

## Evaluation of the Relationship between Air Bubbles Depth and Pregnancy Rate in ICSI Cycles

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### ABSTRACT

**Objective:** to determine the relationship between embryo transfer outcome and the distance between fundal endometrial surface and air bubbles assessed by trans-abdominal ultrasound

**Design:** Prospective (cohort) study

**Setting:** This study was conducted in assisted reproductive technology (ART) unit of Ain Shams University Hospital from April 2015 to October 2016.

**Patient(s):** Eighty two women undergoing ICSI were enrolled in and a written informed consent was obtained from each participant.

**Intervention(s):** no patient received any additional procedure or intervention.

**Outcome measurers:** The primary outcome was biochemical pregnancy rate based on serum beta-hCG level at 14 days after ET. Secondary outcome was clinical pregnancy rate using trans-vaginal US examination at 6 – 8 weeks of amenorrhea to detect the presence of fetal sac & pulsation.

**Results:** Implantation, biochemical and clinical pregnancy rates were significantly higher in distance < 10 mm group than  $\geq$  10 mm group.

**Conclusion:** air bubble used as an identifier of the position of the embryo at ET can be determinative for pregnancy rates. Clinical PRs were higher in cases with air bubbles < 10 mm from fundal endometrial surface.

**Keywords:** embryo transfer, air bubble, ultrasound, ICSI outcome.

### INTRODUCTION

One of critical steps during the process of in vitro fertilization (IVF) is the embryo transfer (ET), as all the preparations during IVF can be ruined by a poorly executed ET procedure<sup>[1]</sup>. The aim should be to meticulously and accurately place embryos within the uterus, to allow for proper implantation and fetal development<sup>[2]</sup>.

Sonographic visualization of a marker air bubble loaded alongside the embryos in the transfer catheter allows visualization of both initial placement of the embryos and their movement just after ET, assuming that the bubble represents the location of the embryos<sup>[3]</sup>.

### AIM OF THE WORK

The aim of this work is to assess the effect of air bubbles localization inside the uterus on embryo transfer outcome in intracytoplasmic sperm injection (ICSI) cycles.

### METHODOLOGY

This study was a prospective (cohort) study conducted in assisted reproductive technology (ART) unit of Ain Shams University Hospital after approval of the research ethical committee from April 2015 to October 2016. Eighty two women undergoing ICSI were enrolled in and a written informed consent was obtained from each participant.

### Sample size justifications:

Sample size was calculated using STATA<sup>®</sup> version 11 program, setting the type-1 error ( $\alpha$ ) at 0.05 and the power ( $1-\beta$ ) at 0.8. Calculation according to values from previous studies produced a minimal sample size of 41 cases for each group. StataCorp. 2001. Statistical Software: Release 7.0. College Station, TX: Stata Corporation.

Inclusion criteria included women in reproductive period (age 18-38 years), infertility due to bilateral tubal block or unexplained infertility programmed for ICSI, BMI < 25 kg/m<sup>2</sup> and FSH level on cycle day 3 of < 12 mIU/mL. Exclusion criteria included previous failed ICSI or IVF attempts, infertility due to male factors, presence of an abnormal uterine cavity due to endometrial polyps, myomas distorting the uterine cavity, Mullerian malformations, endometrial synechiae, etc. (assessed by transvaginal US and/or hystero-graphy), patients with hydrosalpinx or pyosalpinx, patients undergoing ET after cryopreservation, patients with blood present on the catheter during the transfer procedure, patients with difficult transfer or with suspicion of touching the fundus and poor responders.

All the patients were subjected to counseling about all the steps of the study, full medical

history including age, obstetric history, menstrual history, duration of infertility, number of previous ART attempts, physical examination (general including weight and height, abdominal and pelvic) and investigations as LH, FSH & E2 levels on cycle day 3.

#### **Stimulation protocol**

Controlled ovarian hyperstimulation with long luteal GnRH agonist (GnRH-a) down regulation protocol in the form of triptoreline acetate (Decapeptyl 0.1mg/ml- Ipsen, S.P.A, Milan, Italy) subcutaneous daily injections from the mid-luteal phase of the previous menstrual cycle at day 21 onwards till the day of human chorionic gonadotropin administration. Human menopausal gonadotropins daily intramuscular injections were given to enhance ovarian stimulation from day 2 of cycle in the form of (Fostimone, IBSA, Luganon, Switzerland). The starting dose was 225 IU/day (3 ampoules) and doses of gonadotropins were continuously adjusted according to ovarian response. Ovarian response was monitored by transvaginal ultrasonography which was performed every other day from day 8 using (transvaginal probe of 7.5 MHz of Mindray China DP 8800 ultrasound machine) in ART unit. The size and number of the growing follicles were accurately registered in the patients' sheets.

#### **Oocytes retrieval**

Human chorionic gonadotropin ( Choriomon 10000 IU/amp, "IBSA", Switzerland) intramuscular injection was administered when there was at least 3 oocytes measuring  $\geq 18$  mm. Trans-vaginal US-guided oocyte retrieval under general anesthesia was performed 36 hours after HCG injection. The transducer was connected to the ultrasound system. The direction of the guide beam was checked. The puncturing needle (single way catheter Cook medical needle "Bloomington, USA") was connected to an aspiration apparatus (pressure adjusted at 100 – 150 mmHg) attached by fixation ring to the front and rear ends of the vaginal transducer, thereby defining the direction of puncture corresponding to the guide beam on the ultrasound image. The uterus, both ovaries and iliac vessels were identified by the visualization in both planes. The distance between the upper pole of the vagina and the ovary was closely evaluated in each patient to avoid intestinal or vascular interposition.

#### **Oocytes microinjection**

Oocytes were subjected to enzymatic decoronation by hyaluronidase (Fertipro NV, Beernem, Belgium). Each sperm, selected according to their mobility and morphology, was transferred to a drop of polyvinylpyrrolidone (PVP, Irvine Scientific, USA). Fertilization of the oocytes was done by microinjection using micromanipulators mounted on an inverted microscope (Olympus IX71). Each oocyte was then cultured in an incubator (Galaxy, R 170-200P, UK) containing culture medium (Global LOT, Life Global, USA). Fertilization was assessed 16-18 hours after injection of oocytes. Normal fertilization was confirmed when 2 clearly distinct pronuclei were present or presence of 2<sup>nd</sup> polar body. Fertilization rate was calculated as the percentage of transformation of microinjected oocytes into 2 pronuclei.

#### **Embryo transfer and luteal phase support:**

On day 5 after oocytes retrieval, patients were in lithotomy position. The cervix was exposed with a bivalve speculum and then the mucus in the cervical canal was removed by a cotton swab. All ET were performed without any anesthesia or sedation with moderate bladder filling. The embryo transfer catheter used was (Labotect Embryo Transfer Catheter Set, Labotect GmbH, Germany). Labotect is a soft catheter that's less likely to induce endometrial trauma and uterine contractions. Embryo loading was done by using a "three-drop technique". First an air bubble was loaded in the catheter followed by media containing embryos then another air bubble was loaded to bring the total volume to 30  $\mu$ L. Abdominal ultrasound guided embryo transfer using (Shenzen Mindray bio medical electronic model DP 8800 plus) was performed. During transfer, all patients had the upper part of the endometrium thicker on abdominal ultrasonography. The tip of the inner catheter was placed approximately 1.5–2 cm from the fundal endometrial surface. The medium containing the embryos was gently released into the uterine cavity. The distance between the fundal endometrial surface and air bubbles was measured just after withdrawal of ET catheter and all cases were categorized as: Group A: distance < 10 mm. and group B: distance  $\geq 10$  mm.

The catheter was slowly withdrawn and examined by the same embryologist under a stereomicroscope to be sure that there were no retained embryos. After the procedure, the patients were kept supine for approximately 60

min. Progesterone supplement for luteal support was given in the form of (Prontogest, Macryl, Egypt) 400 mg daily vaginal pessaries started 1 day before embryo transfer and continued till a pregnancy test was performed 14 days after embryo transfer (biochemical pregnancy). Clinical pregnancy was assessed using trans-vaginal US examination at 6 – 8 weeks of amenorrhea to detect the presence of fetal sac & embryonic heart pulsations. Implantation rate was measured as the number of gestational sacs found by trans-vaginal US divided by the number of embryos transferred.

**Data management and statistical analysis**

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

**Descriptive statistics:**

1. Mean, Standard deviation ( $\pm$  SD) and range for parametric numerical data
2. Frequency and percentage of non-numerical data.

**Analytical statistics:**

**Student T Test** was used to assess the statistical significance of the difference between two study group means.

**Chi-Square test** was used to examine the relationship between two qualitative variables

P- value: level of significance:

-P>0.05: Non significant (NS).

-P< 0.05: Significant (S).

**RESULTS**

This study included 82 cases undergoing ICSI, the distance between fundal endometrial surface and air bubbles was measured in all cases immediately after withdrawal of ET catheter and they were classified into two groups. Group A included 41 cases with distance < 10 mm. Group B included 41 cases with distance  $\geq$  10 mm.

**Table (1) :** Demographic data in the two studied groups:

Variable	Group A (n=41)	Group B (n= 41)	P value
Age (years)	28.1 $\pm$ 5.06	29.73 $\pm$ 3.84	0.103*
BMI (kg/m <sup>2</sup> )	23.27 $\pm$ 1.28	22.6 $\pm$ 1.56	0.226**
Duration of infertility (years)	4.66 $\pm$ 3.37	5.46 $\pm$ 3.14	0.266*
Cause of infertility :			
PCOS	29 (70.7%)	29 (70.7%)	1.0**
Unexplained	12 (29.3%)	12 (29.3%)	
AFC	14.80 $\pm$ 7.35	13.32 $\pm$ 5.96	0.513*

AFC : antral follicular count, PCOS : polycystic ovarian syndrome

Data presented as mean  $\pm$  SD (standard deviation) or number (percentage)

\*Analysis using student T test for quantitative variables

\*\*Analysis using Chi-square test for qualitative variables

**Table (2):** Hormonal profile in the two studied groups:

Variable	Group A(n=41)	Group B(n= 41)	P value
Day 3 FSH (mIU/ml)	6.97 $\pm$ 2.02	7.02 $\pm$ 1.74	0.906
Day 3 LH (mIU/ml)	11.87 $\pm$ 4.92	13.17 $\pm$ 3.22	0.572
Day 3 E2 (pg/ml)	52.85 $\pm$ 22.02	48.08 $\pm$ 28.83	0.402

Data presented as mean  $\pm$  SD (standard deviation)

Analysis using student T test for quantitative variables.

**Table (3):** Characteristics of ovarian stimulation in the two studied groups:

Variable	Group A(n=41)	Group B (n=41)	P value
Total dose of gonadotrophins (IU)	2313.41 $\pm$ 384.71	2262.20 $\pm$ 683.26	0.677
Days of stimulation	10.56 $\pm$ 1.05	10.27 $\pm$ 0.75	0.159
Endometrial thickness at day of ET(mm)	10.44 $\pm$ 2.83	10.39 $\pm$ 2.5	0.934
No. of oocytes retrieved	8.76 $\pm$ 3.06	9.46 $\pm$ 2.67	0.267

No. of metaphase II oocytes	6.81±2.18	7.09±1.88	0.540
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Data presented as mean ± SD (standard deviation)

**Table (4):** ICSI outcome in the two studied groups:

Outcome	Group A (n=41)	Group B (n=41)	P value
Fertilization Rate	(70.77 %)	(71.53 %)	0.811
Implantation Rate	(21.95 %)	(17.07 %)	0.037
Biochemical pregnancy rate	23 (56.1%)	19 (46.3%)	0.043
Clinical pregnancy rate	18 (43.9%)	14 (34.1%)	0.048

Data presented as number (percentage)

Analysis using Chi-square test

## LIST OF ABBREVIATIONS

**ICSI** :intracytoplasmic sperm injection,**ART**: assisted reproductive techniques,**HCG** :human chorionic gonadotropin,**AFC**: antral follicular count,**BMI** :body mass index, **ET**: embryo transfer,**LH**: lutening hormone,**FSH**: follicular stimulating hormone,**E2**: estradiol PR pregnancy rate,**GnRH**: gonadotropin releasing hormone agonist,**US**: ultrasonographic.

## DISCUSSION

This study included 82 cases undergoing ICSI in assisted reproductive technology unit of Ain Shams University Hospital from April 2015 to October 2016.

In this study, both groups were matched regarding age, BMI, duration, cause of infertility, AFC, day 3 LH, FSH, E2, total dose of gonadotrophins, days of stimulation, endometrial thickness at day of ET, number of oocytes retrieved and number of metaphase II oocytes.

In the current study, there was no significant difference between both groups regarding fertilization rate. Implantation rate was significantly higher in distance < 10 mm group (21.95%) than distance ≥ 10 mm group (17.07%). Biochemical pregnancy rate was significantly higher in distance < 10 mm group (56.1%) than distance ≥ 10 mm group (46.3%). Clinical pregnancy rate was significantly higher in distance < 10 mm group (43.9%) than distance < 10 mm and distance ≥ 10 mm group (34.1%).

These results agree with *Lambers et al.* [4], who reported that the highest pregnancy rates were found when the air bubble ended up closer to the fundus. Pregnancy rates were significantly higher in cases where the position of the air bubbles was in the fundal

half (43.9%) than in the lower half (24.4%) of the endometrial plate.

These results are consistent with *Friedman et al.* [5] who found that the pregnancy rates were significantly higher in the group in which the air bubble was <10 mm from the fundus (62.5%) than 10-20 mm group (42.0%) and > 20 mm group (38.3%) after controlling for age, parity, FSH and frozen transfers, and accounting for repeated cycles per patient.

These results are similar to *Cenksoy et al.* [6] who subdivided US guided fresh ETs according to the distance between fundal endometrial surface and air bubble to < 10 mm, 10 – 20 mm and > 20 mm. The clinical intrauterine PRs were 65.2%, 32.2% and 2.6% in < 10 mm, 10-20 mm and > 20 mm groups respectively. The PR was dramatically reduced in cases with ≥ 10 mm distance between the fundal endometrial surface and the air bubbles.

An explanation for the higher pregnancy rates with air bubbles closer to the fundus may be that the window of implantation is not only a temporal window but also a spatial window: the expression of important factors in implantation differs throughout the endometrium. Therefore, it can be hypothesized that the expression of implantation factors in the fundal endometrium is more optimal for implantation [6].

Studies have also demonstrated that endometrial wavelike activity decreases with progression into the luteal phase, with fundal endometrium demonstrating relative quiescence, possibly enhancing implantation rates. Studies of implantation sites in spontaneous and IVF pregnancies have revealed that gestational sacs most often implant near the uterine fundus compared with middle and lower uterine segment

implantation. Significantly higher endometrial tissue blood flow in the fundus can enhance the ability of blastocysts to implant in this location<sup>[5]</sup>.

In contrast, *Kovacs et al.*<sup>[1]</sup> evaluated transfer depth assessed by air bubble location after ET in relation to pregnancy outcome and reported that air bubble location didn't affect implantation and pregnancy rates when it was in the middle or upper third of the uterus. The proportion of successful and unsuccessful cycles when the embryo was transferred into the upper third (68% of ongoing pregnancies vs 72% of cycles without pregnancy) or middle third of the cavity (32% of ongoing pregnancies vs 28% of cycles without pregnancy) was similar.

In the current study, ET was performed US guided and embryos were transferred at fixed distance (the distance between fundal endometrial surface and the tip of inner catheter was 1.5 – 2 cm). *Kovacs et al.*<sup>[1]</sup> used clinical touch technique for ET. Uterine length, cervical length and endometrial thickness were measured before ET and summed then embryos were transferred to the upper or mid third of cavity.

In another study<sup>[2]</sup>, it was concluded that the optimal distance between fundal endometrial surface and the tip of inner catheter is 1.5–2 cm. Another study found that PRs were higher when the catheter was placed >5 mm from the fundus and for every additional millimeter of placement from the fundus, the odds of clinical pregnancy increased by 11%<sup>[7]</sup>.

## CONCLUSION

From this study, we conclude that the final position of air bubble used as an identifier of the position of the embryo at ET can be determinative for pregnancy rates. Clinical PRs were higher in cases with air bubbles < 10 mm from fundal endometrial surface.

## RECOMMENDATIONS

We recommend that:

Further studies with larger number of groups would be useful to evaluate whether blastocyst morphology parameters such as inner cell mass size, shape and fragmentation, or measures of cell number, blastocyst diameter and blastulation timing could contribute to provide a more precise prediction of pregnancy rate.

The final position of air bubble after ET can be used as a predictive of outcome in ICSI cycles.

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