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Research Article

# Comparison of the efficacy of follicular and endometrial response on metformin – clomophene citrate and metformin – letrozole on polycystic ovarian syndrome women

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**ABSTRACT**

To compare the effects of metformin - Clomiphene citrate (CC) and metformin – letrozole on follicular and endometrial response in infertile polycystic ovary syndrome patients. The patients were visited infertility clinic and examined by gynecologists. Healthy normal cycled women conclude as a control ( group I), untreated PCOS patients selected for (group II), All patients of both groups (III and IV) were received 1500 mg metformin (Glucophage, Merck, West Drayton, UK) per day (500 mg three times a day) for 6–8 weeks. If pregnancy occurred, the patient was excluded from the study. However, in case of failure of pregnancy at the end of this period, the patients in Group III were received 100mg of clomophene citrate and Group IV were received 2.5 mg letrozole (Femara, Novartis, Quebec, Canada) for 5 days from day 3 of their menstrual cycle. In this study, the mean levels of glucose, Insulin, FSH and LH decreased significantly ($p<0.001$) in treated groups. The mean levels of estrdiol ($E_2$), progesterone (p) were significantly ($p<0.001$) suppressed and inhibin B were significantly ($p< 0.001$) improved after the treatment period. The result demonstrates that the total number of matured follicles and endometrial thickness also significantly ($p< 0.001$) increased in treated subjects. The combined effect of metformin - letrozole showed a significantly better follicular and endometrial response compared with metformin - Clomiphene citrate (CC).

**Keywords:** Letrozole; Clomiphene citrate; follicle size; Endometrial response

## INTRODUCTION

Polycystic ovary syndrome (PCOS) affects 4–7% of reproductive age women, making it one of the most common endocrine disorders in this age group (Knochenhauer et al., 1998; Diamanti-Kandarakis et al., 1999; Asuncion et al., 2000). The polycystic ovary is characterized by an increased number of small antral follicles compared with normal ovaries, with 10–12 follicles in a single plane on ultrasound (Jonard et al., 2003, Adams et al., 1986). These follicles are not atretic; rather, their growth is prematurely arrested, resulting in failure of dominant follicle development and ovulation. The cause of this follicular arrest is unknown. In normal women, cyclic follicle development is dependent on circulating FSH and LH levels as well as locally produced growth factors and hormones (Erickson and Danforth 1995; Erickson and Shimasaki 2001). Because FSH levels are normal and should be sufficient to permit ovulatory follicle development in women with PCOS (Taylor et al., 1997), and PCOS granulosa cells respond robustly to FSH in culture (Mason et al., 1994), investigators have begun to assess whether the locally produced ovarian products could contribute to the follicular arrest. Indeed, previous studies have suggested that ovarian inhibin, activin, and/or follistatin (FS) have roles in modulating normal follicle development, raising the possibility that disorders of synthesis or secretion of these proteins could contribute to abnormal follicle development in PCOS (Welt et al., 2002).

Progesterone (P) and estradiol (E2) are required for successful conception, both to prepare the endometrium for blastocyst implantation and pregnancy. Ovarian hyperstimulation results in excessive follicular development and supraphysiologic serum concentrations of E2 and P. Such derangements raised concerns about the impact of such abnormalities on the luteal phase and a possible adverse impact on endometrial tissue (Smitz et al., 1998; Albano et al., 1998). E2 initiates hypertrophy and hyperplasia of endometrial epithelia, but its role in the luteal phase remains poorly understood. How E2 influences endometrial synchronization and blastocyst implantation is also not well described (Ng Hung Yu et al., 2000). In contrast, the role of P in

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the luteal phase is better examined Csapo *et al (*Csapo et al., 1973; Csapo et al., 1973) showed that luteectomy leads to miscarriage in almost every case if performed before seven weeks of gestational age. P transforms the E2-prepared endometrium into a secretory tissue and creates a hospitable environment for embryo attachment (Weitlauf, 1994).

Recently, inhibins have attracted the interest of many researchers. Inhibins are heterodimeric gonadal peptides containing an _ subunit and _A subunit (inhibin A) or _B subunit (inhibin B) that have been suggested to negatively regulate follicle-stimulating hormone (FSH) secretion, even though there is no direct evidence of this phenomenon (Hayes et al., 1998). Locally, inhibins act by enhancing follicle development, thus reflecting the reserve of small antral follicle growth (Hayes et al., 1998; Welt et al., 2001). Interestingly, in girls affected by anorexia nervosa, weight gain, with an increase in adipose tissue and leptin levels, is paralleled by an increase in inhibin B levels (Popovic et al., 2004).

Inhibin A and inhibin B display different secretory patterns throughout the menstrual cycle. Serum and follicular fluid inhibin B levels are maximal in the early to midfollicular phases, most likely under the stimulation of FSH, whereas peak inhibin A levels are observed in the late follicular and luteal phases (Groome et al., 1996). In particular, _A subunit (inhibin A) is expressed mainly by the dominant follicle and the corpus luteum, whereas _B subunit (inhibin B) expression predominates in the granulosa cells of the preantral or small antral follicles (Roberts et al., 1993). Little is known about factors regulating the shift in inhibin production when a follicle becomes dominant.

In polycystic ovary syndrome (PCOS), inhibin B levels are inversely correlated with BMI (Cortet-Rudelli et al., 2002) and insulin levels, suggesting that 1) both body mass and hyperinsulinemiamay inhibit inhibin B secretion, and 2) granulosa cell activity or follicular production of inhibin B, and possibly follicle health, are decreased in obesity. Remarkably, insulin suppression has been shown to result in increased inhibin B in the absence of changes in luteinizing hormone (LH), FSH, BMI, sex hormone-binding globulin, and leptin in PCOS women, thus confirming that insulin negatively regulates inhibin B in these patients (Cortet-Rudelli et al., 2002; Welt et al., 2002; Anderson et al., 1998). However, insulin has been demonstrated to have no direct effect on inhibin B secretion in in vitro studies using human granulosa cells from small antral follicles (Welt et al., 2001).

Therefore, the current study was planned to compare the combined effects of metformin- clomophene citrate and metformin – letrozole on endocrinological changes, endometrial and follicle maturation in south Indian women with polycystic ovary syndrome.

**Patients and Methods**

All patients between the age of 27 and 37 years who attended an infertility clinic with a suspicion of PCOS (specifically, complaining of infertility, menstrual dysfunction or dermatological problems), were included in the study. The study sample was collected from various infertility clinics in Tamilnadu, India. The study was approved by the Scientific Ethics Committee, Coimbatore, Tamilnadu. An informed written consent was obtained from each patient.

The inclusion criteria were, infertility for at least one year, having patent tubes on hysterosalpingogram, and normal semen analysis of the patients' husbands. None of the women had received any hormonal or infertility therapy for at least 6 months before enrollment to the study. Exclusion criteria were organic pelvic diseases, previous pelvic surgery, suspected peritoneal factor infertility, or tubal or male factor infertility or sub fertility as excluded by hysterosalpingogram and semen analysis, respectively. This present study, also excluded women who intended to start a diet or a specific program of physical activity.

**Study design**

The patients were visited and examined by gynecologists. Healthy normal ovulatory cycle women's conclude as a control (group I), untreated PCOS patients selected for (group II). The patients were randomly allocated to metformin-clomiphene citrate (Group III) and metformin–letrozole (Group IV) groups. All the patients of both groups were received 1500 mg of metformin (Glucophage, Merck, West Drayton, UK) per day (500 mg three times a day) for 6–8 weeks. If pregnancy occurred, the patient was excluded from the study. However, in case of failure of pregnancy at the end of this period, the patients in the Group III were taken 100 mg clomiphene citrate for 5 days starting from day 3 of their menstrual cycle, and those in the Group IV received 2.5 mg letrozole (Femara, Novartis, Quebec, Canada) for 5 days from day 3 of their menstrual cycle.

**Hormonal assay**

Serum inhibin B, $E_2$ and P were measured by enzyme-linked immunosorbent assay and automated Chemiluminescence immunoassay systems were used for the determination of LH, FSH (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA), and insulin (IMMULITE 2000, DPC Biermann, Bad Nauheim, and Germany). A level of serum glucose was determined by the calorimetric method using a Cobas Mira Plus autoanalyser (Roche Diagnostics, Mannheim, Germany). These levels were obtained on day 3 of spontaneous or withdrawal cycle.

**Ultrasound examination**

Transvaginal ultrasound examination were performed prior to starting the treatment on day 3 and 10-12 of the cycle, and more often as needed with a 5-MHZ vaginal transducer attached to a Aloko Scanner (Model SSD-500, Aloka Co, LTd, Tokyo, Japan). Follicular di-

**Table 1: Compare the biochemical and hormonal levels in control, baseline and treated women**

| Groups | Parameters | | | |
|---|---|---|---|---|
| | Glucose mg/ dl | Insulin µu / ml | LH U/ L | FSH U/ L |
| Group I - Control | 82.5 ± 7.7 | 7.4 ± 1.6 | 5.4 ±2.1 | 7.2 ± 1.4 |
| Group II - Un treated | 129 ± 32.7$^{\$\$\$}$ | 16.4 ± 5$$$ | 25.6± 6.8$$$ | 12.2± 2.0$$$ |
| Group III - Metformin - Clomiphene citrate | 89.6 ± 6.8$^{\#\#\#***}$ | 9.0 ± 1.7$^{\#\#\#***}$ | 15.2 ± 3.8$^{\#\#\#***}$ | 10.9 ± 1.9$^{\#\#\#**}$ |
| Group IV - Metformin - Letrozole | 84.8 ± 5.7$^{NS‡‡‡,†††}$ | 8.2 ±1.3$^{**‡‡‡,†}$ | 11.2 ± 2.0$^{***‡‡‡,†††}$ | 9.9 ±1.7$^{***‡‡‡,†}$ |

**Table 2: Compare the E2, P and Inhibin B levels in control, baseline and treated women**

| Groups | Parameters | | |
|---|---|---|---|
| | E2 pg/ml | P ng/ml | Inhibin B pg/ml |
| Group I- Control | 35.78+/-5.81 | 0.73+/-0.15 | 140.8+/-22.19 |
| Group II - Un treated | 70.88+/-18.32,$^{\$\$\$}$ | 1.82+/-1.08,$^{\$\$\$}$ | 69.5+/-33.96,$^{\$\$\$}$ |
| Group III - Metformin – Clomiphene citrate | 47.78+/-10.57### *** | 1.24+/-0.48### *** | 134.1+/-25.85$^{NS\ ***}$ |
| Group IV - Metformin - Letrozole | 41.51+/-6.59$^{***‡‡‡\ ††}$ | 0.99+/-0.31$^{***‡‡‡}$ †† | 120.4+/40.09$^{**‡‡‡}$ † |

ameter was determined by calculating the mean of two perpendicular diameters measured at the largest plane of the follicle. The endometrial thickness was taken as the widest distance from the reflective interface between the endometrium and myometrium of one side to the opposing side on a sagittal view of the uterus using a 5-MHz linear-array vaginal transducer (Model SSD-500, Aloka Co, LTd, Tokyo, Japan).

**Statistical Analysis**

The data are reported as the mean +/- SD or the median, depending on their distribution. The differences in quantitative variables between groups were assessed by means of the unpaired t test. One way Analysis of variance [ANOVA] was performed followed by multiple comparisons using the scheffe test. Comparison of a variable between two groups was assessed by Mann-Whitney Test. A p value of <0.05 using a two-tailed test was taken as being of significance for all statistical tests. All data were analyzed with a statistical software package (SPSS, version 13.0 for windows).

**RESULT**

The population consisted of 200 subjects (Female population) divided into four groups was selected. Treated polycystic ovarian syndrome patients with metformin – clomiphene citrate (Group III) and treated polycystic ovarian syndrome patients with metformin - letrozole (Group IV) PCOS were compared with untreated PCOS patients (Group II) and control subjects were collected from normal ovulatory female patients (Group I). Patients visited with infertility problem in various hospitals in various cities, Tamil Nadu, India with suspected PCOS patients was selected as source of population based on the inclusion and exclusion criteria. The control subjects were selected based on inclusion and exclusion criteria. They were not receiving any drugs at the time of the study. General health characteristics such as age, body weight, BMI, hirsutism, menstrual status were investigated by a self-administered questionnaire.

The demographic characteristics like body weight, BMI, hirsutism, menstrual cycle status were significantly increased in after treatment (data's not shown).

Table 1 shows the elevated level of serum glucose was found in group II compared with group I ($p<0.001$).The mean levels of glucose, Insulin, FSH and LH decreased significantly in treated groups (III & IV) compare with group II.

Group IV compare with Group I significant at the present -*P <0.5, **P<0.01, ***P<0.001, NS –Non significant

Group III compare with Group I significant at the present -$^{\#}$P <0.5, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, NS –Non significant

Group II compare with Group I significant at the present -$^{\$}$P <0.5, $^{\$\$}$P<0.01, $^{\$\$\$}$P<0.001, NS –Non significant

Group III compare with Group II significant at the present -*P <0.5, **P<0.01, ***P<0.001, NS –Non significant

Group IV compare with Group II significant at the present -$^{‡}$P <0.5, $^{‡‡}$P<0.01, $^{‡‡‡}$P<0.001, NS –Non significant

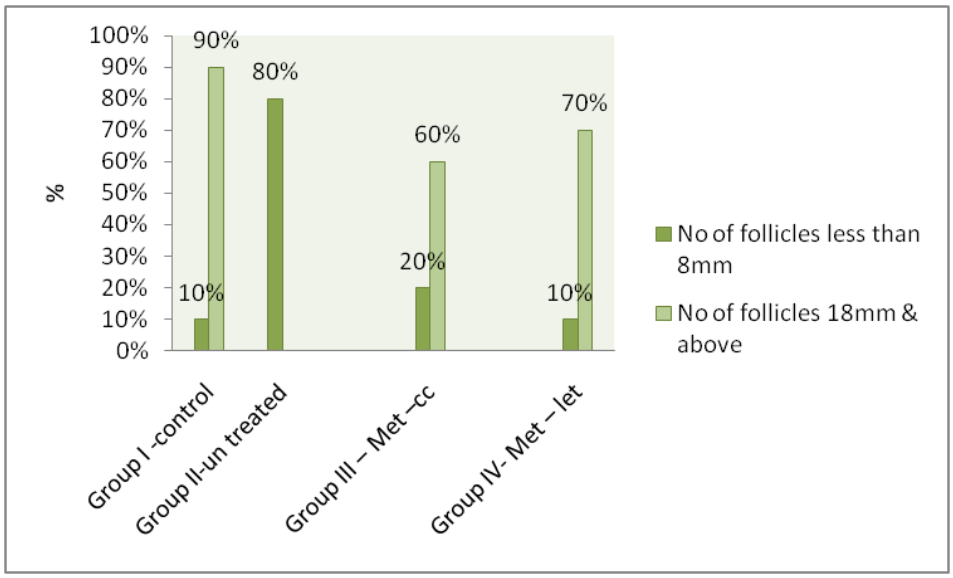


**Figure 1: Comparison of different follicular size in control, baseline and treated groups**

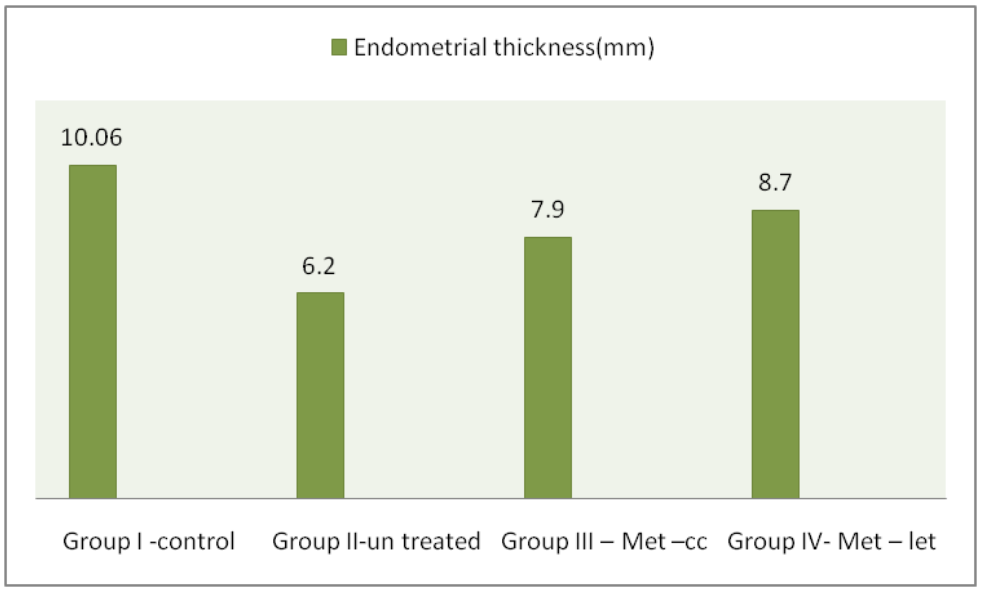


**Figure 2: Comparison of endometrial thickness in control, baseline and treated groups**

Group IV compare with Group III significant at the present -$^{\dagger}$P <0.5$'$ $^{\dagger\dagger}$P<0.01, $^{\dagger\dagger\dagger}$P<0.001, NS –Non significant.

Table 2 shows the significant changes in estrdiol ($E_{2)}$, progesterone (p) and inhibin B levels. The mean levels of estrdiol ($E_{2)}$, progesterone (p) were significantly suppressed in after treatment (III and IV) compared with group I and II. But, after treatment the level of inhibin B was significantly (p< 0.001) improved.

Values are given as mean ± SD from fifty subjects in each group

Group IV compare with Group I significant at the present -*P <0.5, **P<0.01, ***P<0.001, NS –Non significant

Group III compare with Group I significant at the present -$^{\#}$P <0.5, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, NS –Non significant

Group II compare with Group I significant at the present -$^{\$}$P <0.5, $^{\$\$}$P<0.01, $^{\$\$\$}$P<0.001, NS –Non significant

Group III compare with Group II significant at the present -*P <0.5, **P<0.01, ***P<0.001, NS –Non significant

Group IV compare with Group II significant at the present -$^{\ddagger}$P <0.5, $^{\ddagger\ddagger}$P<0.01, $^{\ddagger\ddagger\ddagger}$P<0.001, NS –Non significant

Group IV compare with Group III significant at the present -$^{\dagger}$P <0.5$'$ $^{\dagger\dagger}$P<0.01, $^{\dagger\dagger\dagger}$P<0.001, NS –Non significant

Figure 1 and 2 shows the follicle size and endometrial thickness. The result demonstrates that the total number of matured follicles were significantly increased in treated subjects (group III and IV). The endometrial thickness also statistically improved in group III and group IV patients. But, the enhanced result of follicle

size and good quality of endometrial thickness was obtained in metformin- letrozole than metformin – clomiphene citrate.

**DISCUSSION**

It is generally accepted that CC reduces uterine receptivity, and thus the chances of conception (Kouta et al., 1997). It is associated with endometrial thinning in 15–50% of patients. This is probably due to estrogen receptor depletion (Atay et al., 2006). The aromatase inhibitor letrozole may be a better alternative in terms of endometrial response in ovulation induction. Because of its rapid elimination and reversibility, letrozole allows the endometrium to respond well to raise estrogen levels in the late follicular phase. In our study, we tried to compare the combined effect of metformin - CC and metformin - letrozole in patients of PCOS with due emphasis on the endometrial response related to hormonal changes.

It was found the mean endometrial thickness was significantly better in the letrozole group (6.9 § 0.74) compared to CC group (5.9 § 0.53) in our study. This could be related to the dose of letrozole (2.5–5 mg) we used. It is reported (Mitwally and Casper, 2001) that using 2.5–5 mg of letrozole has a better endometrial response compared to endometrial response using CC. This has been reported (Al-Fozan et al., 2004) that when letrozole is used in the dose of (7.5 mg) and compared with 100 mg of CC, there was no difference in endometrial thickness. The mean number of dominant follicles (¸18 mm) in present study was comparable in both the letrozole and CC groups. Similar findings were reported by Fozan et al., in a randomized control trial of letrozole versus CC in women undergoing super ovulation (Al-Fozan et al., 2004).

A second important issue is the relationship between insulin resistance and the pituitary-ovary axis. Insulin is commonly believed to inhibit folliculogenesis (Roberts et al., 1993). In the present study, insulin levels were decreased significantly after the treatment. These findings are consistent with previous in vitro studies on human granulosa cells from small antral follicles showing that insulin has no direct effects on inhibin B secretion (Lockwood et al., 1998). These data, obtained in women with normal FBG and normal glucose tolerance, seem to exclude a direct role of hyperinsulinemia on inhibin B or estradiol production, at least in women without PCOS.

We demonstrate the improvement in insulin levels after the treatment period. Some studies noticed that, Insulin resistance accompanied by compensatory hyperinsulinemia is a common feature of PCOS and both obese and non-obese women with the syndrome are more insulin resistant and hyperinsulinemic than age and weight matched normal women (Legro et al., 1998). Metformin decreases fasting glucose level by decreasing hepatic glucose output. Its use in PCOS patients, corrects the response to oral glucose tolerance, thus decreasing insulin level (Bailey, 1992; Bailey and Turner et al., 1996).

In this study, the metformin – letrozole treated patients LH, FSH levels were significant decline after the treatment period.In a previous study of 10 subjects, Fruzzetti et al., Observed a statistically non-significant trend toward lower LH level. Since hyperinsulinemia and hyperandrogenism may alter the secretion of gonadotrophins in favor of an increase in LH, these drugs were lower LH secretion by reducing insulin and/or androgen levels (Fruzzetti et al., 2002).

We believe that aromatase inhibitors also act locally in the ovary to increase follicular sensitivity to FSH. This may result from accumulation of intraovarian androgens because conversion of androgen substrate to estrogen is blocked by aromatase inhibition. Recent data support a stimulatory role for androgens in early follicular growth in primates (Weil et al., 1998 35). Testosterone was found to augment follicular FSH receptor expression in primates, suggesting that androgens promote follicular growth and estrogen biosynthesis indirectly by amplifying FSH effects (Weil et al., 1999). In addition, androgen accumulation in the follicle may stimulate insulin-like growth factor I (IGF-I), along with other endocrine and paracrine factors, which may synergize with FSH to promote folliculogenesis (Vendola et al., 1999).It is likely that women with PCOS already have a relative aromatase deficiency in the ovary leading to increased intra ovarian androgens (Agarwal et al., 1996). The increased androgen levels likely lead to the multiple small follicles responsible for the polycystic morphology of the ovaries in these women.

Some other studies showed the level of ovarian aromatase is low in these patients. Multiple small ovarian follicles are due to high androgen level. In addition, androgens increase FSH receptors, and therefore increase FSH sensitivity. Aromatase inhibitors cause growth of one or more ovarian follicles by increasing FSH or deceasing estrogen production (Sohrabvand et al., 2006).

In this present effort were originate that the serum levels of E2 were significantly lower and the number of mature follicles levels were increased in the metformin-letrozole treatment. This concurs with the findings of a previous report (Mitwally and Casper RF, 2002). For normal endometrial morphology to occur, an E2 priming phase is required followed by P. In the pre-GnRH agonist era, the alteration of the E2/P ratio was considered a main cause of luteal-phase inadequacy and IVF failure, possibly mediated by the luteolytic action of E2 (Lessey et al., 1988 ). The action of estrogen is required for up-regulation of P receptors. In the follicular phase of a E2and P receptors are found in glandular and stromal compartments (Lessey et al., 1988 ). P antagonizes the proliferative effects of E2 on the endometrial glands by down-regulating estrogen recep-

tors and is followed by a subsequent disappearance of P receptors (Lessey et al., 1988 ; Garcia et al., 1988).

LH stimulates granulosa cell E2 and P4 secretion earlier in follicle development in PCOS compared with normal follicles (Willis et al., 1998). The insufficient production of inhibin could have functional consequences for antral follicle maturation.In this present finding were showed significant increase in Inhibin B and decrease in P levels were obtained after the treatment. Some study confer, Inhibin signals via a type II activin receptor and type III TGF_ receptor (betaglycan) complex (MacConell et al., 2002), and both have been identified on granulosa and thecal cells (Corrine et al., 2005) of growing antral follicles. This deficient inhibin biosynthesis in PCOS follicles points to a potential mechanism for follicular arrest in women with PCOS and emphasizes the important roles of inhibin B in normal follicle development.

**CONCLUSION**

From the present study, we speculate that metformin – letrozole gives fine outcome on, improved result in androgen metabolism, inhibin B and lower serum estrogen concentration is associated with a matured follicles and good endometrial measurement. It gives better pregnancy rate with lower incidence of multiple pregnancies than metformin - clomiphene citrate. Potential advantages of Aromatase inhibitor of letrozole include reduced the adverse effect such as multiple pregnancies, absence of anti estrogenic adverse effects, and the subsequent need for less intensive monitoring. The outcomes of the study propose that, combined therapy of metformin-letrozole will replace clomiphene citrate in the future as the new primary treatment for follicular development in infertile PCOS patients.

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