

Local injury to the endometrium does not improve the implantation rate in good responder patients undergoing in-vitro fertilization

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Abstract

Background: Despite numerous developments in the field of assisted reproduction the implantation rate remains low. Recent studies suggested that local injury to endometrium in controlled ovarian hyper stimulation cycle improves implantation rate. Studies have attempted to intervene in the development of endometrium.

Objective: The aim of the present study was the exploration of the possibility that local injury of the endometrium increases implantation rate.

Materials and Methods: In this interventional study, 100 good responders to hormone stimulation patients were divided into control group (n=50) and experimental group (n=50) which undergo endometrial biopsy by biopsy catheter (pipelle) on day 21 of their previous menstrual cycle with use of contraceptive pills before the IVF-ET treatment. In total, 26 patients were removed from the study because the number of stimulated follicles were below 3, or there was no embryo or there was the risk of OHSS. The remaining patients were 33 in experimental group and 41 in the control group.

Results: There were no significant differences between the two groups in terms of the age of the patients, duration of infertility and BMI, base line FSH level and responses to hormone stimulation. The rates of embryo implantation, chemical and clinical pregnancy in the experimental group were 4.9%, 18.2% and 12.1% with no significant differences with the control group (6.7%, 19.5%, 17.1%). Cancellation rate was 26%.

Conclusion: In our study, endometrial biopsy didn't increase the chances to conceive at the following cycle of treatment.

Key words: Endometrial injury, Assisted Reproductive Treatment, ART, Implantation, Endometrial biopsy.

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Introduction

Embryo implantation in the human is still a poorly understood process. Implantation begins 6 to 7 days after fertilization (1). "Implantation has been classified into three stages: apposition, adhesion, and penetration" (2). The uterus plays a critical role to the success of each stage. Multiple signals are needed to synchronize blastocyst maturation and uterine receptivity, including sex steroid and peptide hormones, growth factors,

cytokines, immunologic, and angiogenic factors (1, 3). The endometrium is receptive to embryonic apposition, attachment, and invasion during a defined implantation window to establish a pregnancy; however, implantation may fail during in vitro fertilization, embryo transfer (IVF-ET) cycle even if good-quality embryo with good shape and cell numbers are used.

The development of receptive endometrium is thus necessary for successful implantation, which is the rate-limiting step for the success of IVF. However, endometrial receptivity is important to pregnancy in assisted reproduction treatment (ART), obtaining midsecretory endometrium for clinical test or research has been difficult among the various etiologies that were described.

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Endometrial regularity played an important role in infertility and success of IVF program. Previous studies demonstrated that removal of polyps or abnormal thickened endometrium significantly increased the incidence of clinical pregnancy rate in IVF-ET (4).

In previous studies local injury to the endometrium in controlled ovarian hyper stimulation cycles improves implantation rates (5). Endometrial biopsies were taken during the spontaneous cycle that preceded the IVF treatment more than doubled the rates of implantation, clinical pregnancies, and live births (6).

Such a favorable influence of local injury on the endometrium was later confirmed by Raziel *et al* (7), who showed a significant increase in the success of IVF treatments in a population of patients with a high order of previous implantation failures. A more recent study, further demonstrated that "local injury of the endometrium executed in IVF patients during their cycle of treatment, before ovum retrieval, gains the same effect of increasing implantation and clinical pregnancy rates" (5).

In the present study we evaluated the effects of local injury of endometrium in controlled ovarian hyper stimulation cycle on the rate of pregnancy.

Materials and methods

Patients

This study was performed from July 2008 to March 2009, at Shariati Hospital Infertility Center on 20- 39 years old infertile women who were referred to this center. After taking history and physical examination; Hysterosalpingogram (HSG) and routine hematological, biochemical and hormonal tests, semen analysis, all infertile women underwent standard Transvaginal sonography (TVS) with convex 7.5 MHz transvaginal ultrasound probe.

We excluded patients above 39 year old, and the ones whose FSH was above 11, had endometriosis and hypothalamic amenorrhea and azoospermic male. Patients with PCO were not excluded. In this interventional study, 100 patients were defined as good responders to hormone stimulation. Patients were divided to control groups (n=50) and experimental group (n=50) whom undergo endometrial biopsy.

Ethics of experimentation

Investigation has been approved and trial has been authorized under the decision of ethical committee of Tehran University of Medical Sciences.

Measurements

We informed all the cases about the technique therapeutic effect and potential risks of biopsy and obtained informed consent.

Protocol of stimulation in subsequent IVF/ICSI attempt

Patients of the experimental group (n=50) were computerized randomly selected on the basis of their agreement to undergo endometrial biopsy expressed in a written informed consent before the beginning of the IVF cycle. Endometrial biopsy in these patients were taken by biopsy catheter (pipille-de Cornier, Prodimed, and Neuilly-en-Thelle, France) on day 21 of their previous menstrual cycle with use of contraceptive pill before the IVF-ET treatment. Patients in the control group (n= 50) did not receive endometrial biopsy. All of the 100 patients were treated with standard long protocol for COH. GnRH- agonist Buserlin (Superfact- Aventis Pharma, Deutchland) 0.5 mg/day was administered from the day 21 of the cycle.

Then all patients were treated with human menopausal gonadotropin (Menogon-Ferring GmbH Germany) from day 2-3 of the next cycle, while continuing Superfact 0.20 mg/day with the control of follicular growth under sonography. HCG (IVF-C:LG Life Sciences Korea) was administered 10000 IU when the minimum of 3 leading follicles reached 16-18mm, and 36 hours later oocyte collection was performed .

Statistical analysis

Descriptive statistics were used to report demographic characteristics; by the means of SPSS package Version 17.0 SPSS Inc., Chicago, IL, USA. Categorical data were expressed as numbers and percentages, and numerical data as mean (\pm SD). Student's t-test, chi-square test and Fisher's exact test were used when appropriate. Significance was defined as $p \leq 0.05$.

Results

There were no significant differences between the two groups in terms of the age, BMI, baseline FSH level and duration of infertility (Table I). Dose of hormonal stimulation, number of retrieved oocytes, number of transferred embryos, and endometrial thickness were not significantly different (Table II).

The rates of implantation, chemical pregnancy, and clinical pregnancy in the operation group were 4.9% 18.2%, 12.1 % and in the control group

6.7%, 19.5%, 17.1% respectively that were not significant differences (Table III). In total 26 patients were cancelled.

The remaining patients in experimental group were (n=33) and in the control group were (n=41). Cancellation rate was 26% (26 patients cancelled): 6 patients didn't have embryo, in 12 patients the follicles were below 3, and 8 patients because of the risk of OHSS (when the estradiol was more than 3000pg/ml at time of HCG injection). No infections were reported among the patients treated by the biopsy catheter.

Table I. Comparison of demographic characteristic in two groups.

Variations	Control group mean \pm SD	Biopsy group mean \pm SD	p-value
Number	41	33	-
Age (year)	30.2 \pm 4.8	29 \pm 4.6	0.267
BMI (kg/m ²)	26.9 \pm 3.2	26.5 \pm 3.8	0.708
FSH level	6.2 \pm 2.3	6.2 \pm 2.0	0.926
Duration of infertility(year)	7.8 \pm 5.5	7.4 \pm 4.7	0.755

Table II. Comparison of quantities outcomes in two groups.

Variation	Control group mean \pm SD	Biopsy group Mean \pm SD	p-value
No. of gonadotropin	31.2 \pm 12.4	32.7 \pm 11.9	0.626
No. of oocytes	12.9 \pm 5.8	10.7 \pm 3.5	0.071
Endometrial thickness (mm)	10.5 \pm 1.8	10.7 \pm 1.9	0.213
No. of transferred embryos per cycle	3.6	3.7	0.516

Table III. Comparison of pregnancy outcomes between two groups.

Variation	Control group	Biopsy group	p-value
Implantation rate	6.7% (10/148)	4.9% (6/122)	0.95
Chemical pregnancy	19.5% (8/41)	18.2% (6/33)	0.88
Clinical pregnancy	17.1% (7/41)	12.1% (4/33)	0.58

Discussion

"The relationship between endometrial injury and improved implantation is based on animal studies. Early studies in mice demonstrated that endometrial injury by injecting oil into the endometrial cavity resulted in decidualization" (8).

"The decidual tissue performs numerous function to support fetal growth and development of fetus" (9). "The injury-induced decidualization could be prevented by administration of antihistamines into the uterine horn or by chronic treatment with chemical histamine releasers that

produced depletion of endogenous histamine resources" (10, 11).

During healing of the endometrial injury, several substances are secreted including cytokines and growth factors such as leukemia inhibitory factor, interleukin-11, and EGF-like growth factor. "These substances could facilitate implantation. Similarly, in response to local irritation by oil, the endometrium releases histamines" (10, 14).

The possible role of endometrial injury on improved implantation was first emphasized by Barash *et al* (6); they studied 45 women who failed to conceive after one or more cycles of IVF-ET. They found that endometrial injury in the cycle before IVF significantly improved the outcome. They also postulated that the injury promotes decidualization of the endometrium making it more receptive for implantation.

In their study, the endometrial injury was performed using a disposable endometrial biopsy instrument (pipelle, Prodimed, euilly-en-Thelle, France) on days 8, 12, 21 and 26 of the cycle preceding IVF. The rates of implantation, clinical pregnancy, and live birth in the endometrial injury group were 28%, 67%, and 49% and in the control group were 14%, 30%, and 23%, respectively. Raziel *et al* (7) induced endometrial injury in the luteal phase on days 21 and 26 among 60 women with implantation failure.

The mean number of previous failed IVF trials was seven. They compared the results in 57 others who did not undergo endometrial injury. The implantation and pregnancy rates in the injury group were 11% and 30%, and in the control group were 4% and 8% respectively. Zhou *et al* (5) performed endometrial injury in women with irregular echo on ultrasound examination until the strong or homogeneous echo disappeared followed by "scratching" the endometrium once or twice. This was performed on days 5-22 of controlled ovarian hyper stimulation cycle. They also found that endometrial injury is associated with increased implantation and pregnancy rates. They postulated that local injury to the proliferative endometrium in the stimulated cycles delayed endometrial development inducing synchronicity between the endometrium and embryo stage (5).

It is possible that IVF patients who fail to conceive with high-quality embryos are unable to increase the expression of genes related to endometrial receptivity in a spontaneous manner. Endometrial injury optimizes endometrial development. However, whether endometrial injury in the luteal phase leads to a better clinical outcome than in the follicular phase is unclear.

It is also unknown whether one endometrial biopsy is sufficient and whether it should be performed in the preceding or in the same stimulation cycle. Regardless, it seems that a few strokes of endometrial sampling are needed. Zhou *et al* (5) used ultrasound findings as one of their criteria, and they treated their patients with antibiotics and homeostatic drugs (cefaclor and adrenobazone). Their study has some confounding factors. In our study patients were taken single biopsy, by a biopsy catheter (Pipelle) on day 21 of their previous cycle, before the IVF-ET treatment. The patient were requested to use hormonal means of contraception during this cycle. The results of our study demonstrate no improvements in the rate of conception of IVF patients who were taken single biopsy.

Conclusion

In our study endometrial biopsy didn't increase the chance to conceive at the following cycle of treatment. Evidence to date suggests that local endometrial injury might improve the pregnancy rate. However, there are still many unanswered questions regarding patient selection, timing, technique and number of biopsies needed.

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References

1. Viganò P, Mangioni S, Pompei F, Chiodo I. Maternal-conceptus cross talk-a review. *Placenta* 2003; 24: 56-61.
2. Enders AC, Schlafke S. A morphological analysis of the early implantation stages in the rat. *Am J Anat* 1967; 120: 185-225.
3. Norwitz ER. Defective implantation and placentalin: laying the blueprint for pregnancy complications. *Reprod Biomed Online* 2006; 13: 591-599.
4. Li R, Gui Y, Lu L, Hao G, Cai Z. Local injury to the endometrium improves the pregnancy rate in patients undergoing in vitro fertilization .18th World Congress on *Fertil Steril* 2004; 1271: 73-76.
5. Zhou L, Li R, Wang R, Huang HX, Zhong K. Local injury to the endometrium in controlled ovarian hyperstimulation cycles improves implantation rates. *Fertil Steril* 2008; 89: 1166-1176.
6. Barash A, Dekel N, Fieldust S, Segal I, Schechtman E, Granot I. Local injury of the endometrium doubles the incidence of successful pregnancies in patients undergoing in-vitro fertilization. *Fertil Steril* 2003; 79: 1317-1322.
7. Raziel A, Schachter M, Strassburger D, Bern O, Ron-El R, Friedler S. Favorable influence of local injury to the endometrium in intracytoplasmic sperm injection patients with high-order implantation failure. *Fertil Steril* 2007; 87: 198-201.
8. Humphrey KW. The effects of some anti-estrogens on the deciduoma reaction and delayed implantation in the mouse. *J Reprod Fertil* 1968; 16: 201-209.
9. Mikhailov VM. Life cycle of decidual cells. *Int Rev Cytol* 2003; 227: 1-63.
10. Basak S, Dubanchet S, Zourbas S, Chaouat G, Das C. Expression of pro-inflammatory cytokines in mouse blastocysts during implantation :modulation by steroid hormones. *Am J Reprod Immunol* 2002; 47: 2-11.
11. Finn CA, Martin L. Endocrine control of the timing of endometrial sensitivity to a decidual stimulus. *Biol Reprod* 1972; 7: 82-86.
12. Sharkey A. Cytokines and implantation. *Rev Reprod* 1998; 3: 52-61.
13. Akita S, Ishihara H, Mohammad Abdur R, Fujii T. Leukemia inhibitory factor gene improves skin allograft survival in the mouse model. *Transplantation* 2000; 70: 1026-1031.
14. Sherer D, Abulafia O. Angiogenesis during implantation, and placental and early embryonic development. *Placenta* 2001; 22: 1-13.