.

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