Original Article

Improvement in pregnancy rate by removal of cervical discharge prior to embryo transfer in ICSI cycles: A randomised clinical trial

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Aims: The present study aimed to evaluate the effect of removing cervical discharge prior to embryo transfer (ET) on pregnancy rates.

Methods: Five hundred and thirty women who were candidates for fresh ET in intracytoplasmic sperm injection (ICSI) cycles were randomly allocated to intervention or control groups. In the intervention group, the cervical canal was cleansed using sterile cotton swabs prior to ET. The control group had routine ET. Multiple logistic regression analysis was used to estimate the adjusted effect of removing the cervical discharge on pregnancy rates.

Results: There was a significant difference in pregnancy rates between the two groups. The clinical pregnancy rate was 104/265 (39.2%) in the intervention group compared with 60/265 (22.6%) in the control group ($P < 0.001$). The intervention group also had a higher implantation rate (20.5%) compared with the control group (12.2%; $P < 0.001$). Additionally, the live birth rate in the intervention group (33.6%) was significantly higher than in the control group (17.4%; $P < 0.001$). The logistic regression analysis indicated that the odds ratio of pregnancy in the intervention group was 2.297 (95% CI, 1.552–3.399) compared with the control group.

Conclusions: Removal of cervical discharge prior to ET may have a significant effect on the rate of implantation, pregnancy and live birth.

Key words: cervical mucus, embryo transfer, ICSI cycle, pregnancy rate, randomised clinical trial.

Introduction

Embryo transfer (ET) is a crucial step for the success of assisted reproductive technology (ART). Better knowledge of the preparation techniques preceding ET or at the post transfer stage may help improve pregnancy outcomes.[1]

Removal of cervical discharge, as a preparation technique before ET, is routinely performed in some ART centres. However, there are few clinical experiments regarding the effect of removing cervical discharge on pregnancy outcomes.

The presence of mucus or blood on the catheter tip can interfere with a successful ET and has been associated with lower pregnancy rates,[2,3] higher incidence of retained embryos[4,5] and higher rate of embryo expulsion into the cervix.[6] The embryo(s) might adhere to the cervical mucus or blood around the catheter, which can then be expelled during the withdrawal of the catheter. In addition, the transfer of mucus or blood into the uterine cavity may lead to an inappropriate endometrium, resulting in lower implantation rate.[6–8]

Although some studies have reported the positive impact of removing cervical discharge on pregnancy rate,[9–11] there are other studies that have not found such an effect.[5,12] Some researchers believe that cervical mucus acts as a lubricant, the removal of which may increase the incidence of difficult ET or produce uterine contractions.[10] Given the conflicting findings, this study was designed to evaluate the effect of removing cervical discharge on the rate of ART success.

Methods

This randomised, parallel design clinical trial was performed at the Royan Institute for Reproductive Biomedicine (Tehran, Iran) over a 12-month period between May 2009 and May 2010.

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A total of 530 women aged ≤35 years who had ≥2 good-quality embryos and were candidate for fresh ET (as inclusion criteria) participated in the study.

Women with uterine factor infertility were excluded from the study. Those with congenital abnormalities such as bicornuate, unicornuate, septate or didelphis uteri as well as those with acquired abnormalities, including uterine fibroids, polyps, hyperplasia, intrauterine adhesions, endometritis or hyperplasia, were considered to have uterine factor infertility. These cases were diagnosed by hysterosalpingography or diagnostic hysteroscopy.

The study was performed in accordance with the Declaration of Helsinki and subsequent revisions and approved by the Ethics Committee of the Royan Institute for Reproductive Biomedicine. Written informed consent was obtained from all the participants prior to the study. The present trial was registered at clinicaltrials.gov as identifier NCT01156181.

Based on a pilot study (0.8 power, 0.05 type I error), 260–265 women in each group were required for this study. According to our preliminary results, the pregnancy rate was 40% in the intervention group compared with 28% in the control group. The sample size was calculated using NCSS and PASS 2000 software.

On the day of ET, the participants were randomly assigned into equal size (n = 265) groups of intervention and control using a permuted block randomisation method (Fig. 1). The length of blocks was four. The allocation ratio was one-to-one, and a random number table was used as the method of selection.

The women in each group remained within the same allocation throughout the study. The physician responsible for ET allocated the next available number on entry into the trial. Random allocation sequence and participant enrolment were performed by a midwife in the operating room.

In the intervention group, the cervical canal was cleaned using sterile cotton swabs before ET, while the control group had only routine ET without additional cervical canal manipulation. The women, the embryologist and those in charge of data analysis were blinded to the results of allocation.

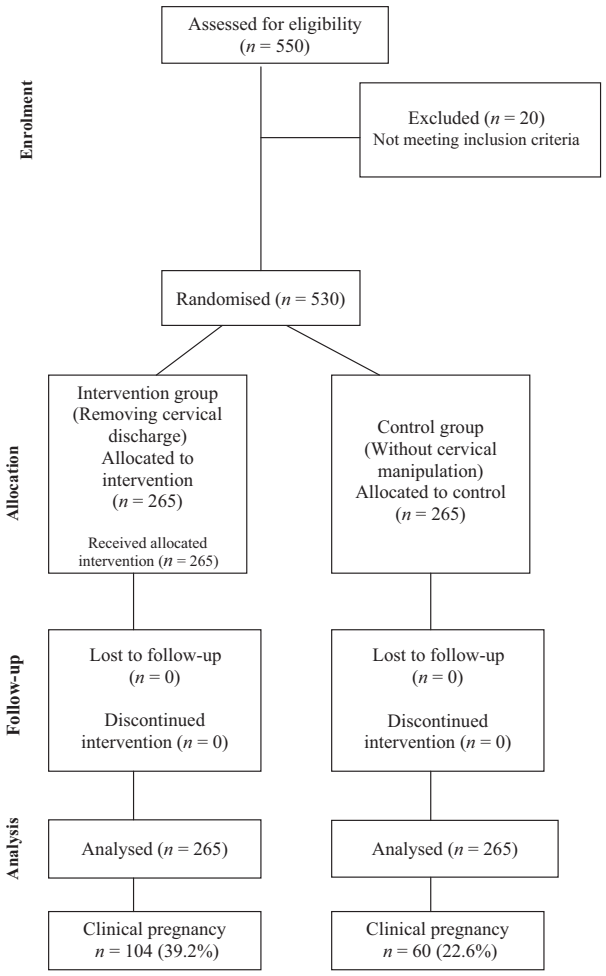

Figure 1 Flow chart of the study design and the primary outcome.

Ovarian stimulation and oocyte retrieval

The stimulation protocol for all women was according to the standard long protocol. All women underwent oral contraceptive suppression starting on the 2nd or 3rd day of the menstrual cycle. GnRH agonist suppression with Busereline (500 μg, Suprefact; Aventis Pharma Deutshlan, Frankfurt, Germany) was carried out via subcutaneous injection starting on the 21st day of the menstrual cycle. Down regulation was confirmed by a serum estradiol concentration <50 pg/mL. Gonadotrophin stimulation by recombinant FSH (150 IU daily, Gonal F; Serono, Geneva, Switzerland) was started 14 days after subcutaneous GnRH agonist injection. The dose and duration of FSH treatment were adjusted by monitoring the follicular development with ultrasound and estradiol levels. The maximum FSH dose was 225 IU/day. In both groups, gonadotropin stimulation was continued until two to three follicles with a mean diameter of ≥17 mm were achieved. Then, 10,000 IU of human chorionic gonadotropin (hCG, Choriomon; IBSA, Lugano, Switzerland) was administered, and oocyte retrieval was performed in the next 34–36 h by a skilled gynaecologist.

Metaphase II oocytes were injected using the ICSI procedure. Normal fertilisation was confirmed when two distinct pronuclei were present 16–18 h after the oocyte injection.

Embryo development and morphology

Maximum two or three good-quality cleaved embryos (grades A, B or AB with four to six blastomeres on the day 2, or with six to eight blastomeres on the day 3) were transferred into the uterus two to three days post injection. Embryo quality was assessed according to morphology, cleavage stage and fragmentation rate. Spare good-quality embryos were frozen.

Embryo transfer technique

All women underwent a mock ET and uterine measurement on the day 2 or 3 of menstruation at the beginning of the ICSI cycle. All ETs were performed using a soft ET catheter (Labotect GmbH, Göttingen, Germany) without ultrasound guidance. Each woman was placed in the lithotomy position with an empty bladder. A sterile bivalve speculum was placed in the vagina, and the cervix was exposed. In the intervention group, prior to ET, excess mucus and debris were removed from the cervical canal using sterile cotton swabs. The cervical discharge was scored as mucosal, bloody, combination of mucosal and bloody or infected. All infections were diagnosed by clinical manifestations based on physical examination by a skilled gynaecologist. The discharge was scored as infected if there were yellow, green or opaque endocervical exudates visible in the endocervical canal or on the swab. Clinically, this type of cervical discharge is an objective characteristic for mucopurulent cervicitis. The control group received no cervical canal manipulation before ET.

To perform ET, the embryos were loaded into the transfer catheter by the embryologist and released into the uterine cavity by an experienced physician. The catheter was slowly rotated and removed. The embryologist flushed the catheter with media to check for retained embryos. The patients were asked to remain at supine position for 20 min after the ET.

Luteal-phase support was provided with vaginal progesterone suppositories (400 mg twice a day; Aburaihan Co., Tehran, Iran) until the day of β-hCG assay. If the β-hCG test was positive, progesterone administration was continued until the tenth week of gestation.

Outcome

The primary endpoint was the clinical pregnancy rate. Implantation, abortion and live birth rates were the other outcomes of interest. Clinical pregnancy is defined as a positive pregnancy test followed by the presence of a gestational sac on trans-vaginal ultrasound four weeks after ET. The pregnancy rate in this study was calculated by dividing the number of clinical pregnancies detected by the number of women (clinical pregnancies per woman). The implantation rate was defined as the number of gestational sacs visualised by transvaginal pelvic ultrasound per embryos transferred. The fertilisation rate was defined as the ratio of the number of embryos formed relative to the number of oocytes injected.

Statistical analysis

Statistical analysis was performed using SPSS (version 13.0; SPSS Inc., Chicago, IL, USA) statistical software. The normality in the distribution of continuous variables was assessed using a Kolmogorov–Smirnov test. Intergroup differences of normally distributed continuous variables were assessed by Student's *t*-test, whereas Mann–Whitney's *U* test was used if the data were not normally distributed. Significant differences were evaluated by the chi-square test to compare the noncontinuous variables. The data were expressed as the mean ± standard deviation (SD), unless otherwise specified. Statistical significance was set at $P < 0.05$. For estimating the adjusted effect of cervical discharge removal on the occurrence of clinical pregnancy, the effect of confounding factors such as age, body mass index (BMI), duration of infertility, number of formed embryos, number of transferred embryos, stimulation duration and endometrial thickness were investigated using multiple logistic regression analysis. The results of regression analysis were presented as odds ratios (OR) and 95% confidence intervals.

Results

The data of 530 eligible women (aged ≤ 35 years) were analysed. The baseline demographic and clinical characteristics of the two groups are shown in Table 1. There were no significant differences between the two groups in age, BMI, duration of infertility and other variables (including the number of oocytes retrieved, fertilisation rate and the number of embryos transferred) that might have impacted upon the pregnancy outcome. The causes of infertility were also similarly distributed between the two groups. The ET procedure was considered as 'easy' if the catheter passed through the cervical canal easily.

There was a significant difference between the two groups in the clinical pregnancy rate. The clinical pregnancy rate was 104/265 (39.2%) in the intervention group compared with 60/265 (22.6%) in the control group ($P < 0.001$). Based on the univariate analysis, the OR and the relative risk were also calculated. The results showed that the OR of pregnancy in the intervention group was 2.21 (95% CI: 1.51–3.2, $P < 0.001$) compared with the control group. The relative risks are shown in Table 2.

The intervention group also had a higher implantation rate (20.5%) compared with the control group (12.2%; $P < 0.001$). The live birth rate in the intervention group (33.6%) was significantly different from the control group (17.4%; $P < 0.001$). The multiple birth rate was also significantly different between the two groups (6.8% in the intervention group vs 1.1% in the control group; $P = 0.001$). The abortion rate was similar between the two groups (Table 2).

The distribution of the scores of different types of cervical discharge in both groups is shown in Table 3. Mucosal discharge was found to have the highest frequency in both groups (56.2% in the intervention group vs 60.4% in the control group). The pregnancy rate was compared based on the type of cervical discharge (mucosal, bloody, combination of mucosal and bloody or infected). There was a higher pregnancy rate in all types of cervical discharges, except in the infected discharge. No pregnancy was observed in the women with infected discharge.

Multiple logistic regression analysis was performed to adjust for the potential confounding effects of age, BMI, duration of infertility, number of embryos available for transfer, number of embryos transferred, duration of

Table 1 Comparison of the baseline demographic and clinical characteristics of the two study groups in the ICSI/embryo transfer cycles

	Intervention group (cleaning the cervical canal) (n = 265)	Control group (no manipulation) (n = 265)	P-value
Age (years)	31 ± 5.11	30.6 ± 4.88	0.466
BMI (kg/m^2)	26.4 ± 4.86	26.2 ± 5.41	0.432
Duration of infertility (years)	8.2 ± 5.15	8.3 ± 4.93	0.918
Duration of stimulation (days)	10 ± 1.64	10.4 ± 2.02	0.147†
Gonadotropin consumption	28.5 ± 13.23	29.5 ± 13.7	0.389
Endometrial thickness (mm)	9.7 ± 1.64	10 ± 1.77	0.080
Follicle number	13.6 ± 6.08	13.1 ± 6.2	0.332
Total number of oocytes retrieved	10.9 ± 5.26	11.7 ± 5.60	0.696
Metaphase II oocytes retrieved	9.2 ± 4.38	8.6 ± 4.6	0.202
Total number of injected oocytes	9.34 ± 4.43	8.9 ± 4.62	0.305
Number of 2PN stage embryos	5.8 ± 3.06	5.5 ± 3.34	0.172
Total number of embryos formed	6.4 ± 3.56	6.2 ± 3.61	0.616
Total number of embryos transferred	2.45 ± 0.50	2.4 ± 0.48	0.187
Total number of embryos cryopreserved	1.6 ± 2.7	1.5 ± 2.56	0.856

Data are expressed as the mean ± SD, unless otherwise specified.
†Mann–Whitney's U test.
BMI, body mass index.

Table 2 The ICSI/embryo transfer cycles outcome in the intervention group following the cervical canal cleaning compared with the control group

	Intervention group (Cervical canal cleaning)† (n = 265)	Control group (No manipulation)† (n = 265)	P-value	Relative risk (95% CI)
Clinical pregnancy rate*	104 (39.2)	60 (22.6)	<0.001	1.733 (1.325–2.267)
Implantation rate*	135/658 (20.5)	78/641 (12.2)	<0.001	1.686 (1.304–2.174)
Abortion rate	13 (4.9)	14 (5.3)	0.843	0.929 (0.445–1.937)
Live birth rate*	89 (33.6)	46 (17.4)	<0.001	1.935 (1.415–2.645)
Multiple birth rate*	18 (6.8)	3 (1.1)	0.001	6.00 (1.789–20.128)
Fertilisation rate	1546/2475 (62.5)	1446/2368 (61.1)	0.316	1.023 (0.979–1.069)

*Significant statistical differences between the two groups.
†Values are the numbers with percentages in the parentheses.

Table 3 The distribution of the scores of different types of cervical discharges in the two groups

	Intervention group (cervical canal cleaning)† (n = 265)	Control group (no manipulation)† (n = 265)
Mucosal discharge	149 (56.2)	160 (60.4)
Bloody discharge	23 (8.7)	48 (18.1)
Mucosal and bloody discharge	55 (20.8)	41 (15.5)
Infection discharge	38 (14.3)	16 (6)

†Values are the numbers with percentages in the parentheses.

stimulation and endometrial thickness. The OR of pregnancy in the intervention group was 2.297 (95% CI, 1.552–3.399, $P < 0.001$) compared with the control group, which was comparable to the univariate analysis before adjustment (OR, 2.21; 95% CI, 1.51–3.2, $P < 0.001$). Hence, there were no confounding effects of the above-mentioned variables on clinical pregnancy rate. In addition to the removal of the cervical discharge, the number of embryos transferred and endometrial thickness were found to be independent factors influencing the treatment outcome (Table 4).

Discussion

There are some reports in the literature suggesting that methods as optimising the ease of ET, ET with ultrasound guidance and use of soft catheters may improve ART outcomes.[9,13,14] However, there is no reliable proof about the effect of the following methods for improving the ART outcomes: mock ET, preparing the women for ET by removal of cervical mucus, depositing the embryos in the

Table 4 Multiple logistic regression analysis of the factors influencing pregnancy rate after ICSI/embryo transfer

Variables	OR (95% CI)	*P*-value
Groups* (Intervention and control)	2.297 (1.552–3.399)	0.000
Age	0.961 (0.915–1.009)	0.107
BMI	0.993 (0.955–1.032)	0.709
Duration of infertility	0.992 (0.949–1.036)	0.708
Type of infertility	1.583 (0.860–2.912)	0.140
Number of embryos formed	1.039 (0.983–1.097)	0.178
Number of embryos transferred*	1.789 (1.154–2.773)	0.009
Stimulation duration	1.023 (0.918–1.139)	0.680
Endometrial thickness*	1.163 (1.039–1.301)	0.009

BMI, body mass index; CI, Confidence interval; OR, Odds ratio. *Wald statistic test. $P < 0.05$ was considered as statistically significant.

mid-portion of the uterus, decreasing the time interval from loading the ET catheter to depositing the embryo and minimising the negative pressure during the withdrawal of the catheter as recommended based on expert opinion.[13]

According to the findings of this single-blind randomised study, significant benefits of removing cervical discharge prior to ET include a higher rate of pregnancy, implantation and live birth. A higher pregnancy rate has also been reported in previous studies.[9–11] As it is possible that cervical mucus or blood may adhere to the embryo, thus preventing its escape from the catheter into the uterus,[15] the removal of cervical mucus provides for more effective delivery of embryos into the uterus. After estimating the adjusted effect of cervical discharge removal on the rate of clinical pregnancy, we demonstrated the efficacy of the intervention. It appears that other factors had no confounding effects on the treatment outcome.

Some researchers have failed to confirm the significant benefits of this procedure prior to ET.[5,16] Different ET techniques such as using different catheters, cervical cleansing by endocervical lavage or cervical brush instead of cotton swab and performance of ET by different physicians may account for the conflicting results.

In the current study, women with mucosal or bloody cervical discharges had significantly higher pregnancy rates. Contamination of the ET catheter by blood or mucus is common after both easy and difficult ETs, although it is more prevalent after a difficult ET. All ETs were easy in the present study. However, 71 women had bloody discharges, and 96 women were found to have a combination of bloody and mucosal discharges before the ET. Normally, during the luteal phase, the cervical mucosa has thin and superficial blood vessels. In addition, in the presence of an inflammatory pathology such as cervicitis, the cervical mucosa may be more fragile. In such situations, cervical manipulation using a cotton swab may lead to mechanical injury and a bloody discharge. During ET, blood from the endocervix may be drawn into the uterus and interfere with embryo implantation.[17,18] However, Moragianni *et al.*[19] have shown that the macroscopic or microscopic presence of blood or mucus in an ET catheter does not affect the rate of implantation or clinical pregnancy.

Bacterial contamination of the ET catheter may also interrupt the process of implantation.[20–25] Different recommendations such as cleaning the cervix and vagina and concurrent antibiotic use at the time of ET have been suggested for reducing bacterial contamination.[26] In the present study, no positive pregnancies occurred in 54 women who had yellow or green endocervical exudates. It appears that mucopurulent discharge interferes with embryo implantation; however, no bacterial culture for confirming the infection was done, and trials with a larger sample size as well as cultures of the catheter tip or cervical swab are needed to confirm this result.

A higher multiple birth rate in the intervention group may be explained by a higher number of transferred embryos in these cases. Transfer of ≤2 embryos and elective single embryo transfer (eSET) in fresh IVF–ET cycles are recommended in most guidelines to prevent multiple pregnancies.[27,28] However, in Iran, most specialists (gynaecologists and embryologists) recommend transfer of ≤3 embryos. Different factors such as the quality of services, uncertainty about the eSET technique and absence of insurance coverage for ART treatment and inadequate options to select couples suitable for eSET may be reasons for different protocols in this regard.

Post-ET procedures may also influence the success rate of ART outcomes. However, there are insufficient studies to support different interventions such as bed rest following ET, mechanical closure of the cervix and the use of a fibrin sealant following ET on the ART outcomes.[29] Therefore, more trials are needed to evaluate the effectiveness of post-ET procedures on the ART outcomes.

In conclusion, cleansing the cervical discharge with cotton swabs prior to ET has benefits including higher pregnancy and live birth rates. In addition, it is a safe method without any compromising effects such as increased uterine contractions.

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