

Correlation of Site of Embryo Transfer with IVF Outcome: Analysis of 743 Cycles from a Single Center

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ABSTRACT

Objective: To investigate the influence of site of embryo transfer (ET) on reproductive outcome. **Materials and Methods:** A retrospective analysis of 743 ultrasound-guided ET in fresh *in vitro* fertilization (IVF) cycles from a single center over a period of 4 years was conducted. The distance between the fundal endometrial surface and the air bubble was measured, and accordingly, patients were divided into four groups (≤ 10 mm; >10 and ≤ 15 mm; >15 and 20 mm; >20 and <25 mm). **Setting:** Tertiary Assisted Reproductive Technology (ART) center. **Patient(s):** All patients enrolled in the IVF program undergoing ET. **Intervention(s):** Controlled ovarian hyperstimulation (OS), IVF, and ET. **Main Outcome Measure(s):** Cleavage rate and clinical pregnancy rate. **Result(s):** Clinical pregnancy rate was significantly more in groups 2 and 3 compared to the other groups. Logistic regression analysis showed that one unit increase in embryos transfer will enhance the pregnancy outcome about 3.7 (adjusted odds ratio) times with 95% confidence limits 2.6 to 5.4. Similarly, pregnancy outcome will be 3.1 (95% confidence limits: 1.5–6.4) times higher for distance group >15 and <20 mm compared to less than 10-mm distance group. Ectopic pregnancy rates were similar in all the four groups. **Conclusion:** The present study demonstrates that site of ET has significant difference on reproductive outcome. **KEYWORDS:** Distance of air bubble, embryo transfer, *in vitro* fertilization, site of embryo transfer

INTRODUCTION

Embryo transfer (ET) is one of the most crucial steps in an *in vitro* fertilization (IVF) cycle. There are several factors that play pivotal roles in influencing the pregnancy outcome in IVF cycles by directly influencing the efficiency of ET, such as ultrasound (US) guidance, transfer catheter type, catheter-loading technique, uterine cavity fluid, blood or mucus effects, retained embryos, mock transfer, myometrial contractions, and facility of the procedure.^[1-8] One of the most debatable point in ET is the influence of the exact site of ET in the uterine cavity on clinical pregnancy outcome.^[9-12] It has been estimated that up to 85% of the embryos placed into the uterine cavity fail to implant.^[13] ET is the last and probably least successful step in ART; however, much less effort has been placed on assessing or maximizing ET procedures, and the technique of ET has remained largely unchanged since it was first described.^[14]

The use of US for ET was first described by Strickler *et al.* in 1985, who found US guidance easier and associated with less catheter distortion than ET without US guidance. US guided ET significantly increases the chance of live birth and ongoing and clinical pregnancy rates (PRs) compared with the clinical touch method.^[15] Since then, several investigators have described an overall trend toward higher PRs with US. It has been traditionally accepted that the embryo should be placed 10 mm below the fundal endometrial surface.^[16] Some authors have suggested that placing embryos rather lower in the uterine cavity may improve pregnancy rate.^[17] On the

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other hand, some investigators showed a significant increase in pregnancy rate and implantation rate where there was a distance >10 <15 mm between the tip of the catheter and uterine fundus.^[11] Extensive research has been done on different aspects of IVF cycle, like-patient selection, age, factor of infertility, stimulation protocol, ovum pickup, number of oocytes retrieved, fertilization rate, and others, but very few studies have been conducted regarding the impact of ET compared with the many other facets of IVF. Therefore, based on the aforementioned data, this retrospective cohort analysis was undertaken with the primary objective to investigate the influence of ET distance on clinical pregnancy, implantation, and ectopic PRs.

MATERIALS AND METHODS

A retrospective, database-searched cohort study was conducted. Data were extracted from the database of the IVF center of a tertiary referral hospital from Jan 2009 to May 2014. The data collected included age, factor for infertility, type of infertility, dosage of gonadotropin, baseline follicle-stimulating hormone (FSH) levels, luteinizing hormone (LH) levels, anti-müllerian hormone (AMH) ovarian stimulation protocol, days of stimulation, number of oocytes retrieved, number of embryo formed, fertilization rate, cleavage rate, distance of ET, the clinical pregnancy rate, implantation rate, and number of ectopic pregnancy.

CONTROLLED OVARIAN STIMULATION

During ovarian stimulation, two types of protocol were used namely, long Gonadotropin-releasing hormone (GnRH) agonist and GnRH antagonist protocol were used depending upon the patient age and ovarian reserve.

The patients in agonist group were given Injection Leuprolide acetate 1 mg (Injection Leuprolide; Sun Pharmaceutical Industries Ltd, Mumbai, Maharashtra, India) starting from day 21 of menstruation for 14 days. After downregulation, dose of Leuprolide was reduced to 0.5 mg/day and patients were started on recombinant FSH (Injection Gonal-f, Merck Serono Specialities Pvt. Ltd., Guidonia Montecelio, Italy). The starting dose was between 150 and 225 IU/day depending upon patient's characteristics and was adjusted according to follicular growth as monitored by US

In GnRH antagonist protocol, Cetrotrel acetate (Injection Cetrotide, Aeterna Zentaris, Montreal, Quebec, Canada) 0.25 mg was added on sixth day of menstrual cycle (fixed dose regime). Oocyte retrieval was done under general anesthesia after 32 to 35 h. ET was done between days 2 and 5 depending upon number of good quality embryo. Oocytes were transferred to pre-equilibrated IVF culture medium and stored in a CO₂ incubator at 37°C

temperatures. Fertilization was done either by IVF or intracytoplasmic sperm injection. Fertilization check was done after 16 to 20 h of insemination and the number of pronuclei and polar body assessed.

EMBRYO TRANSFER

Embryos were graded according to the cleavage rate and the amount of fragmentation. Usually two to three (rarely four) embryos were replaced in each patient according to age, indication of IVF, number of previous attempts, and number and quality of embryos available.

All the ETs were done using Cooks catheter under US guidance and the measurement was done by a single person. All patients underwent diagnostic hysteroscopy and Mock ET prior to recruitment for IVF. It helps in deciding the type of catheter (soft/rigid) and ease of transfer.

On the day of ET, patient was laid in lithotomy position with full bladder. Uterus, endometrial cavity, cervix, and cervical canal were visualized using transabdominal US. No anesthesia was used. Under sterile condition, Cusco's speculum (Biochrom AG, Germany) was inserted to expose the cervix. Mucus plugs were aspirated from external ovarian hyperstimulation (OS) and cervical canal flushed with media (modified Earles Balanced Salt solution (EBSS) medium with SSR and Human Albumin serum (HAS) (Baxter (India) Pvt Ltd.) buffered with bicarbonate and Hydroxy ethyl Piperazine Ethanesulphonic Acid (HEPES) (Biochrom AG, Germany)). Outer sheath of the catheter was advanced toward the internal os, under transabdominal sonographic guidance. Once the position of the catheter was confirmed inner sheath of the catheter was loaded with embryos and advanced through the outer sheath under sonographic guidance toward the endometrial cavity and the embryos were pushed inside the endometrial cavity, which were seen as "air bubble", screen was frozen at this step, and slowly inner catheter was withdrawn, outer sheath was kept stable at the internal os. Embryologists checked the inner catheter for any retained embryos, once they confirmed clear catheter, outer sheath was gradually withdrawn. Tip of catheter was checked for presence of any blood or embryos.

Distance of the air bubble from the fundus of the endometrial cavity was measured in the frame frozen on sonography and was classified into four groups. In group 1, embryos were transferred at or within 10 mm of the fundal endometrial surface, at a distance >10 mm but <15 mm from the fundal endometrial surface in group 2, at a distance >15 mm but <20 mm in group 3, and in group 4, embryo were transferred at a distance >20 mm but <25 mm from the fundal endometrial surface.

The patient was kept in this position after freeing of her legs for at least half an hour and then transferred to her bed to remain in supine position for 2 h.

All patients were given luteal phase support by im injection progesterone 100mg/day. Urine pregnancy tests and β HCG levels were done 14 days after ET. US for confirmation of intrauterine pregnancy was done 28 days after ET. Clinical pregnancy was defined as presence of intrauterine gestational sac on US. Ongoing pregnancy was defined as presence of fetal heart rate at about 6 to 8 weeks Period of Gestation (POG).

Statistical analysis

Data were computerized and analyzed using the statistical package SPSS IBM version 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). Descriptive statistics, such as mean, standard deviation, and median values, were computed for baseline characteristics of patients, ovarian stimulation factors, hormonal profile, ET on the day of human chorionic gonadotropin (HCG) trigger, and embryological variables for each distance group. After determining whether the data met the normality assumption, one-way analysis of variance (ANOVA) test followed Bonferroni post hoc test was carried out to test whether the means of continuous variables were significantly different between the groups. Nominal or frequency data were analyzed using Chi-square test or Fishers's exact test as appropriate. Further, logistic regression analysis was performed to decide the significant distance that will enhance the pregnancy rate. Pregnancy outcome was taken a dependent

variable and baseline characteristics such as age, body mass index (BMI), AMH, Day 2 Follicle stimulating hormone (D2FSH), D2LH, stimulation days, endometrial thickness at the time of HCG injection (ETHCG), Estrogen at the time of HCG injection (E2HCG), progesterone at the time of HCG injection (P4HCG), oocytes retrieved, number fertilized, number cleaved, distance (group variable), and number of embryos transferred were taken as independent variables. Backward likelihood ratio procedure was adopted to find out significant variables and adjusted odds ratio with 95% confidence limits was calculated. For the entire statistical tests $P < 0.05$ was considered to be statistically significant.

RESULTS

The four groups in our study were similar to each other with respect to patients' ages [Table 1]; however, the four groups were significantly varied with regard to BMI, as revealed by one-way ANOVA test ($P < 0.001$). The *post hoc* test demonstrated that the BMI of group 1 was significantly ($P < 0.05$) higher than that was observed in groups 3 and 4. Day 3 FSH levels and LH were not shown significant ($P > 0.05$) variation between the groups [Table 1].

Table 2 summarizes ovarian stimulation characteristics such as oocyte retrieval, fertilization rate, cleavage rate, and number of embryos transferred. The total dose of gonadotrophins administered in each group was comparable but the groups were significantly different from each other with regard to mean E2 level in picograms per milliliter measured on the day of HCG

Table 1: Mean (\pm SD) of baseline characteristics by distance group

| Variables | Group 1 (<10 mm) (N = 99; 13.3%) | Group 2 (>10 mm <15 mm) (N = 278; 37.4%) | Group 3 (>15 mm <20 mm) (N = 305; 41%) | Group 4 (>20 mm <25 mm) (N = 61; 8.2%) | P value |
|-------------|-------------------------------------|--|---|---|---------|
| Age (years) | 31.89 \pm 3.21 | 30.59 \pm 4.14 | 31.90 \pm 4.14 | 30.37 \pm 2.60 | NS |
| BMI | 27.57 \pm 3.36 | 26.26 \pm 4.43 | 25.55 \pm 3.25 | 23.9 \pm 2.9 | 0.00 |
| Day 2 FSH | 6.00 \pm 2.04 | 5.85 \pm 2.04 | 6.11 \pm 2.06 | 6.03 \pm 1.52 | 0.697 |
| Day 2 LH | 4.62 \pm 2.44 | 4.86 \pm 5.1 | 4.56 \pm 2.6 | 4.42 \pm 2.2 | 0.749 |

Table 2: Ovarian stimulation characteristics in four groups studied

| Parameter | Group 1 (N = 99; 13.3%) | Group 2 (N = 278; 37.4%) | Group 3 (N = 305; 41%) | Group 4 (N = 61; 8.2%) | P value |
|----------------------------|----------------------------|-----------------------------|---------------------------|---------------------------|--------------|
| Total dose of gonadotropin | 3265 \pm 1175.4 | 3385 \pm 1087.5 | 3451 \pm 1187.6 | 3423 \pm 1232.3 | 0.697 |
| Days of stimulation | 11.08 \pm 1.49 | 10.62 \pm 1.78 | 11.24 \pm 1.56 | 10.76 \pm 1.51 | 0.001 |
| E2 on day of HCG | 3461 \pm 2190.8 | 3140 \pm 2036.8 | 3649 \pm 2160.7 | 4387 \pm 3346.1 | 0.001 |
| ET on day of HCG | 9.2 \pm 1.92 | 9.1 \pm 1.95 | 9.5 \pm 1.81 | 9.1 \pm 1.46 | 0.171 |
| Oocyte retrieved | 8.5 \pm 4.0 | 9.35 \pm 6.0 | 9.4 \pm 5.2 | 11.6 \pm 6.6 | 0.001 |
| Embryo transfer | 2.29 \pm 0.90 | 2.68 \pm 0.71 | 2.76 \pm 0.72 | 2.48 \pm 0.95 | 0.001 |
| Fertilization rate | 70.8 | 69.2 | 67.9 | 69 | 0.563 |
| Cleavage rate | 93.1 | 91.6 | 91.2 | 91.6 | 0.671 |

injection (hCG, Ovitrelle; Merck Serono, Italy) ($P < 0.001$) [Table 2]. The groups were also different from each other with regard to days of stimulation ($P < 0.001$) but were comparable with regard to endometrial thickness. The groups were varied significantly ($P < 0.001$) with regard to the mean number of oocytes retrieved during oocyte pick-up [Table 2]. The post hoc test revealed that the mean number of oocytes retrieved in groups 4 was significantly ($P < 0.05$) higher compared to the other groups.

The mean number of embryos transferred in each group showed a significant variation ($P < 0.001$) between the four study groups [Table 2]. *Post hoc* analysis by Bonferroni test showed that the mean number of embryos transferred in groups 2 and 3 were significantly ($P < 0.05$) higher compared with the mean values of group 1.

The clinical PRs were significantly more in groups 2 and 3 (27 and 33%, respectively) as compared to groups 1 and 4. Fitting of logistic regression equation was found to be adequate (Chi-square = 164.2; $P < 0.001$) with three significant variables, namely, age, number of embryos transferred, and distance group. One unit increase in embryos transfer will enhance the pregnancy outcome about 3.7 (adjusted odds ratio) times with 95% confidence limits 2.6 to 5.4. Similarly, pregnancy outcome will be 3.1 (95% confidence limits: 1.5–6.4) times higher for distance group >15 and < 20 mm compared to less than 10 mm distance group.

In the entire series of 743 ETs, 16 (2.2%) ectopic pregnancies occurred [Table 3] but the ectopic pregnancy rate was not shown significant variation between the four groups.

DISCUSSION

The goal of ET is to successfully deliver the embryos atraumatically to the desired location in the uterine cavity to maximize implantation. Although accepted as the easiest part of the entire procedure by most reproductive endocrinologists, ET is obviously the rate-limiting step for ART and should be undertaken with utmost precaution.

Various studies have been carried out analyzing various aspects of ET procedure and factors affecting and influencing it. Several researchers suggest higher PRs with ET lower in the uterine cavity,^[18,19] whereas other

investigators report better success with ET higher in the cavity^[20] closer to the uterine fundus lead to higher PRs,^[21,22] whereas study by Franco *et al.*^[12] claimed that this question was of no importance as the site of ET did not influence implantation rates.

Therefore, the question regarding the best site for ET for higher implantation and success rates remains unresolved.

Till date, the largest retrospective study^[21] done in 5055 cycles depicted that PRs and ongoing PRs were higher if the embryos are replaced at a distance >10 mm from the fundal endometrial surface.

Further, a prospective cohort study comparing IVF outcome after upper uterine cavity vs. lower-to-middle uterine cavity ETs showed significantly better results when the embryos were released in the middle of the uterine cavity, approximately from 15 to 20 mm from the fundal endometrium^[22].

In a recent study by Peter Kovacs *et al.*,^[23] to evaluate whether transfer depth, assessed by air bubble location after ET is associated with clinical outcome concluded that transfer depth does not affect implantation and PRs when the ET is in the middle or upper third of the uterus.

The data in our study seem to show a significant increase in implantation rate and pregnancy rate in groups 2 and 3, where there was a distance 1.0 to 1.5 and 1.5 to 2 cm, respectively, between the air bubble and the uterine fundus. But in these groups, more number of embryos were transferred as compared to groups 1 and 4. Therefore, higher PRs in groups 2 3 might be due to contributing factor of higher number of embryos transferred compared to the other groups

In our study, number of ETs at a distance <10 mm from the fundal endometrial surface (group 1) was very small compared with the ETs at other distances. This was obviously the effect of previously mentioned studies, as most studies investigating the best site for ET suggested that ET high in the endometrial cavity near the fundal endometrial surface were associated with lower PRs.

The lower PRs found in the group transfers directed toward the uterine fundus may be a result of more traumatic transfers, resulting in more frequent uterine contractions, negatively affecting PRs

Table 3: Pregnancy rates and outcome of gestation in the four groups studied

| Variable | Group 1 (N = 99; 13.3%) | Group 2 (N = 278; 37.4%) | Group 3 (N = 305; 41%) | Group 4 (N = 61; 8.2%) | P value |
|-------------------------|----------------------------|-----------------------------|---------------------------|---------------------------|---------|
| Clinical pregnancy rate | 12.1 (12/99) | 27.3 (76/278) | 33.4 (102/305) | 11.5 (7/61) | 0.001 |
| Ectopic pregnancy rate | 1.5 | 2.6 | 2.2 | 1.5 | 0.884 |



Figure 1: Embryo transfer is enhanced by the use of ultrasound guidance to place the embryos at the optimal uterine location. The small hyperechoic areas distal to the catheter tip represent microbubbles of air expelled from the transfer pipette and serve to visualize embryo placement. (Image courtesy of Dr Roger Stronell.) Reference: Mansour, R. T. and M. A. Aboulghar (2002). "Optimizing the embryo transfer technique." *Hum Reprod* 17(5): 1149-53.



Figure 2: Abdominal Ultrasound showing Embryo transfer catheter and presence of air bubble at the fundus. Reference: Woolcott R, Stanger J. Potentially important variables identified by transvaginal ultrasound-guided embryo transfer. *Hum Reprod*,1997;12(5):963-6.

Cochrane reviews and randomized trials have shown that significantly higher PRs are obtained when ET is performed under US guidance and the embryos are deposited in the middle part of the uterine cavity.^[24] In our study, all ETs were done under US guidance.

Previous studies have reported an increased risk of ectopic pregnancies in cases of transfers close to the uterine fundus.^[2,25] However, in the present study, difference in the number of ectopic pregnancy among four groups was nonsignificant Figure 1.

As the embryo cannot be visualized by US, most studies use the location of the air bubble deposited with the embryo at the time of ET as a surrogate marker for embryo location. Woolcott and Stanger found that in

94% of cases the air bubble did not move from its immediate after-ET position.^[26] Baba *et al.*, using three-dimensional US, found that 81% of the pregnancies implanted where the embryos were transferred.^[27] So it can be interpreted that literature is not uniform regarding this issue as Confino *et al.* reported random bubble movement following ET, with no movement in only 11% of the cases Figure 2.^[28]

CONCLUSION

Although the position of the air bubble at ET was found to be relevant for PRs, but unfortunately, it is at present not possible to predict and/or control the position of the air bubbles and is dependent on various factors like, the resistance of the plunger, the pressure used to press the plunger, and a possible intrauterine resistance.

We concluded after analysis of 743 cycles that the site of ET (measured by the distance of the air bubble from the fundus) has influence on the reproductive outcome.

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Conflicts of interest

There are no conflicts of interest.

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