

Clomiphene Citrate versus Letrozole for Ovulation Induction in PCOS: A Comparative Study

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ABSTRACT

Polycystic ovary syndrome (PCOS) affects 20–33% of women of reproductive age and the leading cause of infertility. Treatment includes lifestyle modification, metformin, ovulation induction, surgery, *in vitro* fertilization. Clomiphene citrate is most commonly used for ovulation induction but is antiestrogenic. Clomiphene resistance occurs in 15–20% of patients. Letrozole, an aromatase inhibitor, inhibits aromatization thus conversion of androstenedione and testosterone to estrogen in the ovary. This releases the hypothalamic/pituitary axis from estrogenic negative feedback, follicle stimulating hormone secretion increases, stimulating ovarian follicular development.

We compared clomiphene citrate vs letrozole for ovulation induction in the treatment of anovulatory infertility in women with the polycystic ovarian syndrome. Two hundred consecutive women were observed for a number of cycles required to achieve ovulation, follicular growth, endometrial thickness, dominant follicle size, pregnancy, multiple, and abortion rates. The groups were matched for age, parity, duration of married life, menstrual regularity and prior history of treatment for infertility.

Clomiphene showed a significantly increased number of follicles. Monofollicular development and endometrial thickness were higher in the letrozole group. The difference in dominant follicle size, number of cycles to achieve pregnancy or difference in ovulation rate per cycle, number of pregnancies and pregnancy losses per cycle lacked statistical significance. Greater number of follicles in the clomiphene group did not result in increased pregnancy rate. A number of pregnancies were marginally higher but not statistically significant in the letrozole group. There were one quadruplet and one twin gestation in the clomiphene group with none in the letrozole group. This data was not sufficient for statistical analysis. There were no instances of ovarian hyperstimulation syndrome or congenital anomalies in either group.

Keywords: Clomiphene citrate, Follicle, Infertility, IVF, Letrozole, Pregnancy.

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INTRODUCTION

Polycystic ovary syndrome (PCOS), also known clinically as Stein-Leventhal syndrome, affects approximately 20–33%,¹⁻⁴ of women of reproductive age, and is a leading cause of infertility. The symptoms and signs are highly variable.¹ There may be a familial predilection with studies suggestive of autosomal transmission of genetic sequences.⁵

Clinical Manifestations

- Obesity, hyperandrogenism,⁶ menstrual irregularities, infertility.
- Increased levels of androgens,⁷⁻⁹ LH, FSH, prolactin, estradiol, hyperinsulinemia¹⁰⁻¹² and decreased the level of sex hormone binding globulin.
- Increased risk of diabetes mellitus, dyslipidemia, hypertension, cardiovascular disease, endometrial carcinoma¹³ and breast carcinoma with advancing age.

DEFINITION

1. 1990, consensus workshop by NIH/NICHD:¹⁴
 - Hyperandrogenism and/or hyperandrogenemia
 - Oligoovulation
 - Exclusion of other related disorders, e.g., Cushing's syndrome, CAH, hypothalamic amenorrhea, premature ovarian failure, etc.
2. 2003, consensus workshop by ESHRE/ASRM in Rotterdam indicated PCOS to be present if 2 out of 3 criteria are met:¹⁵
 - Oligoovulation [follicular arrest", i.e., several follicles develop to a size of 5–7 mm, but not further. No single follicle reaches the preovulatory size (16 mm or more)] and/or anovulation.
 - Excess androgen activity
 - Polycystic ovaries (by gynecologic ultrasound), and other causes of PCOS are excluded.

According to the Rotterdam criteria, 12 or more small follicles should be seen in an ovary on ultrasound examination. The follicles may be oriented in the periphery,

giving the appearance of a 'string of pearls'. The numerous follicles contribute to the increased size of the ovaries, that is, 1.5–3 times larger than normal.¹⁶

3. Androgen Excess Society in 2006 states as PCOS if:

- Hirsutism and/or hyperandrogenemia.^{6,17-19}
- Oligoanovulation and/or polycystic ovaries by ultrasonogram.
- Exclusion of other androgen excess or related disorders.

The Rotterdam definition, however, is more inclusive, unlike NIH/NICHD definition where androgen excess is a prerequisite. Treatment of PCOS should preferably be tailored according to the patient's symptoms. Accordingly, options include weight loss, insulin sensitizers like metformin,²⁰⁻²³ ovulation induction²⁴ and laparoscopic surgery.^{25,26} Studies to decide the best of these are not available. PCOS is a risk factor for ovarian hyperstimulation syndrome²⁷ hence ovulation induction needs careful monitoring with serial ultrasonograms.²⁸

Clomiphene citrate and gonadotropins alone or used synergistically as agents of ovulation induction. However, their use is fraught with risks of multiple pregnancies,^{29,30} antioestrogenic effects like hot flushes, breast tenderness, dizziness, nausea, ovarian cysts, multiple pregnancies, OHSS, etc. Search for alternatives has led to the use of aromatase inhibitors, e.g., letrozole which claims to lack these negative effects.

AIM

There are no studies authoritatively quantifying the effects of letrozole compared to clomiphene citrate, but Sammour and colleagues showed a higher pregnancy rate than clomiphene citrate (16.7 % versus 5.6% per patient), i.e., more than three times higher pregnancy rates.³¹ Hence, in our study, we go ahead with the hypothesis that letrozole has at least 2.5% higher rates of ovulation induction and pregnancy than clomiphene citrate in view of its shorter half-life and consequent negligible antiestrogenic action on the endometrium and cervical mucus.

OBJECTIVE

To compare the efficacy of the aromatase inhibitor letrozole vs clomiphene citrate in controlled ovarian hyperstimulation (COH)-the parameters considered being: ovulation, pregnancy, and abortion rates, follicular size, number, and endometrial thickness. We also observed for the occurrence of multiple pregnancies, congenital anomalies, and ovarian hyperstimulation syndrome.

REVIEW OF LITERATURE

Stein and Levinthal³² in 1935 reported seven women who presented with problems of amenorrhoea, anovulation

and bilateral enlarged polycystic ovaries with thickened tunica and were treated by wedge resection. Stein also reported 75 women who underwent wedge resection; 33.34 90% of resumed regular menstruation and 65% became pregnant.³⁵ The disease itself may be traced as far back as 1721 as an Italian printout which reads as: "Young married peasant women, moderately obese and infertile with two larger than normal ovaries, bumpy, shiny and whitish, just like pigeon eggs" (A Vallisneri). Similar ovarian changes were described by Chereau³⁶ in 1844.

Clomiphene citrate introduced in 1961, with around 70% ovulation and 30% pregnancy rates,³⁷⁻³⁹ began to be used for ovulation induction in women with PCOS. Greenbhatt²⁴ report successful ovulation followed by pregnancy with this drug. Gonadotropins,⁴⁰ selective estrogen receptor modulators, aromatase inhibitors are also used for ovulation induction. Surgery includes ovarian drilling and diathermy²⁷ with 70–90% ovulation and 40–70% pregnancy rates.²⁵ Medical induction is however favored due to fear of expenses and operative morbidity.

Letrozole is an aromatase inhibitor that prevents androgen to estrogen conversion. The antioestrogenic effects of clomiphene are not found with letrozole.^{38,41-43}

DESCRIPTION OF DRUG

Clomiphene Citrate

It inhibits the action of estrogen on the pituitary gland, competes with estrogen for estrogen-receptor-binding sites and delays replenishment of intracellular estrogen receptors. This results in increased release of FSH from the pituitary leading to a higher rate of ovulation and hence pregnancy. Dose: 100 mg orally, once daily.

Treatment is usually commenced on day 2 of the cycle and given for 5 days. The starting dose of clomiphene is usually 50 mg. The dose should only be increased if there is no response after three cycles. All women who are prescribed clomiphene should be carefully monitored with an ultrasonographic assessment of follicular growth and ovulation because of the risk of multiple pregnancies.

Earlier reports had suggested an association between clomiphene and ovarian cancer with more than 12 months' therapy; although in most cases of prolonged use the indication was unexplained infertility rather than anovulation.⁴⁴

Side Effects

Hot flushes, abdominal discomfort, visual blurring, and/or reversible ovarian enlargement and cyst formation, abnormal uterine bleeding, nausea, and/or vomiting.

Letrozone

An oral nonsteroidal aromatase inhibitor⁴⁵ that was used for the adjuvant treatment of hormonally-responsive breast cancer. Letrozole blocks the production of estrogens in this way by competitive, reversible binding to the heme of its cytochrome P450 unit. It does not downregulate the ER compared to CC46 and by lowering circulating estrogen releases hypothalamus from negative feedback of estrogen causing an increase in (gonadotropin-releasing hormone (GnRH) which in turn leads to an increase in follicle stimulating hormone (FSH) and leutinizing hormone (LH). These stimulate the growth of ovarian follicles and increase the ovarian follicle sensitivity to FSH.

- *Dose used:* 2.5 mg orally, bid
- *Side effect:* Nausea, vomiting, tiredness, headache, muscle aches, joint pains, hot flushes, hair thinning, vaginal dryness.

Letrozole inhibits estrogen synthesis, thereby causing enhanced GnRH pulsatility and consequent FSH and inhibin stimulation. This results in normal or enhanced follicular recruitment without the risk of multiple ovulation and OHSS. Letrozole has a very short half life⁴⁶ and is quickly cleared from the body thus minimizing antioestrogenic effect on the endometrium and cervical mucus. Incidences of congenital anomalies have been found to be equal with both drugs.⁴⁷

Mitwally and Casper reported the success of aromatase inhibition in inducing ovulation in women with PCOS.^{45,48} In another study, the same group reported the comparable success of an aromatase inhibitor in inducing ovulation in 12 women with PCOS in addition to success in augmenting ovulation in a group of 10 ovulatory women. Sammour et al.³¹ carried out a double-blinded randomized trial that compared an aromatase inhibitor with clomiphene citrate for stimulation in 49 women with unexplained infertility where the patients receiving the aromatase inhibitor achieved a better uterine environment including increased blood flow as determined by Doppler ultrasound and increased endometrial thickness compared with that receiving clomiphene citrate. The aromatase inhibitor was associated with a higher pregnancy rate than clomiphene citrate (16.7% *vs.* 5.6% per patient).

Mitwally and Casper also studied the benefits of using aromatase inhibitors along with gonadotrophins in ovulatory (unexplained infertility) and anovulatory PCOS women.⁴⁹ This study concluded that cotreatment with an aromatase inhibitor significantly reduced the FSH dose⁴⁹ required during controlled ovarian hyperstimulation as did clomiphene citrate. The aromatase inhibitor, however, was not associated with the antioestrogenic effects seen with clomiphene citrate treatment^{41,50} as demonstrated by

the significantly lower endometrial thickness noted with clomiphene citrate treatment³⁹ despite the significantly higher estradiol levels.⁵¹

MATERIALS AND METHODS

This study was carried out in the Department of Obstetrics and Gynecology, Kerala Institute of Medical Sciences. This hospital is a privately run tertiary referral center and teaching hospital, which serves the districts of Trivandrum, Kollam, Pathanamthitta in Kerala and Kanyakumari and Tirunelveli districts of Tamil Nadu.

Our study was a prospective randomized controlled trial. The randomization unit was the individual drug and the schedule of allotment of the drug to each patient was determined by computer-generated random numbers. The drug to be prescribed was placed in sealed envelopes along with the mode of consuming the same. The doctor was not aware of the drug prescribed, but the patient was aware of the same, with clomiphene having once daily and letrozole twice daily dosage schedule. The analysis was performed by an independent analyzer.

Inclusion Criteria

- All patients satisfying criteria for PCOS according to the Rotterdam criteria.^{14,15}
- Seeking treatment for infertility.
- Willing to participate in the study.

Exclusion Criteria

- Women with hyperthyroidism/hyperprolactinemia/pituitary causes of infertility.
- Pregnancy.
- Male factor abnormalities
- *Women with a tubal block:* This was ruled out with hysterosalpingography. Some doctors recommend a laparoscopy to exclude tubal blockage before the commencement of treatment.

Another approach, in the absence of any symptoms or signs of pelvic disease, is to undertake treatment and only perform laparoscopy if pregnancy has not occurred after six months of treatment. This approach had to be taken in our study in view of the unwillingness of most patients to attempt any surgical modality before the use of medications.

SAMPLING PROCEDURE

In our study, we go ahead with the hypothesis that Letrozole has at least 2.5% higher rates of ovulation induction and pregnancy than clomiphene citrate.³¹ Hence, we arrived at the sample size with the help of the following formula:

$$N = \frac{[Z_{\alpha}\sqrt{2\bar{p}\bar{q}} + Z_{\beta}\sqrt{p_1q_1 + p_0q_0}]^2}{(p_1 - p_0)^2}$$

$$\text{where } P_1 = \frac{P_{0R}}{1 + P_{0(R-1)}}$$

$$\text{and } \bar{q} = 1 - \bar{p} = \frac{1}{2}(p_1 + p_0)$$

$$q_1 = 1 - p_1$$

$$q_0 = 1 -$$

P_0 = Percentage of pregnancies usually achieved with clomiphene citrate = 40% with an ovulation rate of 80%

$$\therefore = 0.40$$

$R = 2.5$ (Letrozole hypothesized to have 2.5 times higher pregnancy rate than clomiphene)

$$Z_{\alpha} = 1.96 \text{ for } \alpha = 0.05$$

$$Z_{\beta} = 1.28 \text{ for } \beta = 90\% \text{ power}$$

$$\therefore N = 101 \approx 100 \text{ women in each group}$$

A sample of 200 consecutive women was taken (after block randomization), assigned to two groups. Group 0—Clomiphene citrate -96 patients and group 1—Letrozole—104 patients.

The period of study decided upon was 1 year 6 months from July 2008 to February 2010. This was preceded by 3 months of treatment with metformin, exercise, lifestyle modification, and folic acid.

CONDUCT OF STUDY

- A thorough history was taken to rule out other causes of anovulation and infertility including galactorrhea, symptoms suggestive of thyroid hormone abnormalities, features suggestive of hyperandrogenism, hyperinsulinemia, etc.
- Complete medical and gynecological examination including complete blood count, liver, thyroid and renal function tests, prolactin, FSH/LH, Testosterone, and DHEAS.
- Baseline transvaginal ultrasonogram (TVS) to assess features of polycystic ovaries was done prior to the commencement of treatment.

PROCEDURE

A sample of 200 consecutive women (after block randomization) was assigned to participate in the study after counseling and obtaining informed consent. Polycystic ovary syndrome was defined as per Rotterdam criteria. Withdrawal bleeding was achieved with 10 mg levonorgestrel tablets for five days before stimulation. Group 0 were subjected to ovulation induction with 100 mg OD of clomiphene citrate and group 1 with 2.5 mg of letrozole BD for five days starting on day 3 of menses.

Follicular monitoring was done by TVS on days 10, 12, 14, 16 of the cycle till a mature follicle of size 1.4–1.8 cm or more (by averaging inner two diameters of the follicle)

was detected. A single injection of hCG 10,000 IU IM was given if at least one follicle was >14 mm and the endometrial thickness at least more than 8 cm. A second TVS was done after 48 hours of hCG to observe the release of the egg. If the follicle was found unruptured, a third TVS was done after 72 hours of the hCG injection to observe a luteinized unruptured follicle.

Ovulation was ascertained by observing rupture of the follicle by TVS—collapsed follicle, fluid in the pouch of Douglas. Endometrial thickness was measured in the plane through the central longitudinal axis of the uterus at a point of maximum distance between the echogenic interfaces of the diameter. A trilaminar diameter of ≥ 8 mm was considered a satisfactory response.

Timed intercourse was advised 36 hours after hCG on two consecutive days and the luteal phase was supplemented with micronized progesterone/dydrogesterone. The main outcome measure considered was the rate of ovulation and pregnancy. Three to four cycles were observed for pregnancy.

If follicular development was poor, the cycle was canceled. If the cycle was canceled, and another cycle to be started, the protocol was followed again from step 1 above.

OUTCOME VARIABLES/MEASURES

Both groups were compared with regard to:

- Successful follicular maturation (14–18 mm).
- Failure of follicular maturation.
- A number of follicles recruited per cycle with each drug.
- Endometrial thickness in each cycle—prior to and after hCG injection.
- Number of cycles following which pregnancy occurred.
- Rate of pregnancy and miscarriage.
- Rate of multiple pregnancies.
- Side effects like OHSS if any.

DATA ENTRY AND ANALYSIS

Using Microsoft excel sheet and analysis by SPSS package.

Ethical Issues

These were tackled by the institutional and local ethical committee. The study commenced after obtaining complete informed consent from the patients.

Analysis of Data

The descriptive statistics—mean, median, standard deviation, percentages were used to summarize the findings. The difference between the two groups was tested by t-test in case of continuous variables and Chi-square test in case of categorical data.

A p value of ≤ 0.05 was considered significant. Results were expressed as the mean and standard error of deviation.

LIMITATION OF STUDY

Laparoscopic evaluation of tubal factor was not found to be feasible in every case in view of the cost factor and the limited time of the study. Some patients were lost to follow-up.

Estimation of serum estradiol and progesterone could not be done in view of the additional expenditure for the patients.

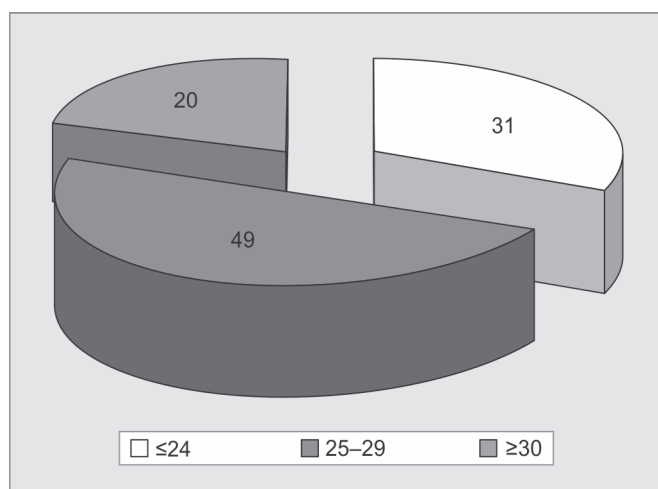
The study was not double-blinded, in that the patient was aware of the drug which she was taking.

OBSERVATIONS AND RESULTS

- **Age:** Of the total population studied, 31% were less than or equal to 24 years of age; 49% between 25–29 years of age and 20% more than or equal to 30 years (Table 1 and Graph 1). It was noted that (Table 2 and Graph 2) in the clomiphene group 28.1% were below or equal to 24 years of age, 53.1% between 25 years and 29 years and 18.8% more than or equal to 30 years of age. In the letrozole group 33.7% were less than or equal to 24 years of age, 45.2% between 25 years and 29 years and 21.2% more than or equal to 30 years of age. The p value was 0.822 hence no significant difference in the age distribution of patients in either group.

Table 1: Percentage distribution of the sample according to age

Age	Count	Percent
≤ 24	62	31.0
25–29	98	49.0
≥ 30	40	20.0
Total	200	
Mean \pm SD	26.7 \pm 4.1	

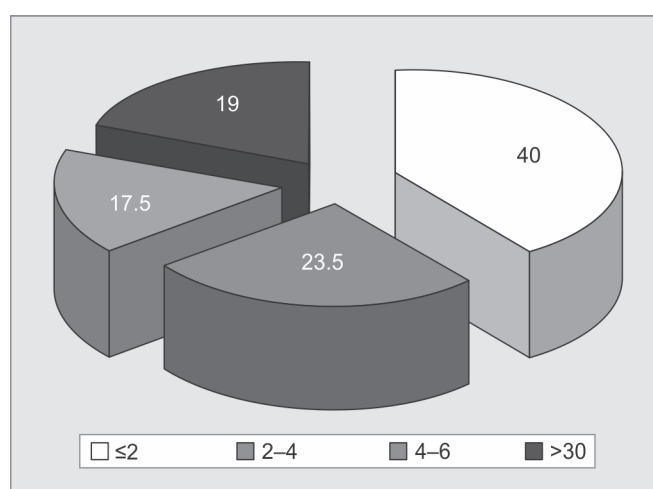


Graph 1: Percent distribution of the sample according to age

- **Duration of married life:** Forty percent of patients were at or past 2 years of married life at the commencement of treatment (Table 3 and Graph 3), 23.5% between 2 years and 4 years, 17.5% between 4 years and 6 years and 19.0% more than 6 years of married life (Table 4 and Graph 4). It was noted that in clomiphene group the duration of marital life prior to commencement of the study was less than or equal to 2 years in 39.6%, between 2 years and 4 years in 21.9%, between 4 years and 6 years in 17.7%, more than or equal to 6 in 20.8% with a mean \pm standard deviation of 4.1 ± 2.7 compared to 40.4%, 25%, 17.3%, 17.3%, respectively in letrozole group with a mean \pm standard deviation of 4.1 ± 3.2 . The p value was 0.984, hence no significant difference in the distribution between the two groups.
- **Parity:** Nearly 61.5% of patients had primary infertility, 30.5% had at least 1 previous pregnancy and 8.0% had 2 previous pregnancies (Table 5 and Graph 5). The percentages of distribution of primary infertility, secondary infertility with one previous pregnancy, two previous pregnancies were 65.6%, 25%, 9.4% respectively in clomiphene group compared to 57.7%, 35.6%, and 6.7%, respectively in the letrozole group (Table 6 and Graph 6). The p value was 0.249 hence no significant difference in the distribution of patients between the two groups.
- **Menstrual pattern:** The percentage of women with regular and irregular menstrual cycles was found to be 12.5% and 87.5%, respectively in the clomiphene

Table 2: Percentage distribution of the sample

Duration of married life	Count	Percent
≤ 2	80	40.0
2–4	47	23.5
4–6	35	17.5
> 6	38	19.0
Mean \pm SD	4.1 \pm 3	

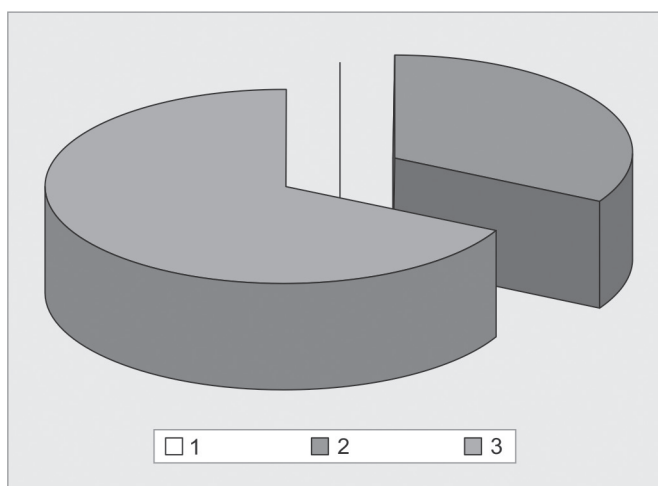


Graph 2: Percent distribution of the sample of duration of married life

group compared to 14.4% and 85.6%, respectively in the letrozole group. The p value was 0.691, again showing comparable distribution of patients between the two groups (Table 7 and Graph 7).

Table 3: Percent distribution of the sample according to parity

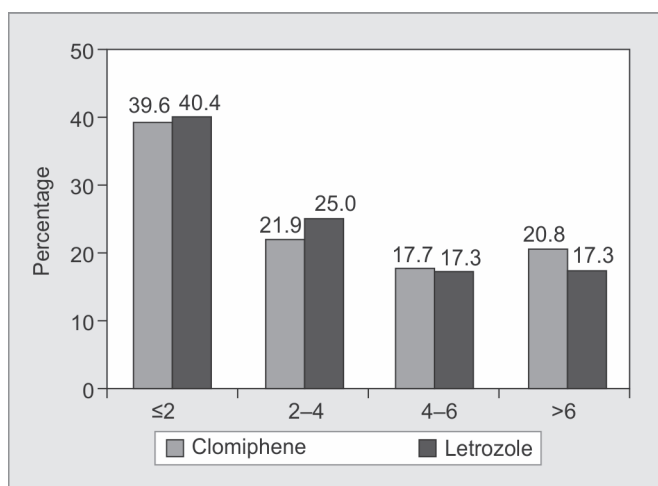
Parity	Count	Percent
0	123	61.5
1	61	30.5
2	16	8.0



Graph 3: Percent distribution of the sample according to parity

Table 5: Comparison of Duration of married life based on drug

Duration of married life	Drug			
	Clomiphene		Letrozole	
	Count	Percent	Count	Percent
≤ 2	38	39.6	42	40.4
2–4	21	21.9	26	25.0
4–6	17	17.7	18	17.3
>6	20	20.8	18	17.3
Mean ± SD	4.1 ± 2.7		4.1 ± 3.2	
t = 0.02	p = 0.984			



Graph 5: Comparison of duration of married life based on drug

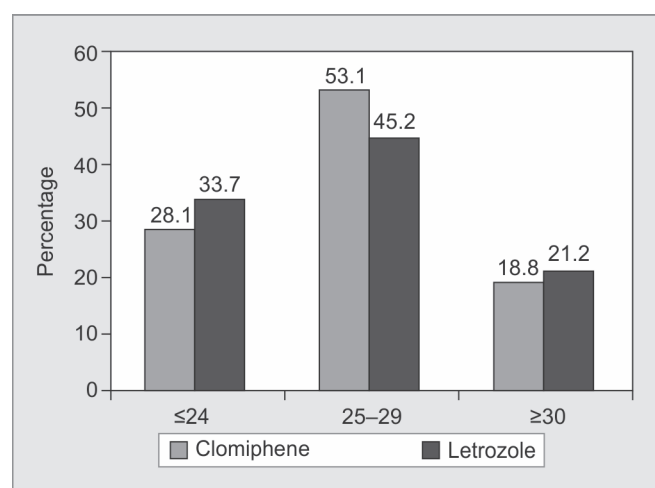
HISTORY OF OVULATION INDUCTION

In the clomiphene group, (Table 8 and Graph 8) 64.6% of patients had a prior history of ovulation induction

Table 4: Comparison of age based on drug

Age	Drug			
	Clomiphene		Letrozole	
	Count	Percent	Count	Percent
≤24	27	28.1	35	33.7
25–29	51	53.1	47	45.2
≥30	18	18.8	22	21.2
Mean ± SD	26.8 ± 3.9		26.7 ± 4.3	

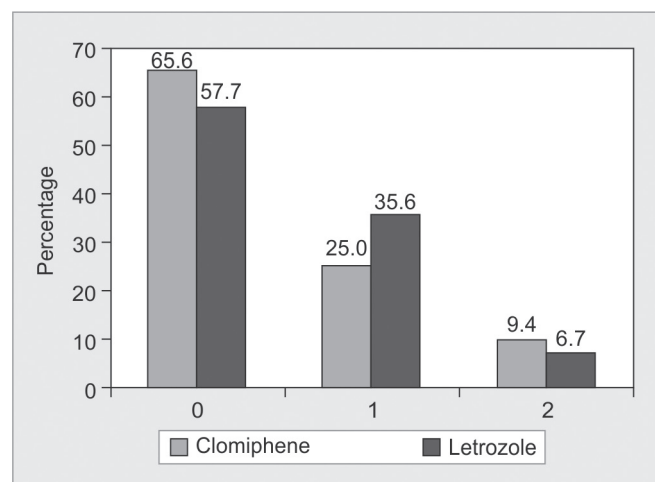
t = 0.22 p = 0.822



Graph 4: Comparison of age based on drug

Table 6: Comparison of parity based on drug

Parity	Drug				χ^2	p
	Clomiphene		Letrozole			
	Count	Percent	Count	Percent		
0	63	65.6	60	57.7	2.78	0.249
1	24	25.0	37	35.6		
2	9	9.4	7	6.7		



Graph 6: Comparison of parity based on drug

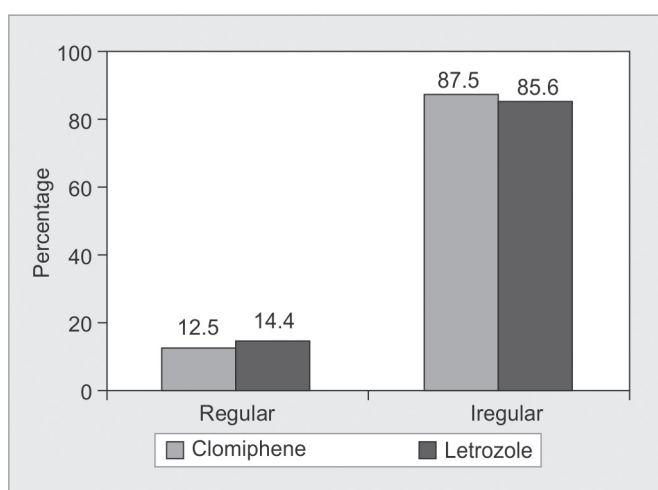
compared to 55.8% in the letrozole group. The p value was 0.204 showing no significant difference between the two groups.

History of Laparoscopy

In the clomiphene group, (Table 9 and Graph 9) 30.2% of patients had a prior history of having undergone lapa-

Table 7: Comparison of type of menstruation based on drug

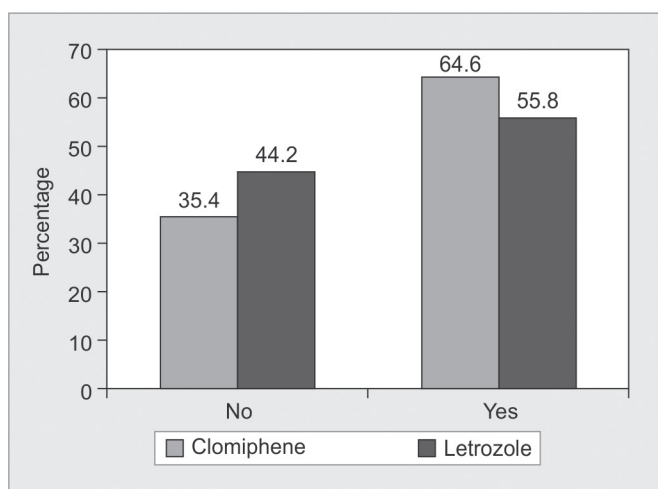
Type of menstruation	Drug				χ^2	p
	Clomiphene		Letrozole			
	Count	Percent	Count	Percent		
Regular	12	12.5	15	14.4	0.16	0.691
Irregular	84	87.5	89	85.6		



Graph 7: Comparison of type of menstruation based on drug

Table 8: Comparison of history of ovulation induction based on drug

History of Ovulation induction	Drug				χ^2	p
	Clomiphene		Letrozole			
	Count	Percent	Count	Percent		
No	34	35.4	46	44.2	1.62	0.204
Yes	62	64.6	58	55.8		



Graph 8: Comparison of history of ovulation induction based on drug

roscopy as part of infertility workup compared to 23.1% in the letrozole group. The p value of 0.254 showed no significant difference in the distribution of patients in the two groups.

History of Intrauterine Insemination

About 40.6% of patients (Table 10 and Graph 10) belonging to the clomiphene group had a history of having undergone IUI compared to 30.8% in the letrozole group, with a p value of 0.146 with no statistically significant difference between the two groups.

Body Mass Index

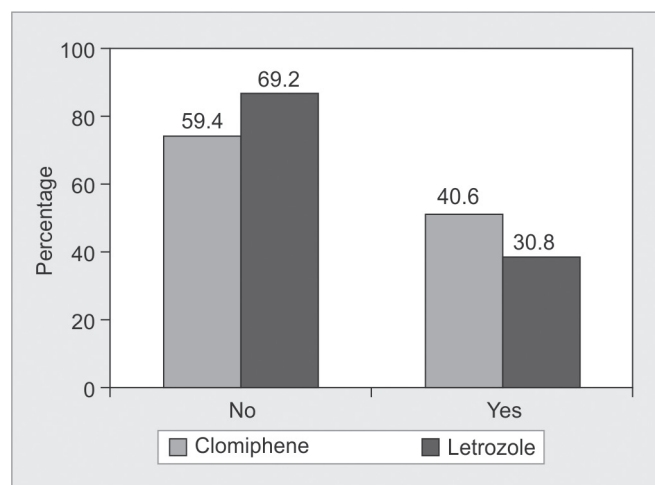
The percentage of patients (Table 11 and Graph 11) with BMI between 19 and 24.9; 25–29.9 and more than or equal to 30 was found to be 42.7%, 41.75 and 15.6%, respectively in the clomiphene group when compared to 41.35, 41.3% and 17.3%, respectively in the letrozole group. The p value was 0.198 showing no statistically significant difference between the two groups.

Hyperandrogenism

In the clomiphene group, (Table 12 and Graph 12) 81.3% of patients showed evidence of hyperandrogenism (in the form of acne, hirsutism) compared to 85.6% of patients in the letrozole group. The p value was 0.410 which was again not significant.

Table 9: Comparison of laparoscopy based on drug

Laparoscopy	Drug				χ^2	p
	Clomiphene		Letrozole			
	Count	Percent	Count	Percent		
No	67	69.8	80	76.9	1.3	0.254
Yes	29	30.2	24	23.1		



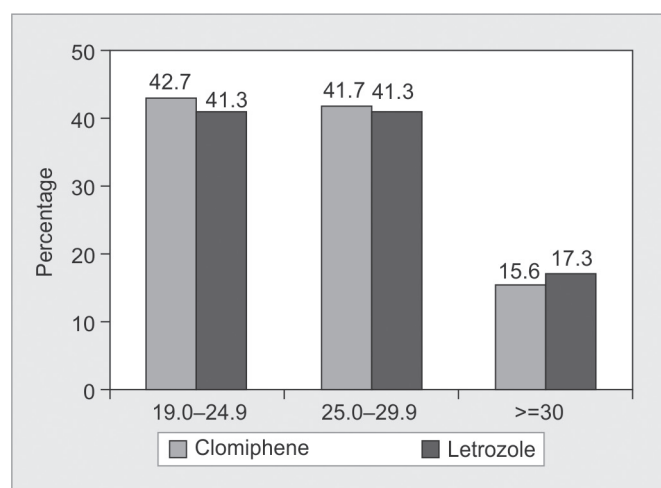
Graph 9: Comparison of laparoscopy based on drug

ULTRASOUND FEATURES

About 84.4% of patients (Table 13 and Graph 13) in the clomiphene group had ultrasonologically defined polycystic ovaries as compared to 80.8% in the letrozole group. The p value was 0.503, not statistically significant.

Table 10: Comparison of IUI based on drug

IUI	Drug				χ^2	p
	Clomiphene		Letrozole			
	Count	Percent	Count	Percent		
No	57	59.4	72	69.2	2.12	0.146
Yes	39	40.6	32	30.8		

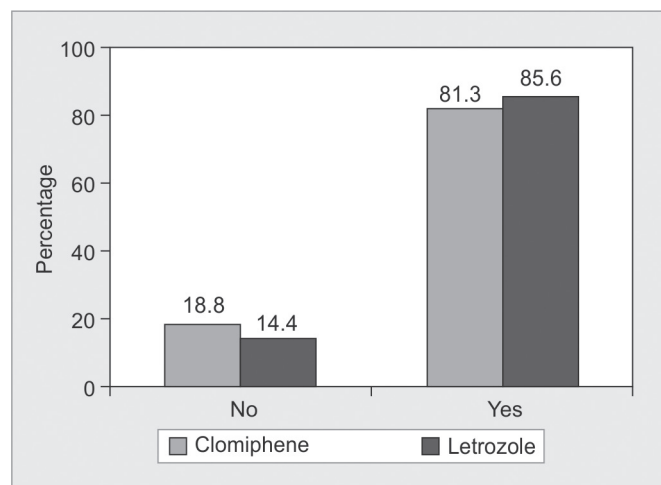


Graph 10: Comparison of IUI based on drug

Table 11: Comparison of BMI based on drug

BMI	Drug			
	Clomiphene		Letrozole	
	Count	Percent	Count	Percent
19.0–24.9	41	42.7	43	41.3
25.0–29.9	40	41.7	43	41.3
≥30	15	15.6	18	17.3
Mean \pm SD	25.6 \pm 3.6		26.3 \pm 4	

$t = 1.28$, $p = 0.198$



Graph 11: Comparison of BMI based on drug

LABORATORY FINDINGS

About 27.1% patients (Table 14 and Graph 14) in the clomiphene group had laboratory findings suggestive of hyperandrogenism compared to 39.4% in the letrozole group with a statistically insignificant p value of 0.065.

Number of Cycles In Which Pregnancy was Achieved

In the clomiphene group, (Table 15 and Graph 15) 30.2% patients achieved pregnancy in 1–2 cycles, 62.55% in 3–4 cycles and 7.3% in 5–6 cycles with a mean \pm standard deviation of $3 + 1.01$ when compared to 30.8%, 66.3% and 2.9% respectively in the letrozole group with a mean \pm standard deviation of 2.92 ± 0.89 . The p value was 0.566, again not statistically significant.

Follicle Size More than or Equal to 14 mm

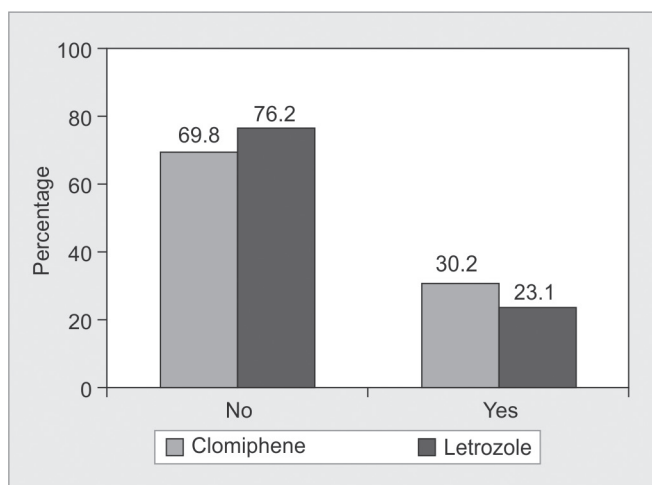
When compared for the total number of follicles more than or equal to 14 mm seen in the clomiphene group, (Table 16 and Graph 16) a mean \pm standard deviation of 2.54 ± 0.77 was seen compared to 1.39 ± 0.53 in the letrozole group. This had a p value of <0.01 , hence statistically significant. The clomiphene group had a statistically significant increase in the total number of follicles more than or equal to 14 mm, i.e., significant multifollicular development.

Monofollicular Development More than or Equal to 14 mm

About 60.2% of patients (Table 17 and Graph 17) in the letrozole group showed monofollicular development

Table 12: Comparison of Hyperandrogenism based on drug

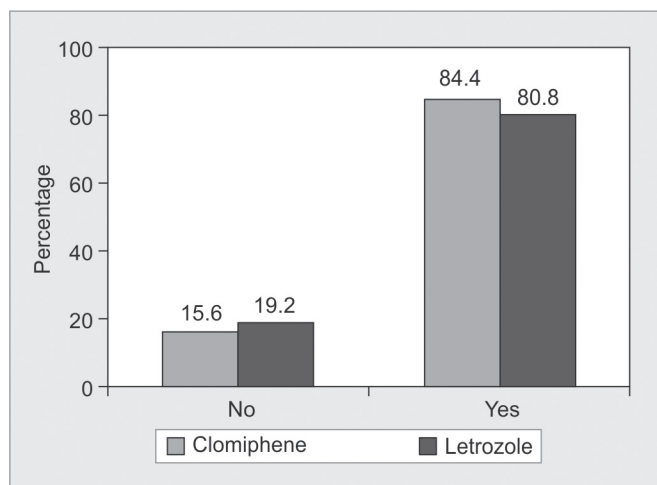
Hyperandrogenism	Drug				χ^2	p
	Clomiphene		Letrozole			
	Count	Percent	Count	Percent		
No	18	18.8	15	14.4	0.68	0.410
Yes	78	81.3	89	85.6		



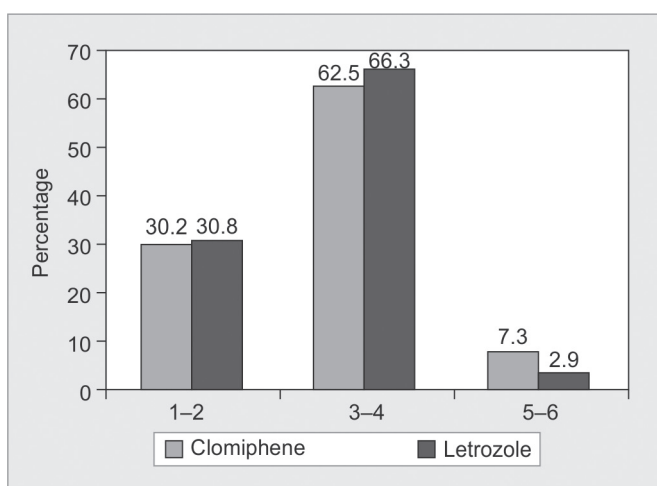
Graph 12: Comparison of hyperandrogenism based on drug

Table 13: Comparison of USS based on drug

USS	Drug				χ^2	p
	Clomiphene		Letrozole			
	Count	Percent	Count	Percent		
No	15	15.6	20	19.2	0.45	0.503
Yes	81	84.4	84	80.8		

**Graph 13:** Comparison of USS based on drugage**Table15:** Comparison of number of cycles based on drug

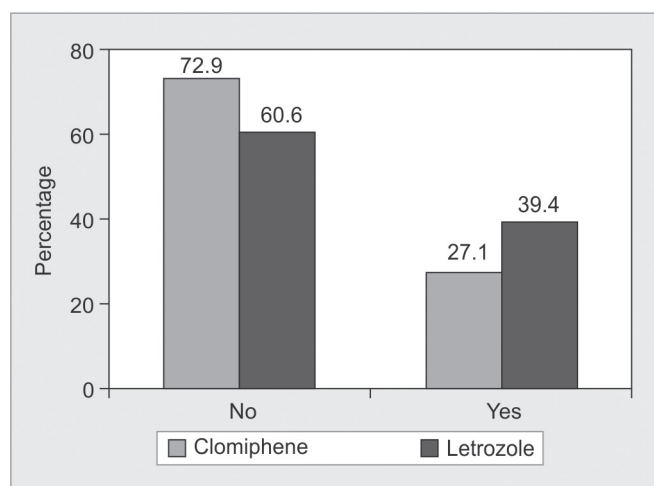
Number of cycles	Drug			
	Clomiphene		Letrozole	
	Count	Percent	Count	Percent
1–2	29	30.2	32	30.8
3–4	60	62.5	69	66.3
5–6	7	7.3	3	2.9
Mean \pm SD	3 \pm 1.01		2.92 \pm 0.89	

**Graph 15:** Comparison of number of cycles based on drug

compared to only 9.4% in the clomiphene group; whereas multifollicular development was seen in 90.6% patients in the clomiphene group compared to 39.8% of the letrozole group. The p value was 0.000. Hence, monofollicular development was found to be very highly statistically significant in the letrozole group, which is one of its major advantages.

Table 14: Comparison of laboratory test abnormalities based on drug

Laboratory test abnormalities	Drug				χ^2	p
	Clomiphene		Letrozole			
	Count	Percent	Count	Percent		
No	70	72.9	63	60.6	3.41	0.065
Yes	26	27.1	41	39.4		

**Graph 14:** Comparison of lab test based on drug**Table 16:** Comparison of number of follicles >14 mm based on drug

Number follicle >14 mm	Drug			
	Clomiphene		Letrozole	
	Count	Percent	Count	Percent
0	0	0.0	1	1.0
1	9	9.4	62	59.6
2	33	34.4	40	38.5
3	47	49.0	1	1.0
4	7	7.3	0	0.0
Mean \pm SD	2.54 \pm 0.77		1.39 \pm 0.53	

$t = 12.4, p < 0.01$

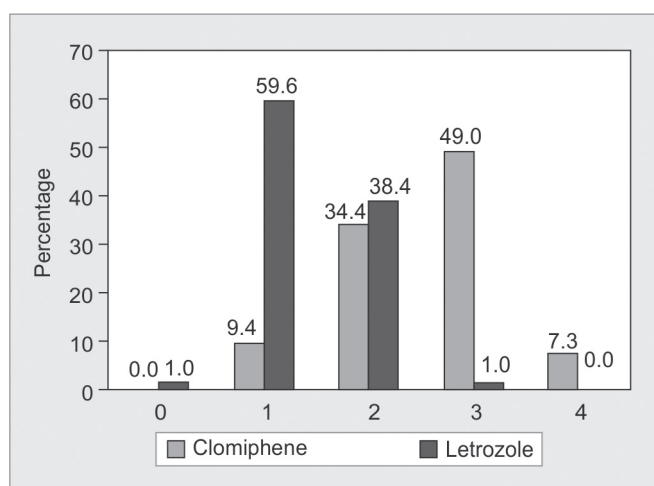
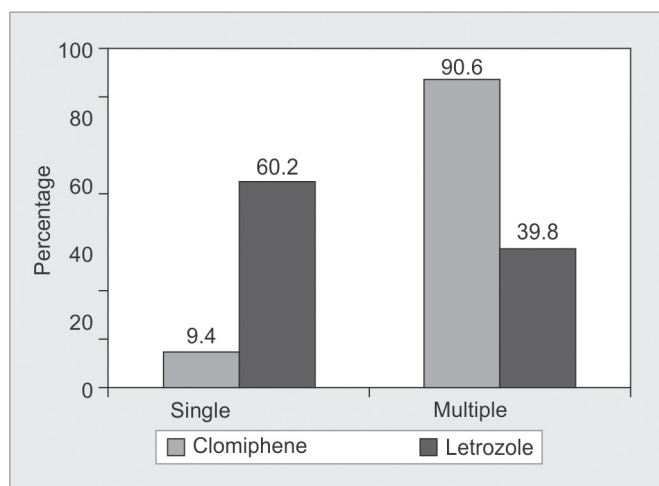
**Graph 16:** Comparison of number of follicles >14 mm based on drug

Table 17: Comparison of follicle ≥ 14 mm based on drug

Number follicle >14 mm	Drug				χ^2	p
	Clomiphene		Letrozole			
	Count	Percent	Count	Percent		
Single	9	9.4	62	60.2	55.9**	0.000
Multiple	87	90.6	41	39.8		

**Graph 17:** Comparison of follicle >14 mm based on drug

Follicle Size More than or Equal to 18 mm

When compared for the total number of follicles (Table 18 and Graph 18) more than or equal to 18 mm seen in the clomiphene group, a mean \pm standard deviation of 1.4 ± 0.7 was seen compared to 1.06 ± 0.6 in the letrozole group. This had a p value of <0.01 , hence statistically significant. The clomiphene group had a statistically significant increase in the total number of follicles more than or equal to 18 mm, i.e., significant multifollicular development.

Monofollicular Development More than or Equal to 18 mm

Eighty percent of patients (Table 19 and Graph 19) in the letrozole group showed monofollicular development compared to only 53.9% in the clomiphene group; whereas multifollicular development was seen in 46.1% patients in the clomiphene group compared to 20% of the letrozole group. The p value was 0.000. Hence monofollicular development was found to be very highly statistically significant in the letrozole group, which is one of its major advantages.

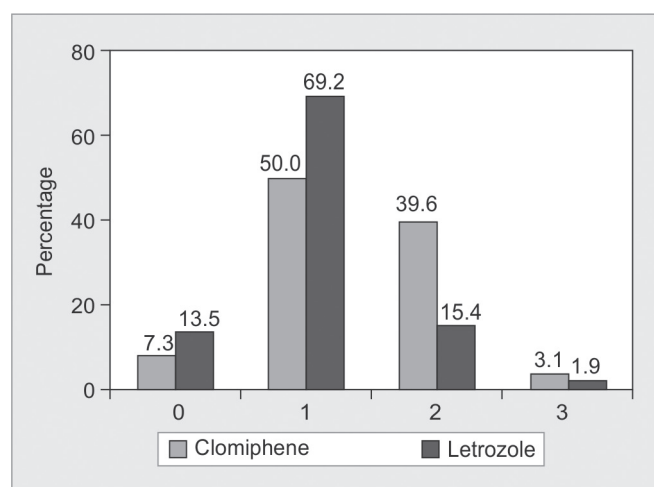
Size of Dominant Follicle

In the clomiphene citrate group, (Table 20 and Graph 20) the size of the dominant follicle was more than 15 mm in 13.5%, between 15 and 19.9 mm in 47.9% and between 20 and 24.9 mm in 33.3% of the patients. In the letrozole group the corresponding values were 11.5%, 39.4% and

Table 18: Comparison of number follicle ≥ 18 mm based on drug

Number follicle >18 mm	Drug			
	Clomiphene		Letrozole	
	Count	Percent	Count	Percent
0	7	7.3	14	13.5
1	48	50.0	72	69.2
2	38	39.6	16	15.4
3	3	3.1	2	1.9
Mean \pm SD	1.4 ± 0.7		1.06 ± 0.6	

$t = 3.63^{**}$ $p < 0.01$

**Graph 18:** Comparison of number follicle ≥ 18 mm based on drug

45.2% respectively. The mean size of the dominant follicle was 18 ± 3.9 mm in the clomiphene group as compared to 19.1 ± 3.5 mm in the letrozole group. The p value was 0.276 and hence deemed not statistically significant.

Number of Ovulations

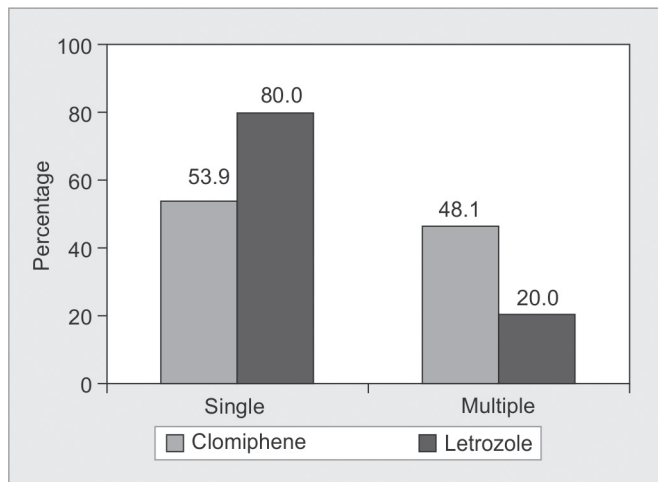
In the clomiphene group 7.3% achieved no ovulation, (Table 21 and Graph 21) 22.9% achieved ovulation in the first cycle, 35.4% in the second cycle, 28.1% in the third cycle and only 6.3 % in the fourth cycle. In the letrozole group, 1.9% achieved no ovulation, 22.1% in the first cycle, 47.1% in the second cycle, 26.0% in the third cycle and only 1.9 % in the fourth cycle. Overall 2.03 ± 1.03 ovulations occurred in the clomiphene group against 2.07 ± 0.85 in the letrozole group, with a p value of 0.787 which was statistically insignificant.

Ovulation Per Cycle

In the clomiphene group 11.5% of patients had less than 25% ovulation per cycle; (Table 22 and Graph 22) 17.7% between 25 and 50%; 31.3% between 50 and 75% and 39.6% achieved more than 75% ovulation per cycle. In the letrozole group, 2.9% of patients achieved ovulation in less than 25% cases per cycle; 25% in between 25 and 50%; 38.5% between 50–75% and 33.7 % achieved more

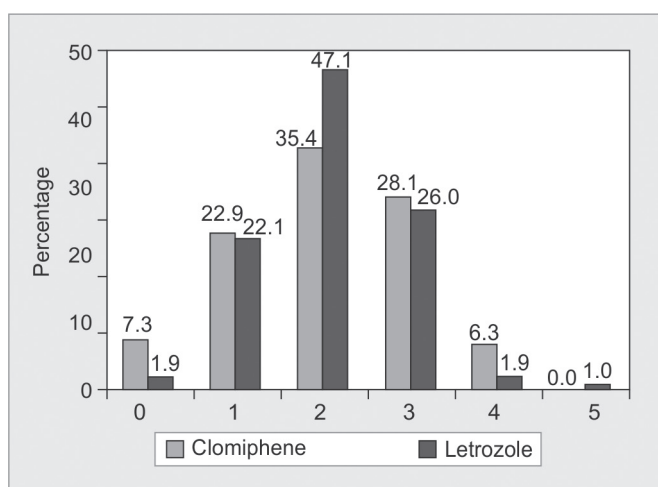
Table 19: Comparison of monofollicle ≥ 18 mm based on drug

Number follicles	Drug				χ^2	p
	Clomiphene		Letrozole			
	Count	Percent	Count	Percent		
>18 mm						
Single	48	53.9	72	80.0	13.76**	0.000
Multiple	41	46.1	18	20.0		

**Graph 19:** Comparison of monofollicle ≥ 18 mm based on drug**Table 21:** Comparison of number of ovulations based on drug

Number of Ovulations	Drug			
	Clomiphene		Letrozole	
	Count	Percent	Count	Percent
0	7	7.3	2	1.9
1	22	22.9	23	22.1
2	34	35.4	49	47.1
3	27	28.1	27	26.0
4	6	6.3	2	1.9
Mean \pm SD	2.03 \pm 1.03		2.07 \pm 0.85	

$t = 0.27$, $p = 0.787$

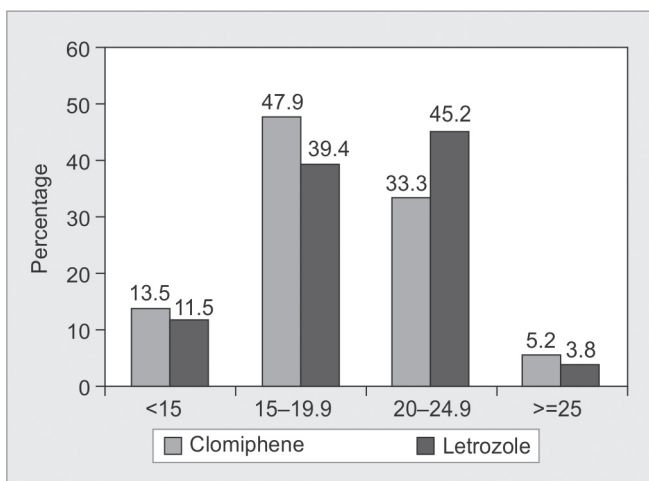
**Graph 21:** Comparison of number of ovulations based on drug

than 75% ovulation per cycle. On an average 69 ± 0.31 % patients achieved ovulation per cycle compared to 72 ± 0.25 % patients in letrozole group. The p value was

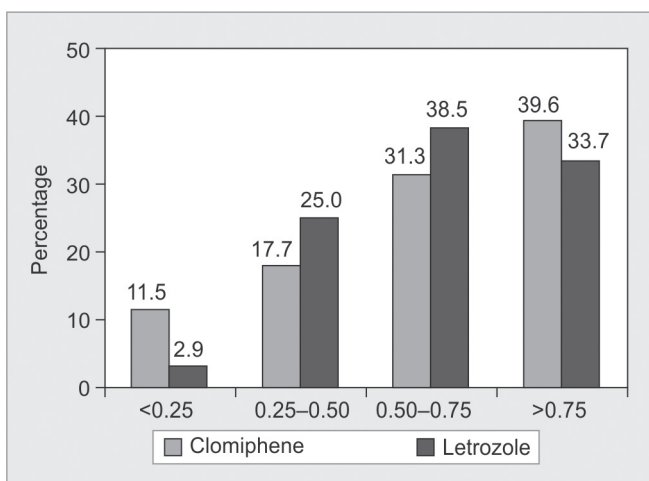
Table 20: Comparison of size of DF based on drug

Size of DF	Drug			
	Clomiphene		Letrozole	
	Count	Percent	Count	Percent
<15	13	13.5	12	11.5
15-19.9	46	47.9	41	39.4
20-24.9	32	33.3	47	45.2
Mean \pm SD	18.6 \pm 3.9		19.1 \pm 3.5	

$t = 1.09$, $p = 0.276$

**Graph 20:** Comparison of size of DF based on drug**Table 22:** Comparison of ovulation per cycle based on drug

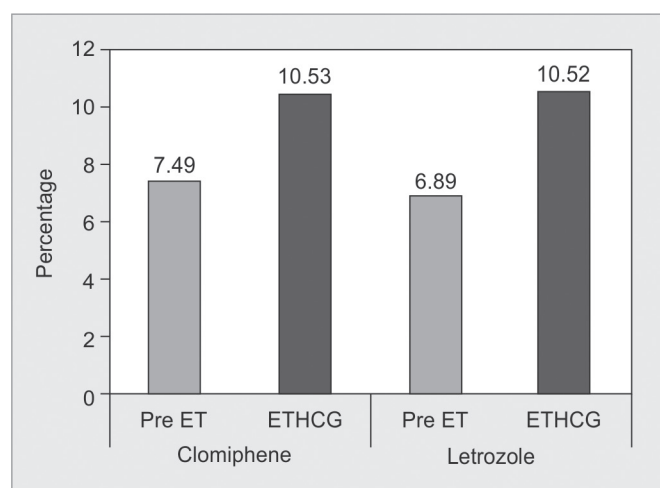
Ovulation per cycle	Drug			
	Clomiphene		Letrozole	
	Count	Percent	Count	Percent
<25 %	11	11.5	3	2.9
25-50 %	17	17.7	26	25.0
50-75 %	30	31.3	40	38.5
>75 %	38	39.6	35	33.7
Mean \pm SD	69 \pm 0.31		72 \pm 0.25	

**Graph 22:** Comparison of ovulation per cycle based on drug

0.526, hence the difference between the two groups did not transform into statistical significance.

Table 23: Effectiveness of drug on ET

Drug	Stage	Mean	SD	N	Mean difference	Paired t	p
Clomiphene	Pre-hCG	7.49	2.76	96	3.03	14.66	0.000
	After-hCG	10.53	3.27	96			
Letrozole	Pre-hCG	6.89	2.32	104	3.64	21.56	0.000
	After-hCG	10.52	2.79	104			

**Graph 23:** Effectiveness of drug on ET

Endometrial Thickness

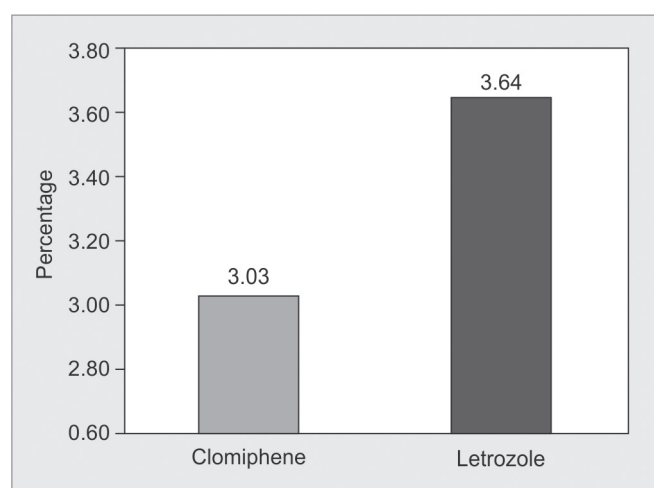
The endometrial thickness is defined to be favorable when it is at least more than 8 mm. (Table 23 and Graph 23) The endometrial thickness before and at the day of hCG administration following the use of either drug was compared. It was found to be 7.49 ± 2.76 pre hCG and 10.53 ± 3.27 on the day of hCG in the clomiphene group compared to 6.89 ± 2.32 pre hCG and 10.52 ± 2.79 on the day of hCG in the letrozole group. The paired t values were 14.66 in the clomiphene group and 21.56 in letrozole group with a p value of 0.000 which is deemed to be highly statistically significant (Table 24 and Graph 24). The mean difference achieved in endometrial thickness with the use of the drugs was 3.03 in the clomiphene group and 3.64 in the letrozole group. The p value was 0.024 which was highly statistically significant.

Number of Pregnancies

In the clomiphene group, (Table 25 and Graph 25) 46.9% achieved no pregnancy, 44.8% achieved pregnancy in at least one cycle, 5.2% in 2 cycles, 3.1% in 3 cycles. In the letrozole group 45.2% achieved no pregnancy, 50.0% achieved pregnancy in at least one cycle, 4.8% in 2 cycles, 0.0% in 3 cycles. The total pregnancies achieved was marginally higher in the letrozole group compared to the clomiphene group, however, the p value was 0.593 hence the difference was not found to be statistically significant.

Table 24: Comparison of increase in ET based on drug

Drug	Mean	SD	N	t	p
Clomiphene	3.03	2.03	96	2.27	0.024
Letrozole	3.64	1.72	104		

**Graph 24:** Comparison of increase in ET based on drug

Pregnancy Per Cycle

In the clomiphene group less than 25% pregnancy rate was achieved per cycle in 58.3%; (Table 26 and Graph 26) between 25–50% in 30.2%; between 50 and 75% in 6.3 %, more than 75% in 5.2%, with a mean pregnancy achieved per cycle in $24 \pm 0.28\%$ of patients. In the letrozole group less than 25% pregnancy rate was achieved per cycle in 56.7%; between 25 and 50% in 34.6%; between 50 and 75% in 1.9 %, more than 75% in 6.7% with a mean pregnancy achieved per cycle in $24 \pm 0.28\%$ of patients. The p value was 0.945 and hence found to be statistically insignificant.

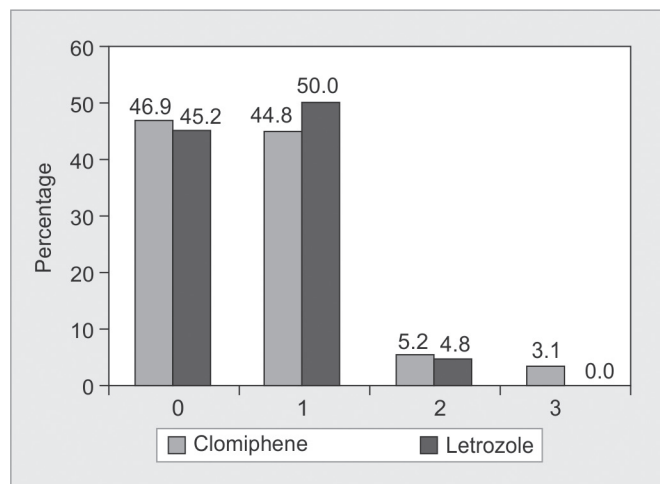
Rate of Miscarriages

In the clomiphene group in 83.3% patients, (Table 27 and Graph 27) there was no pregnancy loss; in 12.5 % the pregnancies achieved per cycle ended in at least miscarriage, and in 4.2% the pregnancies achieved ended in miscarriage in 2 or more cycles.

In the letrozole group in 84.6% patients, there was no pregnancy loss; in 14.4% the pregnancies achieved per cycle ended in at least miscarriage, and in 1.0% the pregnancies achieved ended in miscarriage in 2 or more cycles.

Table 25: Comparison of Number of pregnancy based on drug

Number of pregnancy	Drug			
	Clomiphene		Letrozole	
	Count	Percent	Count	Percent
0	45	46.9	47	45.2
1	43	44.8	52	50.0
2	5	5.2	5	4.8
3	3	3.1	0	0.0
Mean \pm SD	0.65 \pm 0.73		0.6 \pm 0.58	

**Graph 25:** Comparison of number of pregnancy based on drug**Table 27:** Comparison of number of miscarriages based on drug

Number of abortion	Drug			
	Clomiphene		Letrozole	
	Count	Percent	Count	Percent
0	80	83.3	88	84.6
1	12	12.5	15	14.4
2	4	4.2	1	1.0
Mean \pm SD	0.21 \pm 0.5		0.16 \pm 0.4	

$t = 0.7, p = 0.482$

On an average in $0.21 \pm 0.5\%$ cases the pregnancy terminated in miscarriage in clomiphene group compared to $0.16 \pm 0.4\%$ cases in the letrozole group.

The p value of 0.482 did not show any statistically significant difference.

DISCUSSION

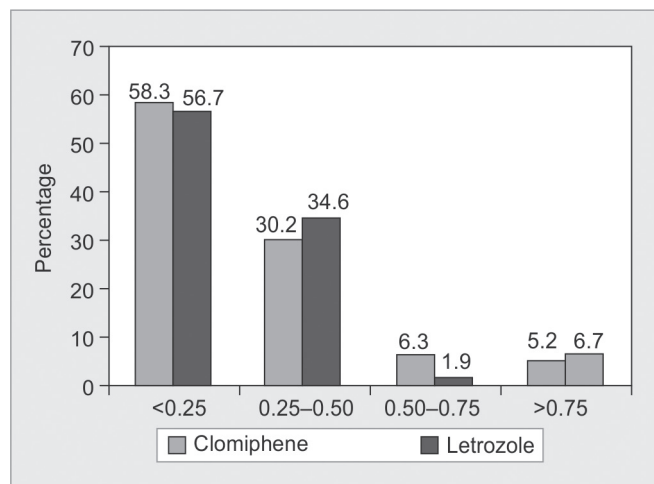
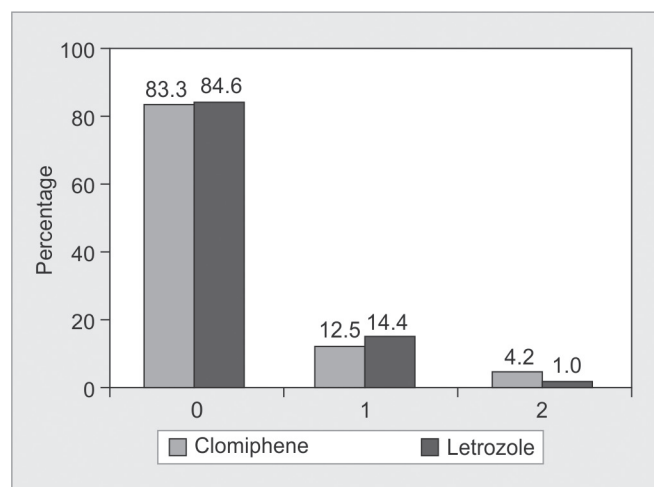
This study was conducted in a tertiary care multidisciplinary corporate hospital. As a result, only patients belonging to higher socioeconomic status were studied.

- **Follicular number and size:** In our study compared with letrozole, the use of clomiphene led to a significant increase in the number of developing and mature follicles (14 and 18 mm follicles). However monofollicular development was highly statistically significant in the letrozole group.

Table 26: Comparison of Pregnancy per cycle based on drug

Pregnancy per cycle	Drug			
	Clomiphene		Letrozole	
	Count	Percent	Count	Percent
<25 %	56	58.3	59	56.7
25 – 50 %	29	30.2	36	34.6
50 – 75 %	6	6.3	2	1.9
>75 %	5	5.2	7	6.7
Mean \pm SD	24 \pm 0.28		24 \pm 0.28	

$t = 0.07, p = 0.945$

**Graph 26:** Comparison of pregnancy per cycle based on drugage**Graph 27:** Comparison of number of abortion based on drug

Endometrial Thickness

The endometrial thickness achieved after letrozole was found to be highly significant statistically.

Mitwally and Casper showed a similar effect of letrozole on the endometrium.⁵² Cortinez et al.⁵³ found normal morphological features of the endometrium and full expression of pinopodes during the implantation window when letrozole was used. On the other hand, no significant difference was noted in other studies in regard to the effect of either drug on the endometrium.^{54,55}

Al Fouzan et al.⁵⁶ reported better results in the letrozole group [for the number of developing and mature follicles] than in the clomiphene citrate group.

The size of the dominant follicle achieved in either group also lacked statistical significance.

- A number of cycles needed to achieve pregnancy: There was no statistically significant difference in the number of cycles needed to achieve pregnancy in either cycle. The ovulation rate per cycle was also not statistically significantly different in the two groups
- Pregnancy rate: In our study, the greater number of mature follicles in the clomiphene group did not result in increased pregnancy rate. On the contrary, the number of pregnancies achieved was marginally higher in the letrozole group, but the difference was not found to be statistically significant. The total number of pregnancy per cycle, however, showed no difference between the two groups

The total number of pregnancy losses was also similar in either group. Bayar et al. reported similar results in women with ovulatory infertility.⁵⁷

There was one quadruplet gestation and one twin gestation in the clomiphene group (0.02%). There was no instance of multiple gestations in the letrozole group. However, this data on multiple gestations was not sufficient for an analysis hence no statistical significance could be attached to the same.

In another study, Mitwally and Casper^{48,58} reported improved response to exogenous FSH stimulation with letrozole cotreatment in poor ovarian responders. Mitwally et al.⁵⁹ reported similar findings; although endometrial thickness was decreased, no negative effect on pregnancy rates was noted in this study. However, when letrozole was compared with clomiphene combined with gonadotrophin, Nejad and Bedaiwy et al.^{60,61} reported that the number of mature follicles and serum E2 level on the day of hCG administration was statistically significantly lower in the letrozole group than the clomiphene group.

Information on the teratogenic capacity of letrozole in humans is lacking, but animal studies have shown that low doses of letrozole are effective in inducing noxious effects on the developing conceptus.⁴⁷ In the rat, teratogenic effects, including fetal domed head and cervical/centrum vertebral fusion, resulted from exposure to 0.03 mg/kg. In the rabbit, embryotoxic effects were induced by exposure to 0.002 mg/kg (approximately 1/100,000 the daily maximum recommended human dose on an mg/m² basis). Mitwally et al.⁵⁹ reported favorable pregnancy outcomes and low multiple gestation rates with aromatase inhibitors for ovulation stimulation. Clomiphene citrate has been reported to elicit various ocular side effects in 1.5–10% of patients.⁵⁸

The cost of letrozole is much higher than clomiphene citrate especially when higher doses of letrozole are

required (Rs 33 per 2.5 mg tablet of letrozole compared to Rs 14.50 per 100 mg tablet of clomiphene citrate).

Bedaiwy et al.⁶¹ have suggested that a letrozole-FSH combination would be more cost effective than FSH alone for ovulation stimulation in intrauterine insemination cycles.

There was no instance of ovarian hyperstimulation syndrome in either group.

There were no incidences of congenital anomalies seen in either group.

CONCLUSION

Our study found the following advantages namely better endometrial thickness, monofollicular development, slightly higher pregnancy rates (though not statistically significant), no multiple gestations in the use of letrozole rather than clomiphene as a first line treatment for inducing ovulation in women with PCOS. There were no incidences of OHSS or congenital malformations in either group.

RECOMMENDATIONS

Letrozole can be used as a first line drug for ovulation induction in women with PCOS, who are at increased risk of hyperstimulation and multiple gestations with drugs like clomiphene citrate and gonadotropins. The doses for letrozole which have been widely studied are 2.5 mg twice daily or 5 mg once daily.

Further studies in this field are however warranted with regard to determining the optimum dose of letrozole, definitive evidence to rule out teratogenic effect of letrozole, if any, means to make the treatment with letrozole more cost-effective and whether or when to combine letrozole with gonadotropins with the aim of reducing the cost as well as increasing the success of induction.

Studies related to the same are underway around the world, to solve the above problems and other issues related to the bane of infertility.

The final word of the ideal drug for ovulation induction in women with polycystic ovarian syndrome, however, remains yet to be said!

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