Article

# Implantation sites after embryo transfer into the central area of the uterine cavity

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## Abstract

A total of 63 pregnancies (47 singleton, 15 twin, 1 triplet) from intracytoplasmic sperm injection cycles were analysed. In all embryo transfers, the catheter was introduced into the endometrial cavity guided by abdominal ultrasound, with the catheter tip placed at the middle point of the endometrial cavity. Gestational sacs (GS) were located 21–24 days after transfer (gestational age = 5 weeks) by two-dimensional and three-dimensional transvaginal ultrasound. The uterine cavity was divided into three parts: upper, middle and lower. Furthermore, the upper region was subdivided into right, middle and left areas, and the middle region was subdivided into right and left areas. The frequency of gestational sacs in each area was evaluated. In singleton pregnancies 66.0% (31/47) of the GS were detected in the upper region, 29.8% (14/47) in the middle region and 4.2% (2/47) in the lower region. In multiple pregnancies (twins and triplet) 45.5% (15/33) of the GS were detected in the upper region, 51.5% (17/33) in the middle region and 3.0% (1/33) in the lower region. In conclusion, the results demonstrate that when embryos are transferred to the central area of the uterine cavity there is an increase in implantation rate in the middle region compared with the rate expected in naturally conceived pregnancies (9–15%).

***Keywords:*** *embryo transfer, gestational sac, implantation site, ultrasound*

## Introduction

Based on surgical uteri or endometrial curettage, it has been reported that the physiological implantation site of a blastocyst is on the endometrium near the centre of the fundus (Hertig and Rock, 1944; Heuser *et al*., 1945). At present, the implantation site can be estimated from the location of the very early gestational sac (GS) by transvaginal ultrasound. Using ultrasound in naturally conceived pregnancies, it has been reported that about 80–90% of the implantations occur in the upper region of the endometrial cavity, 9–15% in the middle region and 1–5% in the lower region (Kinoshita, 1994; Minami *et al*., 2003). Baba *et al*. (2000), who transferred embryos to the midfundal area of the uterine cavity in IVF/intracytoplasmic sperm injection (ICSI) cycles (60 embryo transfers, 22 pregnancies and 32 GS), found 84.4% of the GS in the fundal area and 9.4% in the middle area, and observed a 9.1% (2/22) rate of ectopic pregnancy. However, these authors did not mention the distance between the fundal endometrium and the catheter tip at the time of transfer.

An increase in implantation and pregnancy rates has been reported to occur when embryos are transferred to the central area of the uterine cavity (Coroleu *et al*., 2002; Frankfurter *et al*., 2003, 2004; Oliveira *et al*., 2004; Pope *et al*., 2004), but there are no studies about the implantation site after using this type of transfer.

Based on the above considerations, the objective of the present study was to investigate where human embryos implanted when a standard procedure placing the tip of the

transfer catheter exactly in the central area of the endometrial cavity was used.

## Materials and methods

A total of 47 singleton pregnancies, 15 twin pregnancies and one triplet (GS total: 80) from 63 patients enrolled in the IVF/ICSI programme of the Centre for Human Reproduction Prof. Franco Junior were included in this study. The patients had no history of Caesarean section, uterine fibroids or major uterine anomalies.

Embryos were transferred with a Frydman catheter (Frydman® Classic Catheter 4.5 CCD; Laboratoire CCD, Paris, France) guided by abdominal ultrasound using a 3.5 MHz convex transducer (Aloka SSD-1100; Aloka Co. Ltd, Tokyo, Japan). The same physician performed all transfers and only transfers with clear visualization of the catheter tip upon ultrasound were considered.

The same transfer technique was used for all patients. The catheter was first filled with Irvine P1 transfer medium (Irvine Scientific, Santa Ana, CA, USA) supplemented with 10% of human serum albumin (Irvine Scientific). Next, the transfer medium containing the embryos was loaded into the catheter between air bubbles, and finally more transfer medium was added (maximum total volume: 30 $\mu$l). Before embryo transfer, the distance between the basal layer of the fundal endometrium and the internal ostium of the cervical canal was measured. This parameter was called endometrial cavity length (ECL). In all embryo transfers, the catheter was introduced into the endometrial cavity guided by abdominal ultrasound, with the catheter tip being placed at the half point of the ECL (**Figure 1**).

In all transfers, the medium containing the embryos was gently expelled into the uterine cavity, with an adequate volume being used to permit the ultrasonographic visualization of the transfer inside the uterine cavity, which was also facilitated by the presence of air bubbles between the embryos ('transfer bubbles'). The catheter was immediately and carefully removed after transfer and examined under a stereomicroscope to ensure that all embryos had been transferred.

GS were located 21–24 days after transfer (gestational age = 5 weeks) by the same physician. Two-dimensional transvaginal ultrasound was applied to measure the distance from the central area of the GS to the basal layer of the fundal endometrium (longitudinal view) and to basal layer of the right wall and the left wall of the endometrial cavity (transverse view). The three-dimensional transvaginal ultrasound was also applied for GS localization.

For localization, the uterine cavity was divided into three regions: upper, middle and lower. Furthermore, the upper region was subdivided into right, middle and left areas, and the middle region was subdivided into right and left areas, making a total of 6 areas (**Figures 2** and **3**).

When the GS was located on a borderline, it was considered to be in the area in which more than half of it resided. If GS was located between two transverse areas (of the upper or middle region), the transverse diameter of the GS was measured and and it was determined in which area more than half the GS diameter was located. If the GS was situated between different regions (upper, middle or lower), the process was the same: the longitudinal diameter of the GS was measured and it was determined in which region (and, of course, also in which area) more than half of the GS diameter was located. It is important to point out that this situation was very infrequent, being observed in only two GS.

The frequency of GS in each area and the miscarriage rates were evaluated. Statistical analysis was performed using the InStat 3.0 program for MacIntosh (GraphPad Software, San Diego, CA, USA). The Fisher exact test was used, with the level of significance set at $P < 0.05$.

## Results

The general characteristics of the study population are summarized in **Table 1**.

### Singleton pregnancies

GS were found in the upper region in 66.0% of cases (31/47), in the middle region in 29.8% of cases (14/47), and in the lower region in 4.2% of cases (2/47). When the frequency of GS detection was compared among the upper three areas, a preponderance of implantation in the middle area was observed. However, no difference was observed between the two middle areas. Miscarriages (until the twelfth week of gestation) occurred in 12 cases (25.5%), with no difference among the upper, middle and lower areas. **Figure 2** shows the frequency of GS in each area and the miscarriage rate.

### Multiple pregnancies

In 45.5% of cases (15/33) GS were detected in the upper region, with 51.5% (17/33) in the middle region and 3.0% (1/33) in the lower region. Regarding the distribution of twin pregnancies, both GS were in the upper region in 13.3% (2/15) of cases, both GS were in the middle region in 26.7% (4/15) of cases, one GS was in the upper region and the other was in the middle region in 53.3% (8/15) of cases, and 1 GS was in the middle region and the other was in lower region in only one case (6.7%, 1/15). Regarding the triplet pregnancy, two GS were in the upper region and one GS was in the middle region. There was no difference in the frequency of GS detection among the three upper areas or between the two middle areas. Only in one twin pregnancy were both GS found in the right middle area. In the triplet pregnancy, two GS were seen in the upper right area. There were only two miscarriages (four GS, all twins). **Figure 3** shows the frequency of GS in each area and the miscarriage rate.

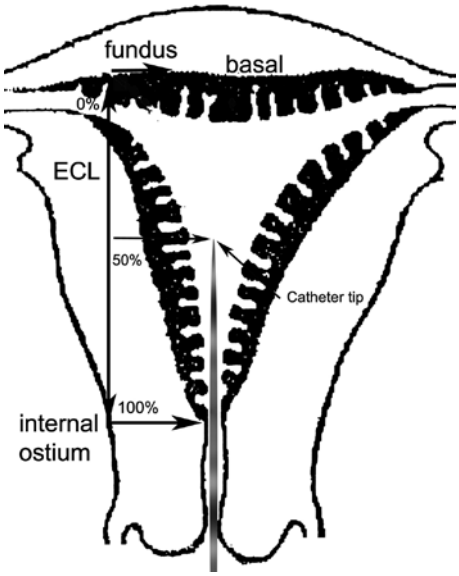


**Figure 1.** Schematic presentation of the measured endometrial cavity length (ECL) and the point of where the catheter tips were placed.

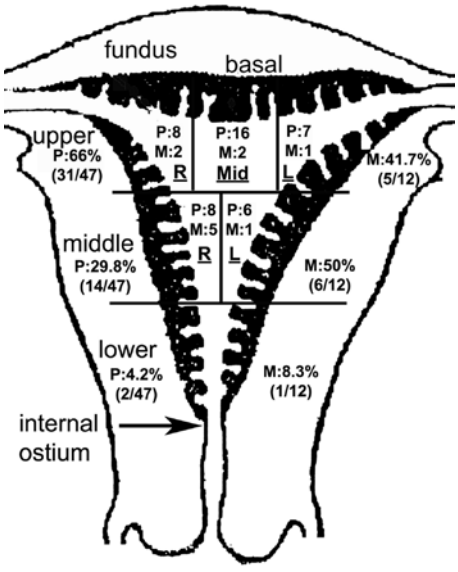


**Figure 2.** Singleton pregnancies: location of the 47 gestational sacs (GS) after embryo transfer with the catheter tip placed at the midpoint of the endometrial cavity. Frequency of GS detection in each area and miscarriage rate: the uterine cavity was divided into three parts: upper, middle, and lower regions. The upper region was subdivided into the right (R), middle (Mid), and left (L) areas, and the middle region was subdivided into the right and left areas. P: pregnancy; M: miscarriage.

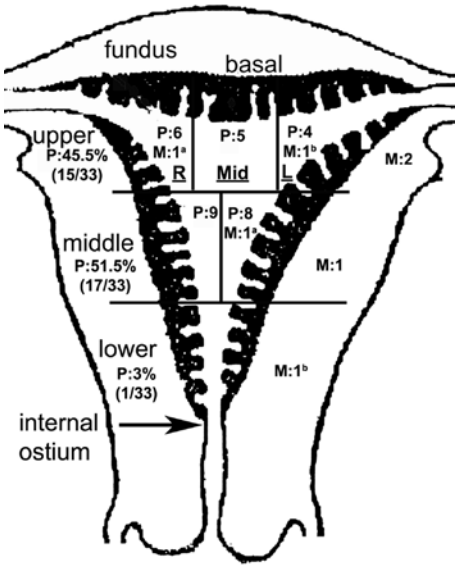


**Figure 3.** Multiple pregnancies: location of the 33 gestational sacs (GS) after embryo transfer with the catheter tips placed at the middle point of the endometrial cavity. Frequency of GS detection in each area and the miscarriage rate: the uterine cavity was divided into three parts: upper, middle, and lower regions. The upper region was subdivided into the right (R), middle (M), and left (L) areas, and the middle region was subdivided into the right and left areas. P: pregnancy; M: miscarriage; [a–b] same twin pregnancy.

**Table 1.** General characteristics of 63 IVF patients after embryo transfer into the central area of the uterine cavity.

| *Characteristic* | *Total* | *Single pregnancy* | *Multiple pregnancy* |
|---|---|---|---|
| No. of patients | 63 | 47 | 16 |
| No. of pregnancies | 63 | 47 | 16 |
| Mean age (range) in years ± SD | | | |
| (22–38) | 34.3 ± 5.5 | | |
| (22–37) | | 34.1 ± 5.4 | |
| (24–38) | | | 34.7 ± 5.9 |
| *Aetiology (%)* | | | |
| Male | 23 (36.5) | 18 (38.3) | 5 (31.3) |
| Idiopathic | 16 (25.4) | 12 (25.5) | 4 (25.0) |
| Tuboperitoneal | 11 (17.5) | 8 (17.0) | 3 (18.8) |
| Endometriosis | 4 (6.3) | 3 (6.4) | 1 (6.3) |
| Male + endometriosis | 4 (6.3) | 3 (6.4) | 1 (6.3) |
| Tuboperitoneal + endometriosis | 4 (6.3) | 3 (6.4) | 1 (6.3) |
| Tuboperitoneal + endometriosis+male | 1 (1.6) | – | 1 (6.3) |
| Miscarriages (%) | 14 (22.2) | 12 (25.5) | 2 (12.5) |

## Discussion

It has been traditionally accepted that embryos should be placed 5–10 mm below the surface of the uterine fundus. However, some investigators have suggested that placing embryos lower in the endometrial cavity more distant from the fundus may improve pregnancy rates in IVF/ICSI cycles. Coroleu *et al.* (2002) concluded that applying the fixed distance of 15–20 mm away from the fundus might optimize the performance of embryo transfers. In their review, Levi Setti *et al.* (2003) stated that the embryos need to be placed in the middle of the cavity, away from the fundus. Frankfurter *et al.* (2003) analysed retrospectively a total of 23 patients who underwent two cycles of ultrasound-guided embryo transfers and the results showed better pregnancy rates when the site of embryo placement was more distant from the uterine fundus. Frankfurter *et al.* (2004), in a prospective study of 666 embryo transfers, detected significantly higher implantation and pregnancy rates for embryo transfers performed in the middle to lower segments of the uterus compared with the upper segment. Pope *et al.* (2004), by performing a multivariate logistic regression analysis on 699 embryo transfers, demonstrated that for every additional millimetre that embryos are deposited away from the fundus, as noted by abdominal ultrasound, the odds of clinical pregnancy increased by 11%. In a randomized study by Franco *et al.* (2004), in which embryos were deposited in the lower or upper half of the endometrial cavity, there was no difference in pregnancy or implantation rates. Oliveira *et al.* (2004) in a prospective study of 400 embryo transfers demonstrated that the site of embryo transfer influences implantation and pregnancy rates and that better results were obtained when the catheter tip was positioned close to the middle area of the endometrial cavity. They concluded that the relative site of embryo deposition is more important than the actual distance from the fundus.

After transfer, it is thought that the embryo is situated between the area in which the tip of the catheter is located and the area in which the air bubbles spread immediately after the transfer (Liedholm *et al.*, 1980; Krampl *et al.*, 1995; Baba *et al.*, 2000). This will probably be the region where the embryo is going to implant. However, a successfully implanted embryo can be found in a different area than expected for different reasons. IVF cycles involve situations in which the endometrium undergoes stimulation that does not occur in the natural process. Even in ultrasound-guided transfers, placement of embryos at higher points might increase the probability of endometrial trauma (Marconi *et al.*, 2003; Murray *et al.*, 2003; Abou-Setta, 2006) and induction of contractions, with potential adverse effects (Liedholm *al.*, 1980; Fanchin *et al.*, 1998; Lesny *et al.*, 1998, 1999; Schoolcraft *et al.*, 2001; Sallam *et al.*, 2002; Bulletti *et al.*, 2005; Abou-Setta, 2006). By positioning the catheter tip close to the midpoint of the endometrial cavity length it is likely that the embryos are transferred to the area that best permits implantation, avoiding lower regions that are inadequate for appropriate nidation and reducing the possibility of endometrial injury and the induction of contractions by minimizing the penetration of the catheter into the endometrial cavity. Nevertheless, there is no study about the influence of this type of transfer on the subsequent implantation site.

Some studies have analysed the implantation site in naturally conceived pregnancies. Kinoshita (1994), using two-dimensional ultrasound, divided the vertical section of uterine cavity into upper, middle and lower regions and examined the detection

sites of GS. Most GS (95.3%) were observed in the upper two-thirds of the uterine cavity (80.8% were in the upper region, 14.5% in the middle region), while only 4.6% were found in the lower one-third (lower region). Minami *et al*. (2003) used three-dimensional transvaginal ultrasound to determine the location of early GS and, as a result, the physiological implantation sites of human embryos. The uterine cavity was divided into three parts: upper, middle and lower regions. The upper region was subdivided into right, middle and left areas, and the middle region was subdivided into right and left areas. Of 138 patients, 123 (89.1%) had GS detected in the upper region, which was found to be the most frequent region; only 13 (9.4%) had GS in the middle region and 2 (1.4%) in the lower region. When the frequency of GS detection was compared among the upper three areas, the right and left upper areas showed a higher frequency than the middle upper area.

The outcomes of this study show that when the catheter tip was positioned in the middle of the endometrial cavity there was an increase in implantation rate in the middle region of the endometrial cavity (single pregnancies: 29.8%) compared with the rate expected in spontaneously conceived singleton gestations (9–15%) (Kinoshita, 1994; Minami *et al*., 2003). Nevertheless, implantation in the upper region still represented the highest incidence (66.0% of singleton pregnancies versus 80–90% in naturally conceived pregnancies). In contrast, no important changes in implantation rate in the lower region of the endometrial cavity were observed (single pregnancies: 4.3%; naturally conceived: 1–5%). When the frequency of GS detection among the upper three areas was compared, in contrast to Minami *et al*. (2003), no difference was observed. The absence of difference was also observed between two middle areas. However, this study intended to be only a descriptive analysis. The absence of a control group represents a limitation indicating that these results should be interpreted with caution. On the other hand, with a power of 80% and a level of significance of 5%, only 57 patients (close to the number of singleton pregnancies analysed) would be required in order to detect a 20% increase (the real increase observed) in the frequency of GS in the middle region when comparing this study to that of Minami *et al*. (2003), who reported the lowest frequency.

The outcome of the embryo transfer procedure depends on a multitude of anatomical, physiological and mechanical aspects. Central placement of the catheter tip and the implantation site were the focus of this study. However, other factors should be mentioned such as the effect of loading air into the catheter, the speed of injection of the transferred embryos, and the posture of the patient at embryo transfer. These factors influence embryo transfer outcome as well as the movement of the embryo in the uterine cavity and thus interfere with the site of embryo implantation independently of the standardized position of the catheter tip during embryo transfer. Although different studies have analysed the correlation between these factors and the implantation and pregnancy rates (Sallam, 2005), there are few observations about its influence on implantation site (Baba *et al*., 2000).

Regarding multiple gestations, a higher than expected occurrence of nidation in lower places in the endometrial cavity may have been influenced by other factors in addition to catheter position. In mammalian species, in which the presence of multiple embryos is more frequent, the allocation of these embryos in the cavity follows a mechanism of placement that regulates the distance among the implantation sites (Abrahamsohon, 1989). Perhaps the same mechanism exists in humans. However, embryo transfer in the mid-fundal area did not show an increase of implantation in lower uterine regions for multiple pregnancies (Baba *et al*., 2000).

As is the case for natural gestations (Minami *et al*., 2003), in this study an increase in the miscarriage rate was observed in lower implantations (middle and lower regions), but without a statistical difference compared to miscarriages in the upper region. The reason was that an increase in miscarriage rate was observed in the upper region (5/31, 16.1%) in this study in comparison with natural gestations (9/123, 7.3%). Variations between the two populations studied may possibly explain that difference (infertile population versus normal population). However, Kinoshita (1994) observed different miscarriage rates in naturally conceived pregnancies; 12.9% (18/139) for the upper region, 28.0% (7/25) for the middle region, and 50.0% (4/8) for the lower region. More data about this are necessary to allow us to draw definitive conclusions.

On the other hand, the overall miscarriage rate observed (22.2%) was similar to that reported in statistical investigations of assisted reproduction [19% (RED LARA, 2003); ~29% (Nyboe Andersen *et al*., 2006)]. In a previous study including only transfer of fresh non-donor embryos, a rate of miscarriage of about 15% was observed when the catheter tip was positioned close to the middle area of the endometrial cavity (Oliveira *et al*., 2004). In addition, in agreement with previous studies that have reported an increased risk of ectopic pregnancies in cases of transfers close to the uterine fundus (Lesny *et al*., 1999; Baba *et al*., 2000; Egbase *et al*., 2000), no ectopic pregnancy was observed in this study, although the number of cases was not sufficient to allow speculation of the significance of this observation. Thus, the increased incidence of implantation in the middle area of the cavity does not seem to have any deleterious effect on the normal evolution of gestation.

In conclusion, when embryos were transferred to the central area of the endometrial cavity there was an increase in implantation rate in the middle region compared with naturally conceived pregnancies. Furthermore, this did not affect the rate of miscarriage and no ectopic pregnancy was observed in this population.

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