The effect of air bubble position after blastocyst transfer on pregnancy rates in IVF cycles

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Objective: To investigate the relationship between air bubble position after blastocyst transfer (BT) and pregnancy rates (PRs).
Design: Retrospective cohort study.
Setting: University-based infertility center.
Patient(s): Three hundred fifteen consecutive nondonor BTs by a single provider.
Intervention(s): Catheters were loaded with 25 μL of culture media, 20 μL of air, 25 μL of media containing the blastocysts, 20 μL of air, and a small amount of additional media. The distance from the air bubble to the fundus, as seen on abdominal ultrasound examination, was measured at the time of transfer. Air bubble location was categorized as <10 mm, 10–20 mm, and >20 mm from the fundus.
Main Outcome Measure(s): Clinical pregnancy rate.
Result(s): After controlling for age, parity, FSH and frozen transfers, and accounting for repeated cycles per patient, the PRs for both the >20-mm (38.3%) and the 10–20-mm (42.0%) from the fundus group were significantly reduced compared with the group in which the bubble was <10 mm from the fundus (62.5%).
Conclusion(s): This study is the first to suggest that BT closer to the fundus is associated with higher PR. Although no ectopic pregnancies occurred in the <10-mm group, this outcome should be monitored closely in larger studies. (Fertil Steril® 2011;95:944–7. ©2011 by American Society for Reproductive Medicine.)

Key Words: Blastocyst transfer, air bubble position, pregnancy rates

Since the introduction of ultrasound guidance for ET, practitioners have debated the optimal location for embryo deposition within the uterus. Studies have used various ultrasonographic parameters at the time of transfer to assess optimal transfer technique. Investigators measuring the distance from the catheter tip to the fundus have found higher pregnancy rate (PR) overall when the catheter tip was further away from the fundus (1, 2), with some suggesting that the odds of clinical pregnancy increase by 11% for every additional millimeter the catheter tip is placed further from the fundus (3).

Ultrasonographic guidance at the time of ET allows for visualization of the exact position of the catheter tip and of air bubbles that have been loaded in the transfer catheter. It is common for the ET catheter to be loaded using a three-drop procedure, in which an air bubble separates the drop of medium containing the embryos from a preceding and a following drop of medium (4). These air bubbles in the catheter have been demonstrated to have no adverse effect on PR (5, 6).

Because air bubbles frequently move away from the catheter tip, the ultimate location of air bubbles may serve as a more exact indication of embryo position. Studies have shown that after a patient stands up after ET, >90% of the air bubbles seen on ultrasound examination do not move in the uterus (7). Moreover, the use of three-dimensional ultrasonography has demonstrated that approximately 80% of gestational sacs are found in the location where the air bubbles were seen immediately after ET (8). Despite this apparent correlation between air bubble position and embryo location in the uterus, few studies have addressed the effect of air bubble position on PR. The limited existing literature has yielded contradictory results (9, 10).

Studies investigating optimal embryo position through catheter tip placement or air bubble location have predominantly analyzed IVF cycles with cleavage stage embryos, without significant focus on blastocyst transfer (BT). With more than one quarter of all fresh, nondonor transfers in the United States occurring at the blastocyst stage, BT has become a common practice in IVF (11). It is unknown, however, whether the position of blastocysts after transfer affects implantation rates, and hence PR. Moreover, because blastocysts are larger and BT occurs closer to the window of implantation, one might expect less embryo migration. No study exists exclusively analyzing the relationship of air bubble position after BT and cycle outcome. Thus, the objective of this study was to investigate the relationship between air bubble position after BT and PR.

MATERIALS AND METHODS

All fresh and frozen nondonor BTs performed by a single physician at a university center from January 2006 through March 2008 were analyzed. Data collected included age, parity, serum FSH, number of blastocysts transferred, number of previous assisted reproductive technique (ART) attempts, and cycle outcome, including number of fetal sacs seen on ultrasound examination. Patients were excluded for FSH >20.

The following ovarian stimulation protocols were used: luteal leuprolide acetate down regulation (long), antagonist protocol, and microdose leuprolide acetate (flare), as previously described (12). HCG was administered

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when at least two follicles reached a mean diameter of >17mm. Transvaginal ultrasound-guided oocyte retrieval was performed 35 hours after hCG administration. Patients with three or more eight-cell embryos on day 3 were offered BT.

Sequential culture with P1/Blastocyst Medium (Irvine Scientific, Santa Ana, CA) or Quinn's Advantage Cleavage/Blastocyst Media (SAGE In-Vitro-Fertilization, Trumbull CT) was used. Blastocysts were chosen for transfer based on the definition of the inner cell mass and trophectoderm, as well as the degree of expansion. Remaining blastocysts were cryopreserved using the Menezo two-step protocol with mHTF and 20% SSS (Irvine Scientific, Santa Ana, CA) as the base medium, as previously described (13). Blastocysts were later thawed using the two-step thaw protocol with the same base medium. Blastocysts were considered eligible for transfer if at least 50% of the inner cell mass and trophoblast were intact after thawing.

For the ET, a Tefcat catheter was used (Cook Ob/Gyn, Spencer, IN) with a 1 mL Norm-Ject Tuberkulin syringe (Henke Sass Wolf, Tuttlingen, Germany). Each catheter was loaded via the three-drop procedure, with air bubbles separating the drop of medium containing the blastocysts from two drops of culture media before and after the embryo drop. The catheter was loaded in the following manner: 25 μL of culture media, 20 μL of air, 25 μL of culture media containing the embryos, 20 μL of air, and a small amount of additional culture media.

In preparation for ET, patients were asked to have a moderately full bladder. Patients were positioned in the dorsal lithotomy position. The cervix was visualized with a bivalve speculum and rinsed with culture media. Any mucus in the cervical canal was removed with a sterile cotton-tipped applicator, and the cervix was rinsed again with culture media. All ETs were then performed under abdominal ultrasonographic guidance with a 3.5C 2–5 MHz probe using a Logiq 5 machine (GE Healthcare, Waukesha, WI).

Transfers were performed with the Tefcat catheter preshaped to a specific bend before loading. The donut-shaped stopper on the catheter was positioned, based on the sounding distance of the uterus performed on a previous visit, so that the catheter tip would be 15 mm short of the fundus when the stopper was at the external os at the time of transfer. After the embryos were deposited in the uterus, the distance from the top of the transfer air bubble to the fundus, as seen on abdominal ultrasound, was measured. Air bubble location was later categorized as <10 mm, 10–20 mm, and >20 mm from the fundus. After embryo deposition, the transfer catheter was slowly withdrawn and checked under a microscope to ensure that no embryos were retained.

Serum quantitative β-hCG levels were obtained at 8–10 days after ET. A clinical pregnancy was defined as the presence of a fetal sac visualized by transvaginal ultrasound examination.

One-way ANOVA was used to test for differences in age, FSH, parity, prior IVF cycles, number of embryos transferred, percentage of frozen ET, and implantation rates. Means were compared using Student's *t* test. Using Stata v. 8.2 (STATA Corporation, College Station, TX), Odds ratios (ORs) were calculated using forward stepwise logistic regression to analyze the effect of air bubble position on PR, adjusting for the potential confounders of age, parity, FSH, and cryopreservation status of embryos. We calculated the effect of air bubble position on implantation rates per transfer, adjusting for the potential confounders, using multivariable ordinary least squares regression analysis. Statistical significance was defined as $P<0.05$. Multiple BTs in individual patients were accounted for in the error terms. The study was approved by the Human Subjects Institutional Review Board.

RESULTS

Of 322 consecutive BTs, the air bubble was visualized in 316 transfers, occurring in 204 individual patients. One patient was excluded for an FSH >20; therefore, 315 transfers were analyzed. Fresh cycles accounted for 56.0% of transfers, and 44.0% were frozen ETs. More than half the transfers were in patients undergoing their first or second BT attempts. Fewer than 12% of BTs occurred in patients undergoing more than four attempts. The mean age of patients was 36.1 years.

Number of blastocysts transferred, number of previous attempts, and cryopreservation status of embryos were not found to be statistically significant predictors of PR. Likewise, between the three distance groups, there were no significant differences in age, FSH, parity, previous ART attempts, number of blastocysts transferred, or percentage of frozen ETs (Table 1).

The overall clinical intrauterine PR was 42%. There was no significant difference between PR among fresh cycles (41.0%) and frozen cycles (43.5%) overall, nor within each distance group. After controlling for age, parity, FSH, and frozen transfers, and accounting for repeated cycles per patient, the PR for bubbles >20 mm from the fundus (38.3%) was reduced compared with bubbles <10 mm from the fundus (62.5%; OR, 0.35; 95% confidence interval [CI], 0.135–0.899; $P=0.03$; Table 2). The PR in the 10–20-mm group (42.0%) was also lower (OR, 0.38; 95% CI, 0.153–0.917; $P=0.03$) compared with the <10-mm group. After analyzing implantation rates in a similar manner, implantation rates appeared lower at distances greater than 10 mm from the fundus, approaching statistical significance. The implantation rate for embryos transferred <10 mm from the fundus was 47.9%. Controlling for potential confounders, the implantation rate in the 10–20-mm distance group (30.3%) was decreased ($P=0.056$), as was the implantation rate in the >20-mm distance group (27.9%) ($P=0.055$) compared with the <10-mm group.

There were no ectopic pregnancies in the <10-mm group. There was one tubal pregnancy in the 10–20-mm group, and one tubal and one cervical pregnancy in the >20-mm group, all from fresh transfers.

DISCUSSION

This study is the first to suggest that the position of air bubbles after BT affects PR. In regard to studies analyzing air bubble position after predominantly cleavage-stage ETs, our results are concordant

TABLE 1

Demographics and distance groups.

	< 10 mm	10–20 mm	> 20 mm	*P* value[a]
Age (y)	35.83 ± 2.92	35.84 ± 3.74	36.61 ± 3.75	0.20
FSH (IU/L)	7.43 ± 3.29	6.52 ± 2.63	6.79 ± 2.23	0.20
Parity	0.46 ± 0.72	0.44 ± 0.61	0.47 ± 0.91	0.93
No. previous ART cycles	1.83 ± 1.46	1.62 ± 1.63	1.75 ± 2.08	0.79
No. of blastocysts transferred	1.92 ± 0.58	1.91 ± 0.76	2.11 ± 1.03	0.15
Frozen transfers per distance group (%)	50.0	47.7	36.5	0.13

Note: Values represented as mean ± SD or percentage.
[a] Calculated using one-way ANOVA.

Friedman. Air bubble position and pregnancy rate. Fertil Steril 2011.