

Therapeutic Efficacy of Endometrial Scratching in Repeated Controlled Ovarian Stimulation (COS) Failure Cycles

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ABSTRACT

Objective: The objective of the study was (1) “to evaluate the therapeutic efficacy of endometrial scratching in repeated controlled ovarian stimulation (COS) failure cycles.” And (2) “to compare differences in pregnancy outcome by endometrial scratching in early (D2–D4) and late follicular phases (D7–D9) of the same stimulation cycle.” **Materials and Methods:** Women attending infertility clinic in a tertiary care center and who have two or more repeated COS failure cycles and planned for COS with intrauterine insemination (IUI) were included in the study which is a prospective parallel, interventional, single-blinded, randomized control study, in 1:1 allocation ratio. A total of 165 patients were recruited and randomly allocated into three groups: Group A ($n = 55$) underwent endometrial scratching on D2–D4 of the same COS cycle, Group B ($n = 55$) on D7–D9, and Group C ($n = 55$) no intervention done. All the patients underwent COS according to standard protocol followed by IUI. **Results:** Clinical pregnancy rate was 12.73% (odds ratio [OR] = 0.87 95% confidence interval [CI] = 0.288–2.55, $P = 1$), 16.36% (OR = 1.15; 95% CI = 0.40–3.23, $P = 1$), and 14.54%, respectively, in Group A, B, and C, respectively ($P = 0.86$), as per intention to treat analysis. Using Chi-square test, P value between Group A and B was 0.787, between Group A and C was 1.000, and between Group B and C was 1.000. As per protocol analysis, clinical pregnancy rate was 13.46% (OR = 0.83; 95% CI = 0.27–2.5, $P = 0.74$), 19.57% (OR = 1.3 95%; CI = 0.45–3.73, $P = 0.41$), and 15.69%. Using Chi-square test, P value between Group A and B was 0.588, between Group A and C was 0.967, and between Group B and C was 0.815. No abortions and multiple pregnancies occurred in either of the groups. **Conclusion:** The effect found was of good quantum in Group B as per protocol analysis which could be of clinical relevance if larger sample size would have been taken. Endometrial scratching is a cost-effective and easy technique which may improve clinical pregnancy rates in previous COS failure cycles, but more trials are needed to be conducted using larger sample size to achieve the improved and significant outcome.

KEYWORDS: *Controlled ovarian stimulation, endometrial scratching, intrauterine insemination*

INTRODUCTION

Implantation is the rate-limiting step in the process of fertilization not only in *in vitro* fertilization (IVF) cycles but also in intrauterine insemination (IUI) cycles. Successful implantation of embryo requires a receptive uterus. Poor endometrial receptivity is an important cause of repeated implantation failure. Endometrial receptivity is modulated by various signaling molecules such

as prostaglandins, cytokines, integrins, and leukemia inhibitory factor. Dysregulation in these factors may lead to repeated implantation failure.^[1-4] Various uterine pathologies, such as thin endometrium, altered expression

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of immunological factors, and adhesive molecules, may decrease endometrial receptivity.^[5] Clinical and basic science data have implied the association of endometrial injury with improved endometrial receptivity and hence improved implantation rates in women undergoing assisted reproductive technology (ART) with repeated implantation failures.

In controlled ovarian stimulation (COS) performed during ART, the implantation rate is decreased due to abnormally advanced endometrial maturation and disturbed endocrine and paracrine milieu. Endometrial scratching by stimulating delay in endometrial maturation corrects asynchrony between endometrium and the conceptus and also promotes wound healing by inducing a significant increase in the local secretion of pro-inflammatory cytokines such as macrophage inflammatory protein-1E, tumor necrosis factor- α , osteopontin, interleukins, growth factors, macrophages, and dendritic cells which in turn promotes successful implantation.^[6,7] Cytokines, growth factors, and natural killer cells are also responsible for increased angiogenesis, thereby providing adequate blood flow to the tissue and preventing embryo rejection.^[8]

Despite maximum studies showing the benefit of endometrial injury in luteal phase of preceding cycle, few studies were conducted in which endometrial injury improved pregnancy outcomes when done in the follicular phase of the same cycle.^[9-11] Very few randomized clinical trials (RCTs) have been conducted that showed the benefit of endometrial scratching in IUI cycles.^[12-14]

In our study, the hypothesis is that endometrial scratching in follicular phase of the same cycle of COS with IUI increases the chances of clinical pregnancy in cases of two or more failed COS cycles as compared to controls by improving endometrial receptivity.

Aims and objectives

1. "To evaluate the therapeutic efficacy of endometrial scratching in repeated COS failure cycles"
2. "To compare differences in pregnancy outcome by endometrial scratching in early (D2–D4) and late follicular phases (D7–D9) of the same stimulation cycle."

Settings and design

This is a prospective, parallel, single-blinded, randomized control study, in 1:1 allocation ratio, conducted in the infertility outpatient department, of a tertiary care center.

MATERIALS AND METHODS

Women who have two or more repeated COS failure cycles and planned for COS with IUI were included in our study. Women who agreed to participate and give a

written informed consent underwent complete infertility workup including husband semen analysis, testing for ovarian reserve, infection screening of the participant and husband, and investigations for tubal patency.

Inclusion criteria

1. All women having two or more repeated COS failure cycles were included in the study
2. Women with age of 20–38 years, primary or secondary infertility, patency of both or either one of the tubes (hysterosalpingography/lap hysteroscopy), and no endometrial scratching done in previous three COS cycles were included in the study.

Exclusion criteria

Women with known pelvic inflammatory disease, bilateral tubal blockage, intrauterine pathology (submucosal fibroid, endometrial polyp, adhesions, Asherman syndrome, bicornuate uterus, and septate uterus) and women with acute vaginal and cervical infection, endometriosis, and hydrosalpinx were excluded from the study.

Participants fulfilling the inclusion criteria were enrolled in the study. Each case was subjected to a detailed history and thorough clinical examination. All participants recruited underwent baseline investigations and hormonal profile (serum follicle-stimulating hormone [FSH], serum luteinizing hormone, serum estradiol, prolactin, and serum thyroid-stimulating hormone), baseline ultrasonography (USG) on day 2/day 3 for antral follicle count, and endometrial thickness. Cases with endometrial thickness <5 mm and follicle size <10 mm on ultrasound underwent COS with IUI according to standard protocol. Follicular growth monitoring was done from day 8 of cycle onward. Ovulation trigger was given once the follicle attained a diameter of 18–20 mm and IUI was done as per standard practice followed by luteal support.

A total of 165 eligible participants planned for COS with IUI cycles were included in the study and were randomly allocated using block randomization into three groups when they were called on day 2 of cycle for COS. Patients were blinded for their allocation. Fifty-five participants were included in each group.

- Group A: Endometrial scratching was done using endometrial aspiration cannula (endocell) in early follicular phase (D2–D4) of the same COS with IUI cycle ($n = 55$)
- Group B: Endometrial scratching was done using endometrial aspiration cannula in late follicular phase (D7–D9) of the same COS with IUI cycle ($n = 55$)
- Group C: No intervention done ($n = 55$).

Procedure

Endometrial scratching was done using endometrial aspiration cannula as an OPD procedure. Endocell of Wallach surgical devices Trumbull, CT 06611 (203) 799-2005 made in the USA was used for the study. After proper written informed consent, patient was asked to lie on the table for a routine pelvic examination. No analgesics were given before the procedure. Sims speculum was inserted, and anterior lip of cervix was held with Allis forceps. The cannula was inserted gently through the cervical canal into the uterine cavity and advanced slowly till just resistance felt. Gentle movement of endometrial aspiration cannula (endocell) along all four walls of the uterine cavity was performed. Within 10 min after the procedure, the women were asked to quantify the degree of pain experienced during the procedure with the help of a visual analog scale (VAS). The VAS consists of a 10 cm long horizontal line with its extremes marked as “no pain” and “worst pain imaginable”.^[15] Each woman was asked to tick her pain level on the line, and the distance from “no pain” on the extreme left to the tick mark was measured in centimeters yielding a pain score from 0 to 10. Endometrial scratching was done in an outpatient setting. Pregnancy was confirmed by beta human chorionic gonadotropin (hCG) in urine/serum 2 weeks after IUI. If participant conceived, she was followed till 20 weeks of pregnancy.

Statistical analysis

A study by Hafiza Ozkurt *et al.* was taken as reference; minimum required sample size with 80% power of study and 5% level of significance is 54 patients per group. To lower margin of error, the sample size is taken as 55 per group and 165 as total sample size.

This study has been part of a thesis, so larger sample size could not be used.

Primary outcome

- Clinical pregnancy rate
- Clinical pregnancy is defined as ultrasound confirmation of gestational sac with fetal cardiac activity. Clinical pregnancy rate is defined as a number of patients with clinical pregnancy divided by a number of cases who underwent COS cycle.

Secondary outcome

1. Miscarriage rate (number of clinical pregnancy losses before 20 completed weeks of gestation divided by number of cases undergoing COS cycle)
2. Multiple pregnancies (presence of more than one fetus with heartbeat)
3. Pain evaluated using a VAS within 10 min after the procedure.

In our study, women of age group 20–38 years were included in the study. Majority of cases were in 26–30-year age group and distribution of cases in this age group was 49%, 54.5%, and 54.5% in Group A, B, and C, respectively [Table 1]. Overall clinical pregnancy rate (CPR) was found maximum in the age group of 21–25 years of age. In this age group, CPR was 20%, 36.3% versus 11.11% in Group A, B, and C, respectively ($P = 0.384$).

A maximum number of cases recruited in our study had a duration of 6–10 years of infertility; distribution of which was 47.27%, 49.09%, and 50.91% in Group A, B, and C, respectively. Mean duration of infertility was similar in all three groups which was 6.22 ± 2.62 , 7.38 ± 3.56 , and 6.67 ± 3.07 years in Group A, B, and C, respectively. In Group A, CPR was 15.38% in 1–5 years, 11.54% in 6–10 years, and 0% in 11–15 years. In Group B, CPR was 27.78% in 1–5 years, 7.41% in 6–10 years, and 11% and 20% in 11–15 years. In Group C, CPR was 19.05% in 1–5 years, 10.71% in 6–10 years, and 16.67% in 11–15 years.

The maximum cases were of unexplained infertility (UI) with distribution of 43.64%, 27.27%, and 30.91%, respectively, in Group A, B, and C ($P = 0.355$) followed by combined etiology (18.8%, 27.27%, and 14.5%, respectively, in Group A, B, and C) and male factor (16.36%, 20%, and 25.45% in Group A, B, and C, respectively) [Table 2].

Antral follicle count and endometrial thickness were measured by USG on day 2 of the cycle. The mean number of antral follicle count in Group A was 10.56 ± 1.62 , in Group B was 10.51 ± 1.41 , and in Group C was 10.51 ± 1.35 . The mean embryo transfer (ET) on day 2 was 3.56 ± 0.89 mm in Group A, 3.83 ± 0.74 mm in Group B, and 3.81 ± 0.75 mm in Group C.

In Group A (early follicular phase, D2–D4), the mean day for taking endometrial biopsy (EB) in Group A was 2.63 ± 0.75 . Pregnancy was maximum in cases with EB done on D2 (13.79%) of cycle, but it was not statistically significant ($P = 0.968$).

In Group B (late follicular phase, D7–D9), the mean day for taking EB was 7.58 ± 0.71 . Pregnancy rate was maximum (28.57%) when EB was done on D7 with $P = 0.274$.

In Group A, out of 55 cases who had undergone EB scratch on D2–D4 in Group A, 63.64% cases received clomiphene citrate (CC), 25.45% cases received CC + gonadotrophins and 10.91% received gonadotrophins. In Group B, 81.82% received CC, 12.73% received CC + gonadotrophins, and 5.45% received

Table 1: Distribution of infertility cases according to demographic profile

	Groups			P
	A (n=55), frequency (%)	B (n=55), frequency (%)	C (n=55), frequency (%)	
Age distribution (years)				
21-25	15 (27.27)	11 (20.00)	9 (16.36)	0.612
26-30	27 (49.09)	30 (54.55)	30 (54.55)	
31-35	10 (18.18)	9 (16.36)	14 (25.45)	
>35	3 (5.45)	5 (9.09)	2 (3.64)	
Mean±SD	28.24±3.88	28.6±4.28	28.58±3.62	
Type of infertility				
Primary	40 (72.7)	44 (80)	36 (65.4)	0.231
Secondary	15 (27.2)	11 (20.)	19 (34.5)	

SD=Standard deviation

Table 2: Etiology of infertility and its effect on pregnancy outcome after endometrial biopsy scratch

Etiology of infertility	CPR group			P
	A, pregnancy (%)	B, pregnancy (%)	C, pregnancy (%)	
Combined	2/10 (20)	2/15 (13.3)	2/8 (25)	0.775
Male factor	1/9 (11.1)	3/11 (27.27)	1/14 (7.14)	0.34
Ovulatory dysfunction	1/4 (25)	2/9 (22.2)	2/9 (22.2)	0.993
Tubal	1/8 (12.5)	0/5 (0)	0/7 (0)	0.45
UI	2/24 (8.3)	2/15 (13.3)	3/17 (17.6)	0.670
Total	7 (12.73)	9 (16.36)	8 (14.55)	0.864

UI=Unexplained infertility, CPR=Clinical pregnancy rate

gonadotrophins. In Group C, 90.91% cases received CC, 7.27% cases received CC + gonadotrophins, and 1.8% cases received gonadotrophins. One case in Group C did not report for USG monitoring after OI with CC. Majority of cases received clomiphene alone in all three groups ($P = 0.012$). In Group A, CPR was 8.57% with CC and 28.57% with CC + gonadotrophin. In Group B, CPR was 15.56% with CC and 28.57% with CC + gonadotrophins. In Group C, CPR was 16% with CC. Although Pregnancy rate was highest with CC + gonadotrophins in all three groups, there was no statistical significance.

The mean length of COS cycle from day of ovulation induction to day of trigger was similar in all three groups. Mean cycle length was 12.18 ± 1.59 , 12.42 ± 1.57 , and 11.98 ± 2.02 , respectively, in all three groups ($P = 0.42$).

In our study, mean number of dominant follicle (DF) >18 mm on date of hCG trigger was similar in Group A, B, and C, i.e., 1.25 ± 0.7 , 1.4 ± 0.87 , and 1.2 ± 0.62 , respectively, and the CPR was not statistically significant with regard to number of follicles on day of hCG trigger.

On the day of hCG trigger, ET was between 7 and 9 mm in 74.55%, 83.64%, and 74.55% women in Group A, B, and C, respectively ($P = 0.65$). Mean ET on day of trigger was 7.98 ± 1.52 , 7.93 ± 1.05 , and 8.19 ± 1.18 , respectively, in Group A, B, and C, respectively. ET

on day of trigger was higher in Group B in our study as compared to controls although it was statistically insignificant. CPR was 33.33% in women with ET 9–11 mm in Group B.

Majority of women had previous two-failed COS cycles in all three groups with (32) 58.2% in Group A, (34) 61.8% in Group B, and (30) 54.5% in Group C. Previous three failed COS cycles in all three groups were (17) 30.9% in Group A, (15) 27.3% in Group B, and (21) 38.2% in Group C ($P = 0.77$). In Group A, CPR in previous two failure cycles was 9.4% and CPR in previous three failure cycles was 11.8%. In Group B, CPR in previous two failure cycles was 14%, CPR in previous three failure cycles was 13.3%, and 33.3% in previous four failed cycles. In Group C, CPR was 16.7% and 14.3% in previous two and previous three COS failure cycles, respectively. PR was similar in cases with previous multiple IUI failure in all three Groups.

As per protocol analysis, pregnancy rate in the same cycle of EB scratch was 6/52 (11.5%), 8/46 (17.39%), and 7/51 (13.7%) ($P = 0.542$). As per intention-to-treat (ITT) analysis, pregnancy rate in same cycle of EB scratch was 6/55 (10.9%), 8/55 (14.5%), and 7/55 (12.7%) ($P = 0.653$).

Pregnancy rate per cycle is 7/62 (11.2%), 9/61 (14.7%), and 8/57 (14.03%) in Group A, B, and C, respectively ($P = 0.67$).

As per protocol analysis, clinical pregnancy rate was 13.46% (odds ratio [OR] = 0.836; 95% confidence interval [CI] = 0.27–2.5, $P = 0.74$), 19.57% (OR = 1.30; 95% CI = 0.45–3.73, $P = 1$), and 15.69%. Using Chi-square test, P value between Group A and B was 0.588, between Group A and C was 0.967, and between Group B and C was 0.815. Abortion rate was 1.92%, 4.35%, and 0% in Group A, B, and C ($P = 0.313$) as per protocol analysis. Ovarian hyperstimulation syndrome rate was 5.77%, 0%, and 1.96% ($P = 0.195$). No multiple pregnancies occurred in any of the three groups [Table 3].

CPR was 12.73% (OR = 0.87 95%; CI = 0.288–2.55, $P = 1$), 16.36% (OR = 1.1587; 95% CI = 0.40–3.23, $P = 1$), and 14.54% respectively in Group A, B, and C, respectively, as per ITT ($P = 0.86$). Abortion rate was 1.82%, 3.64%, and 0.00% in Group A, B, and C, respectively ($P = 0.36$). OHSS rate was 5.45%, 0%, and 1.82% in Group A, B, and C, respectively ($P = 0.16$). There were no multiple pregnancies in each of the three groups [Table 4].

DISCUSSION

Impaired endometrial receptivity may also lead to implantation failure in cases of women trying to conceive with COS with or without IUI cycles. Endometrial injury is a promising intervention, which promote an active T1 inflammatory response that is necessary for acquisition of endometrial receptivity and successful embryo implantation.

Endometrial scratching may have a beneficial effect in improving implantation rate and pregnancy rate.

Table 3: Effect of endometrial scratch on pregnancy outcome (intention to treat applied)

Outcome	Group			P
	A (n=55), frequency (%)	B (n=55), frequency (%)	C (n=55), frequency (%)	
CPR	7 (12.73)	9 (16.36)	8 (14.54)	0.864
Abortion	1 (1.82)	2 (3.64)	0 (0.00)	0.361
OHSS	3 (5.45)	0	1 (1.82)	0.166
Multiple pregnancy	0	0	0	-
Total	55	55	55	

CPR=Clinical pregnancy rate, OHSS=Ovarian hyperstimulation syndrome

However, there are still many unanswered questions regarding patient selection, timing, technique, and number of interventions needed to achieve an optimal effect. Thus, the current study was planned which evaluates the hypothesis that endometrial scratching improves clinical pregnancy rate in repeated COS cycles.

Detrimental effect of advancing age on ART outcomes is well established. In our study, women of age group 20–38 years were included in the study. Majority of cases were in 26–30-year age group and distribution of cases in this age group was 49%, 54.5%, and 54.5% in Group A, B, and C, respectively [Table 1]. Overall CPR was found maximum in the age group of 21–25 years of age. A similar study by Abdelhamed^[12] in IUI cycles included women in the age group of 22–35 years. RCT conducted by Parsanezhad *et al.*,^[10] included women with age group of 23–35 years in their study in COS cycles. Guven *et al.*^[11] recruited women of age <35 years undergoing IVF/intracytoplasmic sperm injection [ICSI] cycles. However, in RCT conducted by Rasheed,^[16] women with UI were recruited with the age group of 20–40 years. In this study, pregnancy rate was higher in control group compared to intervention group (48.3% vs. 45.5%). CPR was higher in endometrial curettage group as compared to controls in women aged 31–40 years having secondary infertility (75% vs. 58.8%). CPR was higher in intervention group as compared to control in women having primary infertility in age groups of both 20–30 years (35% vs. 5.4%) and 31–40 years (25% vs. 5.5%).

In our study, majority of couples had primary infertility in all three groups, and CPR was more in women with secondary infertility. In RCT conducted by Rasheed^[16] also, it was found that, in women with secondary infertility, early cleaving led to significantly higher pregnancy rates as compared to control (68.8% vs. 62.8%; $P = 0.034$). Although cases with primary infertility were more (87 vs. 59.5%; $P = 0.04$) in this study, pregnancy rate was less in intervention group in women having primary infertility (38.5% vs. 34.6%; $P = 0.03$).

A maximum number of cases recruited in our study had a duration of 6–10 years of infertility; distribution of which

Table 4: Effect of endometrial biopsy scratch on pregnancy outcome (protocol analysis)

Outcome	Group			P
	A (n=52), frequency (%)	B (n=46), frequency (%)	C (n=51), frequency (%)	
Pregnancy rate	7 (13.46)	9 (19.57)	8 (15.69)	0.711
Abortion	1 (1.92)	2 (4.35)	0	0.313
OHSS	3 (5.77)	0	1 (1.96)	0.195
Multiple pregnancy	0	0	0	-
Total	52	46	51	

OHSS=Ovarian hyperstimulation syndrome

was 47.27%, 49.09%, and 50.91% in Group A, B, and C, respectively. Mean duration of infertility was similar in all three groups which was 6.22 ± 2.62 , 7.38 ± 3.56 , and 6.67 ± 3.07 years in Group A, B, and C, respectively. In similar study conducted by Parsanezhad *et al.*,^[10] mean duration of infertility was less as compared to our study that is 3.4 ± 1.1 and 3.6 ± 1.4 years ($P = 0.34$) in intervention and control groups, respectively. In an RCT by Zarei *et al.*^[13] conducted in IUI cycles, mean duration of infertility in intervention group (EB done on D6–E8 of preceding cycle) and control group was 4.4 ± 3.1 and 5.6 ± 4.7 years, respectively ($P = 0.075$). In an RCT conducted by Guven *et al.*^[11] in long agonist protocol cycles, mean duration of infertility was 6.08 ± 4.36 and 5.83 ± 4.42 ($P = 0.76$). Association of pregnancy outcome was not analyzed in any of the above-mentioned studies. In our study, maximum pregnancy was found in women with duration of infertility between 1 and 5 years with a pregnancy rate of 15.38%, 27.78%, and 19.05% in Group A, B, and C respectively. Minimum duration of infertility was 2 years in all three groups, and maximum duration of infertility was 12.11 and 13 years in Group A, B, and C, respectively. Thus, it was observed that clinical pregnancy rate was higher in young women with shorter duration of infertility in similar studies.

In our study, maximum cases were of UI with distribution of 43.64%, 27.27%, and 30.91%, respectively, in Group A, B, and C ($P = 0.355$) followed by combined etiology (18.8%, 27.27%, and 14.5%, respectively, in A, B, and C) and male factor (16.36%, 20%, and 25.45% in A, B, and C, respectively).

In similar RCT conducted by Zarei *et al.*^[13] in IUI cycles, maximum cases were of UI, with distribution of 73.6% and 63.9% in intervention and control group respectively, followed by mild male factor and mild endometriosis. Abdelhamed^[12] compared the effect of endometrial sampling in follicular phase of same cycle and previous cycle with control group in women undergoing IUI cycles. UI was most common etiology in all three groups (50%, 46%, and 50% in Group A, B, and C, respectively) followed by ovulatory dysfunction, male factor, and combined. Yeung *et al.*^[17] conducted an RCT to study the effect of endometrial injury in unselected subfertile women undergoing IVF. Male infertility was the most common cause of infertility in both intervention and control group (22 and 23.3%) followed by UI and tuboperitoneal factor. In most of the studies, correlation of etiology with pregnancy outcome was not done. In our study, pregnancy rate was highest in combined etiology (20%, $P = 0.7$) in Group A and Group C (25%, $P = 0.7$). In Group B, pregnancy was maximum in male factor (27.2%, $P = 0.34$), but values were statistically insignificant [Table 2].

Majority of cases enrolled in Group A, B, and C underwent ovulation induction using CC alone (63.64%, 81.82%, and 90.91%, respectively), followed by clomiphene with gonadotrophins and pure gonadotrophins. Although pregnancy rate was highest with CC + gonadotrophins in Group A and B (28.5%; $P = 0.47$), the results were not statistically significant. In other studies, also, ovulation induction was done with CC and gonadotrophins, and significant improvement in pregnancy outcomes was found after local endometrial injury.^[10] In RCT conducted by Zarei *et al.*^[13] in women undergoing IUI cycles, ovulation induction was done by clomiphene with recombinant FSH (rFSH). Abdelhamed^[12] used letrozole with pure gonadotrophins in IUI cycles and found significant improvement in pregnancy outcome after EB.

The mean length of COS cycle from day of ovulation induction to day of trigger was similar in all three groups. Mean cycle length was 12.18 ± 1.59 , 12.42 ± 1.57 , and 11.98 ± 2.02 , respectively, in all three groups ($P = 0.42$). In COS cycles, it is known that the endometrial maturation is advanced, and cycle length hence may shorten. In our study, even after endometrial scratching on D7–D9, cycle length was not reduced and remained optimum for endometrial maturation. In our study, mean number of DF >18 mm on date of hCG trigger was similar in Group A, B, and C, i.e., 1.25 ± 0.7 , 1.4 ± 0.87 , and 1.2 ± 0.62 , respectively, and the CPR was not statistically significant with regard to number of follicles on day of hCG trigger.

On the day of hCG trigger, endometrial thickness (ET) was between D7 and D9 in 74.55%, 83.64%, and 74.55% women in Group A, B, and C, respectively ($P = 0.65$). Mean ET on day of trigger was 7.98 ± 1.52 , 7.93 ± 1.05 , and 8.19 ± 1.18 , respectively, in Group A, B, and C, respectively. ET on day of trigger was higher in Group B in our study as compared to controls although it was statistically insignificant. CPR was 33.33% in women with ET 9–11 mm in Group B. The good endometrium development after scratching in the same phase may be due to increase in angiogenesis and inflammatory mechanism which in turn may be one of the contributing factors for better implantation. Furthermore, endometrial injury retards the already advanced endometrial maturation due to COS cycle, which may lead to the better endometrial thickness and better synchronicity.^[18]

In a study by Hafiza Zepnep *et al.*,^[19] ET on day of trigger was also more in intervention group (10.3 ± 1.4 and 10.8 ± 1.6 in control and study group), but observation was statistically insignificant with P value (0.09). In a similar study conducted by Parsanezhad *et al.*,^[10] in ovulation induction with natural contact cycle, mean ET on day of trigger in intervention group was similar to

control group, respectively (8.94 ± 1.21 and 9.18 ± 1.3 in intervention and control, respectively; $P = 0.16$). In RCT by Zarei *et al.*,^[13] ET on day of trigger was 7.6 ± 1.2 and 7.7 ± 0.9 mm in EB group and control group, respectively ($P = 0.6$). In the RCT conducted by Guven *et al.*,^[11] mean ET on day of trigger was 9.26 ± 2.27 and 9.63 ± 2.15 in intervention and control group ($P = 0.38$). In all above-mentioned studies, even after endometrial scratching, ET on the day of trigger was comparable between intervention and control group.

In our study, we used endometrial aspiration cannula (Endocell, Wallach surgical devices, USA), which is cheap, easily available, and nontraumatic. Barash *et al.*,^[20] Parsanezhad *et al.*,^[10] Yeung *et al.*,^[17] and Guven *et al.*^[11] used pipelle. Karimzade *et al.*,^[21] Shohayeb and El-Khayat,^[9] and Zarei *et al.*^[13] used Novak's curette. Tao brush was used by Abdelhamed,^[12] whereas Rasheed (2014)^[16] used endometrial curette. Nossair *et al.*^[22] used intra uterine contraceptive device (IUCD) insertion tube and infant feeding tube in a prospective cohort study to perform scratch suction in women undergoing repeated IVF/ICSI cycles. Maged *et al.*^[23] used no. 8 neonatal feeding tube to perform endometrial scratch injury. In our study, endometrial scratching was done by gentle movement of endocell around all four walls of uterus. Parsanezhad *et al.*^[10] performed mild local injury by pipelle on posterior wall. Abdelhamed^[12] used a Tao brush, rotating it 4–5 times collecting tissue from entire uterine lining. Zarei *et al.*^[13] took small biopsies from anterior and posterior wall.

There is no clear consensus on whether to perform endometrial scratching in preceding luteal phase or follicular phase. The most common timeframe chosen for endometrial scratching in literature is the luteal phase of preceding cycle, and luteal phase endometrial injury has been reported to be associated with the highest decidualization and pregnancy rates.^[19,20,24-27] Yet, there are many unanswered questions regarding timing of injury and many randomized controlled trials have come up in recent years that demonstrate a significant improvement in implantation rate and clinical pregnancy rate when scratching was done in follicular phase of the same cycle.^[22,10-13] Performing endometrial scratching in follicular phase of the same cycle instead of luteal phase of previous cycle has two advantages. It is more convenient for the women to undergo scratching after transvaginal ultrasound for folliculometry in the same cycle, thus avoiding the need to come in the cycle before IUI. There is a theoretical advantage of a recent inflammatory response and release of cytokines in the same cycle that may lead to better implantation.

In Group A (early follicular phase, D2–D4), the mean day for taking EB in Group A was 2.63 ± 0.75 . Pregnancy was maximum in cases with EB done on D2 (13.79% of cycle, but it was not statistically significant ($P = 0.968$). Endometrial scratching was done on D3 of menstrual cycle following downregulation in ICSI cycle by Guven *et al.*, 2014 and D1–D3 of the same cycle by Rasheed *et al.*, 2013. Wadhwa *et al.*, 2015, performed intervention before day 6 in same COS with IUI cycle and Bahaa Eldin *et al.*^[28] performed endometrial injury on day 5, 6, or 7 of COS with IUI cycle. Thus, only a few studies have evaluated the role of endometrial scratching, done in early follicular phase of the same non-ART cycle [Table 5].

In Group B (late follicular phase, D7–D9), the mean day for taking EB in was 7.58 ± 0.71 . Pregnancy rate was maximum (28.57%) when EB was done on D7 with $P = 0.274$. The scratching of endometrium was done during preovulatory days when LH surge was positive by Parsanezhad *et al.*, 2013, D8–D9 of same and preceding IUI cycle by Abdelhamed 2013, and on D6–D8 of preceding cycle by Zarei *et al.*, 2014, in IUI cycles [Table 5]. A study by Nossair *et al.*, 2014, has been done in failed IVF cycles, in which endometrial scratching was done in late follicular phase (D6–D7) of the same cycle. Maged *et al.*^[23] performed endometrial scratch injury when LH surge was found positive in women undergoing COS with IUI cycles.

No study has been conducted in non-ART cycles to evaluate the benefit of endometrial scratching in women having repeated COS failure cycles.

Our study is the first study, in which endometrial scratching is done in repeated COS failure cycles along with IUI. Women with ≥ 2 failed COS cycles with IUI were recruited and there was an improvement in pregnancy outcomes when EB scratch was done in late follicular phase on D7–D9 of menstrual cycles although results were statistically insignificant.

Clinical pregnancy rate was 12.73% (OR = 0.87; 95% CI = 0.288–2.55, $P = 1$), 16.36% (OR = 1.15; 95% CI = 0.40–3.23, $P = 1$), and 14.54%, respectively, in Group A, B, and C, respectively ($P = 0.86$) as per ITT analysis. Using Chi-square test, P value between Group A and B was 0.787, between Group A and C was 1.000, and between Group B and C was 1.000. Abortion rate was 1.82%, 3.64%, and 0.00% in Group A, B, and C, respectively ($P = 0.36$).

As per protocol analysis, clinical pregnancy rate was 13.46% (OR: 0.83 95% CI: 0.27–2.5, $P = 0.74$), 19.57% (OR = 1.3 95%; CI = 0.45–3.73, $P = 0.41$), and 15.69%. Using Chi-square test, P value between Group A

Table 5: Comparing effect of EB scratch in follicular phase on clinical pregnancy rate in non-ART cycles

Studies (EB scratch)	Design	Participants	Timing of intervention	Method of intervention	CPR/RR/P	ITT analysis	LBR	IR	OR	ITT
Parsanezhad <i>et al.</i> ^[10]	Prospective RCT	Patients with UI <i>n</i> =217 Intervention=114 Mock pipette biopsy=103	Preovulatory days (when spontaneous urinary LH surge was detected)	Pipelle	14.9% versus 5.8% OR: 2.8395% CI: 1.07-7.49, <i>P</i> =0.03	No				
Abdelhamid (2013) ^[12]	RCT	Patients undergoing IUI cycles <i>n</i> =150 Group 1 <i>n</i> =50 Group 2 <i>n</i> =50 Group 3 <i>n</i> =50	Group 1 (control) Group 2-day 8-9 of the preceding stimulation cycle Group 3-day 8-9 of the same IUI cycle	Tao brush	18%, 38% and 36% Control group and Group 2 (<i>P</i> =0.001) Group 3 and the control group (<i>P</i> =0.002)	Yes				
Zarei <i>et al.</i> ^[13]	RCT	UI in IUI cycles, mild male factor and mild endometriosis <i>n</i> =144 Intervention=72	Days 6-8 of the previous menstrual cycle before IUI	Novak's curette	23.6% versus 19.4% <i>P</i> =0.686	No			2.27	
Rasheed ^[16]	RCT	Patients with UI <i>n</i> =197 Intervention=110	Days 1-3 of same cycle	Endometrial curette	48.3% versus 45.5% Control versus EB group respectively	No				
Wadhwa <i>et al.</i> ^[14]	RCT	<i>n</i> =71 Intervention=Group A=86, Group B=90, Group C=75 (control)	Group A: Days 19-24 preceding cycle Group B: Days 2-6 same cycle	Endocell	19.77%, 31.11%, and 9.3% for Group A, Group B, and Group C, respectively <i>P</i> =0.000957 (B vs. C)	Yes				No
Maged <i>et al.</i> ^[23]	RCT	COS with IUI cycles <i>n</i> =154 Intervention <i>n</i> =77)	Group C: control Group S: Endometrial scratch injury done on day of LH surge positive	<i>n</i> =8 neonatal feeding tube	Cumulative pregnancy rate of 39% versus 18.2% respectively in Group S and C respectively (<i>P</i> <0.05)	Yes				
Bahaa Eldin <i>et al.</i> , 2016 ^[28]	RCT	COS with IUI cycles <i>n</i> =349 Intervention=174	Day 5, 6 or 7 of same cycle	Pipelle	18.93% versus 7.42% (<i>P</i> =0.003)	Yes				
Our study	RCT	≥2 failed COS cycle with IUI <i>n</i> =165)	Group A <i>n</i> =55): Days 2-4 of same cycle Group B <i>n</i> =55): Days 7-9 of same cycle Group C <i>n</i> =55): Control	Endocell	12.73%, 16.36% and 14.54% in Group A, B, and C, respectively (ITT) (<i>P</i> =0.8) 13.46%, 19.57%, and 15.69% (protocol analysis) (<i>P</i> =0.71)	Yes				

CI=Confidence interval, RCT=Randomized clinical trial, IUI=Intrauterine insemination, EB=Endometrial biopsy, ITT=Intention to treat, LBR=Live birth rate, IR=Implantation rate, CPR=Clinical pregnancy rate, RR=Relative risk, OR=Odds ratio, COS=Controlled ovarian stimulation, UI=Unexplained infertility, LH=Luteinizing hormone

and B was 0.588, between Group A and C was 0.967, and between Group B and C was 0.815. Abortion rate was 1.92%, 4.35%, and 0% in Group A, B, and C (*P* = 0.313)

as per protocol analysis. OHSS rate was 5.77%, 0%, and 1.96% (*P* = 0.195). No multiple pregnancies occurred in any of the three groups.

Endometrial scratching has been known to improve endometrial receptivity not only in ART cycles but also non-ART cycles. Many studies have come up in recent years that have shown a beneficial effect of endometrial scratching in women undergoing natural cycle/COS cycle/COS with IUI cycles [Table 5].

In IVF, it has been proven that endometrial injury has a positive impact on pregnancy outcome in cases of recurrent implantation failures (RIF). Numerous studies have been conducted that demonstrate a definite improvement in CPR in women with RIF [Table 6].

A meta-analysis conducted by El-Toukhy *et al.*^[31] suggested significant improvement in clinical pregnancy rate in women undergoing endometrial injury done in luteal phase of cycle preceding the IVF cycle (with or without ICSI) in both the randomized (relative risk [RR], 2.63, 95% CI = 1.39–4.96, $P = 0.003$) and nonrandomized studies (RR 1.95, 95% CI = 1.61–2.35, $P < 0.00001$).

A meta-analysis by Potdar *et al.* (2012)^[32] demonstrated a beneficial effect of EB and hysteroscopy in significantly improving clinical pregnancy rates in women with RIF in IVF/ICSI cycles when intervention was done in luteal phase of preceding IVF cycle. Clinical pregnancy rates were twice as high with biopsy/scratch (RR: 2.32, 95% CI = 1.72–3.13) as opposed to hysteroscopy (RR: 1.51, 95% CI = 1.30–1.75).

In a paper published by Simón and Bellver,^[33] the quality of evidence-based data supporting endometrial scratching as a means to improve pregnancy rates in ART was criticized and concluded that well-designed studies and well-performed meta-analysis are needed to generate good quality scientific information regarding endometrial scratching.

Cochrane review^[7,34] conducted (2015) demonstrated that endometrial injury was associated with increased clinical pregnancy rate when done between day 7 of preceding cycle and day 7 of ET (RR: 1.34, 95% CI 1.21–1.61; $P = 0.002$; 13 RCTs; 1972 women; $I^2 = 45%$; moderate-quality evidence), live birth rate or ongoing pregnancy rate (RR: 1.42, 95% CI = 1.08–1.85; $P = 0.01$; 9 RCTs; 1496 women; $I^2 = 53%$; moderate-quality evidence) in women undergoing more than two previous ETs. Thus, it was observed that endometrial injury improves pregnancy outcomes not only when done in luteal phase of preceding cycle but also when done in follicular phase of the same cycle.

Cochrane review (2016)^[34,35] concluded that it is uncertain whether endometrial injury improves the probability of pregnancy and live birth/ongoing pregnancy in women

undergoing IUI or attempting to conceive through sexual intercourse.

In our study, endometrial scratching did not lead to significant improvement in clinical pregnancy rates in women with repeated failed COS cycles. RCTs conducted by Wadhwa *et al.*, Maged *et al.*, and Ahmed *et al.* (2016) reported a significant improvement in clinical pregnancy rate when endometrial scratching was done in follicular phase of same COS with IUI cycle. However, in study conducted by Wadhwa *et al.*, most women underwent scratching in first IUI cycle and Ahmed *et al.* (2016) did not include women with repeated COS failure cycles. Since in our study, women with ≥ 2 failed COS cycles were recruited, this led to inclusion of older women with longer duration of infertility that might have contributed to lower clinical pregnancy rate. In addition, some occult and unexplained factor might be responsible for low pregnancy rate in repeated COS failure cycles.

Pregnancy rate per cycle in our study was more when scratching was done between D7 and D9 (17.39% per protocol analysis), as compared to worldwide reported clinical pregnancy rate of 7% in clomiphene with IUI cycles and 12% in gonadotrophins with IUI cycles. Although our result was not statistically significant when compared to control group,^[35,36] the effect found was of good quantum in Group B as per protocol analysis which could be of clinical relevance if larger sample size would have been taken. The effect found was of good quantum in Group B as per protocol analysis which could be of clinical relevance if larger sample size would have been taken.

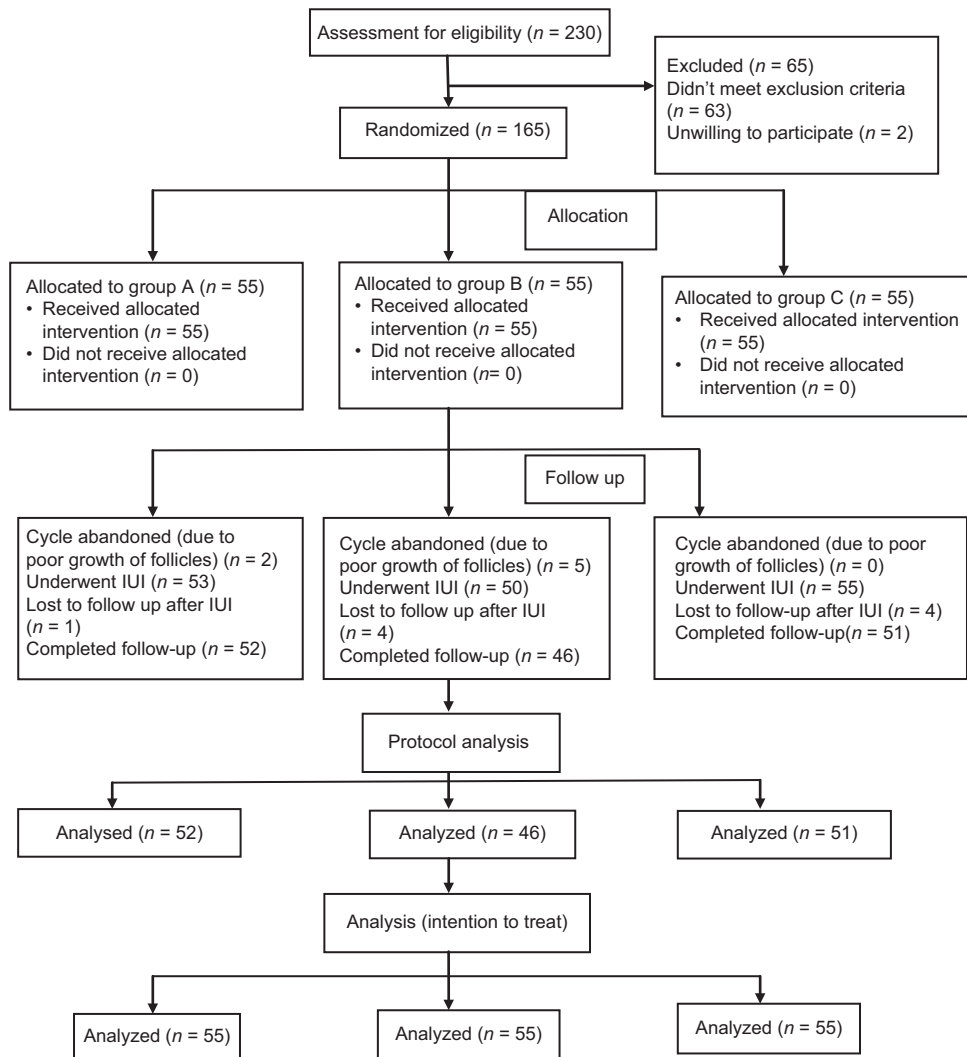
Pain was assessed with the help of a VAS 10 min after the procedure. Pain in all three groups was found comparable. Mean score of pain was 3.67 ± 0.7 , 3.84 ± 0.96 , and 3.6 ± 0.71 cm in Group A, B, and C, respectively ($P = 0.29$). Thus, EB scratching on D2–D4 or D7–D9 is not associated with significant pain as compared to controls as per our study. Nastri *et al.*^[7] found a significant increase in pain score in women undergoing endometrial scratching as compared to controls (6.42 ± 2.35 cm vs. 1.82 ± 1.52 cm, $P < 0.001$).

There were few limitations in our study. Our inclusion criteria mentioned an age group of 20–38 years, which might have led to low pregnancy rates due to the inclusion of older age group. Ovulation induction in majority of cases was done by clomiphene alone, and very few study in literature has been conducted to show the effect of endometrial scratching in clomiphene alone cycles. Inflammatory markers were not measured, so we cannot comment on the role of inflammation in

Table 6: Comparing effect of endometrial biopsy on clinical pregnancy rate in previous failed *in vitro* fertilization cycles

Studies (EB scratch)	Design	Participants	Timing of intervention	Method of intervention	CPR/RR/P ITT analysis	LBR	IR	OR
Barash <i>et al.</i> ^[20]	NR	Previous 1 failed (n=134) Intervention=45	8, 12, 21, 26 (4 times) preceding cycle	Pipelle	66.7% versus 30.3% 2.20 0.00009	Yes 48.9 versus 12.5%; P=0.016		
Raziel <i>et al.</i> ^[24]	NR	Previous failed (n=120) Intervention =60	Days 21 and 26 two times in preceding cycle	Pipelle	30% versus 12% 2.44 0.02	Yes		
Karimzadeh <i>et al.</i> ^[29]	RCT	2-6 previous failed (n=115) Intervention=58	Luteal phase of preceding cycle (21-26)	Pipelle	27.9% versus 8.9% 3.05 0.02	No 10.9% versus 3.38%, P=0.03		
Tiboni <i>et al.</i> ^[25]	Prospective, no control	≥3 previous failed	Day 21 of preceding cycle	NA	45.94% No			
Hafiza Zepnep <i>et al.</i> ^[19]	RCT	Previous 1 failed (n=100) Intervention=50	Luteal phase 2 times with 1 week interval	Pipelle	60% versus 34% 0.009	No 44% versus 24%; P=0.03	34.67% versus 30.88%; P=0.1384	
Singh <i>et al.</i> (2015) ^[26]	RCT	Previous >1 failed IVF-ET	Days 14-21 of preceding cycle	Karmans cannula	Ongoing pregnancy rate (16.7% vs. 0.0%; P=0.052	No 3.3% versus 10%, P=0.612	19.4% versus 8.1%; P=0.028	
Gibreel <i>et al.</i> ^[27]	RCT	Previous 1 failed IVF (n=387) Group A=193	EB procedure twice from Days 21-26	Pipelle		No 47.2% versus 38.1%, P=0.08		(OR) 3.4, P=0.005 in previous 2 failed IVF
Baum <i>et al.</i> ^[30]	RCT	Previous 3 failed IVF-ET (n=36) Intervention=18	Days 9-12 and D21-24 of preceding cycle	Pipelle	0 versus 31.25% (P<0.05)	No 0 versus 25% (P=0.1)	2.08% versus 11.11% (P=0.1)	
Shohayeb and El-Khayat ^[9]	Prospective RCT	Previous ≥2 failed IVF/ICSI cycles (n=200) Intervention=100	Group A: Endometrial curettage on Days 4-7 of the preceding ICSI cycle with hysteroscopy Group B: Only diagnostic hysteroscopy	Novak's curette	32% versus 18% (P=0.034)	No 28% versus 14% (P=0.024)	12% versus 7% (P=0.0015)	
Nossair <i>et al.</i> ^[22]	Observational prospective cohort experimental study	Previous failed IVF (n=30) Intervention=30	Days 6-7 of same ICSI cycle	Scratch-suction technique using insertion tube of (IUCD) and infant feeding tube 8F	66.66% -		80%	

IVF=*In vitro* fertilization, EB=Endometrial biopsy, ITT=Intention to treat, LBR=Live birth rate, IR=Implantation rate, CPR=Clinical pregnancy rate, RR=Relative risk, OR=Odds ratio, NA=Not available, ET=Embryo transfer, ICSI=Intracytoplasmic sperm injection, OR=Odds ratio, IUCD=Intrauterine contraceptive device, NR=Non randomized, RCT=Randomized clinical trial



Flow Chart 1: Consort flow diagram

implantation. Although we have recruited women with previous two or more failed IUI cycles, unselected group of patients was recruited including patients with male factor, tubal factor, ovulatory dysfunction, and combined infertility. In IVF cycle, the role of endometrial injury in improving endometrial receptivity is established as embryonal factors, leading to RIF are excluded to an extent by transfer of good quality embryos. In previously failed COS cycles, many unknown factors besides poor endometrial receptivity may be present that may lead to repeated failure cycles and reduced CPR. Endometrial scratching is hypothesized to improve endometrial receptivity, but other factors such as autoimmune conditions, embryonal defects, and immunological incompatibility might be responsible for cause of RIF despite endometrial scratching. In our study, we only performed endometrial scratching by gentle movement of endocell, but suction was not done and neither attempt to take biopsy. This might have had led to a theoretical

effect of less inflammatory response as compared to if biopsy was taken. In other studies, like Nossair *et al.* (2014), endometrial scratch along with suction was done, which led to a better pregnancy outcome.

IVF is the leading fertility treatment for women having RIF, but it is expensive and has a moderate success rate of approximately 30% per cycle.^[37]

The apparent increase in probability of pregnancy in RIF following endometrial scratch suggests that this procedure might also be beneficial in women who are trying to conceive naturally, or who are undergoing OI or both.^[36,37] Endometrial injury is a simple, low-cost procedure which can be performed on an outpatient basis. Its benefit in women with repeated COS failure cycles or couples trying to conceive with IUI and OI remain unclear. If endometrial injury improves reproductive outcomes in this situation, it could provide a low-cost treatment alternative for some couples before

they consider undergoing IVF. In our study, we found a slight increase in pregnancy outcome in women with repeated COS failures undergoing endometrial scratching in late follicular phase, but the result was not statistically significant. Larger and adequately powered studies are needed to elucidate the effects of endometrial scratching on the outcome of repeated failed COS cycles.

CONCLUSION

Endometrial scratching is a cost-effective and easy technique which may improve clinical pregnancy rates in previous COS failure cycles, but more trials are needed to be conducted using larger sample size to achieve the improved and significant outcome.

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Conflicts of interest

There are no conflicts of interest.

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