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## Association of endometrial thickness, zone 3 flow, endometrial layering, luteal phase defect and subsequent endometrial scratching with improved chances of implantation and conception in a study conducted in a tertiary care centre, India

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### Abstract

A prospective study was carried out in the Department of Obstetrics and Gynaecology in a tertiary care centre, India from February 2019 to July 2019. The parameters documented in the study were endometrial thickness, zone 3 flow and endometrial layering. Among patients with unexplained infertility, 58% had ET  $\leq$  7mm, 42% had ET  $>$  7mm, 33% had sparse (none conceived) and 67% had adequate zone 3 flow (out of which 25% conceived). 25% had poor (none conceived) and 75% had adequate endometrial layering (out of which 22% conceived). Among 48 patients who underwent endometrial scratching, 67% had luteal phase defect (none conceived). Rest 33% who did not have luteal phase defect conceived. Statistical analysis was done using SPSS software 19.0 to find out association, if any. Conclusion of the study was that endometrial scratching enhances chances of conception. Optimum endometrial thickness, zone 3 flow and endometrial layering are also necessary for successful implantation.

**Keywords:** Endometrial scratching, luteal phase defect, endometrial thickness

### 1. Introduction

Infertility is defined as inability to conceive after one year of unprotected intercourse. In India, 16% of married women aged 15-49 are infertile [1]. Unexplained infertility refers to failure to conceive in a couple for whom no definitive cause for infertility can be found. About 10% couples have unexplained infertility [2]. Altered endometrial function due to low endometrial progesterone receptor concentrations, inadequate estrogenic induction of progesterone receptors and suboptimal expression of integrins or pinopode formation in the endometrium leading to poor implantation is a cause of unexplained infertility [3]. For implantation to occur, a genetically normal blastocyst should hatch, appose, adhere, penetrate and finally invade a well synchronised endometrium under the influence of estrogen and progesterone [4]. A no. of locally acting molecules including growth factors, cytokines, metalloproteinases, adhesion molecules and homeobox genes play a role [5]. Implantation failure which is mainly a major barrier in infertility is attributed in most cases to failure of uterus to acquire receptivity. The transition into a receptor uterus includes cellular changes in the endometrium and modulated expression of growth factors, cytokines, an elevated level of pro inflammatory cytokines and immune cells [6]. It is believed that endometrial scratching causes damage to the uterine lining which causes a type of "repair reaction" in which pro inflammatory cytokines are released, genes of implantation are switched on and there is better synchronicity between the endometrium and the embryo leading to better implantation and enhanced conception rates [7]. With the above background, the objective of this study was to see the effect of endometrial scratching in prior cycles on improving chances of conception in next cycle and to see luteal phase defect by endometrial dating on day 22 of cycle.

### 2. Material and Methods

A prospective study was carried out in the Department of Obstetrics and Gynaecology from February 2019 to July 2019. A pre-designed pre-tested questionnaire was used. Ethical clearance was obtained from Institutional Ethics Committee.

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In the previous year, approximately 2000 patients with infertility attended in the OPD. Among them, 20 percent of infertility patients was taken as sample size ie. 400. The interview was carried out three days in a week, 6 patients per day. So, 70 patients per month were interviewed for 6 months to reach the required sample size of 400. Females with unexplained infertility who underwent endometrial scratching on day 22 of cycle were taken as cases and those who did not undergo scratching were taken as controls. All patients were interviewed with questionnaire after obtaining their written informed consent. Endometrial samples were obtained from anterior wall of uterine cavity using Novak's cannula during natural mid secretory phase after LH surge (LH surge + 7 days, day 22 of cycle) and send for evaluation of HPE for endometrial dating and to rule out Luteal Phase defect. Patients underwent Color Doppler study to see endometrial thickness, zone flow and lines of endometrium on day 22. All procedures were performed in compliance with relevant laws and institutional guidelines.

**2.1 Inclusion criteria:** Females of age group of 15-49 years with unexplained infertility after ruling out tubal factors, ovarian factors, endometriosis, genital TB, anatomical factors and male factors.

**2.2 Exclusion criteria:** Females with tubal block, PCOS, genital TB, fibroid, endometriosis and male factors.

**2.3 Statistical analysis:** The collected data were entered into Excel spreadsheets and analysis was carried out. The procedures involved were preliminary data inspection, content analysis and interpretation. The statistical analysis was done using SPSS for Windows version 19.0 software. The mean and standard deviation of the parameters studied during observation period were calculated for various treatment groups and were compared. The critical value of 'p' indicating the probability of significant difference was taken as <0.05 for comparisons.

### 3. Results

**Table 1:** Demographic profile of study population in relation to infertility

Age group (in years)	Number of cases
21 – 25	140
26 – 30	176
31 – 35	68
> 35	16
<b>Total</b>	<b>400</b>
Socioeconomic Status	Number of cases
Upper high	20
Upper Middle	252
Lower middle	128
<b>Total</b>	<b>400</b>
BMI (kg/m <sup>2</sup> )	Number of cases
< 18.5	44
18.5-24.9	304
25-29.9	40
> 30	12
<b>Total</b>	<b>400</b>

Among patients coming for infertility evaluation, 44% were in the age group 26 - 30 years. and 35% were in the age group 21 - 25 years. i.e. young reproductive age group. Only 4% were of age > 35 years. According to modified B G Prasad socioeconomic status scale (modified in 2018), majority of patients (63%) belonged to upper middle class, 32% belonged to lower middle class and 5% belonged to upper high class. Seventy six percent of patients had normal BMI, 11% were

underweight, 10% were overweight and 3% were obese.

**Table 2:** Causes of Infertility

Cause of Infertility	Percentage
Tubal	36
PCOS	12
Uterine	2
Male	26
Unexplained	24
<b>Total</b>	<b>100</b>

In maximum patients (36%) tubal factor was the major cause of infertility. Male factor infertility was present in 26%, PCOS in 12% and uterine factor in 2%. In 24% cases, etiology was unexplained after complete workup.

**Table 3:** Association of endometrial thickness with chances of conception (n = 96)

ET (mm)	Conceived	Not conceived
< = 7	0	56
>7	16	24
<b>Total</b>	<b>16</b>	<b>80</b>

Among 96 patients with unexplained infertility, 56 (58%) had ET < = 7 mm out of which none conceived. 40 (42%) had ET > 7 mm out of which 16 conceived in next cycle. On applying Chi square test, calculated  $\chi^2$  was 26.911 with p value < 0.00001 which was statistically significant at p < 0.05.

**Table 4:** Association of zone 3 flow with chances of conception (n = 96)

Zone 3 flow	Conceived	Not Conceived
Negative	0	0
Sparse	0	32
Adequate	16	48
<b>Total</b>	<b>16</b>	<b>80</b>

Among 96 patients with unexplained infertility, 32 (33%) patients had sparse Zone flow among which none conceived even after endometrial scratching. 64 patients (67%) had adequate Zone 3 flow among which 16 (25%) conceived and 48 (75%) did not conceive. The  $\chi^2$  cal was 9.609 with p value 0.008193 which was statistically significant at p < 0.05.

**Table 5:** Association of no. of lines in the endometrium with chances of conception (n=96)

Lines in endometrium	Conceived	Not Conceived
No lines	0	0
< 5 lines	0	24
≥ 5 lines	16	56
<b>Total</b>	<b>16</b>	<b>80</b>

Among 96 patients with unexplained infertility, 24 patients (25%) had < 5 lines in endometrium on USG Doppler evaluation among which none conceived. 72 patients (75%) had ≥ 5 lines among which 16 conceived (22%) in next cycle and 56 did not conceive (78%). The  $\chi^2$  cal was 6.396 with p value 0.040844 which was statistically significant at p < 0.05.

**Table 6:** Association of luteal phase defect with chances of conception (n = 48)

Luteal phase defect	Conceived	Not conceived	Total
Absent	16	0	16
Present	0	32	32

Among 48 patients who underwent endometrial scratching, luteal phase defect was present in 32 patients (67%) out of which none had conceived. The glands were poorly convoluted and narrow. The lumen were dilated. Secretory activity was decreased. Decidualization had not occurred. 16 patients (33%) showed normal secretory phase. Sub nuclear vacuolation was seen in glandular epithelium. There was linear arrangement of nuclei in at the base of the cells. Cytoplasm was homogenous. Intraluminal secretions were present in vacuoles. All 16 conceived in next cycle. The  $\chi^2$  cal was 47.999 with p value <0.00001 which was statistically significant at p <0.05.

**Table 7:** Correlation of endometrial scratching with chances of conception (n = 96)

Endometrial scratching	Conceived	Not conceived	Total
Done	16	32	48
Not done	0	48	48

Out of 96 patients with unexplained infertility, 48 patients underwent endometrial scratching on day 22 of cycle. Out of them 16 had conceived (33%) in next cycle. Remaining 32 patients did not conceive even after endometrial scratching (67%). 48 patients who did not undergo endometrial scratching

did not conceive. The  $\chi^2$  cal was 19.2 with p value < 0.000012 which was statistically significant at p < 0.05.

**4. Discussion**

In my study, 44% patients were in the age group 26 - 30 years and 35% were in the age group 21 - 25 years i.e. young reproductive age group. Only 4% were of age > 35 years and the mean age was 30.21 ± 3.83 years. In previous similar studies,

**Table 8:** Mean age of female patients

Study	Country	Mean age of female patients (years)
Adamson <i>et al.</i> [8], 2011	Mysore, India	24.0 ± 3.4
Aghahoseini <i>et al.</i> [9], 2007	Iran	31 ± 5.4
Zaidi <i>et al.</i> [10], 2007	London	32.3 ± 3.5
Present study	India	30.21 ± 3.8

So, my results showed similar trends as in the studies mentioned above. In my study, tubal factor was the major cause of infertility in maximum patients (36%), male factor (26%), PCOS (12%), uterine factor (2%) and unexplained infertility (24%). In previous similar studies,

**Table 9:** Show the Cause

Cause	Neeta Singh <i>et al.</i> [11], (%) India (1996)	A. Khanna <i>et al.</i> [12], (%) India (2011)	Present study (%)
Tubal factor	57.4	45.9	40
Male factor	21.7	26	32
PCOS	8.9	24.13	15
Unexplained	6.9	11	10

So, in my study, percentage of females with unexplained infertility was more as compared to previous studies. In my study, maximum patients (58%) had ET ≤ 7mm. 42% had ET > 7mm. In my study, maximum rate of conception was

seen with endometrial thickness between > 7mm and no conception was seen when endometrial thickness was ≤ 7 mm. In previous studies,

**Table 10:** Minimum Endometrial Thickness

References	No. of cycles	Minimum Endometrial Thickness (mm)	Mean Endometrial Thickness (mm)	
			Conception	Non-conception
Coulam <i>et al.</i> [13], 1994	100	6	11.9 ± 2.7	11.5 ± 2.8
Al-Shawaf <i>et al.</i> [14], 1993	77	8	9.30 ± 0.36	10.00 ± 0.24
Strohmer <i>et al.</i> [15], 1994	76	7	11.7 ± 2.6	11.8 ± 2.8
Oliveira <i>et al.</i> [16], 1993	139	6	8.0 ± 1.7	8.6 ± 2.0
Gonen and Casper [17]	85	6	8.7 ± 0.4	7.5 ± 0.2
Alam <i>et al.</i> [18], 1993	111	7	10.5 ± 3.5	9.6 ± 4.2
Abdalla <i>et al.</i> [19], 1994	59	5	10.24 ± 2.63	8.62 ± 3.49
Bustillo <i>et al.</i> [20], 1995	88	6	10.2 ± 2.3	9.7 ± 2.7

No consensus has been reached with regard to the minimum endometrial thickness required for successful pregnancy. In study by Neeta Singh *et al.*, the thinnest endometrial lining for successful pregnancy was 5.8 mm and maximum number of conceptions occurred when the thickness was 8–10 mm. Among 96 patients with unexplained infertility, 64 patients had adequate Zone 3 flow (67%) among which 16 conceived (25%) as compared to 40% patients in previous study by Gupta and Chandra *et al.* [21], with adequate zone flow out of which 54.5% had conceived. 32 patients had sparse Zone 3 flow(33%) among which none conceived as compared to 32.7% with sparse zone flow in the previous study out of which 35.8% had conceived. So, my study was only partially comparable to the previous studies. Among 96 patients, 24 patients (25%) had < 5 lines in endometrium on USG Doppler evaluation among which none conceived as compared to a previous study by M. Aghahoseini *et*

*al.*, in which 23.6% had < 5 lines in endometrium on USG Colour Doppler study. 72 patients (75%) had ≥ 5 lines among which 16 conceived i.e. 22% as compared to 58.9% conception rate in previous study. In a study by Barash *et al.*, [22] in 2003, after endometrial scratching, 66.7% women with unexplained infertility and 30.3% with normal infertility had conceived. In a similar study by Narvekar *et al.* [23], in Bangalore in 2010, after endometrial scratching, 32.7% women with unexplained infertility and 13.7% with normal infertility had conceived. In present study, out of 96 patients with unexplained infertility, 48 patients underwent endometrial scratching on day 22 of cycle. Out of them 16 had conceived (33%). In a study done by T.C. Li, P. Dockery and A.W. Rogers [24] in 2005, endometrial dating was done in 30 women with unexplained infertility and 70 women with normal fertility.

Histopathological findings showed luteal phase defect in 20% women with unexplained infertility and 3% women with normal infertility. In my study, 32 patients out of 48 patients of unexplained infertility who underwent endometrial scratching had luteal phase defect (67%).

## 5. Conclusion

Chances of conception improves after endometrial scratching because scratching causes local injury followed by a repair process which involves release of growth factors, cytokines, immunological factors, implantation factors, etc which causes angiogenesis, improves blood flow in endometrium thus increasing endometrial receptivity and increases chances of implantation and conception.

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