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Effect of endometrial thickness on clinical pregnancy rate in stimulated intrauterine insemination cycle

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Abstract
Background: The preferred therapy for subfertility caused by non-tubal reasons is intrauterine insemination (IUI). For this reason, injectable gonadotropins are the recommended extra strategy for promoting ovarian follicle growth during IUI cycles. Mature, developing follicles emit the hormones estrogen and progesterone, which causes endometrial thickness (ET). ET is often used as supplemental stimulation to encourage ovarian follicle development during IUI cycles. Progesterone and estrogen are produced by the developing follicles, which causes endometrial thickness and proliferation.
Aim of the Study: To investigate the effect of ET on clinical pregnancy rate on IUI stimulated cycles.
Patients, Materials and Methods: The study was included seventy-four women who were agreed to use letrizole-gonadotropin IUI cycles in private clinic at Wasit Province from June 2020 to June 2022. Positive and negative cycles were compared in terms of mean endometrial thickness. After that, ET values were separated into two groups: 7.0–10 mm and >10 mm. Version 22 of the Statistical Package for Social Sciences (SPSS) was used to analyze the data. ANOVA, independent sample t-tests, chi square tests, and Fisher's exact tests were used in the main investigation of the effect of ET on the clinical pregnancy rate.
Results: The study population included 74 IUI cycles, which corresponded to 74 patients and had a clinical pregnancy rate of 27%. The mean ET for cycles that resulted in clinical pregnancy and those did not achieve pregnancy did not vary significantly. The two groups of ETs were significantly different from one another (P=0.031), with the greatest incidence of clinical pregnancy occurring between 7 and 10 mm. The size, number of dominant follicles, and progesterone level on the day of trigger (P=0.013), (P=0.003), and (P=0.000) are additional parameters that have a substantial impact on conception.
Conclusion: In addition to having a numerical trend toward greater clinical pregnancy rates, ET in the 7–10 mm range was significantly more successful in increasing conception rates. Positive predictors include the size and number of dominant follicles, and progesterone levels on the day of trigger. In contrast, ET was found to be a poor predictor of IUI pregnancy rates and it is not suitable to rely it as the only indication for cycle cancellation. To ascertain if the results of this study are genuine, further investigation is required.

Keywords: Endometrial thickness, intrauterine insemination, gonadotropins, clinical pregnancy rate

Introduction
Intrauterine insemination (IUI) itself is a treatment which can be used for the treatment of subfertility. Measures that have been said to influence IUI success includes the age of the couple, the period of sub fertility and the body mass index (BMI) [1]. It has been shown that the thickness of the endometrium (ET) is related to endometrial receptivity and, therefore, embryo implantation and successful pregnancy [3]. ET is one of the most sensitive indicators of a continued pregnancy, as was previously established [3]. Reduced chances of conception are associated with a thin endometrium, and conception improves with increased ET [4]. ET greater than 8 mm is associated with a high clinical pregnancy rate [5]. BMI (body mass index) [1, 2]. Endometrial thickness (ET) and receptiveness, are also essential components for a continuing pregnancy and linked to the successful implantation of an embryo [3]. Thin endometrium is linked to a lower likelihood of conception, whereas ET of >8 mm is linked to a greater clinical pregnancy rate [5] and increased ET facilitated conception [4]. However, there is no correlation between ET and the frequencies of pregnancy in IUI with ovarian stimulation cycles, according to a meta-analysis of 23 studies [6]. Although clomiphene citrate-CC is known to produce lower ET than gonadotropin

stimulation, it is currently unclear whether this lower ET contributes to reduced pregnancy rates [7]. When comparing CC with the different medications used in ovarian stimulation, these differences in ET are rather minor and may likely be due to chance [6].

To further stimulation for the development of ovarian follicles during an IUI cycle, gonadotropin injections, aromatase inhibitors, or clomiphene citrate are used. Growing follicles secrete estrogen, which causes the endometrial stripe thickness as measured by transvaginal ultrasonography to increase. Although prior research has largely focused on CC-stimulated cycles, pretreatment assessment of ET has been examined in connection with prediction of pregnancy prognosis in IUI. This is because the uterus's lower absorption of radioactive estradiol suggests that CC is a selective estrogen receptor modulator with a clear antiestrogenic impact at the endometrial level. As a result, it may change the endometrium in a way that is both measurable and of high quality. ET is lower in CC-stimulated cycles than in spontaneous cycles, according to many studies. [1, 3].

The reduction in ET may negatively affect the receptivity of the endometrium in CC-stimulated cycles. CC does not alter the kinetics of the estrogen receptor or reduce the endometrial wall as injectable gonadotropins do. We have previously shown that peak EST concentrations are higher in gonadotropin-stimulated cycles than in CC-stimulated cycles [6]. Therefore, a same degree of thin endometrial thickness may not be comparable in clomiphene and FSH cycles, respectively. In fact, a thin FSH lining may have pathological abnormalities compared to a thin clomid EST. Nonetheless, the influence of peak estrogen levels on pregnancy outcomes in gonadotropin-stimulated intrauterine insemination has been examined to a limited extent. Numerous studies have examined individuals receiving both CC and gonadotropins, with contradictory results for peak EST and pregnancy outcomes [7, 8, 9].

A meta-analysis by Weiss *et al*., examining the efficiency of EST in IUI cycles, did not identify any link between EST and clinical pregnancy. Numerous studies have examined individuals treated with a combination of CC and gonadotropins, yielding inconsistent findings about peak EST and pregnancy outcomes [7, 8, 9].

A recent meta-analysis conducted by Weiss et al. investigating EST in IUI cycles revealed no correlation between EST with the likelihood of clinical pregnancy [10]. This trial used a combination of ovarian stimulation medications, such as letrozole and injectable gonadotropins. Moreover, distinguishing between ET for pregnancies and negative cycles was feasible only for certain trials included in the study.

Aim of the Study

To investigate the effect of ET on clinical pregnancy rate on IUI stimulated cycles.

Patients, Materials and Methods

A study was conducted for 74 women who had intrauterine insemination (IUI) at a private clinic in Wasit Province from June 2020 to June 2022. Ladies with infertility who had already undergone follicular monitoring or used medication without insemination, as well as ladies above the age of 42, were excluded .

Infertility was determined in accordance with the criteria established by the infertility team and the worldwide Department of Health standard [10]. Letrozole used in the first IUI cycle using a letrozole and gonadotropin injection regimen. The first round of 'IUI' involves ovarian stimulation with Letrozole. On the third day of the menstrual cycle, Letrozole pills (Letro-Denk; Denk Pharma, Germany) were administered in dosages twice daily for a duration of five days. The FSH used in this research was recombinant FSH sourced from Folisurge-PFS, manufactured by INTAS, India, administered starting on the fifth day of the menstrual cycle at a dose of 75 IU per vial, adjusted based on response. The quantity and dimensions of follicles, as well as the advancement of follicular and endometrial lining development, were evaluated using transvaginal ultrasound imaging. Highly pure human chorionic gonadotropin (Ovunal SC 5000; INTAS, India) 5000 IU/vial was self-administered subcutaneously in the morning on day 12 or 13 to induce ovulation when the smallest follicle measured at least 17 mm, with the largest follicle ranging from 17 to 23 mm. On the second day of the cycle and the day of trigger before IUI, we assessed the blood concentrations of estrogen and progesterone. Transvaginal scanning was used to monitor the follicles with the Philips iU22 ultrasound equipment. The sagittal plane, at the broadest point of the uterus, was used to assess ET. The IUI technique was conducted twenty-four to forty-eight hours thereafter. If an ultrasound indicated the presence of more than three follicles, the cycle would be halted. Vaginal progesterone suppositories (400 mg twice day) were used to support the luteal phase after intrauterine insemination (IUI). A serum ß-HCG test was conducted on the fourteenth day post-IUI. An ultrasound was conducted on the lady, who subsequently tested positive, to objectively ascertain the presence of one or two gestational sacs, indicative of clinical pregnancy [11]. Data analysis was conducted using ANOVA, independent sample t-tests, chi-square tests, and Fisher's exact tests using SPSS version 22.0. The findings were considered statistically significant with a p-value equal to 0.05 or below.We compared patients who were clinically pregnant with those who were not. The Chi-square test was used for categorical variables, while the Student's t test was employed for continuous variables. The area under the receiver operating characteristic curve was used to evaluate the predictive power of ET for pregnancy in IUI. Our patient data and prior research informed the calculation of clinical pregnancy proportions for two ET subgroups: 7–10 mm and >10 mm [8, 13, 14]

Results

From the years 2020 and 2022, 74 percent IUI cycles were conducted with Letrizole and gonadotropin stimulants. Approximately 27% of these patients had a single cycle that resulted in conception, with 27% of those cycles culminating in a clinical pregnancy (Figure 1).

The results indicated no statistically significant differences in the demographic characteristics between the study's pregnant and nonpregnant participants, except for the following: the number of dominant follicles on the trigger day, the size of dominant follicles measured in millimeters, and the progesterone hormone level expressed in nanograms per milliliter (Table 1) .

The average age of the pregnant group was 30.35±5.83 years, while the non-pregnant group had an average age of

33.06±6.28 years, yielding a *P*-value of 0.113. Table 1 indicates that the pregnant group exhibited an average body mass index of 25.23±2.80, while the non-pregnant group had an average of 26.55±3.70, yielding a *P*-value of 0.193.

No obvious variations were seen in the baseline hormone levels of the two groups upon comparison. Table 1 presents the levels of P4, E2, LH, and FSH, accompanied by *P*-values of 0.209, 0.091, 0.491, and 0.639, respectively.

Table 1 indicates substantial disparities in the size of dominant follicles (mm) between the pregnant and non-pregnant groups, measuring 19.72± 0.77 and 18.51± 1.85, respectively, with a *P*-value of 0.013.

The quantity of dominant follicles on the trigger day varies considerably between the pregnant and non-pregnant cohorts (2.6±1.4; 2.2±1.1), with a *P*-value of 0.003, as seen in Table 1.

Table 1 indicates substantial differences in progesterone levels (ng/ml), with values of 2.24±0.84 for the pregnant group and a distinct value for the non-pregnant group, yielding a *P*-value of 0.000. Conversely, there is no notable disparity in estradiol levels (pg/ml) between the pregnant and non-pregnant groups, recorded at 240.58±95.43 and 265.51±71.06 pg/ml, respectively, with a *P*-value of 0.260. This is also seen in Table 1.

Table 1 indicates that there were no significant differences in the mean duration of infertility, with values of 6.81± 3.45 for the pregnant group and 6.56± 3.62 for the non-pregnant group with a *P*-value of 0.802.

Table 1 indicates that the pregnant group had 12 instances of primary infertility (60% of the total), whereas the non-pregnant group had 27 instances of secondary subfertility (50% of the total), with a *P*-value of 0.650.

Table 2 indicates that there were no significant differences in endometrial thickness between the pregnant and non-pregnant groups on the day of the trigger (9.30±3.86; 9.27±2.69) and on the day of intrauterine insemination (9.86±1.65; 9.54±3.68), respectively, with a *P*-value of 0.739.

In a study of pregnant women based on endometrial thickness on the day of intrauterine insemination, the majority conceived (13, or 65%) when the thickness was between 7 and 10 mm, while only 7 women, or 35%, conceived when the thickness exceeded 10 mm. Statistically significant differences were observed between the two groups (*P*=0.031), as seen in Figure 2 .

We used receiver operating characteristic (ROC) analysis to evaluate the efficacy of ET in predicting clinical pregnancy rates. Figure 3 indicates that ET is an inadequate predictor of clinical pregnancy outcomes, since the area under the curve for this variable was 0.547 (*P*=0.496).

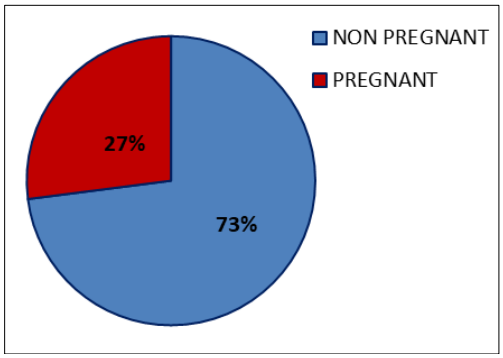

Fig 1: Pregnancy rate in the study participants

Table 1: Comparison of Demographic characteristics of the study participants between the pregnant and non-pregnant groups.

Parameter		Pregnant	Non Pregnant	*P*-value
Age(years)	Mean± SD	30.35± 5.83	33.06± 6.28	0.113
Body Mass Index (BMI) (kg/m2)	Mean± SD	25.23± 2.80	26.55± 3.70	0.193
Basal Progesterone(ng/ml)	Mean± SD	0.45± 0.19	0.52± 0.21	0.209
Basal Estradiol (pg/ml)	Mean± SD	45.03± 34.68	57.86± 23.34	0.091
LH(IU/L)	Mean± SD	5.10± 1.79	5.53± 2.25	0.491
FSH(IU/L)	Mean± SD	7.51± 2.52	7.18± 2.49	0.639
Size of dominant follicles(mm)	Mean± SD	19.72± 0.77	18.51± 1.85	0.013*
Number of dominant follicles at day of trigger	Mean± SD	2.6±1.4	2.2±1.1	0.003*
Progesterone at day of trigger(ng/ml)	Mean± SD	1.19± 0.20	2.24± 0.84	0.000*
Estradiol at day of trigger(pg/ml)	Mean± SD	240.58± 95.43	265.51±71.06	0.260
Duration of infertility(YEARS)	Mean± SD	6.81± 3.45	6.56± 3.62	0.802
Type of infertility	Primary (Percentage)	12(*60%*)	27(*50%*)	0.650
	Secondary (Percentage)	8(40%)	27(*50%*)	
SD; Standard deviation, FSH; Follicle-stimulating hormone, LH; Luteinizing hormone, *; Significant P value<0.05				

Table 2: Comparison of Endometrial thickness of the study participants between the pregnant and non-pregnant

Parameter		Pregnant	Non-Pregnant	*P*-value
ET at day of trigger(mm)	Mean± SD	9.30±3.86	9.27±2.69	0.491
ET at day of IUI (mm)	Mean± SD	9.86± 1.65	9.54±3.68	0.739
SD; Standard deviation, ET; Endometrial thickness, *; Significant P value<0.05				

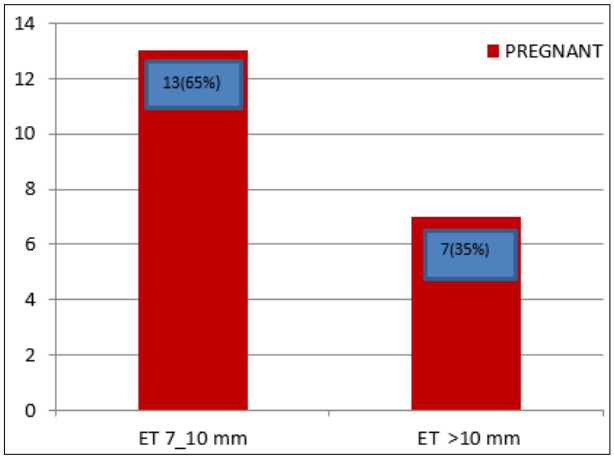

Fig 2: Comparison of pregnancy rate between 2 endometrial thickness groups of the study participants.

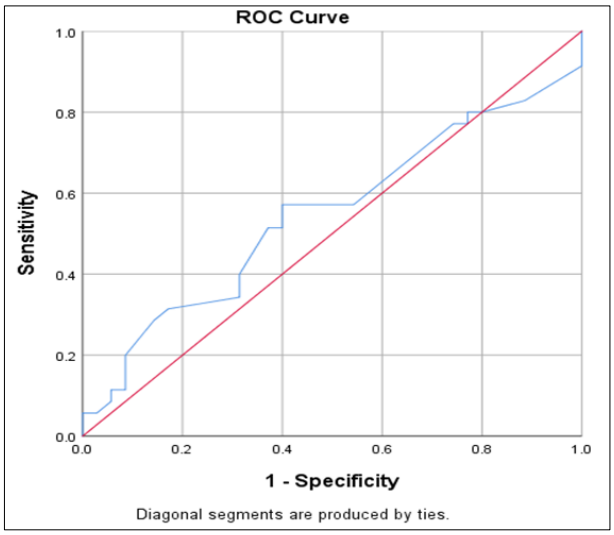

Fig 3: Receiver operating characteristic curve of endometrial thickness to predict positive pregnancy test

Discussion and conclusions

In this study focused on the clinical pregnancy rate specific to both ET and those cycles stimulated with letrozole-gonadotropins and intrauterine insemination. As clinicians overseeing IUI cycles have insufficient data to provide appropriate advice to patients with a thin endometrial lining, this relationship needs to be investigated. In this discussion, clinical pregnancy rates and the related variables were described for seventy-four cycles and ovarian stimulated IUI. The proportion of pregnant women that presented at our facility was 27% in aggregate. IUI conception rates obtained with IUI accordingly have usually averaged 8-22%, or rarely within 2.7%-66% [15, 16]. It is often difficult to explain why clinical pregnancy rates differ significantly due to differences in ovarian stimulation and semen preparation.

In terms of study endpoints, we observed a marginal reduction in the success rate of IUI done/COH as we got older. The female patient's age plays a significant role in this aspect because as postulated and as revealed by other scholars [17, 18], the quality of oocytes decreases with age. COH/IUI cannot be compared to ART-IVF no matter how successful, as it cannot reverse the effects of aging as stipulated

Kim *et al*., concluded that, contrary to expectations, a higher female BMI did not influence IVI treatment outcomes. However, with regard to the present study, it is suggested that women with higher BMI, which are the candidate for IVF procedure, to be counselled for weight reduction in order to reduce obstetric morbidity which is observed with this procedure [20]. Contrary to the basal hormonal levels, the current study did not record any statistically significant difference. Since the mean basal hormone levels of the pregnant and non-pregnant patients are similar, it is reasonable to compare the samples employed to test the two groups' responses to the stimulation protocol in total approximate ovarian reserve in accordance with multiple studies [21,22]. In hCG-triggered letrozole-IUI cycles, the ideal size of the dominant follicle was 19.1 to 21.0 mm; follicles that were too big or too tiny might reduce the success rate of the procedure. This study is in line with previous research that found that follicle size is a better predictor of pregnancy outcomes when estrogen levels are below 200 pg/ml on the day of the hCG trigger [22, 23]. While one study found a correlation between the size of the dominant follicle on hCG day and improved pregnancy outcomes in gonadotropin and IUI cycles, another found no such correlation [24].

Our study found that the clinical pregnancy rate was much higher in cycles of COH/IUI that achieved two or more lead follicles compared to one on the day of trigger, and the rate was acceptable overall. Consistent with the study by Beliveau, L.N. *et al*., [25], this offers helpful information for counseling patients undergoing IUI.

In regards to the progesterone hormonal level on the day of trigger, this study confirms what Ashmita J. *et al*., [26] found: patients with P4 <1.5 ng/ml had a significantly higher clinical pregnancy rate compared to those with elevated levels, P4≥ 1.5 ng/ml. This suggests that lower clinical pregnancy rates may be linked to premature progesterone elevation in assisted reproductive technology cycles.

Similarly, the current study did not observe a relationship between pregnancy after ovarian stimulation and intrauterine insemination and the level of serum estradiol on the day the trigger [27] Kutlu *et al*., also reported that results suggesting estradiol hormonal levels was not dominant in determining endometrial receptiveness in the trigger day [28].

The simplest and most frequently used way of obtaining a vague idea of endometrial receptivity is through determining the E thickness; the perceptions must be accurate for success. The authors, however, found out that the use of endometrial thickness as the only predictor had low specificity and a positive predictive value [29]. Both the influence of estrogen and progesterone are incorporated into the growth of the endometrium as we know. Our data rejected the null hypothesis and found a positive correlation of the progesterone hormone and no correlation of the estradiol hormone on the trigger day of IUI. It was established that any premature rise in progesterone concentrations within stimulated cycles would significantly reduce the chances of pregnancy. Previous data revealed that progesterone/estradiol ratio at the periovulatory phase was a predictor of ovulatory success in IUI cycles [30] , what causes the progesterone concentration to rise at the end of the follicular phase is not well understood, one possibility being an overstimulation response. Furthermore, our result pointing to a strong positive predictive value for progesterone on the trigger day was consistent with the data indicating that this premature rise is associated to changes in the gene profile and impaired endometrial receptiveness [31]. Regarding the main objective of this study, it is necessary to

describe the results with respect to the primary aim of a study, which is to establish the relationship and effect of endometrial thickness among women with endometrial disorders on the clinical pregnancy rate of women who underwent the procedures. Investigations conducted by Palatnik *et al*., and Rachmawati, A. *et al*., also showed that clinical pregnancy rates were increased in women with larger follicle size and more endometrial thickness. A lower pregnancy rate was discovered if the endometrial thickness and the follicular size and thickness were either less or little. This affords support to our study because it brought out the fact that follicular activity was directly linked to the growth of endometrial thickness [32, 33]. The clinical pregnancy rate was found to be high by Yavuz *et al*., [34] for patients with endometrial thickness < 8mm, whereas, improved clinical pregnancy rates were observed where the endometrial thickness was ≥ 10mm by Kovac *et al.,* [35]. However, the existing evidence is still silent on clear information relating to the likely endometrial thickness required to promote pregnancy rate. The findings are in tandem with the hypothesis of this study that endometrial thickness was not a significant factor. There are three potential explanations for why comparing ET between the pregnant and nonpregnant groups did not yield statistically significant results. First, the fact may be that all study groups' ET levels are equivalent and mean values were obtained. Furthermore, it might also have been the case that the sample size was insufficient to achieve a statistical significance although a clinically relevant difference of ET between the study groups may exist. A criticism that can be made of the analysis that formed the basis of earlier literature is that the relationship between endometrial thickness and the success rate was treated as linear [30]. Consequently, we categorized the ET values into two groups and regressed them against chronic diseases, for uniformity of statistical comparison, as the hypothesis raised expected no significant association. Not only did we compare ET between the two groups, but we also considered the pattern of outcome rates across them. During the initial graphical comparison, we observed that the ET category of 7-10 mm yielded the highest clinical pregnancy and conception rates. In a previous study, Dinelli *et al*., established a link between pregnancy rate and endometrial thickness with the maximum pregnancy rate achieved at 10 mm ET [36]. Of course, larger investigations are required to substantiate such trend, however, it can contribute to increase in endometrial pathology rates, or, possibly, to the decrease in the receptivity beyond the certain point. Although the aforementioned analyses did show a relationship between ET and cycle outcome, that the ROC curve analysis did not find any such relationship suggests that the measurement of ET is not a very good indication of cycle outcomes. In accordance with other study by Kolibianakis *et al.,* who was stated that it was not possible to identify endometrial thickness as a parameter which predetermined the success of the IUI cycles stimulated with CC [37].

Last in summary, in gonadotropin stimulated IUI cycles, ET seems not to play any role in the outcome of conception. Moreover, current clinical PRs increase with increasing ET up to 14 mm, and then decrease. It has been noted that both low and high ET are deleterious to the conception rate and clinical pregnancy rates in FSH-IUI cycles though this study did not have a large number of cycles in these extremes. As a result, ET cannot be used alone as indicator to distinguish whether an IUI will be successful or not. By applying ROC analysis, we provided evidence to state that ET measured in absolute value is not the best means of estimating the CPR. The results of the current study also separated the 'Others' category where patient and cycle factors, including the size of the dominant follicle, the number of dominant follicles, and the progesterone hormone level on the day of the trigger are comparable with clinical pregnancy rates. This is a problem because when the ET is used as the only factor for continuing or canceling a cycle it has very little predictive value on its own. Furthermore, using our data, clinical pregnancies are also achieved at a reasonable rate below this reporting criterion, so our data do not support such a common and stubborn practice of cancelling cycles with 'thin' lining below 7mm. We also do not support cancellations due to a 'thick lining' if the ET is greater than 14mm. If the ET scores are found within this range however, then endometrial pathology has to be considered.

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Conflict of Interest:
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