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Injection of embryo culture supernatant to the endometrial cavity does not affect outcomes in IVF/ICSI or oocyte donation cycles: a randomized clinical trial

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ABSTRACT

Objective: To evaluate whether intrauterine injection of embryo culture supernatant before embryo transfer has any impact on pregnancy and implantation rates.
Study design: A total of 400 cycles, of which 200 IVF/ICSI and 200 oocyte donor (OD), were randomly assigned to have their uterine cavity injected (group I) or not (group II). Primary endpoints to be studied were pregnancy and implantation rates.
Results: Clinical pregnancy rate per transfer (47.87%, 90/188 versus 48.45%, 94/194) based on transvaginal scan findings at 7 weeks of gestation and implantation rate (25.6% versus 26.5%) were similar in the two groups. The day of embryo transfer, day 3 or day 5, did not affect the final outcome.
Conclusion: Injection of embryo culture supernatant into the uterine cavity, 30 min before the embryo transfer on either day 3 or 5, neither improves nor adversely affects the pregnancy rate in IVF/ICSI or oocyte donation cycles.

1. Introduction

The implantation of human embryos in IVF/ICSI cycles represents a black hole in current knowledge, and among other factors the embryo transfer (ET) techniques have been considered important determinants of success rates. Although 30% of IVF failures have been related to poor ET techniques [1], the number of published studies on ET techniques is very low. Ultrasound-guided ET and new catheter materials have been used in order to avoid endometrial lesions caused by catheters [2,3]. Since the reported better pregnancy rates in IVF cases where the endometrium was accidentally flushed prior to ET [4], ET techniques have been given additional attention. Injection of the endometrial cavity with embryo culture supernatant prior to ET was reported to improve implantation and pregnancy rates [5]. However, other similar studies using different flushing medium (culture medium for IVF or day 2 embryo culture supernatant) did not report any improvement in pregnancy rates [6,7]. Therefore this study was set up to determine whether in IVF-ICSI and oocyte donation (OD) cycles injection of the endometrial cavity with their embryo culture supernatant at the time of ET performed on either day 3 or day 5, may affect the implantation and pregnancy rates.

2. Materials and methods

A prospective, randomized study was performed. IVF-ICSI and OD cycles were randomized into group I (injection of the uterine cavity with embryo culture supernatant) or group II (no injection) by an allocation sequence generated from a computerized random number table. Group I consisted of two subgroups, IA (ET on day 3) and IB (ET on day 5), and group II consisted of IIA (ET on day 3) and IIB (ET on day 5). The study was approved by the Institutional Review Board and informed consent was obtained from all women.

Two hundred women participating in the OD program and another 200 in the IVF/ICSI program at the Iakentro IVF centre from June 2009 through November 2010 who underwent ET on either day 3 or day 5 were included in the study. Due to difficult transfer 12 cases from group I and 4 cases from group II were not included in the statistical analysis. All women had a history of at least one previous unsuccessful IVF/ET. The inclusion criteria for the study were age $\leq$ 38 years for the IVF women and $\leq$50 years for the oocyte receivers, without known endometriosis, hydrosalpinx or uterine anomalies, including small submucosal myomas or polyps. Four hundred women were allocated into group I or II when they were called to be informed about the day of their embryo transfer. All cases had had a mock transfer in a cycle previous to IVF and if

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difficulty was encountered a cervical dilatation was performed [8]. All cases included in the statistical analysis had at least one good quality embryo.

2.1. Stimulation protocol and endometrial preparation

A fixed GnRH antagonist protocol was used to stimulate the ovaries in both IVF and OD cycles. Briefly, stimulation started on the day 2 of the menstrual cycle with 225 IU/day of recombinant FSH (Puregon, NV Organon) administered subcutaneously for 4 days. Before the initiation of gonadotrophin treatment, a baseline ultrasound was performed to exclude the presence of ovarian cysts. Cycles were monitored by vaginal ultrasound and oestradiol measurements starting on the 4th day of stimulation, according to which the daily dose of gonadotrophin was adjusted. A GnRH antagonist (Orgalutran 0.25 mg; NV Organon) was added in the afternoon of the 6th day of stimulation administered until the day before human chorionic gonadotrophin (HCG) injection: 10,000 IU of HCG (Pregnyl; NV Organon) were administered when at least three follicles with a mean diameter $\geq$ 17 mm were present on ultrasound scan. No antagonist was injected on the day of HCG administration. Transvaginal ultrasound-guided oocyte retrieval was performed 34–36 h after HCG injection under local anaesthesia and i.v. sedation. ICSI was used to fertilize all oocytes 4 h after retrieval. Fertilization was assessed 16–20 h post-ICSI by visualization of the two pronuclei. Embryo quality was assessed on day 3 and day 5. Classification of embryos was based on the criteria of Veeck [9] for day 3 and Gardner et al. [10] for day 5. Briefly, the following were considered top quality embryos: on day 3, those with blastomeres of equal size and no cytoplasmic fragments while on day 5 those with thin zona pellucida, smooth trophoectoderm, equality and close adhesion of blastomeres, clearly visible blastocyst cavity and well developed inner cell mass. All cases included in the study had at least one high quality embryo for transfer. Luteal phase was supported with vaginal progesterone (Utrogestan; Laboratoires Besins, Iscovesco, France) 600 mg per day, in three divided doses.

The recipient's endometrial preparation started with injection of GnRH analogue (Arvecap 3.75 mg) on day 20 of the cycle prior to ET. On the day of the donor's menstrual period, the recipients were informed to start taking oestradiol valerate in a dose of 2 mg per day for the first four days, 4 mg per day for the next four days and 6 mg per day until the results of the pregnancy test. Twelve hours before the oocyte retrieval, 200 mg of progesterone (Utrogestan) was administrated intravaginally and continued with 200 mg three times a day, until a foetal heartbeat was observed by ultrasound. Endometrial development was evaluated by ultrasound scan and was considered sufficient when the endometrial thickness was $\geq$9 mm.

2.2. Oocyte and embryo culture

After their retrieval, cumulus-oocyte complexes (COCs) were maintained in fertilization media (Sage Inc., A Cooper Surgical Company™). A group of maximum 10 COCS were put in a 0.4 ml drop covered with mineral oil (FertiPro NV, Belgium). When necessary, more than one dish was used per patient. Two hours after retrieval, COCs were enzymatically denuded in 75 IU hyaluronidase (Sage Inc.) and the MII oocytes were maintained in a 0.2 ml drop of fertilization media under oil for 1 h. Then, ICSI was performed. For ICSI, oocytes were put in 20 μl microdrops of Quinn's Advantage Medium with Hepes (Sage Inc.) supplemented with 10% Serum Protein Substitute (SPS) (Sage Inc.). The inseminated oocytes were cultured separately in 25 μl microdrop of cleavage medium (Sage Inc.) overlaid with mineral oil (FertiPro N.V., Belgium) in MINC benchtop incubators (COOK Medical) until day 3.

On the morning of day 3, embryos were washed and put in separate microdrops of blastocyst medium (Sage Inc.) covered with mineral oil and cultured until day 5. Dishes with 9 microdrops of blastocyst medium were prepared on day 2 and overnight equilibrated. When necessary, more than one dish was used per patient. All embryo culture was performed at 37 °C, 6% CO_2, 5% O_2 and 89% N_2, in a humidified atmosphere.

2.3. Embryo transfer

In all cases the ET was carried out by the same examiner (YP) under ultrasound guidance. The tip of the catheter during the ET was approximately in the middle of the endometrial cavity. Cervical canal irrigation was not performed. For women in group I, 30 min before ET the patient was placed in the lithotomy position and a sterile speculum was placed to expose the cervix, which was swabbed using sterile gauze dampened with culture medium while the endocervical canal was gently swabbed with cotton-tipped applicators moistened in the culture medium. Under ultrasound control the Wallace catheter was then inserted into the lower part of the uterine cavity and a 0.4 ml of their supernatant embryo culture medium was injected into the cavity. The patient was left in the same lithotomy position and 30 min later the ET was performed using another Wallace catheter. Except for the flushing of the uterus, all of the rest clinical and laboratory procedures were identical between the study and the control group. The decision to transfer on day 3 or day 5 was based on either the patient's request or the number of good quality embryos on day 3. Cases with $\geq$4 good quality embryos on day 3 were advised to have their transfer on day 5. In all our cases, at most three good quality embryos were transferred. A pregnancy test was performed 15 days after the ET, and, if positive, an ultrasound scan was scheduled after two weeks to determine the number and status of implanted embryos. The concurrency of a positive beta-HCG test and a foetal heartbeat (seen by ultrasound) was defined as a clinical pregnancy.

2.4. Statistical analysis

Power statistical analysis suggested that in order to detect a statistical significant difference of 20% in pregnancy rates as reported by Letterie et al. [4] with power = 90% between the non-injection and injection groups respectively a sample size of 134 subjects per group would be needed. The x^2-test was used to compare pregnancy and implantation rates between different groups. Concerning continuous data of the two groups of the study, normality of their distribution was checked with the Kolmogorov–Smirnov test, which indicated that distribution was normal. Therefore, the Student *t*-test was used for the comparison of mean values between the continuous data, including maternal age, number of oocytes, endometrial thickness, embryos transferred and embryo quality. $P < 0.05$ was considered statistically significant. STATA 11.2 (StataCorp, College Station, TX) was used for power calculations and for data analysis.

3. Results

Data were prospectively collected for 200 IVF-ICSI and 200 OD cycles performed at the Iakentro IVF centre from June 2009 through November 2010. A flow chart of inclusion, randomization and drop-out cycles is shown in Fig. 1.

A total of 384 cycles had their ET on either day 3 or 5. In 188 cycles the uterine cavity was injected with their embryo culture supernatant before embryo transfer (group I) while in 196 cycles