



RESEARCH ARTICLE

Role of Endometrial Scratch in Enhancing Pregnancy Rate of ICSI Cycle Through Its Effect on TNF- α

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Abstract

Background: The overall success rate of assisted reproductive techniques is only about 30% due to certain limiting factors. Endometrial scratching could induce inflammatory response which manifested by the up-regulation of different cytokines and chemokines with subsequent accumulation of immune cells in the injured site. One of the most important cytokine in this regard is TNF- α which was found to induce tissue remodeling by stimulating the production of many other pro-inflammatory cytokines.

Objectives: To investigate the role of endometrial injury during the luteal phase before ICSI cycles in the enhancement of pregnancy outcomes.

Materials and Methods: This prospective study was undertaken in the High Institute of Infertility diagnosis and Assisted Reproductive Techniques / Al-Nahrain University. All women were undergone ICSI cycles. Women in the intervention group underwent endometrial scratching with a Pipelle catheter in luteal phase preceding cycle. Blood sample was taken before scratch. All patients undergo controlled ovarian hyperstimulation, oocyte retrieval and embryo transfer, another blood sample was taken at day of embryo transfer to measure level of TNF- α . Clinical pregnancy rate then evaluation after luteal support of two weeks.

Results: The patients in scratch group has higher pregnancy rate (14/23) 60.9% than non intervention group (11/33) 33.3% although not significant (p value 0.057).

The results showed that the TNF- α after scratch (54.53 ± 10.2) was significantly higher than before scratch (39.26 ± 14.74) in the same group, as p value was (< 0.001). In addition the TNF- α was significantly higher in scratch group (54.53 ± 10.2) than control (25.91 ± 9.08) as p value was (< 0.001).

Conclusion: Induced endometrial local injury is an easy, simple and cost-effective technique that can be used to improve the uterine receptivity and enhance embryo transplantation.

Keywords: Endometrial Scratch, ICSI, pregnancy rate, TNF- α .

Introduction:

Assisted reproductive techniques (ART's) is now considered the favorable treatment for many cases of fertility both in male and female. However, the success rate with this technique is relatively low despite the remarkable advances in in-vitro fertilization (IVF). In fact, many fertility disorders have been overcome by ART's; yet the implantation remains the rate-limiting stride for successful IVF (1). Embryo implantation is initiated with the blastocyst contact to the implantation site (opposition) followed by the attachment of trophoblast to the epithelium of conceptive uterus (adhesion), and then the invasion of endometrial stroma by the trophoblast.

During mid-secretory phase, which lasts from 19 to 23 day of the menstrual cycle (known as window of implantation), the human uterus becomes receptive (2). Thus, for successful implantation, the genetically intact blastocyst has to undergo hatching, opposition, adhesion, penetration and invasion mainly under the control of two sex hormones: estrogen and progesterone (3). A large number of studies have been achieved in order to overcome the difficulties facing the implantation. Many of these studies investigated the impact of scratching of endometrium as a potential method for this purpose. Barash *et al.* in 2003 were the first who studied the effect of endometrial injury on implantation. They reported an increase in pregnancy rate after IVF when endometrial biopsy was performed during the luteal phase of menstrual cycle (4). Accordingly, they assumed that injured endometrium could induce an up-regulation in cytokines and growth factors during the subsequent healing, and these factors may increase the implantation rate. Three possible mech-

anisms have been proposed to explain the effect of endometrial scratching on the enhancement of pregnancy rate of IVF-ET. Firstly, endometrial injury provokes endometrial decidualization which in turns promotes implantation of the blastocyst (5). Secondly, there are several cytokines, growth factors and immune cells accompanying endometrial healing, and these humoral factors and cells have a very important role in embryo transplantation (5,6). Finally, the abnormal advancement of endometrium maturation under the control of ovarian stimulation during ART's could be retarded through endometrial injury, and hence the degree of synchronicity between the transferred embryo and endometrium is increased (5,7,8).

The human endometrium has a unique characteristic among other female organs; that is the presence of ongoing remodeling process throughout the reproduction stages. For example, there is a noticeable fluctuation in TNF- α expression from various endometrial cell types between pregnant and non-pregnant conditions (6). Actually, this cytokine is a principle regulator of many other cytokines like RANTES, MCP-1 and IL-8 (mediators of acute and chronic inflammation) which are released in a time and coordinated pattern in the endometrium. Such a particular pattern indicates the implication of these cytokines in several vital process, among which angiogenesis, differentiation, proliferation, apoptosis and leukocyte trafficking. It was found that the transcription factor NF κ B signaling pathway is involved in the regulation of inflammatory-induced TNF- α gene expression as well as the expression of TNF- α -dependent cytokines in endometrial and cells and chorio-decidua,



suggesting immunological role in pregnancy (7).

Materials and Methods:

A randomized prospective study was conducted in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, from October 2017 to April 2018. Fifty six women were included in this study.

These fifty six infertile women undergoing ICSI-ET cycles were randomized into two groups. Intervention group: 23 ICSI patient women had undergone endometrial scratching once between days 19 –23 of Oral Contraceptive Pills menstrual cycle (in the cycle prior to ET) and blood samples taken to measure TNF-alpha
Control group: 33 ICSI patient women were the scratching had not been done.

For both groups either long agonist protocol or antagonist protocol for COH was used , follow up by ultrasound till at least 3 follicles reaching a size of 17 -18 mm, were hCG trigger was given . 34-36 hours later ova- pickup was done.

At day of ET, for both groups blood samples were taken to measure the levels of TNF-alpha serum.

Fourteen days later, B-HCG test was done in blood to check pregnancy.

Inclusion criteria:

- 1) Age between 18-39 years.
- 2) No uterine congenital abnormalities.
- 3) No endometrial pathology (fibroid or polyp).
- 4) Transfer of at least one grade 1 embryo.

Exclusion criteria:

- 1) All cases of endometrioses.
- 2) Cases of congenital anomalies of the reproductive system.
- 3) Patients aged more than 40 years.
- 4) Those not having G1 embryos.

Results:

Demographic parameters Regarding the demographic parameters, the statistical analysis showed that there are no signifi-

cant differences between scratch and control groups of ICSI patients, concerning age, BMI of the patients and duration of infertility, number of trials , cause of infertility ,type of infertility, p value (0.902, 0.185, 0.124, 0.284, 0.073, 0.761 respectively) , as in table (1).

Table (1): Comparison of demographic data between scratch and control groups.

Parameters		Scratch group N=23 mean±SD	Control group N=33 mean±SD	P value
Age (yr)		29.74±5.88	29.55±5.55	0.902*
Body mass index (kg/m ²)		27.55±4.58	29.08±3.46	0.185*
Duration of infertility (yr)		6.65±3.24	8.3±4.67	0.124*
		No. (%)	No. (%)	
No. of trials	1	19 (82.6)	33 (100)	0.284**
	2	3 (13.0)	0 (0)	
	3	1 (4.4)	0 (0)	
Cause of infertility	Combined	9 (39.1)	4 (12.1)	0.073**
	Female factor	2 (8.7)	13 (39.4)	
	Male factor	11 (47.8)	14 (42.4)	
	Unexplained	1 (4.4)	2 (6.1)	
Type of infertility	Primary	16 (69.6)	25 (75.8)	0.761**
	Secondary	7 (30.4)	8 (24.2)	

* unpaired t-test, ** Yates chi square

Pregnancy Rate Despite of non-significant results of pregnancy rate between the two groups, p value (0.057). Out of 23 patients with endometrial scratching 14 patients get pregnancy (60.9%), while we have 11 out of 33 patients with control group get pregnancy (33.3%) as shown in figure (1).

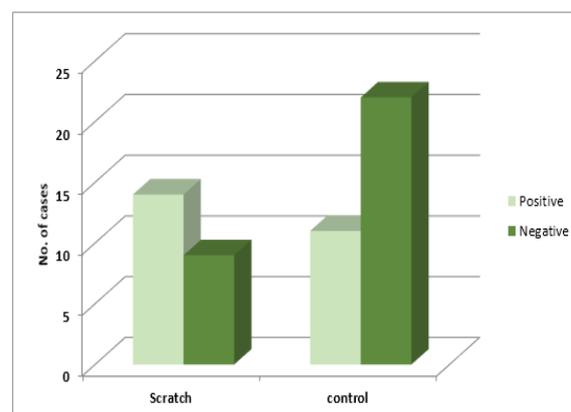


Figure (1): Pregnancy rate in scratch and control groups

Tumor necrosis factor- alpha (TNF- α)

The figure (2) showed that the TNF- α after scratch (54.53 ± 10.2) was significantly higher than before scratch (39.26 ± 14.74) in the same group, as p value was (< 0.001). In addition the TNF- α was significantly higher in scratch group (54.53 ± 10.2) than control (25.91 ± 9.08) as p value was (< 0.001), figure (3).

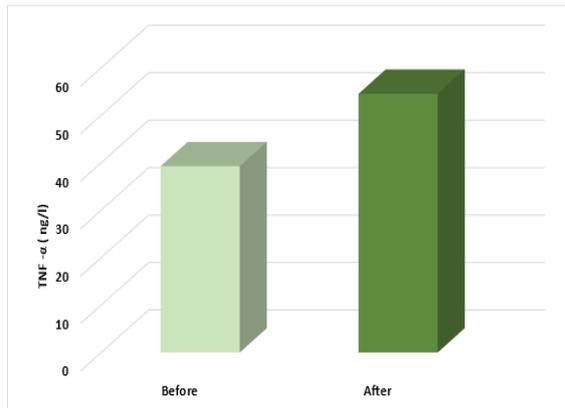


Figure (2): Comparison of TNF- α before and after scratching

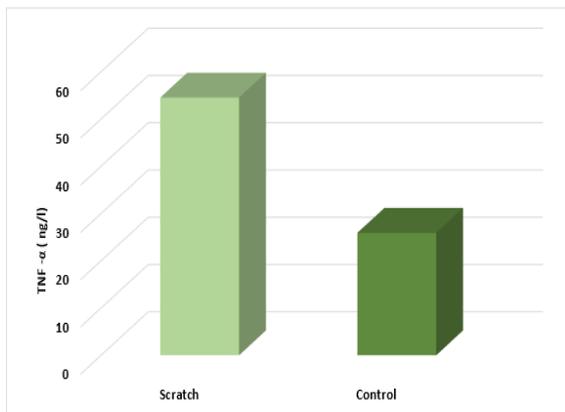


Figure (3): Comparison of TNF- α between scratch and control groups

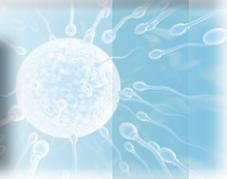
Discussion:

Endometrial injury is a promising intervention that promotes an active Th1 inflammatory reaction which is essential for endometrial receptivity and successful embryo implantation. Endometrial scratching might be an advantageous technique to improve implantation and pregnancy rate. However, for optimal results, many aspects like timing, the required trials of intervention and scratching nature should be well adjusted.

There is some evidence that receiving of hysteroscopic biopsies prior to IVF cycle by infertile women has resulted in higher rate of pregnancy. The first study which highlighted the beneficial effect of endometrial injury on implantation in human was conducted by Barash *et al.* (10). Those authors showed a significant improvement of IVF outcomes following endometrial injury.

The subsequent study has demonstrated that TNF- α (a pro-inflammatory cytokine, the secretion of which is enhanced upon endometrial biopsy) could be a crucial factor that contributes in the up-regulation of other endometrial pro-inflammatory cytokines following endometrial injury (11). This inflammatory milieu induced by TNF- α stimulates the cells in the endometrial stroma to release a group of cytokines associated with activation of immune cells. Activated cells can influence the expression of some adhesive molecules from endometrial epithelium (11).

In the same regard, some evidence has indicated the direct involvement of some pro-inflammatory cytokines in the implantation. In particular, IL-6 and MIP-1 β have been shown to have a chemo-attractant activity towards the trophoblast, while IP-10 was found to have a regulatory function for blastocyst migration, apposition and primary adhesion. Up-regulation of these three cytokines was found to significantly increase the implantation rate in IVF patients (12,13).



Another effect of local endometrial injury throughout the proliferative phase of menstrual cycle is the substantial elevation of the expression of estrogen receptors from endometrial epithelium in the subsequent secretory phase. This mutual effect of estrogen and pro-inflammatory cytokines suggests that the estrogen-induced endometrial production is, at least, partially mediated by pro-inflammatory cytokines.

According to these findings, it is reasonable to assume that IVF patients with recurrent failure have insufficient estrogen to induce endometrial receptivity. Thus, in such patients, the induction of inflammatory response though endometrial biopsy could be a reasonable choice for obtaining a successful implantation (14).

Conclusions:

1. Endometrial scratching induced by Pipelle biopsy sampling is a safe and simple outpatient intervention associated with significant improvement in implantation, also the clinical pregnancy rate in patients undergoing ICSI.

2. Endometrial injury might induce local inflammatory response since; The TNF- α after scratch was significantly higher than before scratch in scratch group. In addition the TNF- α was significantly higher in scratch group than control.

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