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Article

Increased implantation and pregnancy rates obtained by placing the tip of the transfer catheter in the central area of the endometrial cavity

João Batista Alcantara Oliveira was awarded his MD degree in 1985 from the Faculdade de Medicina–Universidade Federal do Rio de Janeiro in Brazil. His speciality degree in Obstetrics and Gynaecology was obtained in the Hospital dos Servidores do Estado do Rio de Janeiro in 1988. MSc and PhD degrees followed in 1992 and 1996 respectively from the Instituto de Ginecologia–Universidade Federal do Rio de Janeiro. He has been involved in the field of human reproduction since 1990 and currently he works as a doctor in the Centro de Reprodução Humana Sinhá Junqueira (Ribeirão Preto- Brazil).

Dr JBA Oliveira

JBA Oliveira[1], AMVC Martins[2], RLR Baruffi[1], AL Mauri[1], CG Petersen[1], V Felipe[1], P Contart[1], A Pontes[3], JG Franco Jr[1,4]

[1]Centre for Human Reproduction 'Sinhá Junqueira', Rua D. Alberto Gonçalves, 1500-CEP 14085-100, Ribeirão Preto, SP-Brazil; [2]Fellow, Department of Gynecology and Obstetrics, Faculty of Medicine of Botucatu, UNESP, Botucatu, SP, Brazil; [3]Department of Gynecology and Obstetrics, Faculty of Medicine of Botucatu, UNESP, Botucatu, SP, Brazil [4]Correspondence: Tel: +55 16 6262909; Fax: +55 16 6283755; e-mail: crh@crh.com.br or franco@crh.com.br

Abstract

The influence of endometrial cavity length (ECL) on implantation and pregnancy rates after 400 embryo transfers was studied prospectively in a population with the indication of IVF/intracytoplasmic sperm injection (ICSI). The tip of the transfer catheter was placed above or below the half point of the ECL in a randomized manner. Two analyses were performed: (i) absolute position (AP); embryo transfers were divided into three groups according to the distance between the end of the fundal endometrial surface and the catheter tip (DTC – distance tip catheter): AP_1 ($n = 212$), 10–15 mm; AP_2 ($n = 158$), 16–20 mm; and AP_3 ($n = 30$), ≥21 mm. (ii) relative position (RP) – embryo transfers were divided into four groups according to their RP [RP = (DTC/ECL) × 100]: RP_1 ($n = 23$), ≤40%; RP_2 ($n = 177$), 41–50%; RP_3 ($n = 117$), 51–60%; and RP_4 ($n = 83$), ≥61%. Analysis based on relative distance revealed significantly higher implantation and pregnancy rates ($P < 0.05$) in more central areas of the ECL. However, analysis based on absolute position did not reveal any difference. In conclusion, the present results demonstrated that implantation and pregnancy rates are influenced by the site of embryo transfer, with better results being obtained when the catheter tip is positioned close to the middle area of the endometrial cavity. In this respect, previous analysis of the ECL is the fundamental step in establishing the ideal site for embryo transfers.

Keywords: *embryo transfer, endometrial cavity length, implantation rates, IVF/ICSI, pregnancy rates*

Introduction

To date, there still is no clear answer to the question regarding the part of the endometrial cavity where embryos should be deposited in order to obtain better implantation and pregnancy rates. Whereas some investigators believe that higher levels in the endometrial cavity closer to the uterine fundus lead to higher rates (Meldrum *et al.*, 1987; Krampl *et al.*, 1995), others have suggested that improved embryo transfer results are obtained when the embryos are placed at lower levels in the uterine cavity (Waterstone *et al.*, 1991; Woolcott and Stanger, 1997; Lesny *et al.*, 1998; Coroleu *et al.*, 2002; Frankfurter *et al.*, 2003, 2004; van de Pas *et al.*, 2003; Pope *at al*. 2004). On the other hand, some authors postulate that the question regarding the site of embryo transfers is of no importance, since it does not influence implantation as long as embryos are placed in the upper half of the cavity (Nazari *et al.*, 1993; Roselund *et al.*, 1996).

When introduced into the uterine cavity, the embryo transfer catheter is usually guided by the manual sensitivity of the clinician or by ultrasound vision. In addition, the location of the embryo transfer catheter in the endometrial cavity is determined on the basis of absolute distance (mm or cm) from a fixed reference point (fundus, internal or external os).

Since various investigators have found a correlation between the site of embryo transfers and implantation and pregnancy outcomes and others have not, such discrepancies may result from the fact that only absolute measurements of the endometrial cavity have been considered.

The objective of the present study was to determine the influence of endometrial cavity length (ECL) on the implantation and pregnancy rates after embryo transfers.

Materials and methods

A total of 400 transfers of 360 patients enrolled in the IVF/intracytoplasmic sperm injection (ICSI) programme of the Centre for Human Reproduction Sinhá Junqueira during the period from August 2001 to October 2003 were included in this prospective study. Transfer cycles of frozen embryos were excluded.

All patients were submitted to the same scheme of ovarian stimulation (Franco Jr *et al*., 2001). First, the patients were down-regulated with nafarelin acetate at a dose of 400 μg/day (Synarel®; Pharmacia, São Paulo, Brazil), started during the second phase of the previous cycle. After 14 days of treatment with the analogue and confirmation of blockade, administration of recombinant FSH (Gonal F®; Serono, Barueri, Brazil) was started at a dose of 150–300 IU depending on the age of the patient, for a period of 7 days. On day 8 of stimulation, follicular development was monitored by 7 MHz transvaginal ultrasound only (Medison Digital Color MT; Medison Co. Ltd, Seoul, Korea) and the FSH doses were adapted according to ovarian response.

On the day of first ultrasound (day 8 of ovarian stimulation), the distance between the basal layer of the fundal endometrium (reference point) and the internal ostium of the cervical canal was measured by transvaginal ultrasound. This parameter was called endometrial cavity length (ECL) (**Figure 1**).

When at least two follicles with a diameter ≥17 mm were observed, human chorionic gonadotrophin (HCG) was administered at the dose of 5000–10,000 IU. Oocytes were retrieved by transvaginal ultrasound-guided puncture 34–36 h after injection of HCG.

ICSI and IVF were performed as previously described (Franco Jr *et al*., 1995; Svalander *et al*., 1995). Embryos were routinely transferred after 48 h in culture and supranumerary embryos were cryopreserved at the end of the second day.

Embryos were then 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).

All transfers were performed by the same physician and only easy transfers (i.e. the catheter passed smoothly through the cervix without the need for uterine fixation clamps) with clear visualization of the catheter tip upon ultrasound were considered for analysis. Patients with a full bladder were placed in the lithotomy position and the cervix was exposed using a bivalve speculum. The exocervix was cleaned and endocervical mucus was removed.

The same transfer technique was maintained 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 μl). The catheter was introduced into the endometrial cavity through the cervix under ultrasound guidance, with the catheter tip being placed above or below the half point of the ECL cavity in a randomized manner by drawing lots using a table previously elaborated. The distance between the basal layer of the fundal endometrium and the catheter tip (DTC – distance tip catheter) was then measured by ultrasound and considered for analysis of the results (**Figure 2**).

In all transfers, the medium containing the embryos was gently expelled into the uterine cavity under ultrasound monitoring, with the volume being sufficient 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 analysed under a stereomicroscope to ensure that all embryos had been transferred. After the procedure, the patient was allowed to rest in bed for 60 min. All patients received luteal phase supplementation with vaginal natural progesterone or additional doses of HCG according to ovarian response.

Pregnancy was diagnosed based on an increase in serum β-HCG concentration 14 days after embryo transfers. Implantation and clinical pregnancy rates were determined based on the presence of a gestational sac accompanied by an image of the embryo/fetal cardiac activity on transvaginal ultrasound 4 weeks after transfer. The frequency of miscarriage and ectopic pregnancy was calculated based on the number of clinical pregnancies found.

Two analyses were performed using the embryo transfer data: (i) Absolute position (AP) (**Figure 2**) – transfers were divided into three groups according to the DTC: group AP_1 (n = 212), DTC 10–15 mm; group AP_2 (n = 158), DTC 16–20 mm; and group AP_3 (n = 30), DTC ≥21 mm. (ii) Relative position (RP) (**Figure 3**) – measurements that express the per cent relationship between DTC and ECL (RP = (DTC/ECL) × 100). Transfers were divided into four groups according to RP: group RP_1 (n = 23), ≤40% of ECL; group RP_2 (n = 177), 41–50% of ECL; group RP_3 (n = 117), 51–60% of ECL; and group RP4 (n = 83), ≥61% of ECL.

The following parameters were evaluated in each group: patient age, aetiology of infertility, number of oocytes retrieved by puncture, number of oocytes in metaphase II retrieved by puncture, fertilization rate, number of embryos transferred, embryo implantation rate, pregnancy rate per transfer, miscarriage rate, rate of ectopic pregnancies, and ongoing pregnancy rate.

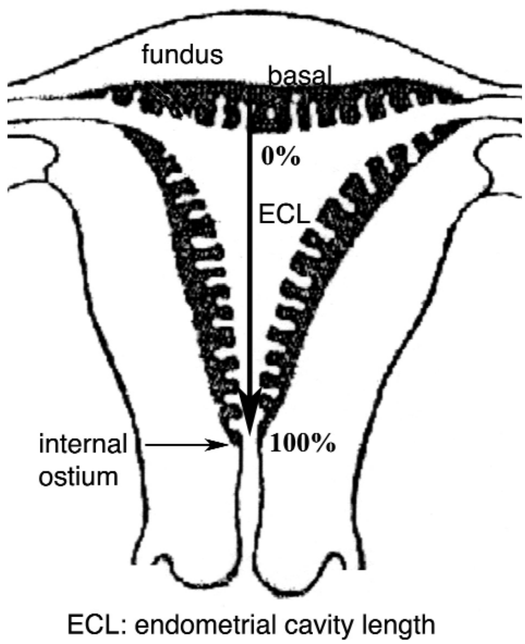

Figure 1. Schematic presentation of the measurement of endometrial cavity length (ECL): distance between the basal layer of the fundal endometrium (reference point) and the internal ostium of the cervical canal.

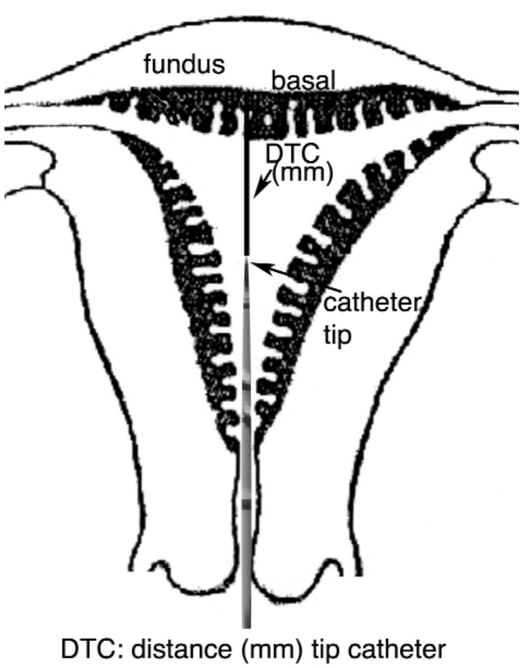

Figure 2. Absolute position (AP) – schematic presentation of the distance between the basal layer of the fundal endometrium and the catheter tip (DTC).

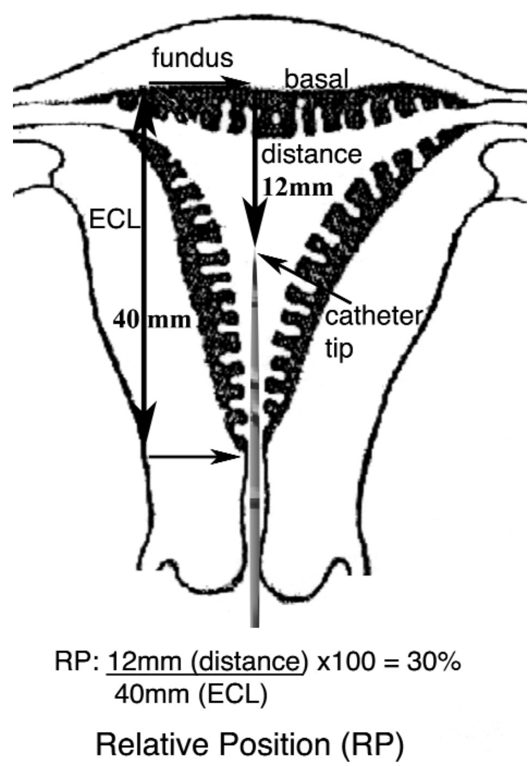

Figure 3. Relative position (RP) – value determined by the ratio between DTC and ECL. Example of a calculation.

Data are reported as means ± SD and were analysed using the InStat 3.0 program for MacIntosh (GraphPad Software, San Diego, CA, USA). Student's *t*-test, the Mann–Whitney test and Fisher's exact test were used when appropriate. The level of significance was set at $P < 0.05$.

Results

Absolute position

Almost all of the 400 transfers (370/400) were observed at a point located 10–20 mm from the uterine fundus, with only a small number (30/400) being situated at a greater distance. None of the transfers occurred at a distance shorter than 10 mm or longer than 34 mm. An equal distribution of the main cycle characteristics was observed for the three groups ($P > 0.05$) (**Table 1**). As expected, a significant difference in DTC was observed between the three groups ($P < 0.001$)

No significant difference in implantation, pregnancy, spontaneous miscarriage, ectopic pregnancy or ongoing pregnancy rates was observed between groups AP_1, AP_2 and AP_3 (**Table 2**).

Relative position

Many of the 400 transfers (294/400) were located between 41 and 60% of the ECL (groups RP_2 and RP_3). Eighty-three transfers occurred at a higher percentage (i.e. more distant from the uterine fundus) and only 23 transfers were observed at a lower percentage of the ECL (closer to the fundus). No transfer was observed at <21% of the ECL or >90.6%. An equal distribution ($P > 0.05$) of the main cycle characteristics was observed for the four groups analysed (**Table 3**). As expected, the position of the catheter tip in relation to ECL differed significantly between groups ($P < 0.001$).

In contrast to the previous analysis, a significant difference in implantation rates was observed between groups, with groups RP_2 (transfer 41–50%) and RP_3 (transfer 51–60%) differing from the other groups ($P < 0.05$). The same trend was observed for clinical pregnancy and ongoing pregnancy rates, but only in comparison to group RP_4 (transfer ≥61%) in which the embryos were placed at a lower point (**Table 4**).

Table 1. Absolute position: general characteristics of the three DTC (distance tip catheter) groups studied.

	Group AP_1 (10–15 mm)	*Group AP_2 (16–20 mm)*	*Group AP_3 (≥21 mm)*
Patients (*n*)	199	154	30
Transfer (*n*)	212	158	30
Age (years)	34.1 ± 4.9	33.7 ± 4.9	35.6 ± 4.1
Aetiology (%)			
Male factor	37.7	37.0	36.7
Idiopathic	25.6	26.0	26.7
Endometriosis	13.6	13.6	13.3
Tubal–peritoneal	11.6	11.7	13.3
Tubal–peritoneal + male	5.0	5.2	6.7
Tubal–peritoneal + endometrial	4.5	4.5	–
Endometrial + male	2.0	1.3	3.3
Endometrial + male + tubo–peritoneal	–	0.7	–
Retrieved oocytes	9.1 ± 5.4	10.2 ± 5.7	11.7 ± 6.5
Oocytes in metaphase II	7.0 ± 4.1	7.7 ± 4.3	9.0 ± 6.3
Fertilization (%)	74.4 ± 20.1	73.4 ± 19.4	67.1 ± 16.5
Embryo transfer (*n*)	2.7 ± 0.9	2.6 ± 0.9	2.9 ± 1.0
DTC (mm)	13.2 ± 1.3[a] (10–15)[b]	17.7 ± 1.2[a] (16–20)[b]	24.3 ± 3.2[a] (21–34)[b]

[a]$P < 0.001$.
[b]Range of values.
It should be noted that the same patient in different cycles might have been included in group AP_1, AP_2 or AP_3.

Table 2. Absolute position in the three DTC groups studied: clinical results.

	Group AP_1 (10–15 mm)	*Group AP_2 (16–20 mm)*	*Group AP_3 (≥21 mm)*
Implantation rate[a] (%)	16.7 (96/576)	16.8 (71/422)	10.3 (9/87)
Pregnancy rate/transfer[b] (%)	34.4 (73/212)	30.4 (48/158)	26.7 (8/30)
Abortion rate[c] (%)	12.3 (9/73)	22.9 (11/48)	–
Ectopic pregnancy rate[c] (%)	1.4 (1/73)	–	–
Ongoing pregnancy rate[c] (%)	29.7 (63/212)	23.4 (37/158)	26.7 (8/30)

[a,b,c]Values in parentheses are numbers of embryos, cycles, patients respectively.
There was no statistical difference among groups.

Table 3. Relative position: general characteristics of the four groups studied.

	Group RP_1 ≤40%	*Group RP_2 41–50%*	*Group RP_3 51–60%*	*Group RP_4 ≥61%*
Patients (*n*)	23	169	111	80
Transfer (*n*)	23	177	117	83
Age (years)	34.5 ± 5.5	34.0 ± 4.9	33.9 ± 4.9	34.6 ± 4.5
Aetiology (%)				
Male factor	39.1	35.5	33.4	38.8
Idiopathic	26.1	26.0	24.3	26.2
Endometriosis	13.0	12.6	22.5	13.8
Tubal–peritoneal	13.0	13.0	11.7	11.2
Tubal–peritoneal + male	4.4	5.9	3.6	5.0
Tubal–peritoneal + endometrial	4.4	5.3	1.8	5.0
Endometrial + male	–	1.8	1.8	–
Endometrial + male + tubo–peritoneal	–	–	0.9	–
ECL (mm)	31.5 ± 3.6 (26–40)[b]	29.5 ± 3.2 (22.3–38.4)[b]	30.6 ± 3.1 (24–39.3)[b]	29.9 ± 4.3 (22.7–44.6)[b]
Retrieved oocytes	11.6 ± 7.4	9.1 ± 5.1	9.7 ± 5.5	10.3 ± 6.1
Oocytes in metaphase II	8.4 ± 6.2	7.1 ± 3.9	7.2 ± 4.0	7.9 ± 5.2
Fertilization (%)	77.1 ± 18.7	74.3 ± 20.5	72.0 ± 20.1	72.8 ± 17.3
Embryo transfer (*n*)	3.1 ± 0.9	2.7 ± 0.9	2.6 ± 0.9	2.8 ± 1.0
(%) of ECL	36.4 ± 2.9[a] (29.4–40.0)[b]	46.1 ± 2.2[a] (40.4–50.0)[b]	55.3 ± 2.7[a] (50.1–60.0)[b]	67.4 ± 6.5[a] (60.6–90.6)[b]

[a]$P < 0.001$.
[b]Range of values.
It should be noted that the same patient in different cycles might have been included in group RP_1, RP_2, RP_3 or RP_4.

Table 4. Relative position: clinical results.

	Group RP_1 ≤40%	*Group RP_2 41–50%*	*Group RP_3 51–60%*	*Group RP_4 ≥61%*
Implantation rate[a] (%)	9.8[d] (7/71)	16.9[e] (82/484)	21.3[d,f] (64/300)	10[e,f] (23/230)
Pregnancy rate/transfer[b] (%)	21.7 (5/23)	36.7[g] (65/177)	35.9[h] (42/117)	20.5[g,h] (17/83)
Abortion rate[c] (%)	20 (1/5)	15.4 (10/65)	14.3 (6/42)	17.6 (3/17)
Ectopic pregnancy rate[c] (%)	20 (1/5)	–	–	–
Ongoing pregnancy rate[c] (%)	13 (3/23)	31.1[i] (55/177)	31.0[j] (36/117)	16.9[i,j] (14/83)

[a,b,c]Values in parentheses are numbers of embryos, cycles, patients respectively.
[d-j]Values within rows with the same superscript letter were significantly different: [d]$P = 0.02$, [e,h,i]$P = 0.01$, [f]$P = 0.0005$, [g]$P = 0.009$, [j]$P = 0.03$.

Discussion

In the present study, the embryos were placed in the uterine cavity in an accurate manner by ultrasonographic observation, a fundamental point of the study. In addition, all embryo transfers were performed by the same clinician, only those performed using the same type of catheter and only those considered to be easy were included in the analysis, and a strict transfer protocol was followed. Thus, the groups studied were protected from possible biases typical of the transfer process.

The data obtained by analysis of transfers based on AP did not show significant differences in implantation or pregnancy rates between the three groups (**Table 2**), in disagreement with data reported by Coroleu *et al*. (2002). In the cited study, in contrast, the authors found statistically significant differences in implantation rates between the embryo transfer groups in which the catheter tip was located 10 ± 1.5 mm (group 1), 15 ± 1.5 mm (group 2) and 20 ± 1.5 mm (group 3) from the uterine fundus. They observed that the implantation rates for groups 2 and 3 were better than those for group 1 (but with no difference between groups 2 and 3). They concluded that applying the fixed distance of 15–20 mm away from the fundus might optimize the performance embryo transfers.

This difference between the present results and those obtained by Coroleu *et al*. (2002) may have been due to variations in the composition of the groups. In the present study, the tip of the catheter was never placed in positions as close to the uterine fundus as done in group I of the study by Coroleu *et al*. However, this does not fully explain the disparity in the results. In addition, even though this study used a group (AP_2: embryo transfers 16–20 mm from the uterine fundus) in which embryo transfers was performed with the catheter tip located at distances similar to those considered ideal in the study by Coroleu *et al*. (15–20 mm), this group was not found to differ from the others.

Analysis of transfers based on RP, which provided a wider variation in transfer sites, indicated better implantation and pregnancy rates when the tip of the transfer catheter was positioned at more central points in the ECL (**Table 4**). This fact suggests that the ideal site for the positioning of the catheter inside the endometrial cavity is better determined by RP rather than by AP. The absolute measurement involves a reasonable potential error because it does not take into account the variations in the dimensions of the endometrial cavity. Determining a fixed measurement may mean placing the tip of the transfer catheter at a higher or lower point of the endometrial cavity, depending on the length of the latter.

On this basis, Frankfurter *et al*. (2003) retrospectively analysed 23 patients who underwent two cycles of ultrasound-guided embryo transfers, considering for each patient a transfer that resulted in pregnancy and one that did not. The results showed better pregnancy rates when the site of embryo placement relative to the ECL was more distant from the uterine fundus. No significant difference was observed when comparing the AP. In addition, Frankfurter *et al*. (2004), in a prospective study of 666 embryo transfers using RP with respect to the ECL to determine the site of deposition, detected significantly higher implantation and pregnancy rates for embryo transfers performed in the middle–lower segments of the uterus compared with the upper segment (21 versus 14; 39.6 versus 31.2%).

These data emphasize the relevance of previous analysis and quantitation of the dimensions of the ECL in order to determine precisely the most adequate site for embryo transfers. The analysis found a variety in ECL within the day of the first measurement (day 8 of stimulation) and the day of embryo transfers. However, the mean difference found was only 0.6 mm (bigger on the day of embryo transfers), with low significance, considering the reported ideal gap for transference (40–60% of ECL, corresponding to an average gap of 6 mm).

Regarding the observation of better implantation and pregnancy rates in the more central regions of the endometrial cavity, a review of the literature shows that other investigators, employing different study designs, have reached similar conclusions. In their review, Levi Setti *et al*. (2003) confirmed that embryos should preferentially be transferred to the middle part of the endometrial cavity distant from the endometrial fundus.

The biological mechanism underlying the better implantation and pregnancy rates obtained when embryos are transferred to a more central area is unknown. Some investigators have suggested that spontaneously conceived embryos implant preferentially on the middle posterior side of the endometrial cavity because of anatomical considerations and gravitational action (Yen *et al*., 1999). In addition, Nikas *et al*. (1995), studying endometrial biopsies, emphasized the presence of pinopodes as markers of the 'nidation window' located 2 cm from the uterine fundus.

On the other hand, Minami *et al*. (2003) observed in natural pregnancies that the preferential site of implantation appears to be in the upper regions of the endometrial cavity. However, IVF cycles involve situations in which the endometrium suffers stimuli that do not occur in the natural process. Stimuli that lead to localized or generalized premature decidualization in animal models may lead to closing of the nidation window *in vivo* (Frankfurter *et al*., 2003). 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) and induction of contractions, with potent adverse effects (Liedholm *al*., 1980; Fanchin *et al*., 1998; Lesny *et al*., 1998, 1999; Schoolcraft *et al*., 2001). It is likely that, by positioning the catheter tip close to the midpoint of the ECL, the embryos are transferred to the area that best permits implantation, avoiding lower regions in the endometrial cavity that are inadequate for appropriate nidation and at the same time, by minimizing the penetration of the catheter into the endometrial cavity, endometrial injury and the possibility of triggering contractions are reduced.

The spontaneous abortion rate observed in the present study was similar to those reported in statistical investigations on assisted reproduction [Red Latinoamericana de Reproducción Asistida, 2001; ASRM/SART Registry, 2004; European IVF-monitoring programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE), 2004]. One interesting aspect, although not statistically significant, was the fact that abortion rates increased with increasing distance from the more central zone of the endometrial cavity (**Table 4**). These data support speculations about an ideal region in the endometrial cavity in which the catheter should be positioned for embryo transfer.

In conclusion, the present results demonstrate that implantation and pregnancy rates are influenced by the site of embryo transfers, with better results being obtained when the catheter tip was positioned close to the middle area of the endometrial cavity. In this respect, previous analysis of the length of the endometrial cavity is the fundamental step in establishing the ideal site for embryo transfers and may reduce inter-observer variability. Based on these data, it has become the norm to place the catheter tip at a position mid-way along the ECL, using transabdominal ultrasound with a two-dimensional image. However, further studies are needed to determine the mechanisms that underlie the effect of the site of embryo transfers on implantation rates in order to provide data that will lead to qualitative improvement in clinical strategies and, eventually, in IVF–ICSI outcomes.

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