International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Selvamani D et al. Int J Reprod Contracept Obstet Gynecol. 2022 Jul;11(7):1956-1962
www.ijrcog.org
pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20221676

Original Research Article

Influence of endometrial thickness and embryo placement distance from fundus in in-vitro fertilization treatment

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Received: 11 May 2022
Accepted: 02 June 2022

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ABSTRACT

Background: *In-vitro* fertilization–embryo transfer (IVF-ET) has become progressively popular as a treatment for different type of infertility issues. Implantation of the embryo is an essential step and depends on various factors that play pivotal role in influencing the pregnancy outcomes in IVF cycle. It directly impacts on the efficiency of embryo transfer procedure, site of embryo deposition, catheter loading technique, embryo placement distance from the fundus and thickness of endometrium.

Methods: In the present retrospective study, data from IVF-ET treatment cycles conducted at Srushti Fertility Centre and Women's Hospital, Chennai, for a period between October 2021 and February 2022 were analysed. Women were divided into four groups according to embryo placement distance from fundus post embryo transfer group 1: <10 mm, group 2: >10 to <15 mm, group 3: >15 to <20 mm and group 4: >20 mm. According to endometrial thickness they were divided into two groups, group A: 6-10 mm and group B: 10-14 mm. Clinical pregnancy were assessed between the groups.

Results: Clinical pregnancy rates were higher in groups 2 and 3 when compared to embryo placement distance from fundus 62.3% and 82.2%, distance of 10 mm and 20 mm and endometrial thickness of more than 10 mm (group B) had higher clinical pregnancy rates 66.3% than (group A) 43.5%.

Conclusion: Ideal ET depth and optimum endometrial thickness were found to influence the clinical pregnancy rates. Embryo placement at 10-20 mm from fundus and endometrial thickness of more than 10 mm is recommended for optimal clinical pregnancy outcomes.

Keywords: Endometrial thickness, Distance from fundus, Embryo transfer, Ultrasound guidance, Clinical pregnancy

INTRODUCTION

In assisted reproductive technology (ART), fertilization and cleavage take place in the *in vitro* fertilization (IVF) laboratory and embryo transfer is the final and crucial step where the cleavage stage or blastocyst embryo is transferred to its *in vivo* environment. Many factors are involved that results in affecting high implantation potential are, embryo transfer catheter type, catheter loading technique, embryo transfer site, uterine receptivity and endometrial thickness.[1] Ultrasound guided embryo transfer allows to visualize the exact position of the catheter tip along with the inclusion of air bubbles loaded in the catheter. Air bubbles were known to be a predictor on the pregnancy rates.[2]

The thickness of endometrium during embryo transfer can be used as a surrogate marker for receptivity and thin endometrium is associated with reduced IVF success rates.

Ovarian stimulation is an important step in fertility treatment and defined as a pharmacological treatment with the intention of inducing the development of ovarian follicles. It can be used to obtain multiple eggs during follicular aspiration. There are several options for ovarian stimulation as some patients may develop few eggs and also would require repeated stimulation, few may overreact with risk of serious complications such as ovarian hyper stimulation syndrome (OHSS).[3]

Conventional IVF cycle is categorized into gonadotropin-releasing hormone (GnRH) agonist cycle and GnRH antagonists cycle. GnRH agonist cycle aims to suppress the premature luteinizing hormone (LH) surge and allows luteinization through pituitary down-regulation and desensitization followed by conventional doses of stimulation with follicle stimulating hormone (FSH) or human menopausal gonadotrophin (hMG). GnRH antagonist administration causes immediate and dose related inhibition of gonadotropins release by competitive occupancy of the GnRH receptors in the pituitary and hence blocks the ability of native GnRH to initiate the release of FSH and LH from pituitary.[4]

The endometrium undergoes cyclic changes in preparation for implantation, during menstrual cycle. In the follicular phase, the growing follicles produce increasing amounts of estradiol that would induce the proliferative endometrial changes. Following ovulation, the corpus luteum will produce progesterone that will initiate secretory changes. If implantation does not occur, during the window of implantation then the endometrium will shed once the corpus luteum regresses.[5]

In assisted IVF technique, fresh and frozen embryo transfer is performed after endometrial preparation using different protocols. In fresh embryo transfer cycle, the estrogen made by the ovarian follicles helps to prepare the endometrium and additional exogenous progesterone (luteal phase support) administered after oocyte retrieval to enhance implantation.[6] Various types of endometrial priming in frozen embryo transfer includes the following.

Hormone replacement therapy (HRT) cycle FET

In HRT, suppression of follicular growth, endometrial proliferation and subsequent secretory transformation is achieved by the timely administration of exogenous estradiol and progesterone.[7]

Natural cycle FET

Monitoring the time of spontaneous ovulation along with frequent endocrine and transvaginal ultrasound assessment.[8]

Modified natural cycle FET

Initial monitoring is same as natural cycle, ovulation is triggered with hCG once the leading follicle reaches 16-20 mm.[9]

Mild ovarian stimulation cycle FET

Mild ovarian stimulation is administered with oral agents such as clomiphene citrate or letrozole and exogenous gonadotropins used to prime the endometrium for FET cycle.[9]

Embryos were allowed to develop in sterile culture dishes until transfer into the uterus for implantation. There are two types of culture media sequential and single step culture media, Sequential media was introduced in order to meet the changing requirements of embryo development *in vitro* (first in the fallopian tube and then in the uterus), involves a combination of two or three different media designed to meet the nutritional needs of the embryo as it advances in time and consists of first pre-compaction media consists of non-essential amino acid, reduced glucose, pyruvate and lactate, compaction media contains essential and non-essential amino acids, elevated glucose, pyruvate and lactate. Single step medium is designed to meet an embryo’s nutritional needs at all the stages, in which the embryos are cultured in a stable medium containing amino acids, glucose, pyruvate and lactate needed for its development.[10]

Embryo transfer is the most crucial and final step in IVF and intracytoplasmic sperm injection (ICSI) treatment, where the embryo will be transferred to its *in-vivo* environment. Factors that influence the clinical outcome of ART procedure includes: embryo quality, experience of the provider, routine uterine quality evaluation, uterine position, hysteroscopy revision of cervical canal, removal of cervical mucus, trial transfer, bacterial contamination, antibiotics prior to embryo transfer, fluid volume and air in the transfer catheter.[11]

The aim of the present study was to determine the optimum distance of embryo placement from fundus and endometrial thickness during embryo transfer procedure and its influence on clinical pregnancy rate.

METHODS

The retrospective cohort study which evaluates the influence of endometrial thickness and embryo placement distance from fundus during embryo transfer assessed by clinical data from IVF cycles performed at Srushti Fertility Centre and Women’s Hospital, Chennai, for a period between October 2021 and February 2022.

A total of 150 women who underwent fresh and frozen–thawed embryo transfer in IVF cycles were included. The data collected includes women age, number of embryos transferred, endometrial thickness, distance from fundus, and clinical pregnancy outcome. Women aged 18-45 years undergoing fresh and frozen embryo transfer were included and presence of blood on the catheter during the transfer procedure, patient with known endometrial polyp or uterine anomaly were excluded.

Endometrial thickness

Endometrial preparation

Endometrial preparations were performed in both fresh and frozen transfer cycles. In fresh transfer cycle patients were administered with luteal phase support was started within 24-72 hours after oocyte retrieval, progesterone taken 400 mg twice a day until biochemical pregnancy test was performed. For frozen transfer cycles all embryos were vitrified and hormone replacement therapy was carried out in women undergoing frozen embryo transfer. Patients administered with oral estradiol twice a day on day 2 or day 3 of the cycle till day 8. The, dosage was adjusted depending on the endometrial thickness measured by ultrasound on day 8, 9 and 10. Transvaginal ultrasound scans were performed to monitor endometrial development. Progesterone in the form of vaginal progesterone gel (90 mg) was administered to the regimen when the endometrial thickness reaches 8-9 mm. Embryo transfer was scheduled on day 3 or day 5 after progesterone supplementation.

Measuring the endometrial thickness

Endometrial thickness of patients was measured using transabdominal ultrasonography in the midsagittal plane of the uterus from the outer edge of the endometrial-myometrial interface to the outer edge of the widest part of the endometrium. Measurements were carried out prior to embryo transfer and noted in mm (millimeters).

Embryo transfer

The highest quality embryos were selected for transfer, the quality of embryos was assessed based on the cell number, form of blastomeres and the percentage of cytoplasmic fragmentation. Embryo transfer were performed on day 3 or day 5.

Patient preparation

Patients were recommended to visit with semi full bladder. Ultrasound monitoring was performed using transabdominal imaging with probe. The patient was placed in lithotomy position on the bed with full bladder. The bladder was filled with the intention to both enhanced imaging of the uterus and endometrial lining and to straighten any uterine flexion that may be present. The cervix was exposed to a bivalve speculum inserted in the vagina followed by vaginal cleansing with saline and cervical mucus was removed using sterile cotton swab.

Catheter preparation

The ET catheter was flushed with culture media and the embryos were loaded in the following manner: 70 µl of culture media, 5 µl of air space, 10-15 µl of culture media containing the embryos, 5 µl of air space, followed by 10 µl of culture media.

Transfer procedure

Embryo transfer was carried out with transabdominal ultrasound guidance. A maximum of 2 or 3 embryos were transferred. The transfer was performed with Labotech ET catheter or Cook catheter. The outer catheter was first inserted in the cervical canal. Once the position of the catheter was confirmed, the inner catheter loaded with embryos was introduced through the outer catheter under sonographic guidance towards the endometrial cavity and the medium containing embryos were gently released inside the endometrial cavity. The air bubbles were visualized on the screen post transfer. The inner catheter was slowly withdrawn followed by the outer catheter and both the catheters were flushed with culture medium and checked for embryos if retained. Both fresh and frozen embryo transfer were carried out.

Measuring the embryo placement distance from fundus

Embryo placement distance from the fundus was measured. When more than one air bubble was observed the one closest to the fundus was considered for measurement. The distance from the lead position of the air bubble to the uterine fundus was measured under transabdominal ultrasonography and reported in mm (millimeters).

Statistical analysis

For the analysis, the subjects were divided into four groups depending on the embryo placement distance from the fundus: group 1: less than 10 mm, group 2: more than 10 mm and less than 15 mm, group 3: more than 15 mm and less than 20 mm, group 4: more than 20 mm. The subjects were divided into two groups depending on the endometrial thickness group A: 6 mm to 10 mm and group B: 10 mm to 14 mm.

The collected data were analysed using IBM statistical package for the social sciences (SPSS) statistics for Windows, version 23.0 (Armonk, NY: IBM Corp). To illustrate the data descriptive statistical frequency analysis, percentage analysis was used for categorical variables and for continuous variables, the outcomes were expressed in mean and standard deviation (SD). The unpaired sample t-test were used to find the significance difference between the bivariate samples in independent groups. One-way analysis of variance (ANOVA) test was used to find the significance difference between more than two groups in independent groups. Chi-square test was used to find the significance in categorical data. In all the above statistical tools the probability value (p value) 0.05 was considered as statistically significant value.

RESULTS

This study included 150 women who underwent US-guided fresh and frozen IVF-ET. Women were grouped into four groups depending upon their embryo placement distance from the fundus and into two groups depending upon their endometrial thickness. Based on their embryo placement distance from fundus they were grouped 1, 2, 3 and 4 as <10 mm (n=38), 10-15 mm (n=77), 15-20 mm (n=29) and >20 mm (n=6) respectively. And based on their endometrial thickness group A: 6-10 mm (n=46) and group B: 10-14 mm (n=104). As shown in Tables 1 and 2, mean ages of the patients considered were 32.26±4.5. Table 1 summarizes the comparison of embryo placement distance from the fundus in relation to the clinical characteristics of study women, (group 1-4) presented by one-way analysis of variance (ANOVA) test. No significant difference in age (p=0.230) and number of embryos transferred (p=0.598), were observed among groups.

Mean values of endometrial thickness were found to be 9.4±1.9, 10.9±1.2, 10.4±1.3 and 11.2±1.0 for groups 1-4 corresponding to distance from fundus respectively, statistically significant difference was found between endometrial thickness and distance from fundus (p=0.0005). Table 2 summarizes the comparison of endometrial thickness in relation to the clinical characteristics of study women, (group A and B) presented by independent sample t-test. No significance difference in age (p=0.875) and number of embryos transferred (p=0.528) were observed among groups.

Mean values of embryo placement distance from fundus were found to be 10.3±3.9 and 12.9±4.3 for group A and B corresponding to endometrial thickness, statistically significant difference was found between distance from fundus and endometrial thickness (p=0.0005). Among the 150 study women, 89 presented with positive for clinical pregnancy test (59.3%) and 61 were tested negative during clinical pregnancy test (40.7%).

Table 1: Comparison of embryo placement distance from fundus in relation to study parameters.

Parameters	Embryo placement distance from fundus				P value
	Group 1 (<10 mm)	Group 2 (10-15 mm)	Group 3 (15-20 mm)	Group 4 (>20 mm)	
Patients	38	77	29	6	
Age (years)	32.1±3.7	32.5±5.0	31.1±4.1	35.0±4.0	0.230#
Number of embryos transferred	2.3±0.6	2.4±0.6	2.5±0.6	2.2±0.4	0.598#
Endometrial thickness	9.4±1.9	10.9±1.2	10.4±1.3	11.2±1.0	0.0005*

Values are expressed as mean±SD, p<0.05 is considered to be statistically significant, *p value considered as significant, #p value considered as not significant.

Table 2: Comparison of endometrial thickness in relation to study parameters.

Parameters	Endometrial thickness		P value
	Group A (6-10 mm)	Group B (10-14 mm)	
Patients	46	104	_
Age (years)	32.3±4.6	32.2±4.5	0.875#
Number of embryos transferred	2.4±0.6	2.3±0.6	0.528#
Embryo placement distance from fundus	10.3±3.9	12.9±4.3	0.0005*

Values are expressed as mean±SD, p<0.05 is considered to be statistically significant, *p value considered as significant, #p value considered as not significant.

Table 3: Comparison of embryo placement distance from fundus in relation to study parameters.

Embryo placement distance from fundus (mm)	Clinical pregnancy rate (%)		Total (%)
	Positive	Negative	
<10	31.6 (12)	68.4 (26)	100 (38)
10-15	62.3 (48)	37.7 (29)	100 (77)
15-20	82.8 (24)	17.2 (5)	100 (29)
>20	83.3 (5)	16.7 (1)	100 (6)
Total	59.3 (89)	40.7 (61)	100 (150)

Table 4: Comparison of endometrial thickness in relation to study parameters.

Endometrial thickness (mm)	Clinical pregnancy rate		Total
	Positive	Negative	
6-10	43.5 (20)	56.5 (26)	100 (46)
10-14	66.3 (69)	33.7 (35)	100 (104)
Total	59.3 (89)	40.7 (61)	100 (150)

Values are expressed as percentage, in brackets number of study subjects mentioned, p value was found to be 0.009 (p<0.05) statistically significant.

Table 3 and Figure 1 show the comparison of embryo placement distance from fundus with clinical pregnancy rates. The clinical pregnancy rates in group 1 was 12/38 (31.6%), group 2 was 48/77 (62.3%), group 3 was 24/29 (82.8%) and group 4 was 5/6 (83.3%). Embryo placement distance from fundus and clinical pregnancy rate was found to be statistically significant with p value of 0.0001. Clinical pregnancy rates were found to be higher in all three groups compared to group 1.

Table 4 and Figure 2 show the comparison of endometrial thickness with clinical pregnancy rates, the frequency of clinical pregnancy rates in group A was 20/46 (43.5%) and group B was 69/104 (66.3%). The difference in endometrial thickness and clinical pregnancy rates were found to be significant with p=0.009. Clinical pregnancy rates were found to be comparatively higher in group B than in group A.

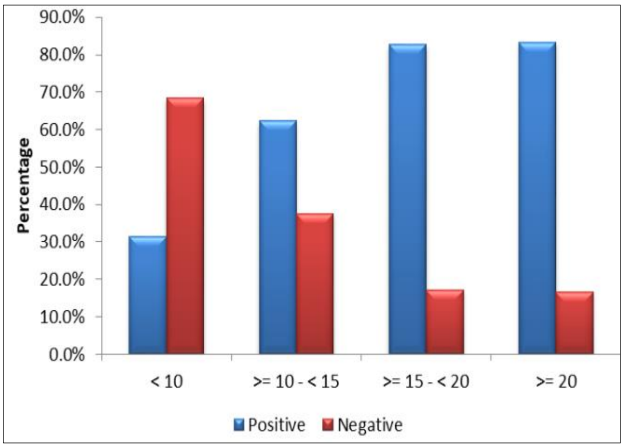

Figure 1: Comparison of embryo placement distance from fundus with clinical pregnancy rate.

Values are expressed as percentage.

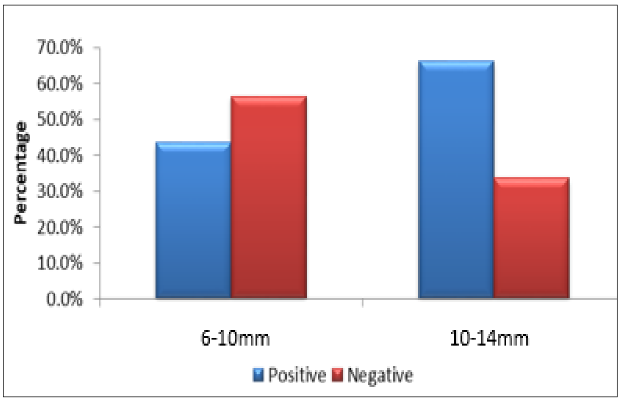

Figure 2: Comparison of endometrial thickness with clinical pregnancy rates.

Values are expressed as percentage.

DISCUSSION

In this present study considering both fresh and frozen IVF-ET cycles, the effect of embryo placement distance from fundus and thickness of the endometrium during embryo transfer treatment procedure, the outcomes were investigated. The results indicated that optimum embryo placement distance from fundus and thickness of the endometrium can enhance the IVF outcomes. Thus, the goal of ET was to successfully transfer the embryos atraumatically to the preferred location in the uterine cavity to maximize the clinical pregnancy rates. Wang et al in a similar study on investigating ideal transfer depth and endometrial thickness suggested that no significance difference in age, other basic information on menstrual cycle, number of oocytes retrieved and number of embryos transferred were found among the groups.[2] As shown in Tables 1 and 2 this study also showed similar results on comparing the embryo placement distance from fundus and endometrial thickness among groups with clinical characterestics of the study subjects revealed that no significance difference in age, number of embryos transferred, with thickness of endometrium and distance from fundus were detected.

As illustrated in Table 3 and Figure 1 clinical pregnancy rates was evaluated in four different groups based on the embryo placement distance from fundus and estimated that clinical pregnancy rates were higher in groups where embryos were placed more than 10 mm in the uterine fundus and maximum where reported at a distance more than 15 mm. Patient underwent ET at a distance of more than 20 mm from the fundal endometrial surface was very small compared with the embryo transfers at other distances. Negative clinical pregnancy rates were found to be higher in group 1 where embryos placed at a distance of less than 10 mm. Thus, it was concluded that embryo placement at a distance of between 10 mm and 20 mm may be the best site of embry transfer to achieve higher clinical pregnancy rates and better implantation that leads to success of IVF treatment. Yarali et al concluded that pregnancy rates and ongoing pregnancy rates where higher if embryos where placed at a distance of more than 10 mm from the fundal endometrial surface, from their discussion based on the number of embryos transferred in each group they deduced that the distance of >10 mm to <20 mm is the appropriate site for embryo transfer procedure.[12] Pope et al in their study suggested that after controlling the potential confounders, the clinical pregnancy rates were significantly influenced by the transfer distance from the fundus, US guided ET also found that pregnancy rates were higher when catheter containing embryos was placed >5 mm from the fundus.[13] Findings of this study were concordant with the literature with regard to the influence of depth of embryo replacement into the uterine cavity concluded that the optimal distance between fundal endometrial surface and the tip of the inner catheter ws found to be 1.5–2 cm.[14] Tanksale et al in a study reported that clinical pregnancy rates were significantly higher in groups where the transfer distance was between 10-20 mm compared to other groups , the results were similar to our analysis.[15]

As shown in Table 4 and Figure 2, the clinical pregnancy rates evaluated in two groups based on the endometrial thickness and estimated that clinical pregnancy rates were found to be slightly higher in group B where the thickness of the endometrium was between 10 and 14 mm. Negative clinical pregnancy rates were found to be higher in group A where endometrial thickness was between 6 mm and 10 mm. Due to smaller sample size and subjects under 7 mm were very less, they could not be considered as a group. Thus it can be concluded that clinical pregnancy rates can be improved when embryos are implanted at endometrial thickness of more than 10 mm. Similar studies that considered endometrial thickness as an influencing factor to achieve better clinical pregnancy rates suggest that endometrial thickness of 7-12 mm might lead to the most promising treatment outcomes in IVF-ET, and is also recommended that endometrial thickness of more than 7 mm is necessary to get ideal clinical pregnancy outcomes.[2] Aydin et al in their study concluded that measurement of endometrial thickness is performed in ART and there are conflicting results regarding the association of endometrial thickness in IVF and ICSI outcomes. They showed the comparison of fresh and frozen found that endometrial thickness less than 7 mm was subjected to lower clinical pregnancy rates, thickness of the endometrium more than 7 mm is optimal for ideal embryo transfer procedure.[16] Holden et al conducted a study with comparitively larger sample size concluded that thicker endometrial lining were associated with increased clinical pregnancy rates.[17]

Limitations

The limitations of this study includes, as the data were collected manually and it was a retrospective study which might inroduce errors. Smaller sample size was another limitation, thus future studies with larger sample size might support the conclusion. Major limitation of this study was due to limited availability of data only few variables were assessed.

CONCLUSION

In conclusion, ideal embryo placement distance from fundus and endometrial thickness is considered as significant variables in influencing IVF-ET and indicated to improve the success of pregnancy rates. This study presents that higher clinical pregnancy rates are obtained when the embryos are selectively placed at a distance between 10 mm and 20 mm from the fundal endometrial surface. Endometrial thickness more than 10 mm is said to be a reasonable parameter for determining the treatment success in ET. Similar studies have reported that optimum endometrial thickness is 7 mm to 12 mm with higher pregnancy rates. The final position of air bubble and endometrial thickness determined by US imaging is used as an identifier during ET. Thus, this study demonstrates that site of embryo transfer and thickness of endometrium during embryo transfer has significant difference on reproductive outcome.

ACKNOWLEDGMENTS

Authors would like to thank the management and staffs of Asia Pacific Institute of Embryology, Mysore (affiliated to University of Mysore), Srushti Fertility Centre and Women's Hospital, Chennai and Pearl Singapore Fertility Centre and Research Institute, Chennai, India for their help and support.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Selvamani D, Gurumoortthy M, Anandaraj A, Elanchezhian M. Influence of endometrial thickness and embryo placement distance from fundus in in-vitro fertilization treatment. Int J Reprod Contracept Obstet Gynecol 2022;11:1956-62.