STUDY PROTOCOL

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Extended Letrozole Protocol Versus Letrozole Plus Inositol in Letrozole-Resistant Polycystic Ovary Syndrome Women

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Extended letrozole protocol versus letrozole plus Inositol for Induction of ovulation in letrozole resistant PCOS Women.

Thesis protocol

Submitted for fulfillment of master's degree in obstetrics and gynecology

By

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder and one of the most frequent causes of infertility in women. **(Li x et al; 2021).** It affects 5–20% of women in childbearing age. Diagnosing PCOS is challenging due to the variability of symptoms. Based on the latest clinical guideline, PCOS should be diagnosed according to the Rotterdam criteria, meaning the presence of at least two of the following criteria: ovulatory dysfunction, hyperandrogenism, or polycystic ovary morphology **(Zhang JQ et al;2022).**

The pathogenesis of PCOS is still not fully understood. On the other hand, insulin resistance (IR) has a central role in its pathogenesis **(Armanini D et al; 2022).** According to a cross-sectional study, IR is present in 75% of lean and 95% of overweight women with PCOS. It is important to emphasize that 60–70% of women with PCOS are overweight. Moreover, IR is more severe in obese women. IR and compensatory hyperinsulinemia can, directly and indirectly, lead to irregular menstrual cycles and hyperandrogenism. Higher insulin levels reduce the sex hormone binding globulin (SHBG) production of the liver. Reduced SHBG levels lead to increased free testosterone levels worsening the symptoms of hyperandrogenism. In addition, hyperinsulinemia stimulates the androgen overproduction of ovarian theca cells (**Notaro ALG et al ;2022**).

In the treatment of PCOS, metformin is the gold standard metabolic treatment. However, metformin may induce mild to severe gastrointestinal side effects such as nausea, diarrhea, vomiting, and flatulence (Rajasekaran **K, et al 2022**). Therefore, alternative treatment with fewer side effects would be beneficial in managing these patients. In recent years, several studies have analyzed the potential effects of

inositol supplementation, suggesting that inositols are potent alternatives for metformin in treating PCOS (**Soldat-Stanković V et al; 2022**).

Inositols belong to the vitamin B complex group, which is synthesized in the human body. There are nine stereoisomers, of which the most important ones are myoinositol and D-chiro-inositol (**Facchinetti F et al; 2020).** Inositols are considered insulin sensitizers, as they modulate the members of insulin signaling pathways. They positively influence menstrual cycle regularity, carbohydrate metabolism, and the clinical and laboratory symptoms of hyperandrogenism (e.g., free testosterone, total testosterone, SHBG). However, to date, the level of evidence has not been satisfactory for accepting them as standard therapy in the guidelines.

Myoinositol is synthesized from glucose-6-phosphate (G6P) endogenously. On the other hand, it can be found in the cell membranes as phosphatidyl-myoinositol as the precursor of inositol triphosphate (PIP2), which plays a crucial role in the signal transduction of various receptors, including FSH, promoting granulosa cell differentiation and follicle maturation. In addition, myoinositol might improve oocyte and embryo quality by encouraging translocation of GLUT4 to the plasma membrane in order to increase glucose uptake and promote aromatase activity. During the secondary signaling mechanisms, inositol triphosphate (IP3) will also be released, which can be converted to free myoinositol by inositol-monophosphat (**Dinicola S. et al 2021**).

letrozole (LE), a specific aromatase inhibitor, was first administered by Mitwally and Casper to women with PCOS who were resistant to clomiphene citrate (CC). LE could prevent the hypothalamic–pituitary axis from receiving estrogen-negative feedback by inhibiting estrogen biosynthesis, thus increasing follicle-stimulating hormone (FSH) production and promoting follicle growth (**Franik S et al; 2018**). Recently, LE gradually has replaced CC as the first-line ovulation induction agent administered to women with PCOS owing to the high rates of live birth and pregnancy **(Ezeh U. et al; 2022).**

The commonly used LE regimen for ovulation induction in women with PCOS is 2.5 mg daily for 5 days. If the initial dose fails to initiate follicular development, it often is increased to 5 mg in the next cycle after a progesterone-induced withdrawal period, and a maximum daily dose of 7.5 mg will be used in the subsequent cycle if still anovulatory (**Wang R. et al ;2019**).

LE resistance is failure of ovulation after a 5-day regimen of 5 mg per day for three cycles.

In accordance with the theory that the "FSH window" is as important as the "FSH threshold" during the selection of dominant follicles, hypothesized that longer treatment with LE could extend the "FSH window", thereby inducing follicle growth in patients who initially do not respond to routine treatment (**Thomas S. et al ;2019**).

In previous study **(Xiuxian Zhu et al;2022)** examined effect of extended letrozole therapy to 7 days and its effect on ovulation rate in letrozole resistant PCOS women was.

Another study **(Sajadeh Pourghasem et al;2019)** examined effect of adding inositol 3 months before letrozole induction in letrozole resistance PCOS women.

. In our study, we will compare between effect of extended letrozole therapy versus adding inositol in letrozole resistant women

Aim of the work

The aim of current study is to compare between extended letrozole protocol and letrozole plus Inositol for Induction of ovulation in letrozole resistant pcos.

Patients and Method

This is a prospective , randamized and comparative study that will be conducted at obstetrics, gynecology department –Kafer Elsheikh University Hospitals during the period from February 2024 to February 2025 after approval of the local ethical committee.

200 candidates will be recruited from gynecology clinic – Kafer Elsheikh University Hospital.

Inclusion criteria

- Women diagnosed with PCOs according to Rotterdam criteria (at least 2 of the following 3 criteria: chronic anovulation or oligomenorrhea, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries on ultrasound (an ovary that contains ≥12 antral follicles measuring 2–9 mm in diameter or at least 1 ovary with an increased ovarian volume (>10 cm^3 without concomitant cysts).
- Women who are resistant to letrozole.
- Age less than 35 years old.
- Women with normal HSG and/or laparoscopy.
- Women whose BMI less than or equal 30 kg/m2.
- Normal semen analysis.

Exclusion criteria

- Women complaining of any endocrine disorders such as active thyroid disorders, adult-onset adrenal hyperplasia, diabetes, hyperprolactinemia, adrenal causes of hyperandrogenemia.
- Women who concomitantly received other insulin sensitizing agents such as Metformin.

- Women with any contraindications to pregnancy.
- Women with any contraindications to inositol as chronic liver diseases and chronic kidney diseases.

Patient evaluation

- Full history taking and clinical examination will be done for all candidates.
- Menstrual cycle will be induced via progesterone in oligomenorrheic women.
- All patients will be evaluated on the 2nd day of their spontaneous or induced menstrual cycles by using transvaginal ultrasonography and hormone tests (Total, Free testosterone, DHEA, TSH, prolactin levels).
- Weight, height, waist circumference, hip circumference, and ferriman gallwey scores (FGS) (to categorize hirsutism) will be measured in the first visit.
- Body mass index (BMI) was defined as the ratio of weight (kg) to height (m2) squared (kg/m2). Waist circumference in cm; hip circumference in centimeters.
- All candidates have a history of LE resistance with a 5-day regimen.
- candidates will be classified into 2 groups.

Group 1

- 100 candidates will receive LE 5 mg daily, starting on day 2 of the menstrual cycle (MC), for 7 consecutive days in women with PCOS and LE resistance.
- Follicular monitoring every 2 days after the last dose of LE using a transvaginal ultrasound examination to record the developing follicles and endometrial thickness until the confirmation of ovulation.
- exogenous human chorionic gonadotropin will be given when at least 1 follicle has a diameter of 18_20 mm .

Group 2

- 100 candidates will receive 2 g of inositol twice daily for 3 months.

- In the last cycle, letrozole will be prescribed at a dose of 5 mg per day from the 2nd day of menstruation for 5 days for the induction of ovulation.
- Follicular monitoring every 2 days after the last dose of LE using a transvaginal ultrasound examination to record the developing follicles and endometrial thickness until the confirmation of ovulation.
- exogenous human chorionic gonadotropin will be given when at least 1 follicle has a diameter of 18_20 mm .

outcome

Primary outcome:

Ovulation rate.

Secondry outcomes:

Spontaneous ovulation rate.

Clinical pregnancy rate.

Ovarian hyperstimulation syndrome rate.

Endometrial thickness.

ETHICS OF RESEARCH

IRB (The Institute for Research in Biomedicine) **approval:**

The clinical research study will be conducted in accordance with the current IBR-approval clinical protocol; ICH GCP Guidelines. (ICH: International Conference on Harmonization) (GCP: Good Clinical Practice) and relevant policies, requirements, and regulations of kafer Elsheikh University.

Declaration of Helsinki:

The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. The committee must have the right to monitor ongoing studies**.** Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information**.** each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study.

Consent procedure:

The investigator will inform all participants about the importance of the study and their role and rights in participation, written informed consent will be signed by the patients after explanation of the research.

Subject Confidentiality:

All evaluation forms, reports, and other records that leave the site would not include unique personal data to maintain subject confidentiality.

Statistical analysis:

The collected data will be coded, processed and analyzed using SPSS program (Version 22) for windows. The appropriate statistical tests will be used when needed. P values less than 0.05 (5%) will be considered to be statically significant.

Sample Size

Based on the results of a previous study1, the mean±SD of Time to ovulation (d) was assumed as 16.19 ± 3.04 in extended letrozole therapy group, and 17.44 ± 3.01 in inositol in letrozole group. To detect the difference between groups with a power of 80%, a level of significance of 5% and with an effect size of 0.41, a total sample size of 186 participants was needed, at ≥93 participants for each study group. The sample size was calculated by G*Power (version 3.1.9.2; Germany).

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