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Ultrasound Guidance Is Not Necessary During Easy Embryo Transfers

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***Purpose*:** To determine whether the use of ultrasound (US) to guide embryo transfer (ET) in a population previously defined as likely to have easy transfer would change the implantation and pregnancy rates in an ICSI program.
***Methods*:** A total of 100 patients identified as likely to have easy transfer after mock transfer were divided into two groups: Group I, US-guided ET ($N = 50$) and Group II, ET without the aid of US ($N = 50$).
***Results*:** Implantation and pregnancy rates were similar ($p = 0.51$, $p = 0.29$) for Group I (19.6%, 42%) and Group II (16.3%, 30%), as also was the abortion rate ($p = 0.55$) (Group I: 1/21; Group II: 2/15).
***Conclusion*:** As long as previous mock transfers are routinely performed during a cycle preceding assisted reproduction and the clinician considers transfer to be easy, ultrasound does not benefit the process of embryo transfer.

KEY WORDS: Embryo transfer; ICSI; implantation; pregnancy; ultrasound.

INTRODUCTION

The main variables that affect implantation and pregnancy rates in a program of assisted reproduction are uterine receptivity, embryo quality, and efficient embryo transfer (ET). Historically, less effort has been devoted to ET execution compared to other aspects of in vitro fertilization (IVF).

The final objective of successful ET is to deposit embryos in an atraumatic manner in the uterine fundus, the site where implantation is maximal. A test transfer performed during a cycle preceding IVF in order to measure the depth and direction of the uterine cavity can be an important step. This previous mock transfer is performed during a cycle preceding ET using the same catheter to be used for the ET procedure.

Mansour *et al.* (1), Knutzen *et al.* (2), and Sharif *et al.* (3) have reported that previous mock transfer favors an increase in pregnancy rates since it minimizes or avoids eventual problems faced during ET. In a prospective randomized study, Mansour *et al.* (1) divided 335 women into two groups, one of them submitted to previous mock transfer, and the other did not submit to this procedure. The cited authors observed that in the group submitted to this procedure 22.8% of the patients became pregnant and all ET were considered easy, whereas in the group not submitted to mock transfer, only 13.1% became pregnant and 29.8% of the ET were considered difficult. In a descriptive study, Knutzen *et al.* (2) assessed 34 IVF cycles, 18 of which involved previous mock transfer while the remaining 16 did not, and obtained a 33% pregnancy rate in the group of women submitted

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to mock transfer, as opposed to no pregnancy in the group not submitted to this procedure. In a prospective study, Sharif *et al.* (3) assessed 261 ET performed after mock in 113 cases and without mock in 148, and obtained respective pregnancy rates of 45.1% and 11.1%.

Our group routinely performs mock transfer in the cycle preceding the assisted reproduction procedure in order to measure the uterine cavity and to determine whether transfer will be easy or not.

Several authors have reported the use of ultrasound to guide ET (4, 5) but a significant increase in pregnancy rates after ultrasound-guided transfer has not been consistently demonstrated. For example, Kan *et al.* (6) did not observe significant differences in pregnancy rates when women submitted to ultrasound-guided ET were compared to a control group, and concluded that ultrasound-guided ET should be used only for patients clinically considered to be likely to have a difficult transfer.

The objective of the present prospective and randomized study was to investigate whether the use of transabdominal ultrasound to guide ET in a population previously defined as likely to have easy transfers would alter the implantation and pregnancy rates in a program of intracytoplasmic sperm injection (ICSI).

MATERIAL AND METHODS

The study was conducted on 100 patients participating in an ICSI program who were submitted to mock transfer during a cycle preceding the process. All patients were considered to be likely to have an easy transfer when a Frydman catheter for easy transfer (4.5 cm, CCD, France) penetrated the uterine cavity with no difficulty, with no need to use a forceps for cervical manipulation. The patients were then divided into two equal groups in a prospective and randomized manner on the day of oocyte retrieval, by drawing lots, using a randomization table previously elaborated for the study: Group I, ultrasound-guided ET (50 patients), and Group II, ET not guided by ultrasound (50 patients).

For ovarian stimulation before the ICSI procedure, the patient was downregulated with nafarelin acetate (Synarel, Searle) at the dose of 400 μg/day by the nasal route from the 21st day of the cycle to the day of human chorionic gonadotrophin (hCG) administration. Fourteen days after the use of the nafarelin acetate and with the patient having menstruated, recombinant follicle stimulating hormone (FSH) was habitually used at a fixed dose of 150–450 IU for 7 days depending on patient age range (7). On the eighth day of ovarian stimulation, follicular development started to be monitored by vaginal ultrasound only, using a Synergy model Diasonic apparatus with a convex endocavitary transducer at the frequency of 7 MHz, always by the same examiner. On the occasion of the first ultrasonographic examination, the size of the endometrial cavity (SEC), corresponding to the distance from the internal os of the uterine cervix to the end of the endometrial cavity, was measured. The doses of recombinant FSH were adapted to the ovarian response and hCG was administered at the dose of 5,000 to 10,000 IU when a minimum of two follicles with a diameter $\geq$17 mm was observed. On the day of hCG administration, the following additional parameters were assessed by ultrasound: endometrial thickness, pulsatility index of the uterine artery in the Doppler mode, and uterine contractions occurring during a period of 2 min. Oocytes were retrieved by ultrasound-guided transvaginal puncture 34–36 h after hCG.

After being identified in follicular fluid, the oocytes were classified according to maturity. The cumulus-corona complex was removed by exposure to type IV hyaluronidase solution (H-4272, Sigma Chemical Co, USA) at the concentration of 40–80 IU/mL. Denuded oocytes were incubated in IVF-50 medium (Scandinavian IVF Science AB, Sweden) until the time for ICSI.

For sperm separation from seminal fluid, discontinuous gradients of Sperm-Prep-100 TM (Scandinavian IVF Science AB, Sweden) were used for the 40% and 90% fractions.

ICSI was performed as described previously (8, 9), with the embryos being routinely transferred after 48 h in culture and the extra embryos being cryopreserved at the end of the second day.

The preparation for ET was the same for the two groups. The patients were placed in the lithotomy position and the cervix was exposed using a bivalve speculum. Mucus and secretions were removed with culture medium. A Frydman transfer catheter (4.5 cm, CCD, France) connected to an insulin syringe was used for all transfers. In Group I, both the insertion and positioning of the catheter were guided by transabdominal ultrasound using an Aloka model SSD-1100 apparatus with a 3.5-MHz convex transducer. The embryos in a $\leq$30 μL volume of culture medium were gently released when the tip of the catheter was positioned 0.5–1.0 cm from the uterine fundus. Ultrasound also permitted the visualization of the "transfer bubble" after the embryos were

expelled. In Group II the embryos were deposited in the uterine fundus according to the measurement made in the cycle preceding ICSI. In addition, all transfers in both groups were performed by the same physician.

The following parameters were assessed: patient age, number of oocytes collected per puncture, number of oocytes in metaphase II, fertilization rate, number of embryos transferred, SEC, endometrial thickness, Doppler velocimetry of the uterine artery, myometrial contraction, implantation rate, pregnancy rate, and abortion rate. Data were analyzed statistically by the Student *t*-test, by the Mann-Whitney test and by the Fisher exact test.

RESULTS

The clinical and laboratory characteristics of Groups I and II are listed in Table I. Mean patient age did not differ significantly ($p = 0.95$) between groups. The number of retrieved oocytes and the number of oocytes in metaphase II were also similar for the two groups ($p = 0.89$ and $p = 0.35$, respectively). The normal fertilization rate also did not differ ($p = 0.71$) between groups.

Furthermore, there were no differences ($p = 0.16$) in the number of embryos transferred for Group I and Group II patients, the implantation and pregnancy rates were similar ($p = 0.51$ and $p = 0.29$, respectively) for Group I (19.6%, 42%) and Group II (16.3%, 30%), and the abortion rate was identical ($p = 0.55$) for the two groups.

The ultrasound characteristics (SEC, endometrial thickness, uterine artery Doppler) for Groups I and II are listed in Table II. There was no difference between groups in these parameters.

The number of myometrial contractions also did not differ significantly between groups (Table III).

Table I. Clinical and Laboratory Results

	Group I (US)	Group II (Control)	*p*
No. of cases	50	50	
Age	32.1 ± 4.1	32.0 ± 3.2	0.95
Collected oocytes (*n*)	11.7 ± 5.7	11.5 ± 5.9	0.89
Oocytes MII (*n*)	9.5 ± 4.5	8.7 ± 4.5	0.35
Fertilization (%)	68.4 ± 22.1	70.0 ± 20.3	0.71
Embryos transferred (*n*)	2.6 ± 0.9	2.3 ± 0.6	0.16
Pregnancy rate (%)	42.0	30.0	0.29
Implantation rate (%)	19.6	16.3	0.51
Abortion rate (%)	4.7	13.3	0.55

Table II. Comparison of the Ultrasonographic Characteristics of the Patients Submitted (Group I) or Not (Group II) to US-Guided ET

Variable	Group I	Group II	*p*
SEC (cm)	3.1 ± 0.32	2.9 ± 0.47	0.31
Endometrial thickness (mm)	9.2 ± 1.57	9.9 ± 1.64	0.07
Doppler of the uterine artery	2.3 ± 0.33	2.4 ± 0.40	0.57

DISCUSSION

ET is a critical phase in any IVF cycle and many aspects of this process have been studied, including the type of catheter used (10, 11), the presence of blood in the catheter (12), the importance of "previous mock transfer" (1, 2), and the need for the patient to rest after the procedure (13). However, what appears to be clear is that the long treatment process will result in pregnancy only if good quality embryos will be properly placed in the uterus.

Up to 1985, ultrasonography (US) was used to assess follicular growth (14) and to direct oocyte retrieval (15). Starting that year, Strickler *et al.* (16) and Leong *et al.* (17) began to employ US to guide ET and suggested that the procedure was facilitated by US because the degree of difficulty of ET was lower, with less catheter kinking and with the removed catheters being cleaner.

The advantages of US-guided ET appear to be: facilitation of the use of softer cannulae and less maneuvering of the latter inside the uterine cavity; with visualization, the tip of the catheter is well positioned in the fundus of the uterine cavity and it is possible to document the ejection of the transfer material containing the embryos into the uterine cavity, and their permanence during catheter removal is documented. In addition, no adverse effect of US was detected in terms of genetic defects of the embryo, teratogenicity or embryotoxicity.

The disadvantages are the need for the presence of a second operator, a longer time of execution, and the inconvenience of filling the patient's bladder.

Table III. Comparison of Myometrial Contraction in the Patients Submitted (Group I) or Not (Group II) to US-Guided ET

Group	0	1	2	3	*p*
I	37	0	10	3	0.72
II	34	1	11	4	0.72

Note. The values are reported as absolute numbers.

In general, the studies conducted to analyze the use of US in ET can be divided into retrospective, prospective, and prospective and randomized types. Regardless of the type of study, however, the data reported about the benefits of US have been contradictory.

In a retrospective study, Strickler *et al.* (16) investigated 16 patients submitted to US-guided ET and 12 patients submitted to ET without the use of US and reported that US-guided ET were easier and involved lower distortion of the catheter. However, no significantly increased pregnancy rates were demonstrated after the use of US-guided ET. Lindheim *et al.* (18), in an egg donation program, studied 137 women submitted to easy to execute ET and compared the rates of embryo implantation between groups submitted or not to US-guided ET. The implantation rate was 28.8% for the group submitted to US-guided ET and 18.4% for the group in which US was not used ($p < 0.05$) and the pregnancy rate was 63.1% and 36.1% ($p < 0.05$), respectively. Thus, the authors concluded that US-guided ET is a simple procedure, which significantly increases pregnancy rates in egg donation programs. Wood *et al.* (19) studied 518 IVF cycles in a retrospective investigation in which they analyzed embryo implantation and pregnancy rates in terms of two important clinical aspects: ET guided or not by US and the quality of the transabdominal ultrasonographic image on the day of ET. The embryo implantation rate was 19.9% for the group submitted to US-guided ET and 14.1% for the group in which US was not used ($p < 0.02$) and the pregnancy rate was 38.4% and 25.4%, respectively ($p < 0.001$). With respect to the quality of ultrasonographic visualization, the pregnancy rate was 41.5% for good/excellent visualization and 16.7% for bad/poor visualization. The authors concluded that US-guided ET with good/excellent visualization resulted in a significant increase in implantation and pregnancy rates.

In a prospective study of 178 cycles, Al-Shawaf *et al.* (20) assessed the pregnancy rates in a group submitted to US-guided ET (29%) and in a group in which US was not used (30.3%), and concluded that US guidance of ET does not change the pregnancy rates. Similarly, Woolcott & Stanger (21) analyzed embryo implantation and pregnancy rates in patients submitted or not to US-guided ET (a total of 93 patients submitted to 101 ET) and obtained a 14.1% embryo implantation rate and a 27.7% pregnancy rate for the group submitted to US-guided ET, as opposed to respective rates of 9.2% and 18.5% for the group not submitted to US-guided ET. However, since the differences were not statistically significant, the authors concluded that US has no significant effect on embryo implantation or pregnancy rate.

In a prospective and randomized study, Hurley *et al.* (22) assessed ET guided by transvaginal US in 94 patients compared to 246 patients not submitted to US-guided ET, with all procedures being considered easy to execute. The pregnancy rate was 20.2% (19/94) for the group submitted to US and 17.5% (43/246) for the group not submitted to US, with the difference being nonsignificant. Even though there was no significant difference, the authors believed that the use of US could be beneficial since couples expressed positive opinions about being able to visualize the transfer bubble during US-guided ET, a fact that might help reduce the degree of anxiety.

Similarly, Coroleu *et al.* (23) assessed the influence of US-guided ET considered to be of easy execution in 362 patients submitted to IVF and divided into two groups: 182 with US-guided ET, and 180 without US-guided ET. The implantation rate was 25.3% in the US-guided group compared to 18.1% in the non-US-guided group ($p < 0.05$). The pregnancy rate was 50% for the US groups and 33.7% in the non-US-guided group ($p < 0.02$). Thus, the authors concluded that the use of US to guide ET significantly increased the implantation and pregnancy rates in IVF programs.

Fanchin *et al.* (24) prospectively studied 209 women in 220 cycles of controlled ovarian stimulation and analyzed the number of uterine contractions under US visualization during ET, also measuring the plasma concentrations of progesterone. The patients were divided into four groups according to number of uterine contractions, i.e., patients with 3 contractions or less, patients with 3.1–4 contractions, patients with 4.1–5 contractions, and patients with more than 5 contractions. The implantation rate was 21%, 20%, 10%, 4%, respectively, and the pregnancy rate was 53%, 46%, 23%, 14%, respectively ($p < 0.001$). The frequency of uterine contractions was negatively correlated with plasma progesterone concentrations. The authors concluded that the high frequency of uterine contractions on the day of ET favors a greater chance of embryo expulsion from the uterine cavity and also observed the uterus-relaxing properties of progesterone. In the present study, even though we assessed the uterine contractions on the day of hCG administration, we did not detect a significant difference in uterine contractile activity during this phase of the process between the 50 patients in Group I (34 had no myometrial contractions, 1 had one contraction, 11

had two contractions, and 4 had three contractions) and the 50 patients in Group II (37 had no contractions, 10 had two contractions, and 3 had three contractions).

In the present study, in which we analyzed variables such as patient age, number of oocytes retrieved, number of oocytes in metaphase II, fertilization rate, number of embryos transferred, size of the uterine and endometrial cavity, endometrial thickness, number of uterine contractions on the day of hCG administration, Doppler pulsatility index of the uterine artery determined by transvaginal US, and implantation, pregnancy, and abortion rates, we did not detect a statistically significant correlation between the groups submitted or not to US-guided ET. In addition, the implantation and pregnancy rates did not differ significantly between the groups submitted or not to US. However, the acceptance of an Ho hypothesis (no difference between groups) when the hypothesis in reality is false would induce a so- called type II error (ß-error) and to avoid this type of error it is advisable to increase the number of patients studied. To detect a difference in pregnancy rates between the two treatment groups (keeping the current rates in both groups) with a power of 80% and a level of significance of 5%, a total number of 267 patients per group would have been required.

We conclude that US was not necessary during the ET process in a subpopulation in which ET was considered to be easy after mock transfer.

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