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Does flushing the endometrial cavity with follicular fluid after oocyte retrieval affect pregnancy rates in subfertile women undergoing intracytoplasmic sperm injection? A randomized controlled trial

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ABSTRACT

Objective: Follicular fluid of mature oocytes is rich in growth factors and cytokines that may exert paracrine and autocrine effects on implantation. The aim of this study was to investigate if flushing the endometrial cavity with follicular fluid after oocyte retrieval improved pregnancy rates in subfertile women undergoing intracytoplasmic sperm injection (ICSI).

Study design: One hundred subfertile women undergoing ICSI between April 2012 and September 2012 at the centre for reproductive medicine, Cairo University, Egypt were enrolled in this open label, parallel randomized controlled study. Patients were randomized into two groups at the start of treatment using a computer-generated programme and sealed opaque envelopes: the follicular fluid group ($n = 50$) and the control group ($n = 50$). Inclusion criteria were: age 20–38 years; basal follicle-stimulating hormone <10 mIU/ml; body mass index <35 kg/m^2; and ostradiol >1000 pg/ml and <4000 pg/ml on the day of human chorionic gonadotrophin administration. Exclusion criteria were: evidence of endometriosis; uterine myoma; hydrosalpinges; endocrinological disorders; history of implantation failure in previous in-vitro fertilization/ICSI cycles; and severe male factor infertility.

Results: Clinical pregnancy and implantation rates were higher in the follicular fluid group compared with the control group [354% (17/48) vs 319% (15/47); $p = 0718$] and (18.6% vs 11.3%; $p = 0.153$), respectively. However, the difference was not statistically significant.

Conclusion: Flushing the endometrial cavity with follicular fluid after oocyte retrieval neither improved nor adversely affected clinical pregnancy and implantation rates in subfertile women undergoing ICSI.

1. Introduction

Implantation of human embryos is the least-understood step in assisted reproductive technology. Successful implantation requires a synchronized interaction between the endometrial secretory pattern and competent embryos in both natural and assisted reproduction cycles [1]. Such cross-talk at the maternal–fetal interface is co-ordinated by local ovarian hormones, oestrogen, progesterone [2], cytokines, chemokines and growth factors [3]. Although embryo quality is an important determinant of implantation, temporally co-ordinated differentiation of endometrial cells to attain uterine receptivity is crucial. Optimizing endometrial receptivity in fertility treatment will improve the success rate.

Follicular fluid of mature oocytes is rich in growth factors (e.g. stem cell factor, vascular endothelial growth factor, transforming growth factor and insulin-like growth factor) and cytokines [4–7] that may exert paracrine and autocrine effects on implantation, as transforming growth factor controls apoptosis, vascular endothelial growth factor promotes angiogenesis, insulin-like growth factor improves endometrial growth, and the immunosuppressive activity of the follicular fluid paves the way for the embryo to implant in the uterine cavity [6,7]. The immunosuppressive activity of follicular fluid is mediated by the specific inhibition of interleukin-1 alpha and interleukin-2 production, and decreased CD25 expression [8].

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Several growth factors and cytokines appear to be involved in implantation. Stem cell factor and its receptor c-kit regulate the progression and survival of germ cells, and play an important role in follicular development in mice. Stem cell factor is present in human follicular fluid, but little information is available on its role and that of its receptor in blastocyst implantation. Mitsunari et al. examined the expression of stem cell factor and its receptor in mouse embryos and in stromal and epithelial cells of uterine endometrium by reverse transcription-polymerase chain reaction. They demonstrated that stem cell factor derived from endometrial cells and the implanting embryo has paracrine and autocrine effects on implantation by stimulating trophoblast outgrowth through c-kit [6].

In 2007, Hunter et al. provided circumstantial evidence that follicular fluid enters the fallopian tubes at the time of spontaneous ovulation in natural cycles and reaches the uterine cavity [9]. Several studies have suggested that follicular fluid could modify the nature of endosalpingeal secretion and transudation. Therefore, the spectrum of gonadal hormones in follicular fluid could influence uterine tissues in a local manner [10].

It has been shown that follicular fluid supplementation of human embryo culture media enhances the cleavage of human embryos during in-vitro fertilization (IVF) to morula and blastocyst stages [11]. Therefore, this study aimed to evaluate the influence of directly flushing the endometrial cavity with follicular fluid immediately after oocyte retrieval in subfertile women undergoing intracytoplasmic sperm injection (ICSI) on clinical pregnancy and implantation rates.

2. Materials and methods

The study protocol was approved by the institutional ethics committee, and informed consent was obtained for each patient. One hundred subfertile women were enrolled for ICSI from April 2012 to September 2012 at the centre for reproductive medicine, Cairo University, Egypt in this open label, parallel randomized controlled study. Patients were randomized into two groups at the start of treatment using a computer-generated programme and sealed opaque envelopes: the follicular fluid group ($n = 50$) and the control group ($n = 50$). Inclusion criteria were: age between 20 and 38 years; basal follicle-stimulating hormone <10 mIU/ml; body mass index <35 kg/m^2; and oestradiol level >1000 pg/ml and <4000 pg/ml on the day of human chorionic gonadotrophin (hCG) administration. Exclusion criteria were: evidence of endometriosis; uterine myoma; hydrosalpinges; endocrinological disorders; history of implantation failure in previous in-vitro fertilization/ICSI cycles; and severe male factor infertility.

A mid-luteal long gonadotrophin-releasing hormone agonist protocol was used for pituitary downregulation in both groups. Controlled ovarian hyperstimulation with a daily dose of 150–450 IU HP-HMG (Merional 75 IU, IBSA, Italy) was initiated once downregulation was confirmed. hCG 10,000 IU (Chorimon, IBSA, Italy) was administrated once at least three follicles reached a diameter of 18 mm or more. Oocyte retrieval was scheduled 35–36 h later, and each follicle was aspirated in a separate tube and examined by the embryologist. A midstream aspirate was collected for each patient and centrifuged. Two millilitres of clear follicular fluid retrieved from a single mature oocyte was injected immediately into the uterine cavity through an intra-uterine insemination catheter (Labotect GmbH, Germany) to flush the endometrium of patients in the follicular fluid group. Embryo transfer was performed 3 days after oocyte retrieval.

The primary outcome measures were the clinical pregnancy and implantation rates. The clinical pregnancy rate was defined as the presence of a gestational sac with fetal echoes and fetal heart rate pulsations. The implantation rate was defined as the ratio of the number of intra-uterine gestational sacs to the number of embryos transferred.

2.1. Statistical analysis

Numerical variables were compared between the study groups using Student's *t*-test for independent samples for normally distributed data and Mann–Whitney's *U*-test for independent samples for non-normally distributed data. Chi-squared (χ^2) test was used to compare categorical data. Fisher's exact test was used when the expected frequency was less than 5. $p < 0.05$ was taken to indicate significance on intention-to-treat analysis. All statistical analyses were performed using Statistical Package for the Social Sciences Version 15 (SPSS Inc., Chicago, IL, USA). For a significance level of 0.05, power of 0.80 and a difference in clinical pregnancy rate between the two treatment arms of 15–20% in favour of the follicular fluid group, a sample size of 2828 treated patients would be required, but this sample size was too large for this single-centre study.

3. Results

One hundred women were randomized into two groups: the follicular fluid group ($n = 50$) and the control group ($n = 50$). Five patients were cancelled (two in the follicular fluid group and three in the control group) due to poor ovarian response, no oocytes available for injection or high risk of ovarian hyperstimulation syndrome (Fig. 1). No significant differences in baseline demographic characteristics (i.e. age, body mass index, basal follicle-stimulating hormone, type and duration of subfertility) were found between the two groups (Table 1).

No significant differences in the serum oestradiol level on the day of hCG administration [mean ± standard deviation: 2668 ± 1099 vs 2536 ± 1110 pg/ml; $p = 0.5$), endometrial thickness (10.1 ± 1.5 vs 10 ± 2.3 mm; $p = 0.7$), number of MII oocytes (10.8 ± 4.2 vs 10.2 ± 5.5; $p = 0.5$); number of available good-quality embryos (3.2 ± 1.4 vs 3.8 ± 1.9; $p = 0.1$) and number of embryos transferred (3.5 ± 0.9 vs 3.2 ± 1; $p = 0.2$) were found between the two groups. The implantation rate per number of embryos transferred and the clinical pregnancy rate per number of women randomized were higher in the follicular fluid group than the control group, but the differences were not significant [186% vs 113% ($p = 015$) and 17/50 (34%) vs 15/50 (30%) ($p = 07$), respectively] (Table 2). There was no significant difference in the miscarriage rate between the two groups.

4. Comments

To the authors' knowledge, this is the first study to examine the effect of flushing the endometrial cavity with follicular fluid on pregnancy and implantation rates in subfertile women undergoing ICSI. Several strategies have been tried to enhance the ability of an embryo to implant, such as endometrial biopsy in a cycle prior to IVF, assisted hatching [12], addition of adherence compounds in embryo transfer media [13], flushing the endometrium with culture media prior to embryo transfer [14], use of cumulus co-culture and cumulus-aided embryo transfer [15] and intra-uterine injection of hCG before embryo transfer [16].

The absence or suppression of molecules essential for endometrial receptivity results in a lower implantation rate. The mechanisms are diverse and include abnormal cytokine and hormone signalling as well as epigenetic alterations. Nanotechnologies have proven to be revolutionary tools capable of dissecting the cellular mechanosensory apparatus, thus allowing the intercellular cross-talk to be decoded, and enabling the vast potential of growth factors and cytokines on embryo survival and growth to be revealed. This presents a fascinating perspective in