The position of transferred air bubbles after embryo transfer is related to pregnancy rate

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Objective: The possibility to visualize the transfer air bubbles is one of the main benefits of ultrasonographic-guided embryo transfer. The objective of this study was to analyze the relation between the position of the air bubbles and pregnancy rates.
Design: Prospective data-analysis.
Setting: University fertility clinic.
Patient(s): IVF and intracytoplasmic sperm injection patients.
Intervention(s): Transabdominal ultrasonographic guidance at embryo transfer.
Main Outcome Measure(s): Pregnancy rate, length endometrial plate, distance catheter to fundus, distance air bubbles to fundus.
Result(s): Analysis of 367 consecutive ultrasonographic-guided embryo transfers following IVF or intracytoplasmic sperm injection treatment. Both absolute and relative position of the air bubbles were significantly closer to the fundus in patients who became pregnant compared with patients who did not become pregnant. When the relative position of the air bubbles was in the fundal half of the endometrial plate pregnancy rates were significantly higher compared with the lower half of the endometrial plate, 43.0% and 24.4%, respectively, $P=.002$. Multiple regression analysis revealed the relative position as an independently associated determinant for pregnancy.
Conclusion(s): The position of the air bubbles after embryo transfer is related to pregnancy rate; the highest pregnancy rates are found when the air bubbles end up closer to the fundus. (Fertil Steril® 2007;88:68–73. ©2007 by American Society for Reproductive Medicine.)

Key Words: Embryo transfer, air bubble position, pregnancy rate, ultrasonographic guidance, IVF

Pregnancy rates following embryo transfer in IVF/intracytoplasmic sperm injection (ICSI) treatment are dependent on multiple factors like embryo quality (1, 2), endometrial receptivity (3), and the technique of the transfer itself (4). The embryo transfer is the final moment in IVF/ICSI that can be influenced by physicians. Therefore, there has been much interest in analyzing and optimizing several aspects of the embryo transfer. One of the main topics in recent studies is the use of transabdominal ultrasonographic guidance during the transfer procedure (5, 6). Although there is no consensus on the effect on pregnancy and implantation rates, there are other important advantages of performing the transfer under ultrasonographic guidance (7). This technique offers the opportunity to visualize the transfer catheter, the air bubbles, the endometrial cavity, and the aspect of the endometrium.

Under ultrasonographic guidance the catheter can be positioned very accurately. A number of studies analyzed the relation between catheter position and pregnancy rates and found that the catheter position does influence pregnancy rates (8–10).

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The transfer catheter is usually loaded using a "three drop technique," in which the drop of medium containing the embryo(s) is/are separated from a preceding and a following drop of medium by an air bubble (11). When standing up directly after embryo transfer, 94.1% of the air bubbles show no movement (12). Of all embryos that implant successfully, 81% does so in the area where the air bubbles were initially seen at embryo transfer (13). Therefore, the air bubbles can be regarded as an indication of the position of the embryos (14).

Surprisingly enough, there are only a few studies analyzing the relation between the air bubble position and pregnancy rates (15–17). The ones that did are contradictive and do not position the catheter at a standardized distance from the fundus. In our clinic we perform embryo transfer at a standardized depth of the transfer catheter according to the findings of Coroleu et al. (8). In this study we analyzed the relation between pregnancy rates and the position of the air bubbles after positioning the tip of the inner catheter 1.5 to 2 cm from the fundus under ultrasonographic guidance.

MATERIALS AND METHODS

All consecutive patients undergoing a fresh embryo transfer after IVF or ICSI treatment during the period November 2004 until February 2005 were included in this prospective

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study. Patients were only included once; if patients had another transfer within this period the first transfer was included. Patients undergoing embryo transfer after cryopreservation were excluded. Oocyte donation cycles were not included in this study.

Stimulation Protocol

Stimulation protocols and IVF procedures were performed as previously described by Roseboom (1) and Goverde (18). In summary, patients <38 years of age or with previous good response in an IVF or ICSI treatment underwent controlled ovarian hyperstimulation with a "long" protocol with GnRH agonist (Decapeptyl [Ferring, Copenhagen, Denmark]) and gonadotropins (Gonal F [Serono, Geneva, Switzerland] or Puregon [Organon, Oss, the Netherlands]). In women >38 years or with a previous poor response a "short" GnRH-agonist protocol was applied.

Ovarian response was monitored by vaginal ultrasonographic and serum estradiol determinations. Human chorionic gonadotrophine (Pregnyl [Organon, Oss, the Netherlands]) 10.000 IU SC was administered, when there was at least one follicle ≥18 mm and three or more follicles ≥16 mm. Ultrasonographic-directed oocyte retrieval was performed 36 hours later.

Embryo transfer was generally executed on day 3 after oocyte retrieval. If only two or fewer embryos were available the transfer was performed on day 2 after oocyte retrieval. In consultation between physician and the couple a maximum of two embryos were transferred.

Embryo Selection and Embryo Transfer

Directly before the transfer procedure, the embryo development and morphology score were determined and the best embryo(s) was/were selected. Each embryo was scored 1 to 4 according to its symmetry and the extent of fragmentation of the blastomeres (11, 19). An optimal quality embryo received a score of 1.

Embryo transfer was performed by one of six experienced physicians. Although ongoing pregnancy rates do vary among the individual physicians, these differences are not statistically significant (20). All embryo transfers were performed under ultrasonographic guidance. Ultrasonographic guidance was performed by one physician, who also performed all ultrasonographic measurements. Patients were instructed to come with moderate bladder filling (last lavatory visit 1.5–2 hours before embryo transfer).

The patient was positioned in the lithotomy position and the cervix was exposed using a bivalve speculum. The mucus in the cervical canal was removed with a cotton swab. The outer catheter of the Cook catheter (K-JETS-7019-SIVF/Sydney IVF® Embryo Transfer Set, Cook Ireland Ltd, Limerick, Ireland) was positioned under the guidance of abdominal ultrasonography. Then the inner catheter was loaded with the embryo(s) by a "three-drop technique" (11), in which the drop of medium containing the embryo(s) is separated from a preceding and a following drop of medium by a bubble of air, and is inserted through the outer catheter. Both air bubble and droplet volume do not exceed 10 μL.

The tip of the inner catheter was placed 1.5 to 2 cm from the fundal myometrium–endometrial interface as measured by ultrasonography. Then the embryo(s) was/were slowly released into the uterine cavity, and the distance between fundal myometrium–endometrial interface and the transfer air bubbles was measured. The catheter was slowly removed and checked under a stereomicroscope to ensure that there were no retained embryos.

During the study we noticed that on abdominal ultrasonography in all patients the upper part of the endometrium was clearly thicker. This was also previously described by Prapas et al. (21). We called this the endometrial plate (Fig. 1A).

In all patients the following was measured (Fig. 1A and B): length of endometrial plate (distance A), distance between fundal myometrium–endometrial interface and the tip of the inner catheter (distance B), and distance between fundal myometrium–endometrial interface and air bubbles (distance C). The distance between the tip of the catheter and the transfer air bubbles (distance D) was calculated by subtracting distance C from distance B (Fig. 1B). The relative position of the air bubbles with regard to the length of the endometrial plate was calculated by dividing the distance between the air bubble and the fundal myometrium–endometrial interface by the length of the endometrial plate (CA ratio). The relative position of the air bubbles was calculated in relation to the endometrial plate because this feature can be measured in most patients, whereas it is more difficult to measure the whole length of the uterus by abdominal ultrasonography.

Data were completed with information obtained from patient records.

The intraobserver variation was monitored using Bland-Altman plots (22). Measurements on 12 patients were carried out three times using blinded procedures. All observed differences fell within the acceptability zone.

Outcome

A serum pregnancy test was performed 14–16 days after oocyte retrieval. Pregnancies were monitored by transvaginal ultrasonography at 6, 9, and 12 weeks gestational age. An ongoing pregnancy was defined as an intrauterine pregnancy with fetal cardiac activity 70 days after oocyte retrieval.

Statistical Analysis

Statistical analysis was performed using SPSS 11.5 software for Windows (SPSS Inc. Chicago, IL). Data were analyzed using an unpaired t test or χ^2-analysis and binary logistic