

## The Differences of Folliculogenesis between Treated with Letrozole and Letrozole-Electroacupuncture in Women with Polycystic Ovary Syndrome

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### ABSTRACT

**Background:** Polycystic Ovary Syndrome (PCOS) is an endocrine disorder that most often causes infertility. It occurs due to the failure of folliculogenesis, thus causing non-ovulation. Letrozole is a 3rd generation drug of non-steroidal aromatase inhibitors, which can increase ovulation rates. Electroacupuncture is the insertion and manipulation of needles into acupuncture points (acupoints) that are selected specifically based on the meridian system and connected to the electrode stimulator. This study aimed to determine the differences of the effects of letrozole and letrozole-electroacupuncture (letrozole-EA) on the improvement of folliculogenesis in women with PCOS.

**Subjects and Method:** This was an analytic observational study with a case-control design. This study was conducted at the Gynecology Polyclinic and Sekar Clinic, Dr. Moewardi Hospital, Surakarta. A sample of 30 infertile women aged 20-45 years was selected by fixed disease sampling. The criteria of the PCOS were determined based on Rotterdam criteria. The dependent variable was the diameter of the follicle. The

independent variables were the administration of letrozole and letrozole-EA. Transvaginal ultrasound of Voluson P6 was used to measure the folliculogenesis. The data were analyzed by independent t-test.

**Results:** On the second day after treatment, the diameter of the follicles in the letrozole-EA group (mean= 6.92; SD= 1.60) was bigger than the letrozole group (mean=5.66; SD=1.11), and it was statistically significant (p=0.004).

**Conclusion:** Combination of letrozole and letrozole-EA therapy is more effective in increasing follicular diameter development than letrozole therapy only.

**Keywords:** polycystic ovary syndrome, folliculogenesis, Letrozole, electro-acupuncture

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### BACKGROUND

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder in women of reproductive age (Hestiantoro et al., 2014). In women of reproductive age and women who had married, PCOS is the most common

cause of infertility due to non-ovulation (75%) (Kini, 2012).

Ovarian disorders in PCOS can be defined as impaired follicular development (folliculogenesis), namely the occurrence of excessive initial follicular growth and disrupt-

tion of follicular growth stages to be mature, thus causing non-ovulation. The pattern of failure to produce dominant follicles is a characteristic symptom of PCOS, namely polycystic ovary morphology (Johansson and Stener-victorin, 2013). It can be seen through ultrasound as the presence of follicles  $\geq 12$ , with a diameter of 2-9 mm, and an increase in ovarian volume  $\geq 10\text{cm}^3$  (Kini, 2012).

One of the therapies used to treat infertile symptoms in women with PCOS is by inducing ovulation. Aromatase inhibitors were first introduced as ovulation induction drugs in women with PCOS who had resistance to clomiphene citrate in 2001. The most commonly used drug is letrozole compared with clomiphene citrate. Based on the Cochrane systematic review, there were some benefits of using letrozole such as increased ovulation rates, increased pregnancy, and increased birth rates life (Now, 2012; ACOG, 2018).

Acupuncture is a treatment method by inserting needles. Acupuncture comes from the words Acus=needle and Puncture=insert and in Chinese, it is called Cen Jiu (Saputra, 2017). The popularity of the use of acupuncture in reproductive endocrinology and infertility worldwide increased. Acupuncture can improve the menstrual cycle, reduce testosterone levels, and improve folliculogenesis in women with PCOS (Johansson et al., 2013; Cable., 2016; Stener-Victorin et al., 2009; Budihastuti et al., 2019).

Nowadays, there is not enough evidence to support the use of acupuncture in the treatment of ovulation disorders in women with PCOS (Stener-Victorin et al., 2018). Therefore, the authors were encouraged to determine the differences of the effects of letrozole and letrozole-EA on the improvement of folliculogenesis in women with PCOS.

## SUBJECTS AND METHOD

### 1. Study Design

This study was an analytic observational study with a case-control design. This study was conducted at the Gynecology Polyclinic and Sekar Clinic, Dr. Moewardi Hospital, Surakarta, Central Java, Indonesia, from February to Mei 2020.

### 2. Population and Sample

The subject of this study had population criteria in the form of a group of 75 women with PCOS. It was filtered using inclusion and exclusion criteria: women aged 20-45 years who had infertility. There were at least 2 of the 3 images found: (1) oligo-ovulation or anovulation, (2) clinical and/or biochemical signs of hyperandrogen, (3) Morphology of polycystic ovaries that were examined using ultrasound. It was found that there were  $\geq 12$  of follicles with a size of  $\leq 9\text{mm}$  that did not use hormonal KB (family planning and birth control), having malignant disease, getting hormone replacement therapy, having bleeding abnormalities, using pacemakers, and getting pregnant. A sample of 30 infertile women with PCOS was selected by fixed disease sampling. The sample was divided into 2 groups, (1) 15 infertile women with PCOS who received letrozole therapy and (2) 15 infertile women with PCOS who received letrozole-EA therapy.

### 3. Study Variables

The dependent variable was the diameter of the follicle. The independent variables were the administration of letrozole and the combination of letrozole-EA.

### 4. Operational Definition of Variables

**The diameter of the follicle** was the diameter of the follicle in the process of follicular development of the ovary.

**Electroacupuncture** was the insertion and manipulation of needles into acupuncture points (acupoints) that were selected specifically based on the meridian system and connected to the electrode stimulator.

**Letrozole** was a 3rd generation drug of non-steroidal aromatase inhibitors. It has potential as an ovulation induction.

**5. Study Instrument**

Follicular diameter was measured using Voluson P6 transvaginal ultrasound on days 2, 6, 8, 10, and 12 of menstruation by using means of the follicular diameter from two perpendicular measurements in units of millimeter (mm) with a numerical scale.

Electroacupuncture involved inserting and manipulating needles into acupuncture points of CV 3 (Zhongji), CV 6 (Qihai), ST 29 (Guilai) bilaterally, SP 6 (Sanyinjiao) bilaterally, LI 4 (Hegu) bilaterally, and ST 36 (Zusanli) bilaterally. They were connected to the electrode stimulator in several ways: before being given letrozole, the patients lay on the bed then the acupuncture points were determined, aseptically gently stabbed, using acupuncture needles (Huanqiu, China), stainless steel, sterile, disposable, using needle size 0.25 x 25 mm or 0.25x40 mm depending on BMI, up to De qi (match-making sensation). After that, the needles were connected to the stimulator electrode (Hwato SDZ V, Shanghai China) which were set for 15 minutes, continuous wave, 2 Hz frequency, then stopped. Electroacupuncture

**1. Sample Characteristics**

**Table 1. Sample characteristics (continous data)**

Characteristics	n	Mean	SD	Minimum	Maximum
Age (years)	30	30.87	4.61	26	44
Height (cm)	30	157.73	4.04	150	167
Weight (kg)	30	64.93	15.14	40	105
BMI (kg/m <sup>2</sup> )	30	25.70	5.24	17	39
Duration of infertility (years)	30	4.87	2.60	1	10

**2. Bivariate analysis**

**Table 2. The diameter of the follicle before therapy**

Treatment group	n	Mean	SD	p
Letrozole	15	5.57	1.18	0.676
Letrozole-EA	15	5.61	1.13	

Table 2 shows the difference of diameter of the follicle between letrozole and

was conducted 2 times a week until it reached 12 times.

The supply of Letrozole used was Lezra with a dose of 2.5mg per tablet. It was given on day 2 to day 6 of menstruation for 5 days at a dose of ® 5mg each day. It was given after electroacupuncture was completed 12 times.

**6. Data Analysis**

The data were analyzed bivariately using independent t-test run on Stata 13.

**7. Ethical Clearence**

The ethical clearance was obtained from the health research ethics commission of dr. Moewardi Hospital in Surakarta, Central Java, Indonesia, No: 137/I/HREC/2020 on 24 January 2020.

**RESULTS**

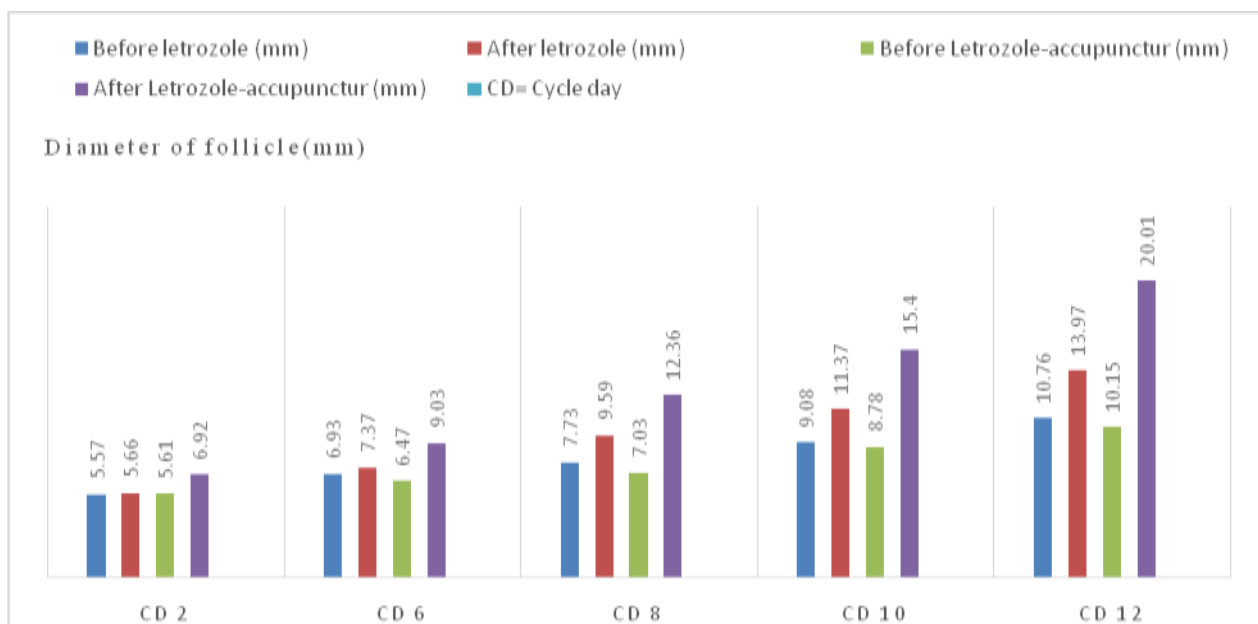
Table 1 shows that the meanof the age of the study subjects was 30 years (Mean= 30.87; SD=4.6). The average of the infertile duration was 4 years (Mean=4.87; SD=2.60). The average of the bodyweight was 64 kg (Mean= 64.93; SD=15.14). The average of the height of the study subjects was 157 cm (Mean= 157.73; SD= 4.04). The average of BMI was 25 kg/m<sup>2</sup> (Mean= 25.70; SD=5.24).

letrozole-EA groups before therapy. Table 2 shows that there was no difference in

follicular diameter in letrozole (Mean= 5.57; SD= 1.13) before therapy with p= 0.676. SD= 1.18) and letrozole-EA (Mean= 5.61; SD= 1.60)

**Table 3. The Difference of follicular diameter after therapy in the Letrozole group and the Letrozole-EA combination group**

Treatment group	n	Mean	SD	p
<b>Day 2</b>				
Letrozole	15	5.66	1.11	0.004
Letrozole-EA	15	6.92	1.60	
<b>Day 6</b>				
Letrozole	15	7.37	1.28	<0.001
Letrozole-EA	15	9.03	1.28	
<b>Day 8</b>				
Letrozole	15	9.59	2.84	<0.001
Letrozole-EA	15	12.36	1.52	
<b>Day 10</b>				
Letrozole	15	11.37	3.25	<0.001
Letrozole-EA	15	15.40	2.45	
<b>Day 12</b>				
Letrozole	15	13.97	3.79	<0.001
Letrozole-EA	15	20.01	1.82	



**Figure1. The comparison of folliculogenesis between the administration Letrozole and the combination of Letrozole-Electroacupuncture from Day 2 to Day 12**

Table 3 shows the differences in follicular diameter after therapy in the letrozole group and the letrozole-EA combination group. Table 3 shows that on day 2, there were differences in follicular diameter sizes

in the two groups. The mean of the follicular diameter in the Letrozole-EA combination group (Mean=6.92; SD=1.60) was bigger than the Letrozole group (Mean=5.66; SD= 1.11), and statistically significant (p=0.004).

On day 6, the mean of the follicular diameter in the Letrozole-EA combination group (Mean=9.03; SD=1.28) was bigger than the Letrozole group (Mean=7.37; SD=1.28), and statistically significant ( $p < 0.001$ ).

On day 8, the mean of the follicular diameter in the Letrozole-EA combination group (Mean= 12.36; SD= 1.52) was bigger than the Letrozole group (Mean= 9.59; SD= 2.84), and statistically significant ( $p < 0.001$ ).

On day 10, the mean of the follicular diameter in the Letrozole-EA combination group (Mean= 15.40; SD= 2.45) was bigger than the Letrozole group (Mean=11.37; SD= 3.25), and statistically significant ( $p < 0.001$ ).

On day 12, the mean of the follicular diameter in the Letrozole-EA combination group (Mean= 20.01; SD= 1.82) was bigger than the Letrozole group (Mean= 13.97; SD= 3.79), and statistically significant ( $p < 0.001$ ).

Figure 1 shows differences in follicular diameter size in the groups given Letrozole and Letrozole-Electroacupoint combination from Day 2 to Day 12. Figure 1 shows that there was an increase in follicular diameter size from day 2 to day 12 in each group.

## DISCUSSION

Based on the result of the study, there was a significant difference in effect between Letrozole and Letrozole-EA therapy and the improvement of folliculogenesis in women with PCOS on day 2 to day 12. However, the Letrozole-EA therapy was better than Letrozole only. It showed that on day 2 to day 6, Letrozole therapy has not shown a significant result. On day 8 to day 12 days, it showed a significant result. However, the Letrozole-EA therapy has shown significant results from day 2 to day 12. The incidence of folliculogenesis improvement in this study was almost the same as the incidence of folliculogenesis improvement in previous studies at dr. Moewardi Hospital, Surakarta, by Budihastuti et al. (2019), that there was an

improvement in folliculogenesis from day 2 to day 12 of the menstrual cycle in electroacupuncture therapy. This was probably occurred due to the similarity in the acupuncture points used, the frequency, and duration of using electroacupuncture, and the examination of follicles in the same menstrual cycle.

Even though the result of this study and study conducted by Budihastuti et al. (2019) proved that there was an improvement in folliculogenesis in patients with PCOS treated with electroacupuncture, the improvement in folliculogenesis in this study was higher than study conducted by Budihastuti et al. (2019). It probably occurred due to additional interventions such as the use of letrozole in this study which functioned as an oral ovulation induction drug. Based on a study conducted by Jain et al. (2018) who compared the use of letrozole with other ovulation induction drugs namely clomiphene citrate in patients with infertility, letrozole showed a higher ovulation rate and a higher endometrial thickness with a trilaminar pattern resulting in higher pregnancy rates. Based on a study conducted by Legro et al. (2014), letrozole was superior to clomiphene citrate as a treatment for anovulatory infertility in women with PCOS. Letrozole was associated with higher live birth and ovulation rates. However, in this study, letrozole only showed significant results on folliculogenesis on the 10th and the 12th day. When the addition of electroacupuncture was carried out, folliculogenesis had improved from day 2 to day 12.

Based on the result of a study conducted by Kusuma et al. (2019) in Dr. Cipto Mangunkusumo Hospital Jakarta, the oocyte maturation index in the EA group was significantly higher than the non-EA group ( $p = 0.002$ ) in patients who were going to do In Vitro Fertilization (IVF). It was evidenced by assessing oocyte maturation which was done microscopically by embryologists. Oocyte



maturation was the final result of folliculogenesis. Oocytes would be mature if folliculogenesis occurred properly. It is in line with this study, that EA significantly helped in the process of folliculogenesis.

According to Johansson et al. (2013), the incidence of ovulation increased in patients with PCOS who received EA. The mean of the incidence of ovulation per month in the electroacupuncture group (Mean= 0.76; SD= 0.27) was higher than the mean of the control group (Mean= 0.41; SD= 0.28), and statistically significant ( $p < 0.050$ ). The result of this study indicated that there was an improvement of folliculogenesis in the acupuncture group.

Increased sympathetic innervation of the ovary could contribute to impaired follicular development in PCOS which was supported by clinical evidence such as the increased density of catecholaminergic nerve fibers, increased production of NGF, and changes in catecholamine metabolism and/or absorption in the ovaries. It has been shown through microneurography, which was a direct and reliable measure of Muscle Sympathetic Nerve Activity (MSNA) that women with PCOS had increased sympathetic nerve activity which correlated with high testosterone levels (Johansson et al, 2013).

Androgens played a central role in the pathology of PCOS. Hyperandrogenemia in PCOS mainly came from the ovaries. It might have a central effect by increasing gonadotropin secretion through the sex-steroid feedback system. It also increased the effects on gonadotropin ovaries which stimulated the production of stimulated sex steroids. Androgen also directly disrupted the development and maturation of follicles, thus contributing to the formation of polycystic ovary morphology. Androgen was also associated with atherogenic blood lipid profile, enlarged adipocyte size, and peripheral insulin resistance. Similar to androgens, insulin resistance

and hyperinsulinemia increased ovarian gonadotropins which stimulate sex steroid production and can contribute to abnormal gonadotropin secretion. Hyperinsulinemia also reduced liver production from Sex Hormone-Binding Globulin (SHBG) which increased the amount of circulatory free sex steroids that were available biologically. PCOS was associated with an increase in Muscle Sympathetic Nerve Activity (MSNA). The interesting part was that testosterone concentration was found to be a strong independent predictor. There was also evidence supporting increased sympathetic nerve activity to the ovaries which could further encourage androgen production and Polycystic Ovarian Morphology (PCOM) (Johansson et al., 2013).

In the science of acupuncture, the human body consists of Qi, blood, and body fluid that makes our bodies alive. The terminology of Qi is used for energy that can move matter. In TCM, energy and matter are a unity known as Jing that consists of 2 aspects, namely: the first aspect, vital energy that has existed since the beginning to carry out life activities; the second aspect, the energy obtained from food, water, and air to carry out the functions of Zang Fu organs and meridians (Saputra, 2017).

Recent studies showed that acupuncture could reduce ovarian Nerve Growth Factor (NGF), corticotropin-releasing factors (CRF), endothelin-1 concentrations, and all markers of sympathetic activity. In addition, it could increase the low concentration of hypothalamic  $\beta$ -endorphins and immune function. Besides, low-frequency electroacupuncture modulated sympathetic activity by expressing mRNA and proteins from  $\alpha 1a$ ,  $\alpha 1b$ -,  $\alpha 1d$ , and  $\beta 2$  adrenoreceptors and p75<sup>NTR</sup> NGF receptors and immunohistochemical growth factors (Saputra, 2012; Yu et al, 2013; Saputra, 2017).

This study also supports a study conducted by Feng et al, (2012) that acupuncture with low-frequency electrical stimulation could reduce testosterone levels in rats with PCOS induced by DHT. There were many PCOS symptoms were associated with opioid disorders or sympathetic activity, including impaired gonadotropin secretion, insulin resistance, and central obesity (Lansdown and Aled, 2012), opioids and/or sympathetic activity that were suspected to play a role in the pathogenesis of PCOS and the effects of acupuncture on PCOS. Women with PCOS had a higher circulating  $\beta$ -endorphin levels that were suspected to interfere with the process of GnRH inhibition in the Central Nervous System (CNS). This is supported by studies in which the  $\mu$ -receptor antagonist and naltrexone could improve the menstrual cycle and increase SHBG, decrease androgen levels, LH/FSH, and LH response to GnRH (Zangeneh et al., 2011).

According to Stener-Viktorin et al, (2008), acupuncture has been shown to modulate  $\beta$ -endorphin, reduce levels of excess  $\beta$ -endorphin plasma, and reduce sympathetic activity. The involvement of the Sympathetic Nervous System (SNS) and changes in ovarian neurogenic control were suspected to be involved in the etiology of PCOS. One mediator that was suspected to be influential was NGF (Stener-Viktorin et al., 2008). Ovarian NGF production increased in follicular fluid in women with PCOS and rats with PCOS as the model induced by estradiol-valerate (EV). Excessive expression of NGF in the ovary caused ovarian hyper-innervation, stoppage of follicular growth, increased ovarian steroid response to gonadotropins, and an increase in ovarian adrenergic receptors which further strengthened the role of the sympathetic nervous system on ovarian function (Dissen et al., 2009). Electroacupuncture has been proved to reduce ovarian NGF levels, adrenergic receptors, and

expression of sympathetic activity markers in rats with PCOS as the model (Manneras et al., 2009). Based on the microneurographic measurements, there was a decrease of MSNA in women with PCOS after 14 sessions of acupuncture therapy, which further reinforced the involvement of the sympathetic nervous system both in the etiology of PCOS and the mechanism of the effect of acupuncture (Sverrisdottir et al., 2008). The effect of giving Naltrexone as a  $\mu$ -receptor antagonist that induced ovulation and decreased LH concentration as discussed above, showed the role of  $\beta$ -endorphin in PCOS. A similar effect could also be achieved through electroacupuncture followed by a decrease in  $\beta$ -endorphin levels implying the involvement of the opioid system in the mechanism of acupuncture effects. This is also supported by an experimental study that electroacupuncture affected the expression of opioid receptors of  $\mu$  and  $\kappa$  in the rat hypothalamus, which was followed by an improvement in the menstrual cycle and a decrease in circulating testosterone levels (Feng et al., 2012).

Based on the result of the analysis above, electroacupuncture, that is the insertion and manipulation of needles into acupuncture points (acupoints) that are selected specifically based on the meridian system and connected with an electrode stimulator is a simple way, cost-effective, and effective to improve folliculogenesis in patients with PCOS. The side effects are minimal. There are differences of folliculogenesis in terms of follicular diameter in women with PCOS treated with Letrozole and Letrozole-EA, where the follicular diameter in Letrozole-EA therapy is bigger.

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## CONFLICT OF INTEREST

None.

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