Original Research Article

The Effect of Endometrial Scratching before Embryo Transfer in Woman with History of Implantation Failure in Previous Intracytoplasmic Sperm Injection Cycle; Clinical and Biological Study

Abstract

Background: The benefit of endometrial scratching in humans has been offered by clinicians as a means of increasing endometrial receptivity was initially reported as a serendipitous finding because a high pregnancy rate was observed in IVF women who had undergone repeated endometrial biopsies during their preceding natural menstrual cycle. The aim of this work was to evaluate the effect of endometrial scratching before fresh or frozen embryo transfer on clinical pregnancy rate in women with history of implantation failure after one or more Intracytoplasmic sperm injection cycle(s) and to predict its value based on measuring the level of peripheral Natural Killer Cells (cd56).

Methods: This prospective randomized study was carried out on 60 women with history of one or more implantation failure of ICSI cycle, and normal uterine cavity. Patients were divided into two equal groups: an experimental group A and a control group B. All patients were subjected to history taking, clinical examination, investigations for infertility, serum Natural killer cells CD56 measure and hormonal profile.

Results: There was significant difference in CD56 measurement between women who got pregnant (decreased CD56) and those who not got pregnant (increased CD56) in group A .Clinical Pregnancy rate was higher in group A after endometrial scratching was done**.**

Conclusions: Performing injury in preceding cycle is more effective as all these processes require time and are controlled by the hormones. This is a simple and inexpensive procedure with lot of benefits as compared to risks of infection and potential of future subfertility. Endometrial immune profiling at the time of implantation window (CD56 measurement) could help in selecting sub-groups of infertile patients who would benefit from a targeted intervention.

Keywords: Endometrial scratching, Embryo transfer, implantation failure, Intracytoplasmic sperm injection

Introduction:

The benefit of endometrial scratching in humans which is also called endometrial biopsy, endometrial injury, and endometrial trauma, has been offered by clinicians as a means of increasing endometrial receptivity was initially reported as a serendipitous finding because a high pregnancy rate was observed in IVF women who had undergone repeated endometrial biopsies during their preceding natural menstrual cycle [1] .

Barash et al. [1] were the first to test the hypothesis that endometrial injury in the natural cycle prior to controlled ovarian hyperstimulation (COH) could increase the chance of pregnancy and found a two-fold increase in live birth rate after multiple endometrial scratches compared to no scratch. (48.9% vs 22.5 %). Subsequently, a number of studies have indicated a potential clinical value of this intervention, particularly in repeated implantation failure (RIF) patients.

Endometrial scratching upregulated the expression of pro inflammatory cytokines involved in the recruitment of monocytes and their differentiation into macrophages and Dendritic Cells (DCs). These cells are known to trigger the expression of pro-implantation genes in endometrial epithelium and stroma, enabling the apposition and adhesion of the blastocyst [2].

DCs and macrophages were shown to secrete an array of cytokines and enzymes allowing tissue remodelling and angiogenesis, required for the endometrial decidualization and regulation of trophoblast invasion [3].

This differentiation of stromal cells within the decidualization process is an essential step for a successful implantation. The scratching induced increase of VEGF (Vascular Endothelial Growth Factor), a central growth factor involved in local angiogenesis and placentation [4].

The uterine Natural Killer cells (uNK) are another essential immune cell population in the human endometrium. Described as CD56bright CD16dim, uNKs are found in the endometrium at the beginning of each menstrual cycle as small agranular cells [4].

The aim of this work was to evaluate the effect of endometrial scratching before fresh or frozen embryo transfer on clinical pregnancy rate in women with history of implantation failure after one or more Intracytoplasmic sperm injection cycle(s) and to predict its value based on measuring the level of peripheral Natural Killer Cells.

Patients and Methods:

This prospective randomized clinical study was carried out on 60 women aged from 18 to 40 years old at time of embryo transfer, with history of one or more implantation failure of ICSI cycle, and normal uterine cavity (assessed by transvaginal sonography at booking and no endometrial abnormalities such as, suspected intrauterine adhesions, uterine septa, submucosal fibroids or intramural fibroids exceeding 4 cm in diameter as assessed by the investigator that would require treatment to facilitate pregnancy). An informed written consent was obtained from the patient. The study was done after ethical approval from the Institute Ethics Committee.

Exclusion criteria were previous trauma/surgery to the endometrium (e.g., resection of submucous fibroid, intrauterine adhesions), body mass index (BMI) of 35 kg/m2 or greater, known grade 4 (severe) endometriosis or any degree of adenomyosis and currently participating in any other fertility study involving medical/surgical intervention.

The patients were divided into two equal groups: an experimental group A (before endometrial scratching, peripheral blood sample was taken for the measurement of level of the natural killer cells CD56 by flowcytometry) and a control group B.

All patients subjected to: History taking **(**full personal, present, past, menstrual and obstetric history), clinical examination **(**general, abdominal and gynaecological examination), investigations for infertility **(**trans vaginal ultrasound, serum Natural killer cells CD56 measure (only for patients of group A) and hormonal profile (basal FSH, LH and E2. TSH

and

Prolactin)).

Blood samples were collected from patients at time of examination. For immunophenotyping analysis, 2 mL of blood were collected in glass tubes containing EDTA and analysed by flow cytometry within 24 h after collection in order to detect CD16+, and CD56+ lymphocytes.

Patients in group A, underwent endometrial scratching once between days 20 and 22 of menstrual cycle in the cycle prior to the planned ICSI cycle or Embryo Transfer. After applying the cusco speculum in the vagina for disinfection of the uterine cervix with betadine, an endometrial curette (Novac Curette) will be introduced through the cervix up to the uterine fundus, after which the examiner slowly retracts the curette while rotating over several ranges of 360° during1–2 min.

Endometrial thickness and blood flow were measured by TVS ultrasonography on the day of HCG administration. Oocyte retrieval was performed 34–36 h after the HCG injection using cook's ovum pick up needle (17 Fr) by TVS route under ultrasound guidance under general anaesthesia. Retrieved oocytes were inseminated or injected with sperms (intracytoplasmic sperm injection). Fertilization check was done after 18 h of insemination and embryos were cultured in sequential medium (Vitrolife, Sweden).

Embryo Transfer (ET) was done on day 2–5 using ET catheter (Cook, Ecotip). Luteal phase support in the form of injection progesterone 100 mg intramuscular daily or vaginal pessary 400 mg twice daily was administered to both the groups.

The outcome measure: clinical pregnancy rate (calculated as the number of patients with clinical pregnancy (by the detection of foetal heartbeat with an ultrasound scan) divided by the number of patients who underwent ET), between the study groups and its correlation to Natural Killer (NK) cells measurement.

Statistical analysis

Data was computerized using excel sheet. All data analysis was carried out using Statistical Package for the Social SPSS IBM version 19.0. Descriptive statistics, such as mean, median, standard deviation, and range value were calculated for continuous variables. After testing for normality assumptions, using appropriate statistics, mean value was compared between two groups using Student's t independent test. Frequency distributions of categorical variables were compared using Chi Square/Fisher's exact test as appropriate. For all statistical tests, the probability of $P < 0.05$ was considered for statistical significance.

Results:

Age, LH, FSH and E2 were insignificantly different between two groups while BMI was significant higher in group B than group A (p = 0.001). **Table 1**

Table 1: Comparison of age, BMI and hormonal profile (LH, FSH, E2) between the two groups

	Group A	Group B	p. value
Age	32.00 ± 3.58	31.93 ± 3.72	0.944
BMI	20.16 ± 1.03	21.34 ± 1.46	0.001*
LH	4.53 ± 2.31	4.19 ± 2.20	0.563
FSH	7.01 ± 1.69	7.32 ± 1.75	0.490
E2	122.01 ± 62.31	149.67 ± 70.84	0.114

Data represented in mean ± slandered deviation, *: statistically significant p value, BMI: body mass index LH: luteinizing hormone, FSH: follicle stimulating hormone, E2: estradiol

TSH was insignificantly different between two groups while prolactin was significant higher in group B than group A (p = 0.030). **Table 2**

Table 2: Comparison of TSH and Prolactin hormone between the two groups.

	Group A	Group B	p. value
TSH	2.40 ± 1.04	2.27 ± 0.88	0.585
Prolactin	16.07 ± 5.09	18.48 ± 3.03	0.030*

Data represented in mean ± slandered deviation, *: statistically significant p value, TSH: thyroid stimulating hormone

There was insignificant difference between the two groups in the number of previous implantation failure after ICSI cycle. **Table 3**

Table 3: The number of previous implantation failure after ICSI cycles, between the two groups

Number of Implantation failure ICSI cycle	Group A	Group B	P-value	X2
1	16 (53.3%)	15 (50.0%)	0.771	0.519
2	12 (40.0%)	14 (46.7%)		
3	2 (6.7%)	1(3.3%)		

Data represented in frequency (%), ICSI: Intracytoplasmic Sperm Injection

Level of peripheral Natural Killer Cells (cd56) was significantly higher in women who not got pregnant than those who got pregnant in patients of group A (p=0.001). **Table 4**

Table 4: Measurement of natural killer cells CD56, between women who got pregnant and women who not got pregnant in patients of group A.

	Pregnant	Non pregnant	t. test	p. value
Natural killer cells (cd56)	77.47 ± 9.29	215.4 ± 91.16	5.830	0.001*

Data represented in mean ± slandered deviation, *: statistically significant p value

Pregnancy rate was insignificantly different between two groups. **Table 5**

Table 5: difference in Clinical pregnancy rate between the two groups

	Group A	Group B	P-value
Pregnancy rate	15 (50.0%)	10 (33.3%)	0.190

Data represented in frequency (%)

Discussion

Intentional endometrial injury is frequently being performed in women undergoing IVF without conclusive scientific evidence on its beneficial effects. Multiple studies have been performed to investigate the effect of endometrial scratching in women undergoing ART

cycles, but the method of scratching, the population being scratch, and the study quality varies widely [8].

The results of the present study suggest that implantation rate is significantly increased after local injury to the endometrium. In this strategy we measured the level of peripheral Natural Killer Cells CD56 in mid-luteal phase to know the relation between it and the effect of endometrial scratching. The endometrial scratching was recommended only if the patient was diagnosed with a lack of peripheral Natural Killer Cells CD56. The endometrial scratching took place during the mid-luteal phase of the preceding cycle to the actual embryo transfer.

Barash et al. [1] in 2003, were first to report that IVF treatment that is preceded by endometrial biopsy doubles the chance for a take-home baby. A total of 134 patients were divided into two groups: An experimental group (n = 45) that included patients from whom endometrial samples were collected and a control group (n = 89).

The implantation rate in the biopsy-treated patients was 27.7%, which is significantly higher than that in the control group. Moreover, 30 of the 45 patients in the biopsy-treated group conceived, exhibiting a doubled rate of pregnancy as compared to that of the control group. Live births rate in the biopsy-treated patients was more than twofold higher than that in the controls [1].

Raziel et al., [9] prospectively studied 120 couples with high-order implantation failure of >4 unsuccessful ET of fresh embryos. Intervention group (n = 60) underwent endometrial biopsy twice on days 21 and 26 of the preceding ovarian stimulation cycle; control group had no intervention (n = 57). Implantation rate was significantly higher for the biopsy group, whereas no statistically significant difference was observed for the ongoing pregnancy and miscarriage rates. Compared with that of the controls: The respective rates were 11% versus 4% for implantation, 30% versus 12% for pregnancy, and 2% versus 8% for ongoing pregnancies. The abortion rate was 28% for each group.

Shohayeb and El Khayat [10] in 2012 found that the single endometrial biopsy regimen performed during hysteroscopy has statistically significant higher implantation rate, clinical pregnancy rate, and live birth rate than hysteroscopy without endometrial scraping.

There were statistically significant differences regarding the implantation rate, the clinical pregnancy rate, and live birth rate. The implantation rate in Group A was 12% while in Group B it was 7%, the clinical pregnancy rate was 32% in Group A while it was only 18% in Group B, and the live birth rate was 28% in Group A while it was 14% in Group B. The abortion rate was 12.5% in Group A while it was 22% in Group B, with no statistically significant difference (P = 0.618).

However, Karimzade et al., [12] evaluated the effect of local injury to the endometrium on the day of oocyte retrieval on implantation and pregnancy rates in assisted reproductive cycles. They concluded that local injury to the endometrium on the day of oocyte retrieval disrupts the receptive endometrium and has a negative impact on implantation and IVF outcomes. Significantly lower implantation rate (7.9 vs. 22.9%, P = 0.002) was noted in the experimental group compared with the controls.

Similarly, Safdarian et al., [13] also found that endometrial biopsy did not increase the chances to conceive at the following cycle of treatment. Patients were divided to control groups (n = 50) and experimental group (n = 50), who underwent endometrial biopsy. Endometrial biopsy in these patients was taken by biopsy catheter on day 21 of their previous menstrual cycle with use of contraceptive pill before the IVF-ET treatment. 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.

Conclusions:

Implantation rate increases significantly after endometrial scratching in patients with previous failed ICSI. Endometrial scratching causes changes within the endometrium, gene expression, and the immune system, leading to enhanced endometrial receptivity and better implantation environment. Performing injury in preceding cycle is more effective as all these processes require time and are controlled by the hormones. This is a simple and inexpensive procedure with lot of benefits as compared to risks of infection and potential of future subfertility. Endometrial immune profiling at the time of implantation window; represented in our study by the level of peripheral Natural Killer Cells CD56, could help in selecting sub-groups of infertile patients who would benefit from a targeted intervention. In our experience, within a large population of patients having a history of unexplained repeated embryo implantation failure, only half of them showed a low endometrial immune activation, potentially justifying an endometrial scratching.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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