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# Effect of Estradiol Valerate on Endometrial Thickness in Infertile Polycystic Ovarian Syndrome Patients- A comparative Study in a Tertiary Level Hospital

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**Abstract:**

***Background:*** *Polycystic ovarian syndrome (PCOS) is a complex heterogeneous endocrine disorder, occurring due to synergistic interaction of genetic, epigenetic and environmental factors, associated with both reproductive and metabolic abnormalities resulting in short-term and long-term consequences on the women's health. The incidence of anovulatory infertility in PCOS is 70% to 8 0% higher than who don't have PCOS. With ovulation induction adequate endometrial development is needed for pregnancy to occur. Improper endometrial thickness i.e. < 7 mm are considered to be less able to sustain implantation and pregnancy.*

***Objective:*** *To study the effect of estradiol valerate on the endometrial thickness in PCOS cases receiving letrozole for induction of ovulation as compared to letrozole alone.*

***Materials & Methods:*** *This Quasi-experimental type of interventional study was conducted in the outpatient department (OPD) of reproductive endocrinology and infertility Unit, Dhaka Medical College Hospital (DMCH), Dhaka for 12 months from July 2021 to June 2022. The study included 65 infertile PCOS patients based on the Rotterdam criteria with improper endometrial thickness. Patients were divided into 2 groups: ovulation induction was given in group A by letrozole 5 mg from day 3 to day 7 of menstruation and 4 mg estradiol valerate from the 8$^{th}$ day of menstruation until 12$^{th}$ day and in group B by 5 mg letrozole from day 3 to day 7 of menstruation.*

***Results:*** *This study showed that endometrial thickness was increased in both groups. In Group A from 6.15±0.48 to 9.70±1.03 and in Group B from 6.29±0.46 to 7.52±0.59. But, Group A showed significantly higher endometrial thickness compared to Group B (p<.001).*

***Conclusion:*** *This study showed that the endometrial thickness achieved with letrozole, estradiol valerate combination was significantly higher than with letrozole alone. This was attributed to the improvement of endometrial thickness by estradiol valerate.*

## Introduction

Infertility is a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse (WHO, 2018). All around the world, 10% of population, 13% of women, 10 % of men, and 15 % of couples of reproductive ages suffer from infertility (Maharloui *et al*., 2021). Among the causes of female factor infertility, tubal and pelvic pathology comprises 30 - 40%, ovulatory dysfunction 20 - 40%. Uterine pathologies are relatively uncommon and the remainder is largely unexplained (Taylor *et al*., 2020).

Polycystic ovarian syndrome (PCOS) is the commonest endocrinopathy resulting in anovulatory infertility in young women. Infertility due to chronic

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anovulation is one of the commonest clinical manifestations of this syndrome (Alnemr *et al*., 2017). The endometrium is a dynamic tissue that responds to changing hormonal signals throughout the cycle (Mohammad, 2018 ). Endometrial thickness is one of the most important factors affecting pregnancy (Harira , 2018). A thin endometrium leads to a risk of inability to achieve pregnancy even if ovulation and fertilization take place, as the endometrium is not thick enough for the blastocyst to be implanted. The pregnancy rate can be very low, especially if endometrial thickness is less than 6 - 8 mm (Cetinkaya and Kadanali, 2012). The minimum thickness needed for implantation is considered 7 mm ( Zadehmodarres *et al*., 2017 ).

The endometrium being a hormone-dependent tissue, proliferates in response to estrogen that further induces the development of progesterone receptors. As a consequence, estradiol (E2) treatments were given to infertile patients who presented with thin endometrium, in an effort to improve endometrial proliferation. Estrogen - induced endometrial proliferation primarily depends on the flow of blood to the basal endometrium ( Dawood et al., 2020 ). This study aimed to evaluate the effect of estradiol valerate, letrozole combination in ovarian stimulation cycle aiming to improve the endometrial thickness in infertile PCOS patients.

**Objectives:**

General: To study the effect of estradiol valerate on the endometrial thickness in PCOS cases receiving letrozole for induction of ovulation.

Specific:

- To measure endometrial thickness by serial TVS in both groups.
- To assess endometrium in terms of triple line by TVS and compare between two groups.
- To see the zone of vascularity with TVS and Doppler in both groups.

**Materials and Methods:**

This quasi-experimental type of interventional study was carried out at the out patient department ( OPD ) of reproductive endocrinology and infertility unit of Dhaka Medical College Hospital (DMCH), Dhaka from July, 2021 to June, 2022 among the infertile PCOS women.

The required sample size was 140, but due to covid-19 pandemic there was limited time, and due to drop out the targeted sample size could not be achieved. Finally, 65 samples were studied. After enrollment, infertile PCOS women aged 20-35 years were assessed by detailed history, clinical examination and routine investigations. Inclusion criteria were diagnosed cases of PCOS according to Rotterdam consensus (presence of two out of three criteria, i.e., oligomenorrhoea and / or anovulation, clinical and / or biochemical signs of hyperandrogenism, and /or polycystic ovaries on ultrasound), no other pelvic pathology, endometrial thickness < 4 mm on baseline (2nd or 3$^{rd}$ day of menstruation) scan and < 7 mm on day 12/13 scan in an unstimulated cycle, no endocrine disorder, husband's semen parameter normal. Exclusion criteria were letrozole hypersensitivity , good endometrial thickness but no mature follicle, contraindication for estrogen treatment, associated male factor infertility .Patients were divided into two groups ( A and B ) , 33 in Group A and 32 in Group B by alternate assignment. In group A, ovarian stimulation was done by letrozole (2.5mg) 2 tablets daily from 3$^{rd}$ day till 7$^{th}$ day of the cycle followed by estradiol valerate (2mg) 2 tablets daily from 8$^{th}$ day till 12$^{th}$ day of the cycle. In group B, ovarian stimulation was done by letrozole (2.5 mg) 2 tablets from 3$^{rd}$ day till 7$^{th}$ day of the cycle .

TVS done on day 12/13 of the cycle for assessment of follicular growth ( pic-1 ), endometrial thickness (pic-2), endometrial pattern (pic-3) and zone of vascularity (pic-4,5,6,7). When there was no dominant follicle (18 mm), dose of letrozole was increased up to 10 mg in next cycle with maximum 3 cycles of treatment. But when the endometrial thickness was good ( >7mm ) but no mature follicle achieved on D12/13 by TVS who were on letrozole and estradiol valerate , patient was excluded from the analysis .

In this study, endometrial thickness was measured from outer margin of anterior layer to the outer margin of the posterior layer of the endometrium in the sagittal uterine plain, perpendicular to the longitudinal axis of the endometrium in its broadest part (1-2 cm below the fundus), excluding the junctional zone (Rao *et al*., 2 019). Endometrial pattern was defined as the type of relative echogenicity of the endometrium compared

with adjacent myometrium and was categorized as follows.

Pattern A (triple-line): hypoechoic endometrium with well defined hyperechoic outer walls and a central echogenic line.

Pattern B (No-triple line): isoechoic endometrium with poorly defined outer walls and central echogenic line.

Pattern C (No-triple line): homogeneous hyperechoic endometrium with a non -prominent or absent central echogenic line. (Chen *et al*.,2010).

The zones of vascularity in this study were defined and categorized as follows Zone 1-when blood vessels reached the hypoechoic endometrio-myometrial junction.

Zone 2- when blood vessels reached the outer hyperechoic line of endometrium. Zone 3- when blood vessels reached the intervening hypoechoic area.

Zone 4-when blood vessels reached the endometrial cavity ( the central echogenic line) (Nagori and Panchal, 2012)

In this study continuous parameters were expressed as mean ±SD and categorical parameters as frequency and percentage. Categorical parameters compared by Chi-Square test. The significance of the results as determined at 95.0% confidence interval (CI) and value of P<0.05 was considered to be statistically significant. Data were presented by tabulation in the form of tables and figures.

**Results:**

In this study most of the cases had primary subfertility (72.7% vs 62.5%) and the rest were secondary (27.3% vs 37.5%). Dominant follicles achieved in maximum cases (72.7%) after 1st cycle of treatment in both groups. In group B, all patients had dominant follicle after 2nd cycle of treatment. The differences were not significant.

**Table-I**

*Comparison of age between two groups (N=65)*

| Age (in years) | Group A N=33 (%) | Group B N=32 (%) | P value |
|---|---|---|---|
| 20-25 | 11(33.3%) | 12(37.5%) | 0.954$^{ns}$ |
| 26-30 | 15(45.5%) | 15(46.9%) | |
| 31-35 | 7(21.2%) | 5(15.6%) | |
| Total | 33(100.0%) | 32(100.0%) | |
| Mean ±SD | 26.8±4.1 | 26.9±3.8 | |
| Range | 20-35 yrs | 20-35 yrs. | |

ns = not significant.

There was no statistical difference regarding age between the groups.

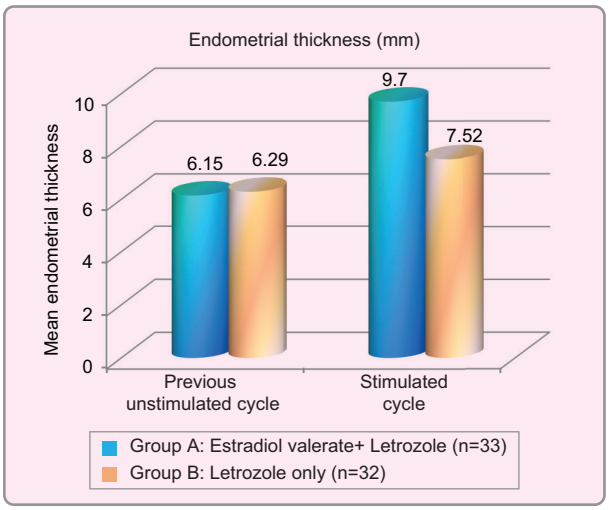


**Figure 1:** *Bar diagram showing the endometrial thickness in two groups*

**Table-II**

*Comparison of endometrial thickness in unstimulated and stimulated cycle (N=65)*

| Endometrialthickness | Unstimulated cycle Mean±SD | Stimulated cycle Mean±SD | P value |
|---|---|---|---|
| Group A | 6.15±0.48 | 9.70±1.03 | <0.001$^{s}$ |
| Group B | 6.29±0.46 | 7.52±0.59 | <0.001$^{s}$ |

Endometrial thickness increased significantly in both groups after stimulation.

**Table-III**

*Comparison of endometrial pattern & zone of vascularity in between the Groups (N=65)*

| Endometrial pattern | Zone of vascularity | Group A N=33 (%) | Group B N=32 (%) | P value | |
|---|---|---|---|---|---|
| Triple line | Zone 1 | 10(41.7%) | 9 (42.9%) | 0.792$^{ns}$ | 0.536$^{ns}$ |
| | Zone 2 | 7(29.2%) | 7(33.3%) | | |
| | Zone 3 | 5(20.8%) | 3(14.3%) | | |
| | Zone 4 | 2(8.3%) | 1(4.8%) | | |
| | Absent flow | 0(0.0%) | 1(4.8%) | | |
| Total | | 24(72.7%) | 21(65.6%) | | |
| Non triple line | Zone 1 | 2(22.2%) | 4(36.4%) | 0.053$^{ns}$ | |
| | Zone 2 | 2(22.2%) | 3(27.3.%) | | |
| | Zone 3 | 0(0.0%) | 0(0.0%) | | |
| | Zone 4 | 0(0.0%) | 0(0.0%) | | |
| | Absent flow | 5(55.6%) | 4(36.4%) | | |
| Total | | 9(27.3%) | 11(34.4%) | | |

Triple line endometrium was more in Group A after stimulation.


**Figure 2:** *TVS showing Dominant follicle*

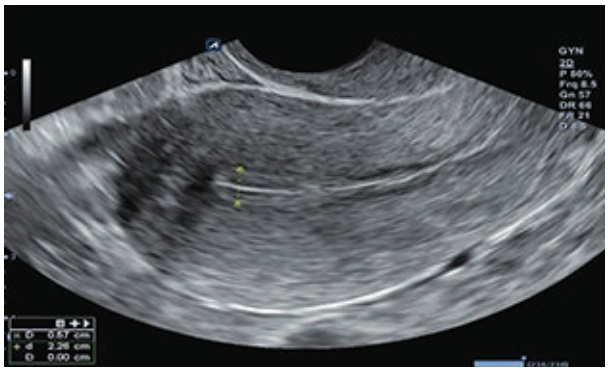

**Figure 3:** *TVS showing Endometrial thickness*

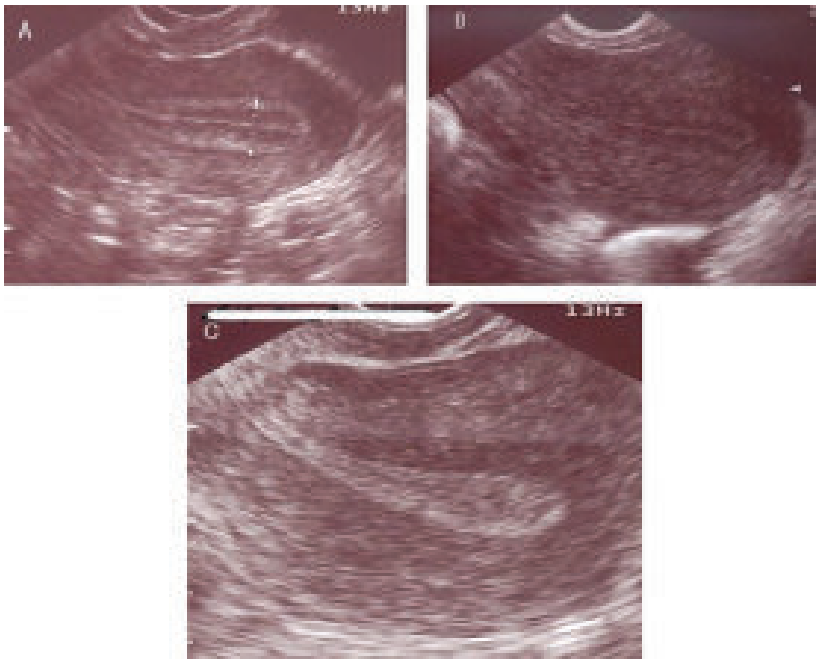

**Figure 4:** *Longitudinal ultrasound images demonstrate the endometrial pattern*

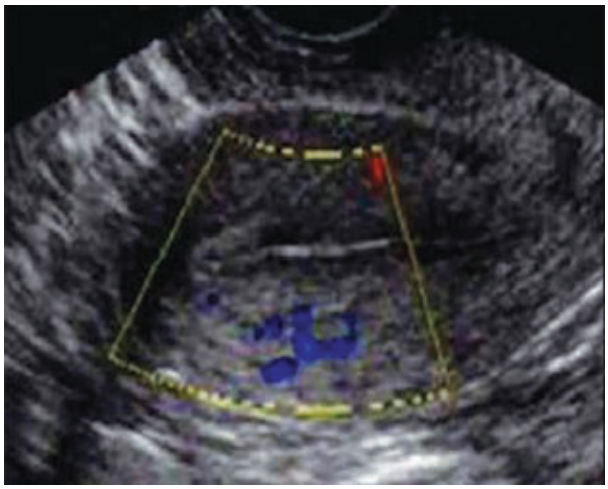

**Figure 5:** *Zone 1 vascularity*

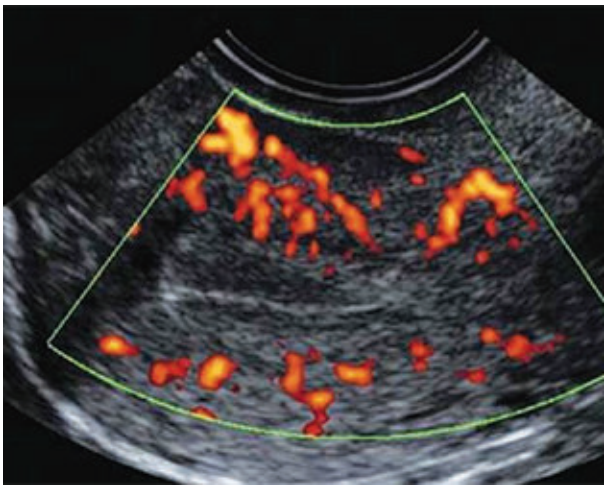

**Figure 6:** *Zone 2 vascularity*

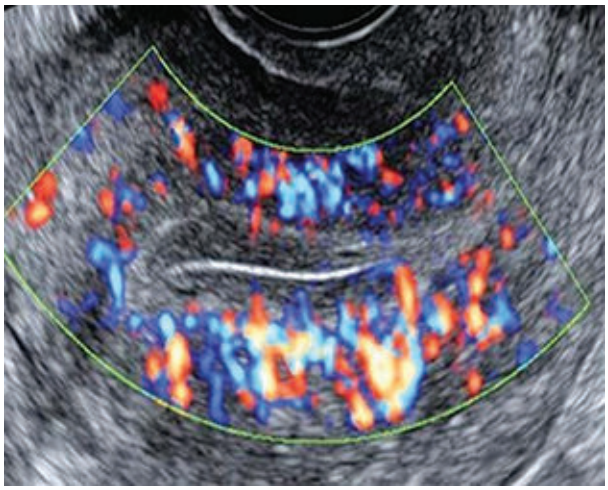

**Figure 7:** *Zone 3 vascularity*

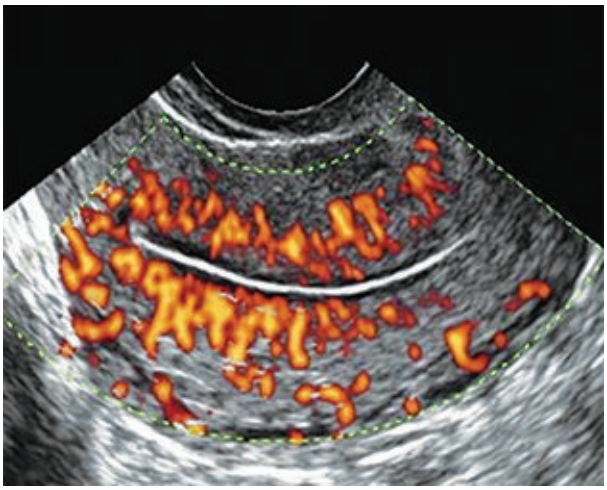

**Figure 8:** *Zone 4*

**Discussion:**

This study showed that endometrial thickness was increased in both groups though with significant difference (p<.001). In Group A, endometrial thickness increased higher than Group B. The explanation of the big difference in endometrial thickness between both groups has also been seen and explained in the study of Groothuis et al. in 2007. They found nuclear translocation in endometrial cells with stromal proliferation and it needed longer duration of estradiol exposure. They concluded that 5 days duration of estradiol exposure is an ideal period to build up thick endometrium and sufficient for implantation. (Groothuis P.G.*et al*, 2007). On the contrary, Gupta et al., did not find any statistically significant difference in endometrial thickness and pregnancy rate with adding estradiol valerate to clomiphene citrate. In that study, one hundred and fifteen patients went into IUI (intrauterine insemination) cycle. They were further divided into three groups. One group received clomiphene citrate alone, the second received clomiphene citrate and estradiol valerate on the day of triggering while the third group received clomiphene citrate and human chorionic gonadotrophins. These results were explained by a steady decline in the antiestrogenic effect of clomiphene citrate by the end of the proliferative phase, while the maximum significant difference in endometrial thickness was on day 8 of the cycle (Gupta *et al*., 2014).

In some cases, the endometrial thickness was not increased after giving estradiol valerate instead of presence of dominant follicles. Those cases need further evaluation.

Endometrial factors other than the thickness have been studied in many literatures. In this study, endometrial

pattern and vascularity was also evaluated at day 12/ 13 (Table-3). Triple line pattern of endometrium in Group A was 72.7% and in Group B 65.6% respectively. Vascularity seen in Zone 1 in both groups were nearly similar if triple line endometrium present. Group A showed maximum vascularity in Zone 1 and it was 10(41.7%) and no case was found to have absent flow in case of triple line endometrium. Though pregnancy rates were not observed in this study, previous studies showed that endometrial vascularity and pattern of blood flow is positively related to implantation rate in patients going for IVF and embryo transfer (Wang *et al*., 2010). Considering this facts Kim et al., studied the effect of endometrial and subendometrial blood flow in pregnant ladies after intrauterine insemination. They found that the vascularization index, flow index and vascularization flow index were significantly higher in the endometrium of pregnant ladies when compared with those who did not achieve pregnancy (Kim *et al*., 2010).

In the study by Chen *et al*. at China, evaluation of combined effect of endometrial thickness and pattern on clinical outcome in patients undergoing in vitro fertilization/ intracytoplasmic sperm injection and embryo transfer (IVF/ICSI-ET) was done. They found that, endometrial thickness and pattern on the day of hCG administration was a better predictor of the outcome of IVF/ICSI-ET (Chen *et al*., 2010).

In meta-analysis conducted by Momeni *et al*., using fourteen articles in Cochrane library, they found that endometrial thickness was significantly higher in the group who achieved clinical pregnancy after IVF cycles when compared with those who did not. Therefore endometrial thickness can be considered one of the simple measurements in prediction of pregnancy on the day of triggering in stimulated cycles (Momeni *et al*., 2011).

**Conclusion:**

An adequately thick, triple layer endometrial pattern environment is essential for embryo implantation and continuation of pregnancy. Letrozole , estradiol valerate increased the endometrial thickness significantly as compared to Letrozole alone in infertile PCOS women who had improper endometrial thickness. Many noninvasive ultrasound criteria related to good endometrial thickness, triple line pattern and vascularity can affect the pregnancy rate in stimulated cycles. Therefore, administration of estradiol valerate is recommended for above mentioned patients. But the study population was selected from only one tertiary hospital in Dhaka city. So, the results of the study may not reflect the exact picture of the community.

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