Endometrial thickness is a limiting factor in achieving a successful pregnancy in infertile women undergoing IVF/ICSI cycle.

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Research Article

Keywords: Transvaginal ultrasonic Scanning (TVS), Human Menopausal Gonadotrophin (hMG)

DOI: https://doi.org/10.21203/rs.3.rs-785729/v1

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Abstract

Introduction: Embryo transfer into the uterus during IVF/ICSI treatment is followed by embryo implantation, which depends on the receptivity of endometrium and is a pre-requisite for the initiation of a successful pregnancy. The endometrium proliferates under the hormonal influence (estrogen and progesterone), which is reflected by its thickness which is measurable by transvaginal ultrasonic scanning (TVS). The thickness of the endometrium less than 8 mm has been linked with implantation failure. Endometrial thickness of 9-14 mm on the day of progesterone supplementation has been shown to have a higher implantation rate. Thus, the implantation potential of a good embryo remains low during IVF treatment despite ovarian stimulation methods of assisted fertilization and improved culture condition.

Objectives: This study was planned to determine the impact of endometrial thickness on ET and pregnancy rate in Pakistani patients undergoing IVF/ICSI.

Methods: This was a prospective, cross-sectional study conducted at a private fertility clinic (Lahore institute of fertility and endocrinology, LIFE) in Lahore, Pakistan, from January 2015 to December 2015. In this study, all those cases were included that reached oocytes pickup and embryo transfer (ET). Two protocols, agonist and antagonist, were used. In all cases, human menopausal gonadotrophin (hMG) and follicle-stimulating hormone (FSH) were used for stimulation. Follicles development was monitored; at least 3 follicles were reached at the diameter of ≥16mm.

Results: Thickness of endometrium on decision day was calculated at various ranges concerning both groups and find pregnancy rates. When endometrial thickness was ≤ 6mm, PR was 2.22%, 7mm pregnancy rate (PR) was 1.11%, 8 mm PR was 3.33%, 9mm PR was 17.78%, 10mm PR was 33.33%, 11mm PR was 32.22%, and more than 11 mm PR was 10%. So, PR was higher from 2.22% to 10%. The overall pregnancy rate was 45.45%.

Conclusion: The study concluded that endometrial thickness has significant effects on embryo transfer and outcomes.

Introduction

Embryo transfer into the uterus during IVF/ICSI treatment is followed by embryo implantation, which depends on the receptivity of endometrium and is a pre-requisite for initiation of a thriving pregnancy. The endometrium proliferates under the hormonal influence (estrogen and progesterone) reflected by its thickness which is measurable by transvaginal ultrasonic scanning (TVS). Endometrial morphology is gauged by the thickness of the endometrium, which is usually assessed by transvaginal ultrasound. Endometrium is specially prepared for the approaching embryo under the hormonal influence during the menstrual cycle. Ultrasound parameters including endometrial thickness, blood flow have been used to assess the endometrium receptivity.
Ovarian steroids increase the thickness and vascularity of the endometrium, and it becomes highly receptive to the signals of the upcoming blastocyst for a brief period. A sort of communication occurs between the embryo and endometrium when the luminal milieu of the uterus is conducive enough nutritionally.

This communication occurs for a short interval between day 20 and 24 during the menstrual cycle, termed a window of implantation. The embryo produces cytokines that direct the implantation process at the molecular level, which is highly synchronized and complex. However, molecular and biochemical events responsible for enhanced receptivity during this short period are still a biological mystery. In addition to embryo quality, another attribute is the receptivity of the endometrium, which plays an essential role in the embryo's implantation. The transformation of the blastocyst into a viable and sustainable ongoing pregnancy is possible only when the luminal environment is hospitable, which requires an endometrium that is receptive and capable. Failure of implantation has a very high rate; the reason for this being lack of endometrial receptivity. Endometrial abnormalities may lead to failure in implantation and thus lead to abortion which has high rates. The thickness of the endometrium less than 8 mm has been linked with implantation failure. Endometrial thickness of 9–14 mm on the day of progesterone supplementation has been shown to have a higher implantation rate. The implantation potential of a good embryo remains low during IVF treatment despite ovarian stimulation methods of assisted fertilization and improved culture condition. This study was planned to determine the impact of endometrial thickness on ET and pregnancy rate in patients undergoing IVF/ICSI.

**Methods**

This prospective, cross-sectional study was conducted at a private fertility clinic (Lahore institute of fertility and endocrinology, LIFE) in Lahore, Pakistan, from January 2015 to December 2015. The ethical committee of Hameed Latif hospital approved this study. Total 689 cases of IVF/ICSI were studied. Inclusion criteria were; the age of the women less than or equal to 38, BMI less than or equal to 28, serum progesterone less than or equal to 4. The patients were divided into two groups; those who got pregnant (group A) and those who did not (group B). Both groups were compared for the various parameters, including age, body mass index (BMI), diagnosis, number of oocytes retrieved, length of stimulation, a dose of hMG, fertilization rate, number of cleaved embryos, and number of transferred embryos. These protocols were administered by clinician choice and patients preference.

**Inclusion criteria**

Patients aged 25–45 years, previously have not more than two IVF/ICSI cycle attempts, on day 2rd of menstrual cycle serum FSH level must be < 9 IU/L were included in this study. The patients with a previous history of PCOS but thoroughly recovered were undergoing for short gonadotropin-releasing hormone agonist (GnRH-a) regime.

**Exclusion criteria**
Patients with any congenital anomaly, pelvic pathology, urogenital surgery, any sexually transmitted disease, habitual abortion, alcoholic addiction, any infectious disease, underwent hormonal replacement therapy during the last three months, any uterine abnormalities, and immunocompromised were excluded in this study.

**Estimation of Clinical Parameters:**

According to standard protocol, clinical parameters such as height and weight were recorded to compute the body mass index (BMI) (8).

**Valuation of endocrine dimensions:**

The blood samples were collected between 8 to 10 am from a cubital vein, and serum was separated instantly and stored at -20°C till the performance of the hormonal assay, including Follicular stimulating hormone (FSH), Luteinizing hormone (LH), estradiol (E2) and anti-mullerian duct hormone (AMH) on 2nd day of the menstrual cycle through electrochemiluminescence Immunoassay according to the manufacturer's instructions (Elecsys® Roche Diagnostics, Indianapolis, USA).

**Therapeutic Regimen:**

**GnRH-agonist long-acting protocol:**

A total of 310 patients were recruited in the long-acting protocol (LAP) group induced by a combination of GnRH-a incorporated with Follicular stimulating hormone (FSH) and Human menopausal gonadotropin (hMG). Women with a regular cycle on day 21 in the mid-luteal phase started with the administration of a single intramuscular dose of 0.1–1.2 mg long-acting decapeptyl® (Triptorelin acetate; Ferring) GnRH-a injection. A complete down-regulation of the pituitary had done when the serum LH level < 2 IU/ml and serum E2 level < 30 pg/mL was achieved. The transvaginal ultrasound scan (TVS) revealed a less than 5mm endometrium thickness, confirming complete pituitary suppression. On cycle day 2, exogenous gonadotropin rFSH (5.5µg; Gonal-F™, Merk Serono) and menotropins hMG (LG™ life sciences, Korea) administration were commenced at doses ranging between 75–220 IU/day and 350–450 IU/day depending upon body weight, age, and follicular size of the patients. A further regular dosage of rFSH and hMG was calculated based on ovarian stimulation monitored through transvaginal ultrasound scan (TVS) and serum E2 levels. The folliculogenesis was consecutively observed through TVS and by measuring the serum E2, LH, and progesterone ratio from the 8th day to the day of Human chorionic gonadotropin (hCG) injection (Pregnyl®, Organon), which is around 14 days post-GnRH-a administration.

**GnRH-agonist short protocol or Flare-up regimen:**

A total of 230 patients in the short-acting protocol group have got a 0.1 mg daily intramuscular dose of decapeptyl® (Triptorelin acetate; Ferring) started from day 3rd of the menstrual cycle and continued till the day of hCG. Controlled ovarian stimulation started from 2nd day of menstrual cycle at the dose of
100–220 IU of rFSH (5.5µg; Gonal-F™, Merk Serono) and 200–450 IU of hMG (LG™ life sciences, Korea) respectively. The daily dosage was adjusted in accordance with ovarian response.

**Ovulation induction:**

In both protocols, recombinant hCG (6500-10,000 IU) was given intramuscularly to trigger the final maturation of follicles or when more than two follicles attained a diameter of 17 mm and increased level oestradiol 2000 pg/ml/mature follicle. While cycle cancelation has been done if there is poor ovarian response during stimulation, i.e., no follicle of 15 mm will be seen on day 9, and E2 level will be < 5000 pg/ml on day 9. The time difference between the last gonadotropin injection and the hCG regimen was no more than 24 hours. After 36 hours of hCG regimen, the transvaginal echo-guided ovarian puncture was done, and oocyte retrieval was performed.

The assessment of oocyte quality has been performed under the inverted microscope after removing cells of the corona radiata. Oocyte maturity has been noted, and mature oocytes of MII are microinjected. The optimal assessment of embryonic grading is based on morphology such as cleavage rate, number of blastomeres, cytoplasmic appearance, extent of a-nucleated fragments, and regularity in the symmetry blastomeres.

**Pregnancy outcomes:**

Fertilization was evaluated 18 to 20 hours after insemination. After 3 to 5 days later, one or two embryos of grade I characterized by smooth and regular blastomeres, devoid of fragmentation and embryo or grade II characterized by equally sized blastomeres, minor fragmentation (< 20%) were transferred to the uterus by using an ultrasound-guided catheter (Cook, Australia). While remaining embryos were frozen, after taking consent of the couplet. Biochemical pregnancies were identified by a high level of β-hCG, i.e., 50 mIU/mL, tested 14 days post-embryonic transfer. A radioimmunoassay kit was used to measure the serum concentration of β-hCG. Clinical pregnancies were confirmed six to seven weeks later using a gestational sac, and heartbeat was monitored through transvaginal ultrasound. Miscarriages or spontaneous abortions were demarcated as termination of pregnancy before 28 weeks. Luteal phase supports were given orally (Duphaston 10mg) or vaginal pessaries (Utrogestan 100mg) from the day of oocyte retrieval until clinical pregnancy had been ruled out.

**Statistical analysis:**

Statistical analysis was done using the statistical package SPSS (version 21; SPSS Inc., Chicago, IL, USA). Values are presented as Means ± SD or n/N (%). The means of the two groups were compared through unpaired Student's t-test, while categorical variables were calculated through χ²-test. Fisher's exact test was applied to relate multiple means from different groups. Significant statistical difference was considered p < 0.05.

**Results**
In this study, 689 patients were included. The clinical pregnancy rate was checked concerning endometrial thickness, which was 45.45%. Compared both pregnant (group A) and nonpregnant (group B) with different variables. In group (A), patients were younger, BMI was almost the same in both groups. No of follicles, no of eggs, fertilized eggs, and cleavage rate was slightly higher as compared to the group B. (Table 1)

| Variables     | Pregnant mean ± S.D | Non-Pregnant mean ± S.D | P-Value |
|---------------|---------------------|--------------------------|---------|
| age           | 30.38 ± 4.395       | 31.44 ± 4.161            | 0.085   |
| BMI           | 26.94 ± 2.815       | 26.16 ± 3.122            | 0.07    |
| Follicles     | 17.22 ± 7.592       | 17.17 ± 7.603            | 0.966   |
| No. of oocytes| 11.700 ± 6.768      | 10.592 ± 5.897           | 0.22    |
| No. of fertilized oocytes | 6.622 ± 5.084 | 5.564 ± 4.506 | 0.123 |
| Cleavage      | 6.04 ± 4.022        | 5.722 ± 6.484            | 0.682   |
| P4            | 3.821 ± 0.9710      | 3.606 ± 1.132            | 0.519   |
| E2            | 2987.96 ± 1441.97   | 2805.18 ± 1339.76        | 0.357   |

In the group, A primary subfertility was reported 80.0% (72), and secondary subfertility was sated as 20.0% (18). While in group B, primary subfertility was 63.9% (69), and secondary subfertility was 36.1 % (39) (P-VALUE 0.013). In those patients who became pregnant, 55.6% (50) were treated with long protocol and 44.4 % (40) with short, while in group B, 65.7% (71) were treated with long protocol and 34.3 % (37) (P-VALUE 0.14). In group A, a single embryo was transferred in 13.3 % (12), two embryos were transferred in 38.8% (35) and 3 or more in 47.8% (43) patients, in group B, single embryo transfer was done in 34.3% (37), two in 37.0% (40) and 3 or more in 28.7% (31), (P-Value 0.001) (Table 2).
Table 2
categorical variables

| Variables            | Pregnant | Non Pregnant | P Value |
|----------------------|----------|--------------|---------|
|                      | n(%)     | n(%)         |         |
| Type of Infertility  |          |              |         |
| Primary              | 72(80.0) | 69(63.9)     | 0.013   |
| Secondary            | 18(20.0) | 39(36.1)     |         |
| Protocol             | 50(55.6) | 71(65.7)     | 0.143   |
| Long                 |          |              |         |
| Short                | 40(44.4) | 37(34.3)     | 0.143   |
| No of ET             |          |              |         |
| One                  | 12(13.3) | 37(34.3)     | 0.01    |
| Two                  | 35(38.8) | 40(37.0)     |         |
| Three                | 43(47.8) | 31(28.7)     |         |

The endometrium thickness on decision day was calculated at various ranges for both groups. When endometrial thickness was ≤ 6mm, the pregnancy rate (PR) was 2.22%, at 7mm endometrial thickness, the pregnancy rate was 1.11%, and at 8 mm endometrial thickness, the pregnancy rate was 3.33%, 9mm, PR was 17.78%, 10mm was 33.33%, 11mm, PR was 32.22%, and more than 11 mm PR was 10%. So, PR was higher from 2.22–10%. The overall pregnancy rate was 45.45%. (Table 3)
Table 3
Evaluation of serum endocrine levels of the two groups presented as mean ± S.D.

| Variables                                      | Group A   | Group B   | P-value |
|------------------------------------------------|-----------|-----------|---------|
| **Basic serum endocrine level**                |           |           |         |
| 2rd day FSH (IU/L)                             | 8.17 ± 2.51 | 8.59 ± 2.32 | 0.34*   |
| 2rd day LH (IU/L)                              | 5.01 ± 1.33 | 5.19 ± 1.98 | 0.31*   |
| 2rd day E2 (pg/L)                              | 50.48 ± 18.96 | 54.84 ± 49.68 | 0.81*   |
| AMH (ng/mL)                                    | 5.99 ± 1.81 | 5.87 ± 2.10 | 0.39*   |
| **Serum endocrine level on Gn stimulation day**|           |           |         |
| FSH (mIU/L)                                    | 2.36 ± 1.89 | 3.99 ± 2.01 | 0.0001* |
| LH (mIU/L)                                     | 1.68 ± 1.01 | 1.96 ± 1.01 | 0.003*  |
| E2 (pg/L)                                      | 20.31 ± 11.01 | 25.01 ± 10.35 | 0.000*  |
| **Serum endocrine level on hCG decision day**  |           |           |         |
| Progesterone (ng/L)                            | 0.89 ± 0.58 | 0.88 ± 0.49 | 0.36*   |
| LH (mIU/L)                                     | 1.02 ± 0.16 | 2.14 ± 0.35 | 0.001*  |
| E2 (pg/L)                                      | 2379.51 ± 1508.25 | 3078.21 ± 1333.36 | 0.001*  |
| Progesterone/Estradiol                         | 0.41 ± 1.99 | 0.50 ± 2.01 | 0.19*   |

**Note:** * = Student’s t-test; FSH = Follicle stimulating hormone; Gn = Gonadotropin; LH = Luteinizing hormone; E2 = Estradiol; hCG = Human chorionic gonadotropin; AMH = Anti-Mullerian hormone.
Table 4
pregnancy rates at different endometrial thicknesses

| Endo Thickness | Pregnant | Non-Pregnant | PR  |
|----------------|----------|--------------|-----|
| ≤ 6            | 2(2.2)   | 2(1.9)       | 2.22|
| 7              | 1(1.1)   | 2(1.9)       | 1.11|
| 8              | 3(3.3)   | 13(12.0)     | 3.33|
| 9              | 16(17.8) | 22(20.4)     | 17.78|
| 10             | 30(33.3) | 36(33.3)     | 33.33|
| 11             | 29(32.2) | 11(10.2)     | 32.22|
| > 11           | 9(10.0)  | 22(20.4)     | 10  |
| Total          | 90       | 108          | 45.45%|

P-Value = 0.003

Discussion

The success of in-vitro fertilization and embryo transfer does not mean that this embryo will be implanted with the subsequent initiation of a viable and sustainable pregnancy. The embryo can be successfully implanted in the endometrium only when crosstalk between both of them is successful. The state of the endometrium at the time of implantation is also very critical, along with the quality of the embryo. Evaluation of the endometrium is essential to gauge the endometrial receptivity. Many sophisticated methods of endometrial assessment are available, from endometrial biopsy to endometrial cytokines in uterine flushing, but a simple non-invasive method of ultrasonic endometrial scan became very popular.

When ovulation occurs, the ovarian follicle turns into a corpus luteum. While the oocytes pass through the fallopian tube, the corpus luteum secretes a steroid hormone, progesterone, which causes structural changes in the endometrium known as decidualization. It actually is the preparation of the endometrium to house the embryo.

A total of 689 patients was included, which were divided into two groups based on pregnancy. Inclusion criteria were; the age of the women less than or equal to 38, BMI less than or equal to 28, serum progesterone less than or equal to 4. Group A was the pregnant group, whereas group B was nonpregnant. Both groups were compared for a number of variables. In group, A primary subfertility was 80.0% (72), and secondary subfertility was 20.0% (18), 55.6% (50) were treated along with long protocol and 44.4 % (40) with short, one embryo was transferred in 13.3 % (12), two in 38.8% (35) and 3 or more in
47.8% (43). Thus, the patients in group A were younger. In addition, they have a slightly higher number of follicles, oocytes, and fertilized oocytes, and the cleavage rate was marginally higher than group B.

In group B, primary subfertility was 63.9% (69), and secondary subfertility was 36.1% (39), 65.7% (71) were treated along with long protocol and 34.3% (37) with short, one embryo was transferred in 34.3% (37), two in 37.0% (40) and 3 or more in 28.7% (31). BMI was similar in both groups. Endometrial thickness was measured on the decision day, and the pregnancy rate was then calculated for the respective thickness of the endometrium. The pregnancy rate was 6.66% with the endometrial thickness less than 8 mm and 93.33% with more than and equal to 8 mm of thickness. In our study, the clinical pregnancy rate in relation to endometrial thickness was 45.45%.

In 1995 Noyes and his colleagues studied the relationship between endometrial thickness and implantation of the embryo after IVF. Implantations of the embryo, clinical pregnancy, and ongoing pregnancy were significantly higher in patients with thicker endometrium P < 0.005). 14

In 2000 De Geyter and his colleagues compared endometrial appearance in 1186 females who underwent assisted reproduction with 205 females who were not treated. The chance of a successful pregnancy was found to be significantly lower in females who showed thinner endometrial thickness. 14

In 2003, Kovacs and his colleagues saw the impact of endometrial thickness on the outcome of in-vitro fertilization/ Intracytoplasmic injection. In pregnant women, there was an increased endometrial thickness and a more significant number of follicles, oocytes, fertilized oocytes, and good quality embryos compared to The pregnancy rate was also higher in women with thicker endometrium. 3

Momeni, Rahbar, and Kovanci, 2011 published a Meta-analysis in which the relationship between the thickness of endometrium and IVF outcome was explored among 14 articles selected from 484 articles, all from authentic resources. They concluded that the thicker the endometrium better the IVF outcome. There was a significant difference between the pregnant and nonpregnant groups (p-value 0.001). 6 Al-Ghamdi and her co-researchers performed a retrospective cohort in 2008 on a total of 2464 cycles. The pregnancy rate was found to be 35.8%. The pregnancy rate increased as the thickness of the endometrium increases. 16

The day of measurement of endometrial thickness might also influence the association between endometrium thickness and cycle outcome. Implantation and pregnancy rate may improve with increasing the endometrial thickness. 17 In 2012, Zhao, Zhang and Li, studied the relationship between ultrasonically measured thickness and pattern of the endometrium and IVF-ET outcome. They found that consistency and pattern of endometrium had an independent effect on IVF and embryo transfer. Implantation rate and pregnancy rates were 13% and 25.5%, in patients who had endometrial thickness lesser than 7 mm, 33.8% and 52.1% with endometrial thickness more than 7 mm and to ≤14 mm and 39.1% and 63.5% with endometrial thickness more than 14 mm. 17
IVF transvaginal ultrasonographic monitoring of endometrial thickness and other changes aids the clinicians while counseling the patients undergoing medically assisted reproduction.6

**Declarations**

**Funding:**

The infertility research center of HLA hospital

**Conflict of Interest:**

The authors declared no conflict of interest.

**Declarations:**

**Ethics approval and consent to participate:**

The study was approved by our Institutional Ethical Committee (IEC). Informed consent was obtained from all subjects before the research and publishing of the results of the investigation.

**Consent for Publication:**

Not applicable

**Availability of data and materials:**

The data set used and analyzed during the current study is available from the corresponding author on reasonable request.

**Competing interests:**

The authors declared that they have no conflict of interest.

**Acknowledgments:**

We acknowledge the research initiative and gratefully thank Professor Dr. RLK, Professor of Emeritus in Obstetrics and Gynecology, for manuscript editing. The study is supported by the infertility research center of HLA hospital.

**Abbreviations**

IVF

*In vitro* fertilization, AFC: Antral follicle count, AMH anti-Müllerian hormone, PCOS: Polycystic Ovarian Syndrome, ff: Follicular fluid. PGD: preimplantation genetic diagnosis, hESCs: human endometrial stromal cells, ROC: receiving operating characteristics, $E_2$: 17β-estradiol, BMI: body mass index, FSH: follicular
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Figures
Figure 1

Endometrial thickness with respect to pregnant and non-pregnant groups.
Figure 2

Endometrial thickness with respect to pregnancy rate.

Fig 2: Endometrial thickness with respect to pregnancy rate
Figure 3

ROC analysis of Endometrial thickness and pregnancy outcome