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Original Research Article

The impact of embryo quality and endometrial thickness on frozen embryo transfer cycles

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ABSTRACT

Background: Endometrial thickness and embryo quality are critical factors influencing implantation rates in IVF cycles. This study aimed to study how endometrial thickness impacts implantation rate in FET cycles and study the relationship between the quality of the transferred embryos and the occurrence of successful pregnancy.
Methods: This study retrospectively analyzed 100 embryo transfer cycles that were performed in fertility centers associated with SPOVUM TECHNOLOGIES PRIVATE LIMITED, Rajajinagar, Bangalore. ET assessment was performed with grayscale ultrasound on the day of embryo transfer. Embryo grading ranked embryos based on morphological features of each embryo indicating its viability and developmental potential.
Results: Out of 100 patients, successful pregnancy was achieved in 56 patients, giving implantation rate of 56% (56/100). Patients with endometrial thickness (ET) <9 mm on the day of embryo transfer had significantly lower implantation rates compared to those with ET ≥9 mm ($p = 4.68 \times 10^{-5}$). Implantation rates by embryo grade were 65% for excellent, 60% for fair, and 14% for poor-grade embryos. Higher implantation rates were observed following transfer of blastocysts with Grade A and B trophectoderm (TE) ($p = 0.002833$). Associations between successful implantation and patient age, as well as embryo grade, were statistically significant ($p = 3.451 \times 10^{5}$ and 3.511×10^{-4}, respectively).
Conclusions: Ultrasound assessment of endometrial thickness and vascularity helps optimize Day 5 embryo transfer timing and implantation. Overall blastocyst quality strongly influences pregnancy outcomes. The study supports incorporating TE and ICM morphological grading to improve embryo selection criteria.

Keywords: Endometrial thickness, Embryo grading, Euploid embryo transfer, Implantation rate

INTRODUCTION

Infertility which is described as inability of the couples to conceive after 12 months of having regular unprotected intercourse.[1] It affects both men and women. Various factors affecting female infertility include issues with ovulation. The ovaries' ability to release eggs is impacted by several diseases. Among them are hormonal issues like polycystic ovary syndrome.[2] Hyperthyroidism, and hypothyroidism can both impact the menstrual cycle and result in infertility. There can be abnormalities in the structure of the uterine cavity which would hinder implantation such as septate or bicornuate uterus. Damage or obstruction to the fallopian tube due to previous surgery or infection can also lead to infertility.[3] Primary ovarian insufficiency, Pelvic adhesions are some of the other reasons which are often diagnosed as the causes during infertility investigations. Female infertility can be diagnosed through a routine investigation workup which

includes taking the past medical and surgical history, hormonal profiling and some ultrasonological tests. These tests often give a clue for any derangement in the patient's female reproductive system anatomy. Previous surgeries can lead to adhesions and scarring which in future hinder the easy passage of oocyte down from the fallopian tube thus resulting in tubal factor of infertility. These adhesions can also lead to partial or total blockage of the tubes blocking the pathway of gamete fertilization. Past medical history also sheds light upon any previous miscarriages or obstetric procedures such as medical termination of pregnancy, which becomes a cause for disturbed environment for future implantations in the uterus. Ultrasound evaluation is crucial to determine the AFC, which gives a major clue about the ovarian reserve of the patient. Ultrasound also helps in evaluating the endometrial thickness and quality.

The uterus has three layers from outermost to innermost consisting of the perimetrium, myometrium and the endometrium. The most crucial layer which helps in maintain a pregnancy is the endometrial layer. It undergoes cyclical variations each menstrual cycle and gets ready to receive an embryo. Endometrial thickness ranges from 1 to 18 mm, depending on the individual's cycle stage.[4] The endometrium in menstrual cycle undergoes four phases- menstruation, proliferative, ovulatory, and secretory. During the mid secretory phase, when the signs of invasion and cell adhesion appear, the endometrial window of implantation (WOI) is open. WOI often occurs between days 19 and 22 of the cycle.[5]

Embryo transfer is the precise and painless placement of embryos using an ET (embryo transfer) catheter in a uterine cavity at a location with the highest likelihood for implantation is known as embryo transfer. There are two types of embryo transfers- fresh and frozen embryo transfers.

Fresh embryo transfer involves placing fertilized eggs (embryos) back into the uterus in the same cycle they were retrieved from the ovaries, at day 3- cleavage stage or day 5-blastocyst stage.[6] In frozen embryo transfers, after the embryo is frozen, a frozen embryo transfer typically takes place as either immediate (first cycle after pickup) or a delayed frozen embryo transfer in any subsequent cycles.[7] Frozen embryo transfers enables us to develop OHSS (ovarian hyperstimulation syndrome) free clinic.

FET mainly includes three types of cycles. Natural cycle FET, here no medications/ hormones are administered prior to ovulation. Embryos are transferred in a woman's natural ovulatory cycle with the help of endocrine and ultrasound monitoring. ARTIFICIAL CYCLE FET/HRT CYCLES- here the endometrial proliferation and follicular growth suppression is achieved by giving estrogen. STIMULATED CYCLE FET- here mild ovarian stimulation with clomiphene citrate, aromatase inhibitors, gonadotrophins with HCG trigger with or without LPS has been advocated to increase serum estrogen levels in turn enhancing endometrial receptivity.

In embryo grading, there has been found a direct correlation between embryo grading and IVF success rates.[8] Higher-grade embryos, such as 4AA blastocysts, have more chances to implant and lead to a successful IVF pregnancy. However, while embryo quality plays a significant role, it is not the only factor influencing IVF success. Age, uterine health, and maternal as well as paternal factors also contribute to the chances of a successful outcome. Even lower-grade embryos may occasionally result in pregnancy, though they generally have a lower chance of success. Evaluating blastocyst morphology evaluates the degree of blastocyst expansion, the consistency of the inner cell mass (ICM), and the cohesiveness of the trophectoderm (TE).

METHODS

A retrospective study was performed consisting of patients from hospitals and fertility centers associated with SPOVUM Technologies Private Limited, located in Rajajinagar, Bangalore from January 2025 to May 2025. Study population consisted of total number of patients (n)=100. Endometrial thickness was assessed with gray scale ultrasound on the day of embryo transfer just before thawing the embryos in patients undergoing Frozen embryo transfer cycle after endometrial preparation. Patient data was collected from medical records and the data was de-identified to maintain patient confidentiality. The data contained the following parameters from each patient- age of the patient, numbers of embryo transferred, day of embryo transfer, grades of embryos transferred, endometrial thickness on the day of embryo transfer, result positive/negative pregnancy.

Inclusion criteria

Inclusion criteria include patients of age range consisting of women of reproductive age between 20-45 years and The endometrium was prepared using HRT protocol.

Exclusion criteria

Exclusion criteria exclude genetic abnormality in either partner, uterine malformations, any intrauterine conditions affecting pregnancy outcomes such as endometrial polyp, hydro salpinx etc.

Ethical clearance was obtained from Spovum technologies for using the data. The patient data was anonymized and used solely for academic purposes.

Data was compiled in Microsoft Excel for statistical computation. Data analysis was done in R SOFTWARE (VERSION 4.5.1). One sample t test, Chi square tests and calculation of p value were performed on the data for obtaining various comparisons between EM thickness, embryo quality, age, and successful implantation.

Ovarian stimulation was started on day 2/day 3 of the cycle. The follicles were monitored by analyzing serum estradiol levels and imaging them by transvaginal ultrasound. Both long agonist and antagonist protocols were used for patients according to the ovarian response rates. After the follicles were visualized as mature on the ultrasound scan then the HCG trigger was given. And subsequently, approximately 36 hours later an ovum pick up procedure was carried out. Ovum pickup procedure was carried out under anesthesia, Conscious sedation included Midazolam (2-5mg), Propofol (1-2 mg). Prophylactic antibiotics were given before OPU procedure. Vaginal preparation- done using povidone iodine and normal saline. OPU needles used- 17-18 mm and the negative pressure maintained 80 to 300mmHg. After oocyte retrieval, oocyte cumulus complex (OCC) is visualized under stereomicroscope. The OCCs were then washed in Hepes media with a glass pipette. Then they are transferred to a center well dish containing a solution with 1:1 (Hyase: Hepes) for 40-60 seconds. The hyase helps to loosen the cumulus cells around the oocyte and becomes easier to denude. Then the oocytes were progressively denuded with 175µm and 140µm denupet. The denuded oocytes were then placed in fertilization media and kept in incubator for 2-3 hours to facilitate cytoplasmic maturation. Later then they were transferred to ICSI dish and sperms are immobilized and injected into the oocyte. Injected oocytes were transferred into the culture dish and kept in the incubator. Fertilization was checked after 18-20 hours post ICSI. The fertilized oocytes were allowed to develop into future embryos for 3-5 days, in suitable culture media and specific laboratory conditions. Then on day 3-cleavage stage or day 5-blastocyst stage, the embryos were assessed and cryopreserved. Day 3 embryo assessment was done using Istanbul consensus. Embryos consisting of equal sized cells and minimum fragmentation were given higher grade. Embryos with severe fragmentation, unequal cells and evidence of multinucleation were assigned poor grade. Day 5 blastocysts were graded according to Gardner's scoring system. The scoring assessed 3 main parameters such as blastocoel expansion, inner cell mass, and trophectoderm cell layer. Fully expanded blastocyst with compact well defined inner call mass and cohesive trophectoderm with multiple cells is graded as excellent blast. Poor expansion of blastocyst with inner cell mass consisting of very few cells and poorly defined trophectoderm cells are graded as poor-quality embryos.

Meanwhile, the endometrium of the patient is prepared in the subsequent cycles. The objective is to increase the chances that the embryo transferred leads to a successful implantation. Most commonly used hormones were estrogen and progesterone. Once the endometrium seemed sufficiently thick, the embryo transfer procedure was carried out. Luteal phase support was started by giving certain medications which would increase the likelihood of a positive implantation to occur. Luteal phase support was continued until βhcg test. Pregnancy can be confirmed through estimation of βHCG in urine and blood after 3-5 weeks of gestation.

RESULTS

Endometrial (EM) thickness

A total of 100 patients were categorized based on endometrial (EM) thickness into Group A (<9 mm, n = 40) and Group B (≥9 mm, n = 60). The mean EM thickness was 8.89 mm, which was significantly lower than the reference value of 10 mm (t = -8.72, df = 99, p<0.001; 95% CI: 8.64-9.15 mm).

According to Table 1, as the p value is less than 0.05, it is statistically significant and helps establish a direct relationship between higher endometrial thickness and higher rates of successful pregnancy.

Table 1: Comparison of group A with group B regarding positive pregnancy rates.

Group category	Positive pregnancy		Negative pregnancy		Total	
	Number	Percent	Number	Percent	Number	Percent
Group A	12	30	28	70	40	100
Group B	44	73.33	16	26.66	60	99.99
				Total (N)	100	

Chi-squared = 16.574, df = 1, p-value = 4.68e-05

Table 2: Comparison of embryo quality with successful implantation.

Embryo quality	Positive pregnancy		Negative pregnancy		Total	
	Number	Percent	Number	Percent	Number	Percent
Excellent	30	65.21	16	34.79	46	100
Fair	24	60	16	40	40	100
Poor	2	14.28	12	85.71	14	99.99
				Total (N)	100	

Chi-squared = 11.733, df = 2, p-value = 0.002833

According to Table 2, we can see that the excellent grade embryos have the highest positive pregnancy rates and the poor grade embryos have the least positive pregnancy rates. The resulting p value is statistically significant.

According to table 3, we can see that the highest positive pregnancy rates were seen in the younger age group. The positive pregnancy rates subsequently declined in advanced maternal age group.

Table 3: Comparison of age of the patient with successful implantation.

Age group in years	Positive pregnancy		Negative pregnancy		Total	
	Number	Percent	Number	Percent	Number	Percent
21-30	39	76.47	12	23.53	51	100
31-40	12	29.27	29	70.73	41	100
41-50	4	50	4	50	8	100
				Total (N)	100	

Chi-squared = 20.548, df = 2, p-value = 3.451e-05

Table 4: Comparison of age of the patient with embryo quality.

Age group in years	Excellent		Fair		Poor		Total	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
21-30	31	60.78	20	39.2	0	0	51	99.98
31-40	12	29.26	18	43.9	11	26.82	41	99.98
41-50	3	37.5	2	25	3	37.5	8	100

Chi-squared = 20.774, df = 4, p-value = 0.0003511

Table 5: Comparison of day of embryo transfer with successful implantation.

Day of embryo transfer	Positive pregnancy		Negative pregnancy		Total	
	Number	Percent	Number	Percent	Number	Percent
Day 3	4	80	1	20	5	100
Day 5	41	55.4	33	44.6	74	100
Day 6	11	52.38	10	47.62	21	100
				Total (N)	100	

Chi-squared = 1.2911, df = 2, p-value = 0.5244

In table 4, the age group 21-30 has zero poor grade embryos whereas the excellent grade embryos are the highest. And the percentage of excellent grade embryos significantly decreases with advancing age.

According to table 5, the p value was not significant and hence according to the data collected there is no significant differences in results on different days of embryo transfer.

DISCUSSION

Ultrasound measurement of endometrial thickness is a simple and reproducible method to evaluate endometrial proliferation.[10] Understanding its significance is even more important in medicated FER cycles since endometrial thickness is the predominant factor determining the timing of P supplementation and cryo-thawed ET.[11]

In the present study we investigated the association between endometrial thickness and treatment outcome in FET cycles. The results of the above study establish the influence of endometrial thickness and embryo quality in successful pregnancies in IVF. By categorizing the patients into two groups based on their endometrial thickness on the day of embryo transfer, a direct relationship was established between the positive implantation rate and the endometrial thickness and blood flow. This agrees with the current literature studies indicating the importance of endometrial thickness and embryo quality on pregnancy outcomes. [12] The above results also agree with the literature stating that highest implantation potential is seen in an eight cell embryo with ≤ 10% fragmentation in the third day following oocyte retrieval.[12] Significant embryo-endometrial interaction indicates combination of EMT and embryo quality might improve the prognostic value in clinical practice for live-birth in patients undergoing transfer of 1-2 fresh cleavage-stage embryos.[13] A recent publication involving 274 FETs suggested that the degree of endometrial compaction (a decline in endometrial thickness between the date of introduction of progesterone and the date of embryo transfer) is an important positive predictor of ongoing pregnancy rates.[14] No evidence was found to support the idea that a lining that is "too thick" is detrimental to live birth rates. An earlier publication from a Canadian group had proposed that an endometrial thickness >14 mm was associated with lower implantation rates, lower pregnancy rates, and higher pregnancy loss rates. [15] However, in our data we find no evidence to support this hypothesis.

However, some studies show that neither attainment of pregnancy nor pregnancy outcome can be predicted by endometrial thickness alone which is contradictory to the

present study.[16] One of the studies also mentions that objective TLIA (Time Lapse Imaging Analysis) is superior for selecting embryos for their propensity to generate a live birth over a conventional, subjective blastocyst morphology scoring system which goes against the results of the present study.[17]

Sub endometrial blood flow has also been linked with endometrial thickness and it is conceivable that when the endometrium reaches a thickness of 9 mm or more after hormone supplementation, improved vascularity may allow markers of endometrial receptivity (such as the expression of fully developed pinopodes) to cover a longer period.[18,19] thereby prolonging the receptive phase and increasing the chance of embryo implantation.[2]

This study has a few limitations. The retrospective design may be associated with selection bias and limits control over confounding variables. The sample size was relatively small and derived from a single geographical region, which may restrict the generalizability of the findings to broader populations. Only morphological embryo grading was used, without incorporating genetic testing such as PGT-A or objective time-lapse imaging, which could provide more precise embryo selection. Endometrial receptivity was assessed primarily by thickness, without evaluation of molecular markers or endometrial receptivity assays. Additionally, live birth rates were not assessed, and pregnancy outcome was limited to biochemical or clinical pregnancy.

CONCLUSION

According to the above tables and data analysis, the p value is significant for tables 1,2,3 and 4 indicating that the results are statistically significant and thus a direct correlation exists between embryo grading and implantation rates. Higher grade cleavage and blastocyst embryos have a higher implantation rate.

Endometrial thickness and embryo quality are important determinants of reproductive success. This study reinforces the importance of these factors and how the results are negatively impacted if either of them is compromised. Endometrial thickness and receptivity have a greater impact on successful implantation rates as compared to embryo quality as we have seen that a good endometrium can make up for a poor-quality embryo. But a poor-quality endometrium will not support the embryo development in later stages of gestation and can lead to miscarriages. The findings of the above study also help us understand the various methods which are used to improve endometrium quality and offers variety of treatment options to ensure optimal treatment.

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