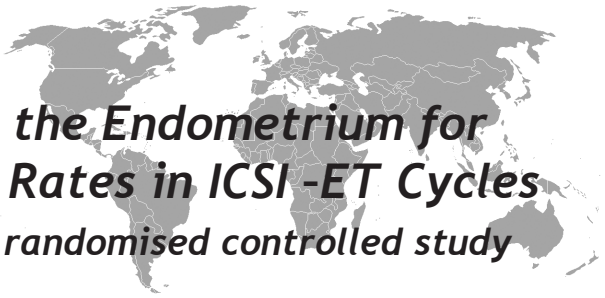

The Effect of Local Injury to the Endometrium for Implantation and Pregnancy Rates in ICSI -ET Cycles with Implantation Failure: a randomised controlled study

Zeynep Hafiza Öztürk İnal[1], Hüseyin Görkemli[2], Hasan Ali İnal[1]

ABSTRACT

To assess the effect of local injury to the endometrium for implantation, pregnancy and live birth rates in ICSI -ET cycles with recurrent implantation failure. The study was prospectively designed and performed in a university hospital. A group of 100 women, who failed to conceive during one or more cycles of IVF and embryo transfer (ET), was treated with a long protocol for controlled ovarian hyperstimulation. The IVF treatment and ET were preceded by repeated endometrial biopsies, in a randomly selected 50 of a total of 100 women. Age of the women, days of stimulation, total dose of gonadotropins, E2 concentrations and endometrial thickness on hCG day, total MII stage oocytes, the total number of high quality embryos for transfer, the day of transfer and the number of embryos transferred were similar for both groups. Transfer of a similar number of embryos (138 and 141 in the experimental and control patients, respectively) resulted in rates of implantation (34.67 % vs. 30.88 %; p= 0,1384) clinical pregnancy (60 % vs. 34 %; p= 0.009), and live births per ET (44 % vs. 24 %; p= 0.03), which were higher in the experimental group as compared to controls. These results suggest that IVF treatment that is preceded by local injury to the endometrium improved the rates of embryo implantation, clinical pregnancy, and live births in asiste reproductive technique.

Key words: *Endometrium, local injury, implantation, pregnancy, in vitro fertilization*

İmplantasyon Başarısızlığı Olan ICSI-ET Sikluslarında Endometriuma uygulanan lokal hasarın implantasyona Etkisi ve Gebelik Oranlarına Etkisi

ÖZET

Tekrarlayan implantasyon başarısızlığı olan ICSI-ET (intrasitoplazmik sperm injeksiyonu-embriyo transferi) sikluslarında endometriuma uygulanan lokal hasarın implantasyon, klinik gebelik ve canlı doğum oranlarına olan etkisini araştırmak. Çalışma bir üniversite hastanesinde prospektif olarak tasarlandı ve geçekleştirildi. İnfertilite nedeniyle başvuran ve ICSI-ET yapılması kararlaştırılan, uzun protokol (GnRH analoğu; rFSH+HMG) yöntemiyle KOH uygulanacak, tekrarlayan implantasyon başarısızlığı olan toplam 100 hasta seçildi. Aralarından randomize olarak seçilen 50 hastaya KOH siklusundan önce tekrarlanan endometrial biyopsi uygulandı. Hastaların yaş ortalaması, indüksiyon gün süresi, kullanılan toplam gonadotropin dozları, hCG günü E 2 değerleri ve endometrium kalınlıkları, siklus başına toplam MII evresindeki oosit sayıları, transfere uygun kalitedeki total embriyo sayıları, transfer günleri ve transfer edilen embriyo sayıları iki grupta da benzerdi. Benzer sayıda embriyo transferleri ile (çalışma ve kontrol gruplarında sırasıyla 138 ve 141) implantasyon oranları (% 34.67'e karşı % 30.8, p > 0.05), klinik gebelik oranları (% 60'e karşı % 34, p= 0.009) ve ET başına canlı doğum oranları (% 44'e karşı % 24, p =0.03) çalışma grubunda kontrol grubuna göre daha yüksek bulunmuştur. Bu sonuçlar IVF tedavisi öncesinde endometriuma uygulanan lokal hasarın embriyo implantasyon, klinik gebelik ve canlı doğum oranlarını artırdığını desteklemiştir.

Anahtar kelimeler: *Endometrium, lokal hasar, implantasyon, doğum, in-vitro fertilizasyon*

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INTRODUCTION

IVF/ICSI-ET (in vitro fertilization/intracytoplasmic sperm injection-embryo transfer); is one of the most important methods in the treatment of female infertility and male sterility, but success rate is only around 30-40 %. How clinical pregnancy rates would be improved in human reproductivity is the main question. Embryo implantation step is a complex and multistage process which may be one of the causes that may explain outcome failures (1). In spite of repeated transfers of embryos with good morphology into a normal uterus in IVF-ET cycles, implantation may fail. Consequently, a successful implantation depends not only on quality of embryo but is also related to endometrial receptivity. Thus, developing a receptive endometrium, which is a limiting step for success in IVF procedure, is at upmost importance for a successful implantation (1,2). The objective of this study is to investigate whether local injury to the endometrium prior to controlled ovarian hyperstimulation (COH) cycle in women with implantation failure would improve the implantation, clinical pregnancy and live birth rates.

MATERIALS AND METHODS

Setting

The study was conducted in assisted reproductive center of Selcuk University Meram Medical Faculty Hospital in Konya, Turkey. A group of 100 women who applied to our clinic due to infertility and had a history of prior failure of fresh IVF cycles were included in our study for ICSI-ET between January 2008 and March 2009.

Inclusion and exclusion criteria

A group of 100 patients, defined as good responders to hormonal stimulation, who failed to conceive during one or more cycles of IVF and embryo transfer (ET) was selected for this study. We excluded women with hydrosalpinx, trombophilia or submucous myoma and factors that are found to have a negative impact on implantation. Written informed consents of the participants were obtained and the study was approved by the ethic committee of our faculty.

Randomisation schedule and allocation

A total of 100 women were randomly assigned to the study, 50 in the study group and 50 control. The randomization was based on a computer generated random numbers. All participants were allocated to long protocol for COH (Figure 1).

The women in the study group underwent two consequtive endometrial biopsies with one-week intervals during the luteal phase of the nontransfer cycle, when they are on GnRH-analogue for down regulation. Endometrial biopsy was performed with a biopsy catheter (Pipelle; de Cornier, Prodimed, Neuilly-en-Thelle, France) introduced through the cervical os and rotated in uterine cavity 3-4 times after withdrawing the piston. Antibiotics were administered after the procedure and no infections or complications occured.

Treatment schedules

All of the women were protected by one of non-hormonal contraception method during this cycle. On about day 21 of their previous menstrual cycles, all participants were administered Leuprolide acetate (Lucrin; Abbott, France) at a dose of 1 mg/day subcutaneously for pituatory down regulation and depletion of endogenous gonadotropins. When down regulation was achieved controlled ovarian hyperstimulation with recombinant gonadotropins (Puregon (Organon)s.c., Gonal F (Serono) s.c) and/or human menopausal gonadotropins (Merional (IBSA) s.c., Menogon (Ferring) i.m.) were started at a dose adjusted according to patient characteristics. When a leading follicle of 18-20 mms or two follicles of 17 mms were achieved, ovulation was triggered by 10 000 IU urinary hCG (Pregnyl amp; Organon, Turkey). Oocytes were retrieved at 35-37 hours following hCG administra-

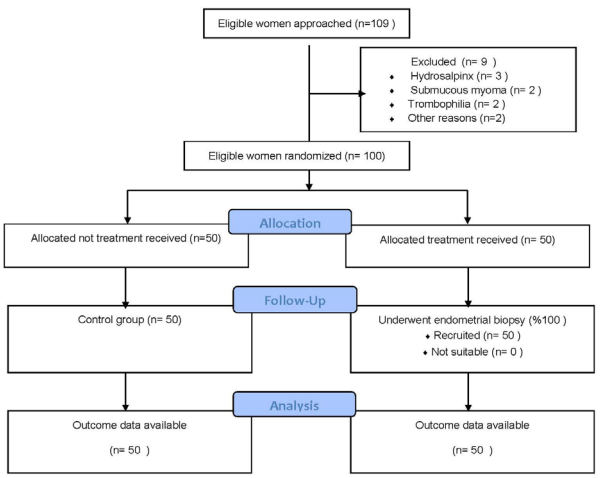

***Figure 1**. Trial flow chart*

***Table 1**. Ovarian hyperstimulation parameters of control and study groups*

	Control	Study	p value
Age(years)	30.8±4.5	29.6±3.8	0.15
Unexplained infertility	14 (28%)	10 (20%)	0.61
Reasons of Tubal Factor	9 (18%)	11 (22%)	0.87
Infertility			
Ovulatory Factor	12 (24%)	13 (26%)	0.96
Male Factor	15(30%)	16 (32%)	0.96
Number of failed			
IVF cycles: 1	38 (76%)	31 (62%)	0.62
2	9 (18%)	16 (32%)	0.30
3	3 (6%)	3 (6%)	0.67
Number of previously			
transferred embryos	2.68+1.3	2.42+1.2	0.30
Duration of hormonal stimulation (d)	10.52±1.1	10.48±1.0	0.84
Total gonadotropin amount(IU)	3087.7±498.2	3075±461.9	0.89
hCG administration (pg/mL)	2003.3±410.9	2025.9±414.2	0.78
Endometrial thickness (mm)	10.3±1.4	10.8±1.6	0.09
Mean MII oocyte numbers per cycle	8.4±1.8	8.5±1.6	0.76

tion with ultrasound guidance. Semen specimens were collected on the day of follicle aspiration several hours before the procedure by masturbation after sexual abstinence of 2-4 days. Sperms were obtained by TESA/ TESE in 3 men in the study and 3 men in the control group. Intracytoplasmic sperm injection was performed in all cycles. Fertilization (exhibition of two pronuclei) was evaluated at the subsequent 18th hour and embryo development was evaluated at 24th-48th-72nd hours. On 2nd-3rd or 4th day following oocyte retrieval, maximum 3 embryos having highest quality scores (grade 3 or 4) were selected, and were transferred into intrauterine cavity with ultrasonographic guidance . Wallace 23 mm soft catheter (Smiths, England) was used for the embryo transfer. Follicle aspiration and embryo transfer procedures were performed by a single senior physician. Luteal phase support was started at the evening of follicle aspiration on all women. Intravaginal micronized progesterone 3x200 mg (Progestan soft capsule; Kocak ilac, Turkey), intramuscular progesterone 25 mg/day (Progestan in oil), prednol tablet 16 mg/day p.o. for 5 days (Mustafa Nevzat, Turkey) daily and Estraderm TTS 100 (Novartis Pharma, Basel, Switzerland) 100 microgram/ on alternate days were recommended until the pregnancy test.

Outcome measures

A serum ß-hCG measured 12-14 days after the embryo transfer was accepted as positive if it was >10 microIU/ ml. Women with a positive pregnancy test were assessed by ultrasonography weekly for the number of gestational sacs and the presence of cardiac activity. A positive cardiac activity was defined as clinical pregnancy and pregnancies reaching 12th gestational week were considered as ongoing pregnancy. While the primary outcome measure was live birth rate, the secondary outcome measure were clinical pregnancy and implantation rates.

***Table 2**. Parameters of control and study groups after ICSI procedure*

	Control	Study	p value
Fertilization rate	55.88%	54.08%	0.47
Rate of number of embryos	43.96%	44.04%	0.98
Average number of embryos	2,82	2,76	0.59
Average transfer day	2,8	2,8	0.60
Transferred embryo quality	3.77	3.67	0.34
Implantation rate	30.88%	34.67%	0.13
Rate of clinical pregnancy	17 (34%)	30 (60%)	0.009
Rate of live births	12 (24%)	22 (44%)	0.03

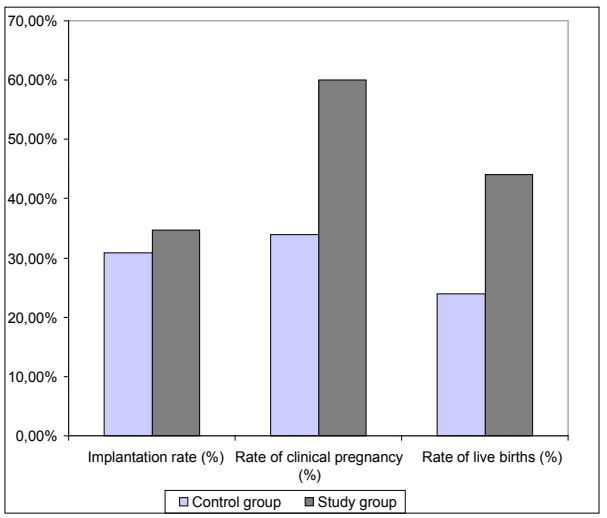

***Figure 2**. Implantation, Clinical Pregnancy and Live Birth Rate*

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (10.0 SPSS Inc.,Chicago). Student's t test, chi-square test and Mann Whitney-U test were used according to distribution characteristics. p value <0.05 was accepted as significant.

RESULTS

Baseline characteristics of the women

Baseline characteristics of the patients and outcomes of controlled hyperstimulation are represented in Table 1 and 2. Two groups were similar with regard to age, cause of infertility, number of failed IVF cycles, number of previously transferred embryos, dose and duration of induction, serum E2 values (pg/ml) measured on the day of hCG, endometrial thicknesses (mm) measured on the day of follicle aspiration by transvaginal ultrasound, number of M-II oocytes collected per cycle, fertilization rate, number of transferred embryos, transfer day and embryo grade .

Outcomes

Implantation rates were 34.67% and 30.88% in the biopsy-treated and control groups respectively (p=0.13). Although the difference between the groups was not statistically significant, implantation rate of 34.67% in the study group was considered as clinically significant. The incidence of clinical pregnancies was significiantly higher in the study group. Cardiac activity was detected in 30 patients (60%) in the study group and 17 patients (34 %) in the control group (p=0.009). Twenty two of the 30 pregnancies in the study group ended in the delivery of 27 healthy babies. This represents a rate of live births in the study group that was significiantly higher than the control (44% vs. 24%) (p= 0.03). The comparison of the outcome measures of the two groups is represented in figure 2.

DISCUSSION

The results of the present study suggests that clinical pregnancy rate and live birth rate is significantly increased after local injury to the endometrium. Implantation rate was also improved but it did not reach a level of statistical significance. Until now several strategies have been suggested to improve implantation and clinical pregnancy rates in cases with recurrent implantation failure. Recently, local injury to the endometrium has been adressed as a promising method by many investigators. Initially, Barash et al.(2) emphasized the possible role of endometrial injury on improved implantation and pregnancy rates. They demonstrated that local injury of the endometrium in IVF patients substantially increased the incidence of pregnancy and more than doubled the rate of a take-home baby. Subsequently better implantation rates after local injury to the endometrium were reported by others like Raziel (3), Zhou(4), Karimzadeh (5) and Narvekar (6). They suggested local injury to the endometrium during a COH cycle improved the rates of embryo implantation, clinical pregnancy and live birth in ART. Our results are consistent with the above-mentioned studies that clinical pregnancy rates and live birth rates are significantly increased after subsequent to endometrial injury. Although it was considered clinically significant, the improvement in the implantation rate was not statistically significant.

However, these studies have differences in design. Our study is a randomized controlled trial like Karimzadeh's and Narvekar's but Barash's and Raziel's studies were case control studies. The other difference in the methodology was the number and timing of endometrial biopsies performed in each patient. Barash et al. performed endometrial biopsies four times in the spontaneos cycle

(days 8, 12, 21 and 26), while Raziel et al. twice on luteal phase in the spontaneous cycle like us. Narvekar et al. made two samplings, one in follicular phase and one in luteal phase. Zhou et al. performed endometrial scratching in women only with irregular echo on ultrasound during controlled ovarian hyperstimulation cycle. They used ultrasound findings as one of their criteia. Because of these their study had some confounding factors. We performed two biopsies with one week intervals during the down regulation period. The study by Narvekar et al. have some confounding factors. They evaluated all patients with hysteroscopy and patients were administered diclofenac prior to endometrial sampling. Hysteroscopy induces some injury to the endometrium and there are studies that support the role of endometrial injury with hysteroscopy in increasing the reproductive outcome indirectly. Demirol, Mooney S and Rama Raju G. found that hysteroscopy increased the clinical pregnancy rate regardless of the findings (7-9). Moreover, the nonsteroidal anti- inflammatory agent might have decreased or prevented the release of different cytokines and growth factors generated by endometrial injury. It is unknown whether one endometrial biopsy is sufficient. Spandorfer et al.(10), performed the endometrial biopsy only once in the luteal phase prior to IVF cycle in a case control study and evaluated the effect of autologous endometrial co-culture and endometrial injury on pregnancy rate. They reported that although autologous endometrial co-culture was a useful adjunct in multiple failed IVF attempts, there was no increase in pregnancy rate. Therefore it seems that a few strokes of endometrial sampling are needed.

It is also unknown whether it should be performed in the preceding or in the same stimulation cycle. There are some possible mechanisms by which endometrial sampling may increase receptivity and conception rate of IVF-ET patients. First mechanism is injury induced decidualization. The initial evidence is based on animal studies. In guinea pigs endometrial injury resulted in desidualization and improved reseptivity of the uterus to implantation (11). The same effect was observed by injecting oil into the endometrial cavity in mice (12). The injury induced desidualization 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 (13,14). After animal studies, clinical studies were made In 1971, Karow et al.(15) reported that only two of 28 women who underwent endometrial biopsy in the luteal phase and who conceived in the same cycle aborted (%7). Endometrial biopsy might be associated with a better decidual reaction. In 1993, Friedler et al.(16) reported 14 patients with repeated implantation failure who were treated by a special protocol including hysteroscopy, dilatation and curetage, triple antibiotics and estrogen. Six of 14 patients conceived (pregnancy rate 43%) in the subsequent IVF-ET cycle. Endometrial injury by curretage might possibly have a role in the improvement of the results. The injury promotes decidualization of the endometrium making it more receptive for implantation. In a rat model, injury- induced decidualization is the most effective under progesterone influence. This is why endometrial injury was induced in the luteal phase in our study. However, whether endometrial injury in the luteal phase leads to a better clinical outcome than in the follicular phase is unclear.

Second mechanism is the wound healing. Endometrial injury might provoke the wound healing, involving a massive secretion of different cytokines and growth factors, including leukemia inhibitory factor (LIF), interleukin-11 and heparin-banding endothelial growth factor-like growth factor (12-14). A high level of endometrial proinflammatory cytokines, such as interleukin-6, LIF, and Tumor Necrosis Factor-α (TNF-α), characterize early implantation (17). These cytokines can be secreted by the endometrial cells as well as by cells of the immune system that are recruited to the site of implantation. Gnainsky et al.18 noted that local injury of the endometrium induced an inflammatory response promoting successful implantation. In inflammatory reaction, the amount of uterine dendritic cells and macrophages (HLA-DR+ CD11c+ cells) increases and may play a role in the development of a receptive endometrium. They also demonstrated that proinflammatory cytokines, TNF-α, growth-regulated oncogene-α (GRO-α), interleukin-15 (IL-15), and macrophage inflammatory protein 1 B (MIP-1B) increased with endometrial injury (18). Third mechanism was postulated by Zhou et al. (4) that local endometrial injury in stimulated cycle delays the endometrial development because of the wound repair processes correcting the asynchrony between endometrial and embryo stage. The last mechanism is endometrial gene modulation. Kalma et al. reported that the expression of 183 genes increased 2- to 10-fold and the expression of 39 genes was down regulated at least two fold in biopsy-treated patients. Genes that were upreg-

ulated in the endometrial samples of the biopsy-treated patients included mucin 1 transmembrane (MUC1), phospholipase A2 (PLA2), cyristallin alpha B, apolipoprotein D (APOD), and uroplakin 1b(UP1b), which had the highest upregulation (19). Song et al.(20) noted that in women lacking PLA2, the initiation of implantation was deferred, shifting the normal window of implantation. Dey et al.(21,22) concluded that the PLA2 pathway is crucial for implantation. MUC1 represents a potential ligand for selectins that are known to be expressed by human blastocysts, and which may have an important role in the adhesion of the blastocyst to the endometrium (23).

As a result; we have demonstrated that the live birth rates, clinical pregnancy rates, and implantation rates are increased after local endometrial injury. Endometrial injury with a biopsy catheter in an outpatient setting seems a cost effective, easily applicable and efficient procedure with almost no complications. The local injury to endometrium may provide clinical and economical advantages by decreasing number of IVF cycles and risk of hyperstimulation. The method of endometrial sampling can be applied as a routine procedure if unanswered questions are solved regarding patient selection, technique, the number of biopsies needed and timing. Further studies are needed to answer these questions and to explain the mechanisms by which endometrial injury improves receptivity.

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Contribution to authorship

ZHOI conceived, designed and initiated the study, HG was the trial coordinator. ZHOI and HAI undertook the statistical analysis. The article was written by ZHOI and HAI. All authors contributed to its content and approved the final version.

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