



COMPARISON OF THE EFFECT OF CLOMIPHENE CITRATE AND LETROZOLE ON THE ENDOMETRIAL PARAMETERS OF PCOS WOMEN

Obstetrics & Gynaecology

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ABSTRACT

Background: Receptive endometrium is fundamental for the successful implantation of an embryo. It is adequately primed for implantation; its growth is regulated by steroid hormones, various growth factors and cytokines. Clomiphene citrate (CC) is an orally active nonsteroidal drug with mixed estrogen agonist/antagonist properties. While Letrozole, a selective reversible third-generation aromatase inhibitor, has the potential to be used for ovulation induction.

Objective: to decide which induce better endometrial receptivity either clomiphene citrate or Letrozole in PCOS patient.

Materials And Method: In prospective study, 120 infertile women with PCOS, undergo basic hormonal profile in cycle day 2, pelvic ultrasound to confirm diagnosis and to rule out presence of cyst, few number of patients get resistance to CC but the resistance to treatment was higher in clomiphene citrate group compared to letrozole group (36.76% vs 21.15%) 120 patients enrolled in the study and they received standard dose of either clomiphene citrate or letrozole as induction protocol so only 80 patients apart from those enrolled in the study of endometrium. On cycle day 12 or 13 another ultrasound done to examine growth of follicle, endometrial thickness, pattern and echogenicity, Human chorionic gonadotropin (hCG) was injected subcutaneously when at least one mature follicle 18 mm diameter was detected and asking couple to have intercourse 34-36 hour, follow up the patient to do pregnancy test after two weeks.

Results: Clomiphene citrate and letrozole achieved follicle maturation with a mean \pm SD of 12.13 \pm 0.52 and 11.9 \pm 0.5 days respectively; the mean number of dominant follicle in clomiphene citrate was significantly higher than letrozole (2.35 \pm 0.92 vs 1.83 \pm 0.38). letrozole group had a significantly greater endometrial thickness, than clomiphene citrate (0.85 \pm 0.07 vs 0.77 \pm 0.09) and significant triple hypoechoic pattern in letrozole compared to clomiphene citrate. The pregnancy rate achieved in letrozole group (30%) was greater than clomiphene citrate group (17.5%) although non-significant difference.

Conclusion: Letrozole induce better endometrial receptivity parameters than Clomiphene citrate.

KEYWORDS

polycystic ovarian syndrome, clomiphene citrate, Letrozole, endometrial parameters.

INTRODUCTION

PCOS is commonest causes of anovulation and amenorrhea. It is classically characterized by infertility, oligo and amenorrhea, hirsutism, acne or seborrhea, and obesity. The Rotterdam consensus defined the polycystic ovary as having 12 or more follicles, measuring between 2 and 9 mm follicle number per ovary, and/or an ovarian volume (OV) >10 cm³.⁽¹⁾ Clomiphene citrate (CC) considered as first line of treatment, it is an isomeric mixture of ~38% zuclomiphene and 62% enclomiphene.

Zuclomiphene is a mild estrogenic activity, it is estrogenic agonist properties occur when endogenous estrogen level are extremely low, as well as antiestrogenic, while enclomiphene is entirely antiestrogenic. CC is a safe and effective oral agent with good ovulatory rates; it is associated with several drawbacks, like adverse antiestrogenic effects systemically and on the endometrium and cervical mucus, low conception rates despite good ovulatory rates. However, since over 75% of ovulations occur within a dose range of 100–150 mg/day for 5 days; CC resistance was defined as the absence of ovulation after using this dose.⁽²⁾

Letrozole is a selective reversible third-generation aromatase inhibitor, has the potential role to be used for ovulation induction with demonstrable endometrial sparing effect. It induces the ovulation by inhibition of the conversion of androgens to estrogen and creating an estrogen-deficient environment; mimic the central reduction of negative feedback through which CC works.⁽³⁾

Some studies revealed that letrozole is superior or at least equal to CC in ovulation and pregnancy rates in PCOS women with anovulatory cycle PCOS and inadequate clomiphene response. Endometrial thickness is known as the maximal distance between the echogenic interfaces of the myometrium and the endometrium, measured in the plane through the central longitudinal axis of the uterus.

There is correlation between endometrial thickness and the likelihood of conception, in the context of assisted conception. However, a very thin endometrium (below 7 mm) seems to be accepted as a reliable sign of suboptimal implantation potential.⁽⁴⁾ Endometrial pattern which is the relative echogenicity of the endometrium and the adjacent

myometrium as demonstrated on a longitudinal ultrasound scan. A researcher recognizes that multilayered pattern to be more predictive of implantation than any other parameter measured.⁽⁵⁾

MATERIALS AND METHODS

A prospective study conducted in Department Obstetrics and Gynaecology, Darbhanga Medical College and Hospital, Laheriasarai, Bihar during the period from (July 2020 to June 2021).

120 PCOS women-part of them show resistance to induction and other got cyst those not enrolled in current endometrial study and 80 patients who response to induction were enrolled in this study and classified randomly in to two group. The diagnoses of PCOS is basis on history taking and physical examination.

The PCOS women included in this study with age range 18-40 years, more than 2 years of infertility, the FSH level less than 10 mIU/ml and have bilateral patent tubes confirmed by hysterosalpingography, in addition their partners have normal seminal fluid analysis according to WHO criteria.⁽⁶⁾ All patients should have no history of ovarian drilling, any uterine pathology or endocrinopathies and not taking any other medications.

All patients undergo assessment by taking full history and examination, hormonal assay (FSH, LH, prolactin, E2 and testosterone) at day two of cycle.

Measurement of body mass index (BMI). Base line ultrasound to confirm polycystic ovaries on day 2 or 3 of cycle and to exclude ovarian cyst and other ovarian.

Fifty two PCOS Patients were given letrozole orally 2.5 mg twice daily 12 hours apart from day 3 for 5 days of menstrual cycle. While sixty eight PCOS patients were given Clomiphene citrate 50mg twice daily in two doses 12 hour apart from Day3 of menses for 5 days long.

Transvaginal ultrasound was done from day 10 of the menstrual cycle and every other day till we reach the optimal size of follicle where at least one follicle with mean diameter more than 18mm. A recombinant HCG (1-2) ampules was administered subcutaneously to trigger

ovulation and timed intercourse (24-36hour after hCG injection) was advised. All patients had a blood-based pregnancy test at 2 weeks after HCG injection. Followed-up by an ultrasound which could document the viability of pregnancy (fetal heart +ve).

Statistical Analysis:

Most of the data was continuous so presented as mean ± standard deviation, and comparison between means of study groups done by using unpaired student t-test. Only non-continuous data presented as frequency and comparison between frequencies of study groups done by using Fisher exact test. Pearson correlation was done between different variables of each group. P value less than 0.5 was considered as significant. Microsoft excels 2016 and SPSS (statistical package for social sciences) version 23 was used as software do the statistics.

RESULTS

The demographic and hormonal parameter of the PCOS patients in both CC and letrozole groups). No significant difference between two groups in all parameter as shown in table (1)

Table 1 : Comparison Of Demographic And Hormonal Parameters Between Clomiphene Citrate And Letrozole Groups

Parameter	CC N = 40 Mean±SD	Letrozole N = 40 Mean±SD	P-value
Age (yr)	29.58±5.81	28.45±5.95	0.395
BMI (Kg/m ²)	24.87±2.85	25.29±2.76	0.505
Duration of infertility (yr.)	3.5±1.88	3.53±1.87	0.953
Type of Infertility			
Primary	19	22	0.655
Secondary	21	18	
S. FSH (mIU/ml)	7.33±2.15	7.38±1.71	0.914
S. LH (mIU/ml)	5.81±3.34	5.69±2.24	0.853
S. E2 (pg/ml)	42.11±19.45	45.37±19.15	0.458
S. Prolactin (ng/ml)	14.31±7.16	14.97±5.93	0.653
S. Testosterone (ng/ml)	0.21±0.2	0.19±0.2	0.746

FSH= Follicle stimulating hormone, LH= Luteinizing hormone, E2= Estradiol , CC= clomiphene citrate, BMI= Body mass index, * Data expressed as frequency & percentage

Regarding stimulation parameters, table (2), letrozole group has significantly lower no. of developing follicle and lower estradiol level at day of HCG, but on the other hand has significantly larger size of follicles, also significant shorter duration of stimulation. In addition letrozole group has thicker endometrium if compared to CC group.

Regarding pregnancy rate, letrozole group have higher although not significant pregnancy rate where 12 patients from 40 became pregnant (30%), if compared with those using CC in which 7 patients get pregnancy out of 40 (17.5%).

Table 2 : Stimulation Protocols Results Parameters By Unpaired T-test

Parameter	CC N = 40 Mean±SD	Letrozole N = 40 Mean±SD	P-value
No. of follicles	2.35±0.92	1.83±0.38	0.001
Size of follicles	17.66±1.46	18.82±1.3	<0.001
Duration of stimulation (days)	12.13±0.52	11.9±0.5	0.05
E2 level at day of hCG (pg/ml)	271.65±142.24	220.8±60.16	0.041
Average endometrial thickness (mm)	0.77±0.09	0.85±0.07	<0.001
Pregnancy outcome No. (%)	12(30%)	7(17.5%)	0.293

CC= clomiphene citrate

The endometrial pattern of PCOS patient using letrozole showed significant more frequent triple endometrial pattern than in CC group, as shown in table (3). Furthermore PCOS patients using letrozole have highly significant more hypoechoic endometrium than those use CC.

Table 3 : Comparison Of Endometrial Pattern And Echogenicity Between Of Clomiphene Citrate Group And Letrozole Group

Endometrium	Parameter	CC N = 40	Letrozole N = 40	P-value
Pattern	NTET	23	12	0.024

	TET	17	28	
Echogenicity	Hypoechoic	10	30	<0.001
	Hyperechoic	30	10	

CC= clomiphene citrate, NTET: Non Triple Endometrium, TET: Triple Endometrium

Table (4) showed that hypoechoic endometrium was highly significant associated with pregnancy in both CC and letrozole groups. The same as the triple endometrium pattern was significantly higher in pregnant than non-pregnant in CC group, while it was also higher in letrozole group but without significance The endometrial thickness shows on significant difference between pregnant and non-pregnant patients of both CC and letrozole groups.

Table 4 : Comparison Of Pregnancy Test Between Categorical Parameters In Clomiphene Citrate And Letrozole Groups By Unpaired T-test

Parameter	CC N=40		Letrozole		
	Pregnant	Non Pregnant	Pregnant	Non Pregnant	
Endometrial Echogenicity	Hyper-echoic	1	29	0	10
	Hypo-echoic	6	4	12	18
	P-value	<0.001		0.019	
Endometrial pattern	NTET	1	24	2	10
	TET	6	11	10	18
	P-value	0.012		0.285	

NTET= Non-triple endometrial thickness, TET= Triple endometrial thickness, CC= clomiphene citrate

DISCUSSION

The data of this study showed that the mean no. of follicle which is observed by transvaginal ultrasound was significantly higher in clomiphene citrate compared to letrozole groups as demonstrated in table (2). This is agreed by other others, there were more follicles ≥16 mm in clomiphene group.^(7,8) Another study show there was no significant difference between the total number of developing follicles in the letrozole and in the clomiphene citrate groups.⁽⁹⁾ In the ovary, aromatase inhibitors increase follicular sensitivity to FSH by the accumulation of intraovarian androgens. As the dominant follicle grows and estrogen levels rise, normal negative feedback occurs centrally, resulting in suppression of FSH secretion and atresia of the smaller growing follicles resulting in single dominant follicle and mono-ovulation⁽¹⁰⁻¹²⁾. Other study explains that, the hypoestrogenic state created by letrozole not last late in the follicular phase of the menstrual cycle due to its short half-life, create a higher likelihood of mono-follicular growth.⁽¹³⁾

Current study showed that average size of dominant follicle was larger in letrozole group than that in clomiphene citrate with highly significant difference. As explained earlier the mechanism of Letrozole action, the selected follicle receive FSH stimulation allowing greater growth of follicle also it increase intrafollicular androgen in ovary which upregulate and sensitize FSH receptors in ovary.^(11,12)

Also a higher level of estradiol on the day of HCG injection as shown in table (2) was noticed in Clomiphene Citrate group than that in letrozole group, the E2 elevation may have a detrimental effect on receptivity of endometrial.

Some study show that excessive estrogen administered post ovulation can prevent implantation of blastocyst, there is little understanding of how varying levels of estrogen in serum and progesterone within the wide normal range, may influence receptivity⁽¹⁴⁾. This explains some of the adverse effects of Clomiphene on the outcome of infertility treatment, although reducing estrogen synthesis by aromatase inhibitor may ameliorate such deleterious effects⁽¹⁵⁾.

In addition, clomiphene citrate affects the endometrial development, causing implantation failure and thinning of the endometrium, this we clarified in our study in table (2).

The mean thickness of endometrium in this study was higher significantly in a letrozole than that of clomiphene groups, these

findings were consistent with other studies reporting that most patients taking letrozole had a thicker endometrium. Endometrial thickness (ET) measurement is a predictor for a successful implantation following ovulation induction, with many studies reporting more success with a thickness of 9 - 10 mm.^(10,18), there is no negative effect on endometrial thickness. This is due to the relatively short half-life of Letrozole, which allows complete recovery of endometrium before implantation.⁽¹⁸⁾

Compared to clomiphene citrate, letrozole is cleared from the circulation more rapidly due to a shorter half-life; and it does not deplete estrogen receptors and is devoid of any antiestrogenic peripheral actions; therefore, it has no adverse effect upon the receptivity of endometrium and the cervical mucus quality⁽¹⁸⁾.

The endometrial echogenicity and pattern were significantly different between pregnant and non-pregnant in both clomiphene and letrozole groups, table (4), on the other hand as mentioned earlier letrozole has significant more hypoechogenic and triple endometrium pattern if compared to clomiphene as shown in table (3), this is agreed with other authors who stated that the triple layer of endometrial pattern was the most suitable for conception^(19,20). Contrary to other research results, Ng et al., reported, no relationship between the endometrial thickness, morphology and pregnancy outcomes⁽²¹⁾. However, this reduction of the endometrial thickness did adversely affect pregnancy or ongoing pregnancy rates. The statistical data of the current study showed in table (4) that PCOS patient in letrozole group has significantly higher hypoechogenic endometrium if compared with clomiphene group that is agreed with Wei Liu et al.⁽²²⁾ The endometrial thickness of 10–12.9 mm with a trilaminar appearance produced a highest clinical pregnancy rate (56.5%) compared to the least clinical pregnancy rate was recorded in the echogenic pattern group (6.5%).⁽²³⁾ This is in agreement with Check et al.⁽¹⁵⁾, who found that no pregnancies occurred in those patients with a homogenous hyperechoic endometrium⁽¹⁵⁾.

Another study disagree with our result demonstrated that the homogeneous hyperechoic endometrial texture on the day of ET was a predictive of implantation.⁽²⁴⁾

In the current study, the trilaminar endometrial pattern was significantly higher in the letrozole group compared to the clomiphene citrate group, as clarified in table (3). This result agreed with the study of Jang and Jee which got significant difference between Clomiphene Citrate and letrozole group, which was more frequent in letrozole.⁽¹⁶⁾

Receptive endometrium is essential for the implantation of an embryo. Endometrial thickness, trilaminar pattern are the essential determinants of endometrial receptivity^(25,26). The pregnancy rate was lower in Clomiphene Citrate group than letrozole group with pregnancy rate 17.5% for Clomiphene Citrate and 30% in letrozole group, this is agreed by⁽¹⁷⁾⁽²⁷⁻³⁰⁾. A likely explanation for the mechanism underlying the lower rate of pregnancy in Clomiphene Citrate group was Clomiphene citrate is an anti-estrogen with a 60% - 80% ovulation rate and a 10% - 20% pregnancy rate. This is due to the antiestrogen effect of Clomiphene citrate, resulting in longlasting estrogen receptor (ER) depletion, also it accumulates in the body due to its long half-life (2 weeks), causing adverse effects on the quality and quantity of cervical mucus, these undesirable effects of Clomiphene citrate on the endometrium may explain the relatively poor pregnancy rate associated with Clomiphene citrate despite the high rate of ovulation⁽³¹⁾.

CONCLUSION:

Letrozole induce better endometrial receptivity parameters than Clomiphene citrate.

REFERENCES

- The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004; 19: 41-7.
- Ornstein RM, Copperman NM, Jacobson MS. Effect of weight loss on menstrual function in adolescents with polycystic ovary syndrome. *J Pediatr Adolesc Gynecol*. 2011 Jun; 24(3):161-5.
- Lumachi F, Rondinone R. Use of cyproterone acetate, finasteride, and spironolactone to treat idiopathic hirsutism. *Fertil Steril* 2003; 79:942-946.
- Nardo LG: Vascular endothelial growth factor expression in the endometrium during the menstrual cycle, implantation window and early pregnancy. *Curr Opin Obstet Gynecol*, (2005) 17:419-423.
- Goldstein SR. Modern evaluation of the endometrium. *Obstet Gynecol* 2010; 116:168-76.
- Agboola A. *Textbook of Obstetrics and Gynaecology*, Heinman Educational Books, Ibadan, vol 1, 2004, 174-176.
- Badawy A, Elnashar A, Totongy M. Clomiphene citrate or aromatase inhibitors for superovulation in women with unexplained infertility undergoing intrauterine insemination: a prospective randomized trial. *Fertil Steril*. 2009;92:1355-1359.
- Hendawy Sherif F, Samaha Hanan E, Elkholi Mohamed F 2011-Mar-14, letrozole versus Clomiphene Citrate for induction of ovulation.
- Zeinalzadeh M, Basirat Z, Esmailpour M. Efficacy of letrozole in ovulation induction compared to that of clomiphene citrate in patients with polycystic ovarian syndrome. *J Reprod Med*. 2010;55:36-40.
- Atay, V., Cam, C., Muhcü, M., Cam, M. and Karateke, A. (2006) Comparison of letrozole and clomiphene citrate in women with polycystic ovaries undergoing ovarian stimulation. *Journal of International Medical Research*, 34, 73-76.
- Holzer H, Casper R, Tulandi T. A new era in ovulation induction. *Fertil Steril* 2006; 85:277-284.
- Topipat C, Choktanasiri W, Jultanas R, et al. A comparison of the effects of clomiphene citrate and the aromatase inhibitor letrozole on superovulation in Asian women with normal ovulatory cycles. *Gynecol Endocrinol* 2008; 24:145-150.
- Polyzos NP, Tziaras S, Badawy AM, et al. Aromatase inhibitors for female infertility: a systematic review of the literature. *Reprod BioMed Online* 2009; 19:456-471.
- Yang JH, Chen HF, Lien YR, Chen SU, Ho HN, Yang YS. Elevated E2: oocyte ratio in women undergoing IVF and tubal ET. Correlation with a decrease in the implantation rate. *J Reprod Med* 2001; 46:434-8.
- Check J.H., D. Lurie, C. Dietherich, C. Callan, A. Baker. Adverse effect of a homogenous hyperechogenic endometrial sonographic pattern, despite adequate endometrial thickness on pregnancy rates following in-vitro fertilization.
- Jang E.J., B.C. Jee. The efficacy of letrozole in women with a poor endometrial response to clomiphene citrate. *Korean J. Reprod. Med.*, 37 (1)(2010), pp. 73-81.
- Payal Banerjee Ray, Amitabh Ray, P. S. Chakraborty, Comparison of efficacy of letrozole and clomiphene citrate in ovulation induction in Indian women with polycystic ovarian syndrome. *Archives of Gynecology and Obstetrics*, 2012, 285, 3, 873 Akbari S, Ayazi Roozbahani M, Ayazi Roozbahani F. Comparing of letrozole versus Clomiphene citrate combined with gonadotropins in intrauterine insemination cycles. *Iran J Reprod Med*. 2012;10:29-32.
- Pritts EA. Letrozole for ovulation induction and controlled ovarian hyperstimulation. *Curr Opin Obstet Gynecol* 2010;22(4):289-94.
- El-Zenneni H, Moustafa R, Abdel-Hafeez M, El-Salally H, Abdel-Kader A, Elnaggar A, et al. Assessment of uterine, subendometrial blood flows and endometrial gland vascular endothelial growth factor (EG-VEGF) in women with unexplained infertility. *Middle East Fertil Soc J* 2014.
- Singh N, Bahadur A, Mittal S, Malhotra N, Bhatt A. Predictive value of endometrial thickness, pattern and sub-endometrial blood flows on the day of hCG by 2D doppler in in-vitro fertilization cycles: a prospective clinical study from a tertiary care unit. *J Hum Reprod Sci* 2011;4:29-33.
- Chan CC, Ng EH, Tang OS, Yeung WS, Ho PC. The role of endometrial and subendometrial blood flows measured by three-dimensional power Doppler ultrasound in the prediction of pregnancy during IVF treatment. *Human Reprod* 2006;21:164-70.
- Wei Liu, Shengnan Dong, Yumei Li, Lihong Shi, Wei Zhou, Yingling Liu, Jie Liu, and Yanzhong Ji. Randomized controlled trial comparing letrozole with laparoscopic ovarian drilling in women with clomiphene citrate-resistant polycystic ovary syndrome 2015 Oct; 10(4): 1297-1302
- Maged AI Mohammady, Ghada Abdel Fattah and Mostafa Mahmoud. The impact of combined endometrial thickness and pattern on the success of intracytoplasmic sperm injection (ICSI) cycles. *Middle East Fertility Society Journal* Volume 18, Issue 3, September 2013, Pages 165-170
- Sanja Kupescic, MD, PhD, Ivanka Bekavac, MD, Drazena Bjelos, MD, Asim Kurjak, MD, PhD. Assessment of Endometrial Receptivity by Transvaginal Color Doppler and Three-dimensional Power Doppler Ultrasonography in Patients Undergoing In Vitro Fertilization Procedures 2001 by the American Institute of Ultrasound in Medicine. *J Ultrasound Med* 20:125-134, 2001 0278-4297/01.
- Kupescic S., I. Bekavac, D. Bjelos, A. Kurjak. Assessment of endometrial receptivity by transvaginal color Doppler and three-dimensional power Doppler ultrasonography in patients undergoing in vitro fertilization procedures. *J Ultrasound Med*, 20(2001), p. 125.
- Singh N., A. Bahadur, S. Mittal, N. Malhotra, A. Bhatt. Predictive value of endometrial thickness, pattern and subendometrial blood flows on the day of hCG by 2D Doppler in in-vitro fertilization cycles: a prospective clinical study from a tertiary care unit. *J Hum Reprod Sci*, 4(2011), p. 29.
- Shahrokh Tehrani Nejad E, Abedialz Z, Rashidi BH, Azimi Nekoo E, Shariat M, Amirchaghmaghi E. Comparison of the efficacy of the aromatase inhibitor letrozole and Clomiphene citrate gonadotropins in controlled ovarian hyperstimulation: a prospective, simply randomized, clinical trial. *J Assist Reprod Genet*. 2008;25:187-190.
- Ibrahim MI, Moustafa RA, Abdel-Azeem AA. Letrozole versus Clomiphene citrate for superovulation in Egyptian women with unexplained infertility: a randomized controlled trial. *Arch Gynecol Obstet*. 2012;286:1581-1587.
- Zadehmodares S, Niyakan M, Sharafy SA, Yazdi MH, Jahed F. Comparison of treatment outcomes of infertile women by Clomiphene citrate and letrozole with gonadotropins underwnt intrauterine insemination. *Acta Med Iran*. 2012;50:18-20.
- Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, et al. Letrozole versus Clomiphene for infertility in the polycystic ovary syndrome. *N Engl J Med*. 2014;371:119-129.
- Baruah, J., Roy, K., Rahman, S., Kumar, S., Sharma, J., and Karmakar, D. Endometrial effects of letrozole and clomiphene citrate in women with polycystic ovary syndrome using spiral artery Doppler. *Arch Gynecol Obstet*. 2009; 279: 311-314