

## Clomiphene citrate or Letrozole as a first line treatment of anovulatory sub fertile polycystic ovarian syndrome women

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### Abstract

**Background :** PCOS is associated with anovulation and a common cause of infertility affecting 4-6 % women in reproductive age , Clomiphene citrate has traditionally used as a drug of choice in treating women with anovulatory PCOS , in the last decade letrozole , an aromatase inhibitor has emerged as an alternative ovulation induction agent .

**Objective :** to compare letrozole and clomiphene citrate as an ovulation induction drug in infertile PCOS women .

**study design :** prospective study .

**Patient and method :** PCOS women were treated randomly with 5mg letrozole or 100mg clomiphene citrate day 2 to day 6 menstrual cycle .

**Main outcome :** ovulation rate , mono or multi follicular rate , days to ovulation , endometrial thickness , serum estrogen and pregnancy rate .

**Results:** *there is statistically significant LH and prolactin levels were significantly higher in Letrozole group but still within normal levels, and letrozole group was higher in number of responsive when compared with CC group but still not significant*

*In letrozole group the number of monofollicle cycle was higher (69.57%) and multifollicles cycle was low (30.43%) when are comparing with that of CC group.*

**Conclusion:**

Letrozole in patients with PCOS is as effective as Clomiphene citrate in inducing ovulation , ovulation rate and the incidence of pregnancy was higher with letrozole than that with Clomiphene citrate.

Apart from that, Letrozole treatment is prone to a production of monofollicles and hence leads to reduced incidences of the adverse pregnancy outcome of multiple fetuses as compared to treatment with Clomiphene citrate.

**Aim of the study**

This is prospective randomized central trial drugs study was carried out to compare the effects of 5mg of letrozole with 100mg CC as ovulation induction drug in treatment of sub fertile PCOS women.

### Introduction

PCOS was first described by Dr. Stein & leventhal in 1935, it is the most common cause of female infertility and ovulatory dysfunction, it is a syndrome of ovarian dysfunction along with cardinal features of hyperandrogenism and polycystic ovary morphology. Its clinical manifestations may include menstrual irregularities, signs of androgen excess and obesity, insulin

resistance and elevated serum LH levels are also common features in PCOS (1). **prevalence:-** (4-12%). The prevalence of polycystic ovaries seen on ultrasound is much higher, around 25% in normal and 87% in patients with oligomenorrhea. (1)

### Pathophysiology

The etiology of PCOS remain unclear. Women with PCOS have abnormalities in the metabolism of androgens and

estrogen and the control of androgen production . High serum concentrations of androgenic hormones , such as testosterone , androstenedione, and dehydroepiandrosterone sulfate (DHEA\_S) may be encountered in these patients however , individual variation is considerable, and a particular patient might have normal androgen levels.(2)

PCOS is also associated with peripheral insulin resistance and hyper-insulinemia , and obesity amplifies degree of both abnormalities . Insulin resistance in PCOS can be secondary to a post binding defect in insulin receptors signaling pathways , and elevated insulin levels may have gonadotropin–augmenting effect on ovarian function . Hyperinsulinemia may also result in suppression of hepatic generation of sex hormone – binding globulin (SHBG) , which in turn may increase androgenicity.(3)

A proposed mechanism for anovulation and elevated androgen levels suggests that , under the increased stimulatory effect of luteinizing hormone (LH) secreted by the anterior pituitary ( a result both of disordered ovarian-pituitary feedback and exaggerated pulses of GnRH from the hypothalamus) stimulation of the ovarian theca cells is increased , these cells in turn increase the production of androgens (e.g. testosterone , androstenedione). Because of the decrease level of (FSH) follicle stimulation hormone relative to LH, the ovarian granulosa cells cannot aromatize the androgens to estrogens, which leads to a decreased estrogen levels and consequent anovulation (2).

(GH) growth hormone and insulin like growth factor -1(IGF-1) may also augment the effect on ovarian function. Some evidence suggests that patients have a functional abnormality of cytochrome P450c17 , the 17

hydroxylase , which is the rate-limiting enzyme in androgen biosynthesis.

PCOS is genetically heterogeneous syndrome in which the genetic contribution remain incompletely described , PCOS is an inherently difficult condition to study genetically because of its heterogeneity. Studies of family members with PCOS revealed that about 50% of first-degree relatives have PCOS suggesting that an autosomal dominant mode of inheritance occurs for many families with this disease.(4)

### Clinical features (5)

Oligomenorrhea / amenorrhea (65-75%)

Hirsutism (30-70%)

Sub-fertility(75%)

Obesity (40%) and metabolic syndrome.

Diabetes .

Obstructive sleep apnea .

Acanthosis nigricans (2%)

### Diagnostic criteria

A 1990 expert conference sponsored by the National Institute of Child Health and Human Development (NICHD) of the united states national institutes of health (NIH) proposed of the following criteria for the diagnosis of PCOS:-

- 1- Oligo-ovulation or anovulation manifested by oligomenorrhea or amenorrhea.
- 2- Hyperandrogenism (clinical evidence of androgen excess) or hyperandrogenemia (biochemical evidence of androgen excess)
- 3- Exclusion of other disorders that can result in menstrual irregularity and hyperandrogenism .(6)

\* In (2003) the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM)

recommended that at least 2 of the following 3 features are required for PCOS to be diagnosed :

1- Oligo- ovulation or anovulation manifested as oligomenorrhea or amenorrhea.

2-Hyperandrogenism:- clinical and /or biochemical evidence of androgen excess commonly Hirsutism, acne and baldness.

3-polycystic ovaries as defined on ultrasonography . (7)

PCOS encompasses a broad spectrum of clinical and biochemical characteristics and the following findings on investigation are supportive of a diagnosis of PCOS.

#### **Laboratory tests**

- 1- Elevated androstenedione and free testosterone levels.
- 2- Elevated LH levels and LH/FSH ratio 2:1 or 3:1.
- 3- GnRH stimulation test.
- 4- Impaired GTT in 35%.
- 5- Increased fasting insulin levels.
- 6- Decreased SHBG levels.(2)

#### **Imaging tests**

Ovarian ultrasound preferably using trans-vaginal approach:- US

Criteria has been defined also by Rotterdam consensus as an ovary with 12 or more follicles 2-9 mm in diameter and increased ovarian stroma and volume >10 ml. It was postulated that the hyperandrogenic environment within PCO results in an increased

recruitment of growing follicles of 2-5 mm, followed by the arrest at 6-9 mm.(8)

Pelvic CT-scan or MRI to visualize the adrenals and ovaries .

#### **Differential Diagnosis**

Exclude all other disorders that can result in menstrual irregularity and hyper-androgenism including adrenal or ovarian tumors , thyroid dysfunction, congenital adrenal hyperplasia , hyper-prolactinemia , acromegaly and Cushing syndrome . So baseline screening laboratory studies for women suspected of having PCOS include the following:

- 1- Thyroid function test (e.g. TSH, free thyroxin)
- 2- Serum prolactin level.
- 3- Total free testosterone level.
- 4-Free androgen index.
- 5-Serum HCG level.
- 6-Cosyntropin stimulation test .
- 7-Serum 17- hydroxyprogesteron (17 OHPG) level.
- 8-Urinary free cortisol (UFC) and creatinine level.
- 9-Low dose dexamethasone suppression test.
- 10-Serum insulin like growth factor (IGF-1) level.(2)

#### **MANAGEMENT of PCOS**

##### **Treatment of Anovulation in polycystic ovaries syndrome .**

**1-Weight loss:-** obesity is observed in more than (50) percent of women with PCOS.The body fat is usually deposited centrally (android obesity ). Normalization of menstrual cycle and ovulation could occur with weight

reduction include diet and life style modification (5) .

### **2- improvement in metabolic function**

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Polycystic ovarian syndrome is associated with metabolic derangements, and treatment of this leads to resumption of ovulation . Insulin resistance is thought to play an important role in the pathogenesis of PCOS in the subset of patient who have either incremental (BMI ) or hyperinsulinemia and /or significant hyperandrogenism . Medications that have been tested in patients with PCOS include metformin, thiazolidine and ions , vitamin D and statins (7) .

### **3- Ovulation induction Agents:-**

The recommended first choice of treatment remains clomiphene citrate (CC) , if CC use fails to result in pregnancy ; the recommended second line intervention is the exogenous gonadotropins or laparoscopic ovarian drilling ( LOD).

### **Clomiphene citrate**

It was first synthesized in 1956, and it was approved for infertility treatment by the United States Food and Drug Administration (FDA) in 1967. It is available as a racemic mixture of two stereo isomers, En(Trans)-clomiphene and Zu(Cis) clomiphene , its zu-isomer exists in tissues for weeks and contributes to its metabolic life where are en- isomer is more potent one and is responsible for its biological action. It is non-steroidal triphenylethylene derivative that exhibits estrogen receptors antagonist properties, most commonly used as first line treatment for IO in women with anovulatory PCOS. CC is readily absorbed with half life of 5 days, and primarily excreted in the feces.(9)

### **Mechanism of action :-**

Clomiphene exerts its major effects on the hypothalamus, pituitary, ovary and uterus .

### **Hypothalamus and pituitary:-**

Most evidence suggests that primary site of CC action is the hypothalamus where it appears to bind to hypothalamus estrogen receptors there by blocking the negative feedback effect of circulating estrogen as a consequence, GnRH pulsatility and gonadotrophin secretion are enhanced, which further results in the stimulation of follicular recruitment, selection, assertion of dominance, and rupture. In vitro data suggest that CC also has a pituitary site of action where it causes an increase in the gonadotrophin response to GnRH.(10)

### **Ovary :-**

The ovarian action of CC are for the most part secondary to the effects of elevated FSH and LH on ovarian follicular development . Direct effects of CC on the ovary are not well understood but probably exist .

### **Uterus and cervix:-**

Clomiphene citrate acts primarily as an anti-estrogen in the uterus , cervix and vagina this may at least partially explain the low pregnancy rates observed cycles . Some investigators have observed significantly decreased sonographic endometrial thickness and altered morphometric endometrial histology (decreased gland number and increased vacuolated cells) in clomiphene cycles. Data on the effect of CC on cervical mucosa are conflicting; while one study found a decrease in the quality and quantity of cervical mucosa at all CC doses, in a meta –analysis , detrimental effect was seen only with doses greater than or equal to 100 mg/day(2) .

### **Indications (2)**

- **Anovulation:-** is the most important indication for CC treatment . In addition, treatment is indicated for women with oligomenorrhea, or amenorrhea, who responded to progesterone treatment with withdrawal bleeding. These women belong to the WHO group II, the majority of these women have the characteristics of PCOS. However, given its hypothalamic site of action, CC ineffective in women with hypogonadotropic hypogonadism (WHO group I).
- **Luteal phase deficiency:-** progesterone levels are typically higher after CC treatment than in spontaneous cycles, reflecting improved preovulatory follicle and corpus luteum development.
- **Unexplained infertility:-** In couples whose infertility remains unexplained, empiric treatment with CC may be justified, particularly in young couples with a short duration of infertility.
- **IVF:-** when multiple follicle development is required.

### **Doses**

The starting dose of CC generally should be 50 mg/day for 5 days, but a dose of 25 mg can be considered for lower weight patients, while a dosage of 100 mg may be more appropriate for obese patients, starting from cycle day 2-5 of a spontaneous menstruation or after progestin induced withdrawal bleeding . CC commenced on day 2 of the menstrual cycle rather than day 5 , result in more rapid follicular growth , a longer CC-free period before ovulation . The dose of CC is increased by 50 mg in every subsequent cycle in the absence of ovulation. Maximum dose of CC which has been reported to be used is 250mg/ day , however the recommended maximum dose is 150 mg/day as there is no clear evidence of efficacy at higher doses and this is in

accord with FDA recommendation of 750mg/ treatment cycle.(1)

### **Monitoring**

The following procedures may be used for monitoring:

- Follicular monitoring with vaginal ultrasound, starting 4-6 days after last pill. Serial transvaginal ultrasound can reveal the size and number of developing follicles.
- Serum estradiol levels, starting 4-6 days after last pill.
- Urine LH surge tests started 3-4 days after last clomiphene pill and continue until ovulation is indicated ( positive test) or through cycle day 18.
- Mid-luteal progesterone, with at least 10ng/ml 7-9 days after ovulation being regarded as adequate.

The practice in many centers is to monitor the first cycle by follicle monitoring or urinary LH surge to allow adjustment of the dose in subsequent cycle. In the absence of complete cycle monitoring an ultrasound is recommended to evaluate ovarian and endometrial morphology on the start of CC. There is no evidence that administrate of (HCG) in mid cycle improves the chances of conception.(11)

### **Results**

Comiphene citrate is a very efficient ovulation inductor ; approximately 75-80% of patients with PCOS will ovulate after CC. However, there appears to be discrepancy between ovulation and pregnancy rates and life table analysis of largest and most reliable studies indicates a conception rat of only up to 22% per cycle in those ovulating on CC.

This discrepancy occurs due to:-

- peripheral anti-estrogenic action on the endometrium.
- Antiestrogen effects on the cervical mucus.

- Decrease of uterine blood flow.
  - Impaired placental protein synthesis.
  - Effect on tubal transport.
  - Detrimental effects on the oocytes.(8)
- Most pregnancy resulting from OI with CC occur during the first 6 month of the therapy, so treatment generally should be limited to six ovulatory cycle and in absence of pregnancy second line therapy should be considered.
- Cumulative live birth rates vary between 50% and 60% for up to six cycles. (12)

"Clomiphene citrate resistance" means PCOS women do not ovulate in response to the highest dose of CC, while if CC fails to achieve a pregnancy despite ovulation called "clomiphene failure". Option for women not response to increasing dose of CC include concomitant use of insulin sensitizers , gonadotrophin, and LOD. Alternative CC regimens have been developed including extended use of CC, pretreatment with oral contraceptives, and adding dexamethasone (10) .

#### ***Side effects***

Common problems associated with CC are :

- 1- Vasomotor flushes 10% .
- 2- Bloating, abdominal distention 5% .
- 3- Nausea and vomiting 2%.
- 4- Visual symptoms (scotomas ) CC should be discontinued promptly.
- 5- Dryness of the vagina .

#### **Letrozole (Femara tradename)**

Is an oral non steroidal aromatase inhibitor drug for the treatment of hormonally–responsive breast cancer after surgery.

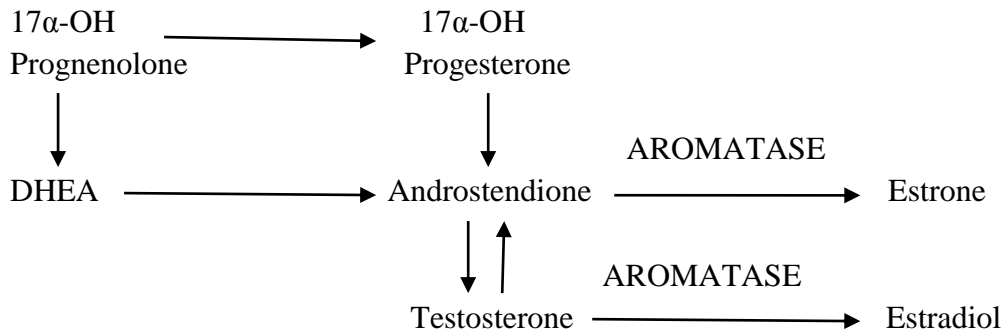
- 6- Headache .(8)
- 7- Significant ovarian enlargement in 5% and ovarian hyperstimulation rare <1%.(13)
- 8- Multiple pregnancy 7- 10% .
- 9- Clomiphene and ovarian malignancy :- The mechanism responsible for initiation of malignancy is possibly repetitive trauma of ovarian surface and exposure of this surface to estrogen rich follicular fluid , which may stimulates mitotic cell proliferation. The risk of ovarian cancer after CC therapy increases with the number of treatment cycles, when only six CC cycles are applied the risk of ovarian cancer will not exceed that of other women, but more than 12 cycles of use in a life time has been associated with increase in risk of future ovarian cancer (relative risk RR=1.5-2.5) . Therefore after 6-9 cycles of CC use this drug should preferably be discontinued (14) .

10- The evidence of fetal and neonatal abnormalities for patients on CC is similar to that seen in the general population. There is no data to suggest a higher rate of congenital anomalies or spontaneous abortions after using this drugs.

#### ***Contraindications***

- 1- Ovarian cyst .
- 2- Pregnancy .
- 3- Liver disease .
- 4- Abnormal uterine bleeding.
- 5- History of breast cancer(15) .

### How do aromatase inhibitors work?



In contrast to the central actions of CC and tamoxifen, letrozole acts in the periphery to inhibit ovarian follicular estradiol production (2).

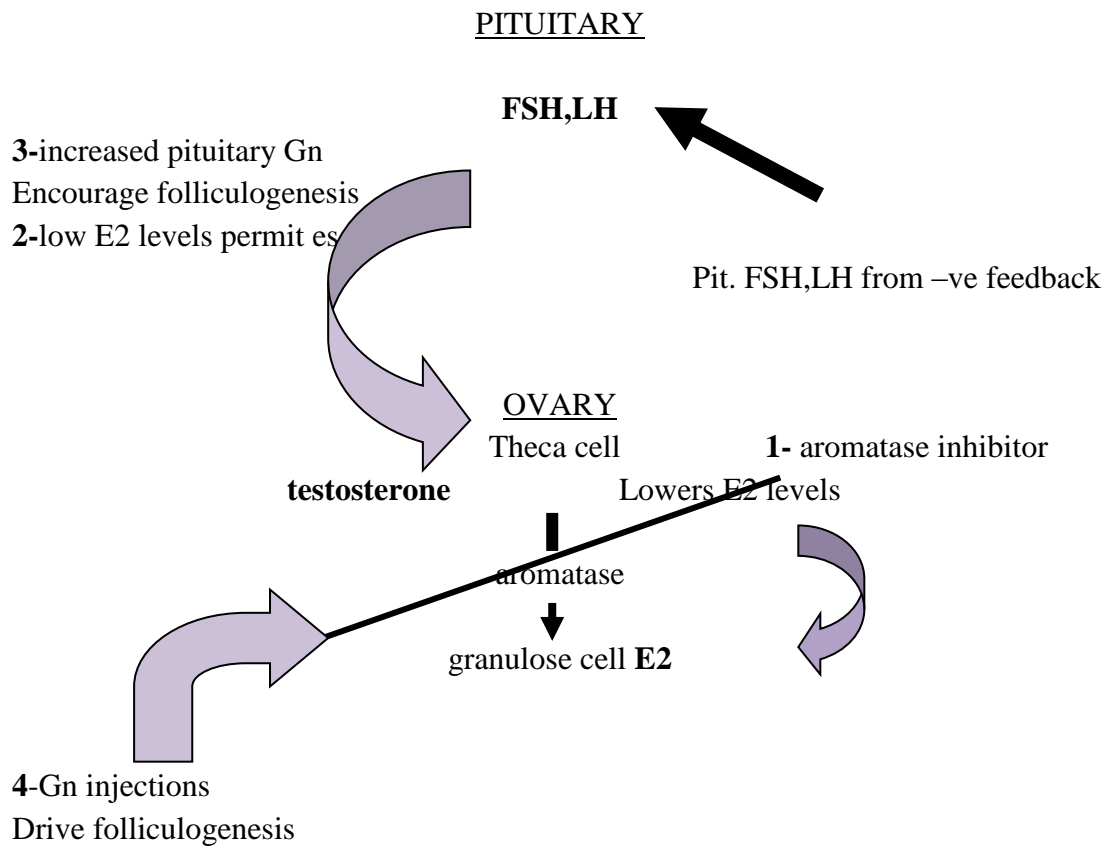
Estrogens are produced by the conversion of androgens through the activity of the enzyme aromatase. E2 produced by the ovary in turn exerts a negative feedback effect (inhibit) on FSH release from the hypothalamic-pituitary axis (16). When letrozole blocks aromatase activity by competitively binding to the heme of the aromatase-cytochrome P450 subunit of the enzyme, resulting in a drop in estrogen biosynthesis in all tissues where it is present, and release of the hypothalamic/pituitary axis from estrogenic negative feedback. The resultant increase in FSH secretion stimulates growth of follicles (8). Because aromatase inhibitors do not deplete estrogen receptors, as does clomiphene, normal central feedback mechanisms remain intact. As the dominant follicle grows and estrogen levels rise, normal negative feedback occurs centrally, resulting in suppression of FSH and atresia of the smaller growing follicles, so single follicle, and mono-ovulation,

should occur in most cases. Mono-ovulation is the major advantage of using aromatase inhibitors for ovulation induction. A drug that consistently results in a single ovulation is particularly desirable in patients with PCOs, who are often hyperresponsive to gonadotrophins.

It also is known to increase intrafollicular androgens which in turn is thought to upregulate and sensitize FSH receptors in the ovary, as well as increase activin secretion, which further increases the secretion of FSH.

Treatment significantly lowers serum estrone, estradiol, and estrone sulfate, and has not been shown significantly to affect adrenal corticosteroid synthesis, aldosterone synthesis, or synthesis of thyroid hormones.

Letrozole, unlike clomiphene citrate, does not decrease the estrogen receptor density so not affect on the cervical secretion or thin the endometrium lining. Letrozole's short half-life (45h) ensures clearance by the body before implantation occurs, unlike clomiphene (17).



### Uses

#### 1- *Chronic anovulation:-*

Letrozole is approved by the United States Food and Drug Administration (FDA) in 1997 for the treatment of local or metastatic breast cancer that is hormone receptor positive or has an unknown receptor status in postmenopausal women.

Letrozole has been used for ovarian stimulation by fertility doctors since 2001 because it has fewer side effects than clomiphene (clomid) and less chance of multiple gestation and its uses are legal in many countries such as the US and UK. PCOS patients who failed to ovulate with clomiphene citrate, the option is to treat with a higher dose of CC or switch to letrozole. It appears that letrozole when used for CC-resistant women, confers superior pregnancy rates. Letrozole also performs as well, if not better

than, gonadotrophin therapy in women with CC-resistant and at a fraction of the cost. Letrozole can be used in women who develop a thin endometrium on CC (18). The first clinical study using an aromatase inhibitor (letrozole) for ovulation induction was published by Mitwally and Casper in 2001 (18). With letrozole use in PCOS patients, 75% ovulated and 25% became pregnant.

#### 2- *Unexplained infertility:-*

Letrozole is also used as ovulation induction in unexplained infertility. When considering ovulation, endometrial thickness, and pregnancy rates, letrozole has similar efficacy to clomiphene or injectable gonadotrophin, and outperforms hybrid treatment with clomiphene citrate and gonadotrophin combination (19).



### 3- *Diminished ovarian reserve:-*

In women with diminished reserve, there is a poor response to ovulation induction medications. In some, it is due to lack of oocytes; in others however, it is due to a decrease in follicular FSH receptors. With the use of letrozole, an increase in intrafollicular androgen is known to increase follicular FSH receptors (20).

### **Dosing**

Letrozole is typically administered on day 3-7 of the menstrual cycle at doses of 2.5-7.5 mg/day in 2.5 mg increments.

Little investigation has been attempted to define optimal dosing in infertile women, most studies utilizing letrozole at 2.5mg daily for 5 days show between one and two mature follicles grown at this dose. Available evidence suggests a dose-response with letrozole, with higher doses (5mg) producing more mature follicles and higher ovulation rates and pregnancy rates as well (21).

The 1<sup>st</sup> randomized controlled trial addressing letrozole dosing was performed in 2007. Badawy et al. utilized either 2.5, 5, or 7.5 mg daily although they found no differences in pregnancy rates, the number of mature follicles was significantly higher in the 7.5 mg group versus 5 or 2.5mg (22).

The optimum length of treatment in a cycle is also unknown at this time. In a recent study letrozole used for 5 days and 10 days, ovulation rates were similar but the number of mature follicles was high in 10 days treatment (23).

### **Contraindications**

Letrozole is contraindication in pregnancy, and lactation .

### **Adverse effects**

Approximately 10-20% of women will experience side effects but because the medication does not directly fit into the estrogen receptor, these effects are usually mild:

- Sweating.
- Hot flashes.
- Blurred vision.
- Headache.
- Arthralgia (joint pain ) and fatigue.
- Nausea.
- Ovarian cyst.
- Multiple pregnancy 3-5% risk of twins which is little than clomid.
- No differences in birth defects observed in children who were conceived using either CC or letrozole (2).

### **Interactions**

Letrozole inhibits the liver enzyme cyp2a6 , and to a lesser extent cyp2cla in vitro, but no relevant interactions with drugs like cimetidine and warfarin have been observed.

### **Patients And Methods**

**Setting:-** This is a prospective randomized study conducted in the fertility center in Al-Sader medical city between March 2014 and September 2014. The study protocol was approved by the institutional ethics committee, and informed consent was obtained from all study participants.

**Patients:-** ( 115) infertile PCOS women were recruited into the study. PCOS diagnosed according to the Rotterdam revised 2003 criteria for polycystic ovary syndrome , patients had a history of oligomenorrhea or amenorrhea, ovaries with at least 12 sub-capsular cysts 2-8mm in diameter, and /or elevated serum testosterone and Hirsutism. Our inclusion criteria included patients in the age of 18-35 years, having infertility for more than one year, and patients of anovulatory PCOS. Patients with hyperprolactinemia, thyroid disorder, male factor, BMI >35,

suspected tubal factor, unexplained infertility were not included in the study.

**Methods:-** Patients were randomized to receive either 100 mg CC (n=60) or 5 mg letrozole (n=55) daily for 5 days beginning on day 3 of the menstrual cycle. Clomiphene citrate (Clomid; Sanofi Aventis, France) and Letrozole ( Femara; Novartis pharma AG, Basle, Switzerland). Transvaginal ultrasound examination was performed on day 2 of the menstrual cycle before treatment was commenced, also hormonal studies like FSH, LH, prolactin, TSH, and testosterone were performed . Follicular development was monitored using transvaginal ultrasound from day 10 of menstrual cycle till a follicle attained >16mm diameter. The endometrial thickness was measured at the greatest diameter perpendicular to the midsagittal plane in the fundus region including both layers of the endometrial cavity . The number of follicles and endometrial thickness in patients of both groups were documented. When at least one mature follicle with a mean diameter  $\geq 17$  mm was observed, HCG (pregnyl) at dose of 10.000 IU was used to trigger ovulation and the ovulation was confirmed by seeing follicle collapse on subsequent ultrasound 48 hrs after the injection. Each woman was asked to have timed intercourse 24 hrs to 36 hrs after HCG injection. Ovulation was diagnosed when the mature DF was approximately 17 to 22 mm followed by evidence of rupture approximately 3 to 4 days later. If the dominant follicle (DF) was absent (DF < 17 mm), a repeat TVS was performed every 3 - 4 days later. The absence of a dominant follicle (DF < 17

mm) by Day 20 was considered as a non-response or anovulation.

Serum E2 levels on day of HCG was done for all patients.

Pregnancy was diagnosed by serum level of  $\beta$ -HCG performed once the patient missed her period.

**Outcome measures:-** Primary outcome measures were the mean number of follicles, endometrial thickness and ovulation rate, while secondary outcome measure was pregnancy rate compared in both the groups. The patients were given only one cycle of treatment for the study.

#### Statistical Analysis

The number of mature follicles, the endometrial thickness, the ovulation and pregnancy rates were compared in the two groups after one cycle of stimulation. A group *t*-test or the Student's *t*-test was used to compare data as appropriate. A *P*-value < 0.05 was considered to be statistically significant.

#### Results

During the study period, a total of 115 patients were analyzed for recruitment, 60 women were in 100mg Clomiphene citrate group and 55 women received 5mg letrozole group.

Table 1 summarizes the demographic profile of patients. There was no statistically significant difference in the mean age, BMI, basal FSH, type and duration of infertility in both groups of patients. Although LH and prolactin levels were significantly higher in Letrozole group but still within normal levels.

**Table (1) Patient characteristics of both CC and Letrozole groups (mean $\pm$ SD)**

Parameters	Treatments		
	Clomid(60)	Letrozol(55)	<i>P</i> value
Age (year)	23.67 $\pm$ 5.01	23.64 $\pm$ 5.02	0.975

BMI (kg/m <sup>2</sup> )	27.36±4.06	27.08±3.71	0.703
FSH (IU/l)	5.34±1.91	5.67±2.21	0.390
LH (IU/l)	5.88±3.25	8.08±6.85	0.049
Prolactine (ng/ml)	17.05±10.87	23.18±13.83	0.011
Duration of infertility (year)	2.62±1.67	3.39±1.84	0.074
Primary infertility no.	43	40	0.742
Secondary infertility no.	17	15	0.724

From 60 patients on CC 17 (28%) ended by no response (no follicle >16mm) and 43 patients continue the program, while 9(16%) from letrozole group not responding to stimulation induction and 46 continue the study. Table (2) below showed that the group of letrozole was higher in number of responsive when compared with CC group but still not significant.

**Table (2) Treatment responses**

Treatments results	CC (N.60) No. &%	Let (N.55) No. &%	P value
Responsive	43(71.67)	46(83.64)	0.125
Canceled (Non-responsive)	17(28.33)	9 (16.36)	0.125

Comparison between responders and non-responders within each group regarding baseline endocrine and demographic variables did not find significant difference in any of them (Table 3).

**Table (3) Comparison between responsive and non-responsive cases within each group.**

Treatment responses	CC (+ve)	CC (-ve)	P	Let (+ve)	Let (-ve)	P
Parameter						
BMI	26.85±3.81	27.99±4.45	0.315	26.74±3.33	27.94±4.54	0.278
Duration of infertility	2.50±1.40	2.90±2.02	0.385	3.34±1.78	3.50±2.03	0.781
FSH	5.46±2.06	5.12±1.73	0.514	5.96±2.41	5.19±1.57	0.306
LH	4.46±2.73	5.49±3.89	0.246	8.36±7.30	7.32±5.65	0.620

Table 4 demonstrates the outcomes of ovarian stimulation in terms of number of mature follicles, ovulation rate, endometrial thickness, pregnancy rate and E2 levels between CC and Letrozole groups. The mean number of dominant follicles (>16mm) in the letrozole group was higher as compared with CC group although not significantly different (Letrozole 1.32 versus CC 1.58,  $P=$ ). The ovulation rate in CC group was 50 from 67 (74.63%) versus 50 of 61 (81.97%) in Letrozole group which was higher but not significant. The mean midcycle endometrial thickness in the CC group was  $7.03\pm 1.39$ mm compared with  $7.21\pm 1.37$ mm in

Letrozole group, which was not significant ( $P=0.496$ ). Out of total 60 cases, 8 women became pregnant (13.33%) in CC group compared with 12 women who became pregnant out of 55 patients (21.82%) in Letrozole group, which was higher although statistically insignificant ( $P=0.230$ ). Pregnancy rate per responder was also higher in letrozole group (26.09%) in comparison with CC group (18.60%), although this difference also was not statistically significant ( $P=0.398$ ). E2 level at day of HCG of CC group was ( $380.79\pm 268.29$ pg/ml) which was significantly higher ( $P= 0.000$ ) in comparison with the level of E2 of letrozole group ( $166.216\pm 84.54$ pg/ml).

**Table (4) Outcome of ovarian stimulation**

Treatments Parameters	Clomid(43)	Femara(46)	P value
No. of follicles > 16 mm	67(mean1.58)	61(mean 1.32)	0.538
Ovulation rate No.(%)	50(74.63)	50(81.97)	0.315
Endometrium thickness (mm)	$7.03\pm 1.39$	$7.21\pm 1.37$	0.496
Pregnancy rate from the total No.(%)	8/60(13.33)	12/55(21.82)	0.230
Pregnancy rate from the responders No.(%)	8/43(18.60)	12/46(26.09)	0.398
E2 level at day of HCG (pg/ml)	$380.79\pm 268.29$	$166.216\pm 84.54$	0.000*

\*significant value

Table 5 shows the comparison between the 2 groups regarding the number of follicles per cycle. In Letrozole group the number of monofollicle cycle was higher(69.57%) and multifollicles cycle was low(30.43%) when are comparing with that of CC group ( 51.16% & 48.84% respectively), but this differences still insignificant. But when we compared in the same group, high significance difference ( $P=0.008$ ) between the numbers of monofollicle cycles (32, 69.57%) and multifollicle cycles (14, 30.43%) of letrozole group.

**Table (5) Outcome of ovarian stimulation**

Treatment Parameters	Clomid(n 43) No.(%)	Femara(n 46) No.(%)	P value
Monofollicles	22(51.16)	32(69.57)	0.899
Multifollicles	21(48.84)	14(30.43)	0.899

\*significant value

## Discussion

Today, CC still remains the first line of treatment in PCOS although aromatase inhibitors are an effective, inexpensive and safe alternative to CC.

Clomiphene citrate is an anti-estrogen with a 60% - 80% ovulation rate and a 10% - 20% pregnancy rate. This is due to the anti-estrogen effect of Clomiphene citrate, resulting in long-lasting estrogen receptor (ER) depletion because of long half life (2wks). It is generally accepted that CC reduces uterine receptivity, and thus reduces the chances of conception. (24) It is associated with endometrial thinning in 15-50% of patients, probably due to estrogen receptor depletion (25) Furthermore, the use of CC may block estrogen receptors in the cervix, producing a negative effect on the quality and quantity of cervical mucus. [4] Inappropriate development of the endometrium is associated with low implantation rate and early pregnancy loss due to luteal phase defect.(26,27)

Letrozole, an aromatase inhibitor, has shown to be effective in inducing ovulation and pregnancies in women with anovulatory PCOS and in patients with CC resistance or failure (18). It also improves the ovarian response to FSH in poor responders.(28)

Aromatase inhibitors are non-steroidal compounds that suppress estrogen biosynthesis by blocking the action of the enzyme, aromatase, which converts androstenedione and testosterone to estrogens. The efficient estrogen-lowering property of letrozole could be utilized to temporarily release the hypothalamus from negative feedback effect of estrogen and thereby inducing an increased discharge of FSH. (18). 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. A single dominant follicle and monoovulation should occur in most cases (29). In the endometrium, estrogen receptors may be upregulated, resulting in rapid endometrial growth once estrogen secretion is restored following clearance of Letrozole because of its short half life (45h) (25,30).

**Response:** In our study the response in Letrozole group was better than CC group, as the anovulation (no follicle >16mm) in 1<sup>st</sup> group was 16.36% while in CC group was 28.33% (**Table 2**). And the data regarding the BMI, duration of infertility and hormonal levels were comparable between the responsive and non-responsive in both groups, this is in agreement with Mervet and Hassan study 2011 (31). While some studies showed that CC resistance is more likely in patients who are insulin resistant and obese (Imani B et al. 2002)(32).

**Number of Ovulations:** In the present study, though the mean number of dominant follicles (>16 mm) was comparable in both groups. Al-Fozan et al. also reported similar result (33).

Ovulation was more frequent in the Letrozole group 50 (81.9%) than Clomiphene citrate group 50 (74.6%) although was not significant, (**Table 4**). Abu Hashim study showed ovulation rates of 82.4% and 63.2% for Letrozole and Clomiphene citrate, respectively which was significant (34). Another study found that among women taking Letrozole, 62.5% had achieved ovulatory cycling as compared to 37.5% of women taking Clomiphene citrate, which was not different significantly (25). Mitwally and Casper (18) using 2.5 mg/day of letrozole achieved 75% and 100% ovulation in anovulatory and ovulatory patients respectively. Higher ovulation rate with letrozole was also reported in other studies (28,35). Ovulation rate was found to be

similar reported by Bayer et al., (36) (81% in letrozole group versus 85% in CC group).

**Endometrial Response:-** In our study, mean ET in the Letrozole group was thicker than in the Clomiphene citrate group at midcycle of menses, with ET values of 7.21 mm and 7.03 mm respectively (**Table 4**). This difference was statistically insignificant. But another studies are reported that using 2.5-5 mg/day of letrozole has a better endometrial response compared with endometrial response using CC in the dose of 50-100 mg/day. (27,Dicky 1996). Similar findings were reported in the study of Baruah et al. 2009, which demonstrated that endometrial thickness and sub-endometrial blood flow were significantly better in cases receiving induction with letrozole than CC despite comparable follicular response (37). This has also been reported with Al-Fozan et al. ( 33,2004). Other studies reporting that most patients taking Letrozole had a thicker endometrium compared to those taking Clomiphene citrate(38). Similar findings showed that Letrozole had an overall greater beneficial effect on the endometrium (29,39).

This is possibly due to an increase in the number of growing follicles and thus a higher level of estrogen and progesterone in CC group in our study, although endometrial thickness in both study groups was >7 mm.

**Number of Pregnancies** In our study, consistent with the number of successful ovulatory cycles, pregnancy rate was notably higher in the Letrozole treatment group compared to the Clomiphene citrate group with 19 (25.3%) and 12 (16.0%) pregnancies, respectively; however, this was not significantly different. The pregnancy rate observed in our study was consistent with other reported studies such as that by Mitwally (18), in which a pregnancy rate of 25% was observed for

PCOS patients treated with 2.5 mg Letrozole. Additionally, Atay (38) also found a pregnancy rate of 21.6% after treatment with 2.5 mg Letrozole and 9.1% after treatment with 100 mg Clomiphene citrate, which was statistically significantly different. There are other studies that showed significantly higher pregnancy rates with Letrozole than with Clomiphene citrate (23,40,41).

With CC, supraphysiologic levels of estrogen can occur without control suppression of FSH because the normal estrogen receptor-mediated feedback mechanisms are blocked. This results in multiple follicular growth and higher multiple pregnancy rates with CC than are found in letrozole cycles (42). This is corresponding with our result, **Table 5**.

It has been reported that serum E<sub>2</sub> level on the day of hCG administration was statistically significantly lower in the letrozole group than the CC group (36,43). In our study, we have also found that the mean total E<sub>2</sub> was significantly higher in CC group as compared with letrozole group **Table 4**. This also indicates the multifollicular response in CC group.

#### **Conclusion:**

Letrozole in patients with PCOS is as effective as clomiphene citrate in inducing ovulation, ovulation rate and the incidence of pregnancy was higher with letrozole than that with clomiphene citrate.

Apart from that, Letrozole treatment is prone to production of monofollicles and hence leads to reduced incidences of the adverse pregnancy outcome of multiple fetuses as compared to treatment with Clomiphene citrate.

#### **Recommendations:**

- Further studies are needed to determine optimal dosing and the long term safety for women treated with the drug.

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