University of Basrah
College of Pharmacy
Department of Pharmacology and Toxicology

Induction Of Polycystic Ovary Syndrome By Letrozole In Female Rats

By :
Mustafa Khazal Halawi
Hussein Ali shareef
Mohammed Abdulkareem abdulhussein

Supervisors
Assist prof: Dr. Muhsin S. Ghalib
Assist Lecture: Ali M. Alrawaq
2020-2021

Abstract

Background: Polycystic ovary syndrome (PCOS) is a widespread endocrine disorder, affecting approximately 20% of women within reproductive age. It is associated with hyperandrogenism, obesity, menstrual irregularity, and anovulatory infertility.

This review will cover functions of the ovary and hypothalamic-pituitary-gonadal axis (HPG-A), in addition to the role of insulin resistance and related hormones in PCOS pathogenesis, female infertility disorders and animal models of induced cystic ovary disease.

Aim: to develop a new animal model for the study of polycystic ovaries by using the non-steroidal aromatase inhibitor, letrozole as inducer for PCOS .

Methods: Sixteen rates were divided into two groups, including a control of eight rats that received normal saline only once daily orally, and induction group of eight rats which administered letrozole 1mg/kg of D.W dissolved. The treatment period was 28 days. On the day subsequent to last letrozole dose administration, rats were killed; uteri and ovaries were then excised and weighed. Histologic changes in ovaries were examined.

Results: When compared to control group, ovaries from study groups showed high incidence of subcapsular ovarian cyst and capsular thickening together with incomplete luteinization and decreased number of corpora lutea. Letrozole treatment brought about dose-dependent suppression of uterine weight despite having no significant effect on ovarian weight.

1- Introduction :

1.1- Letrozole:

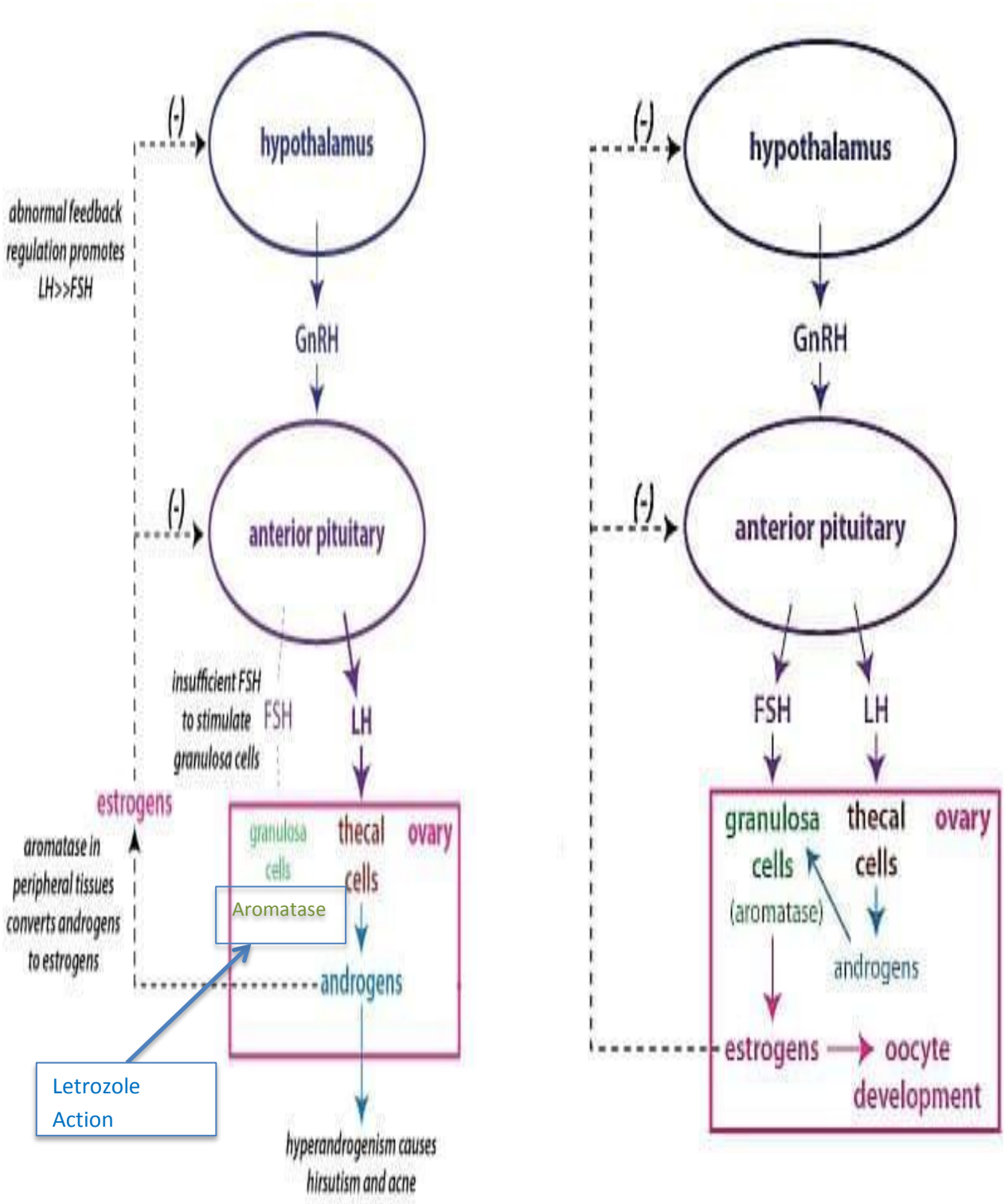
Letrozole is a highly potent and selective aromatase inhibitor (AI) that inhibits the enzyme activity of intracellular aromatase at the major sites where it is found, resulting in almost complete suppression of whole body aromatization. chemical structure of letrozole is (4,4′-[(1H-1,2,4-triazol-1-yl) methylene] bis-benzonitrile). This modern third-generation aromatase inhibitor (AIs) effectively block the production of estrogen without significantly affect cortisol, aldosterone, and thyroxine.

1.1.1-Indications :

Letrozole is an aromatase inhibitor used in the treatment of breast cancer. As breast tissue is stimulated by estrogens, decreasing their production is a way of suppressing recurrence of the breast tumor tissue, extended adjuvant treatment of early breast cancer after 5 years of tamoxifen , treatment of advanced breast cancer with disease progression following anti estrogen therapy. Adjuvant treatment of hormone receptor-positive early breast cancer , first-line treatment of hormone receptor-positive or hormone receptor-unknown ,also For locally-advanced, or metastatic breast cancer [1].

1.1.2-Mechanism of action:

Letrozole inhibits cytochrome P450 enzyme (aromatase) by the catalytic action of which estrogen is synthesized by the androgens. The absence of aromatase enzyme causes disturbance in the steroidogenesis, thus causing increase in the production of androgens developing PCOS [2]. Aromatase (cytochrome P-450 [CYP] 19) catalyzes the rate-limiting step (conversion of steroidal C-19 androgens to C-18 estrogens) in estrogen biosynthesis [3]. Aromatization is the final step in steroid biosynthesis, and therefore, aromatase is an attractive target for selective inhibition . Aromatase is expressed primarily in the ovary and also in central and peripheral tissues, fat, muscle, liver, and breast [4]. With increasing age, as ovarian estrogen production declines, and the contribution of peripheral production of estrogens increases (from the adrenal gland).



Mechanism of action of letrozole **Normal Axis**

***http://courses.washington.edu/pbio376/pcos/pcos-376.html**

1.2- Polycystic ovary syndrome (PCOS):

Polycystic ovary syndrome is an endocrine disorder characterized by hyperandrogenism , hyperinsulinemia, abnormal menstrual cycle , and a small cysts that appear on one or both of the ovaries with size up to 8 mm . This health problem affects 1 in 10 women of childbearing age. The clinical features of PCOS include causing inhibition of follicular development, anovulation, a group of micro cysts in the ovaries, and menstrual changes .

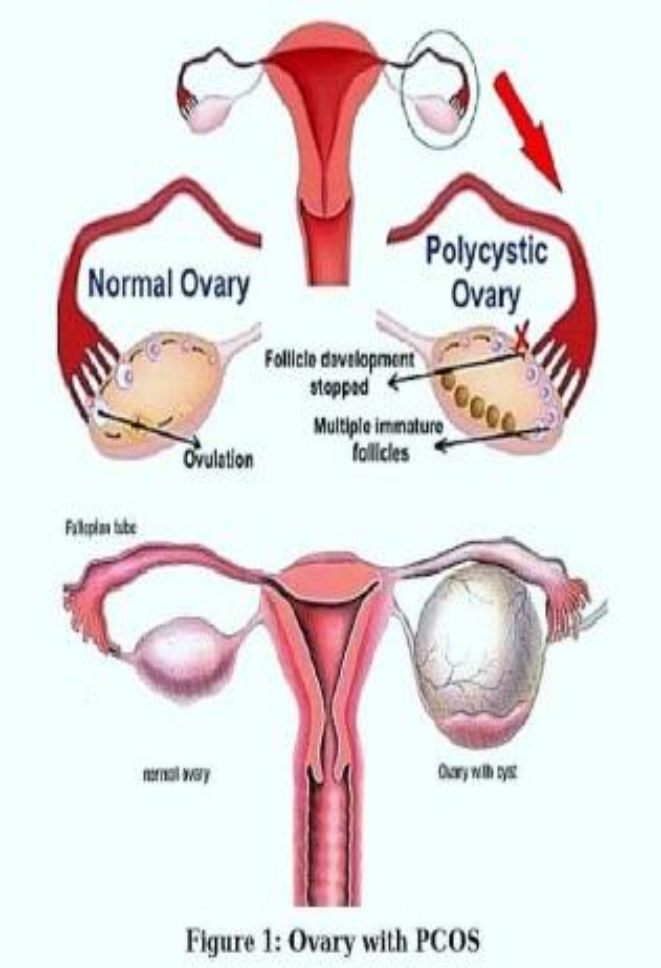


Figure 1: Ovary with PCOS

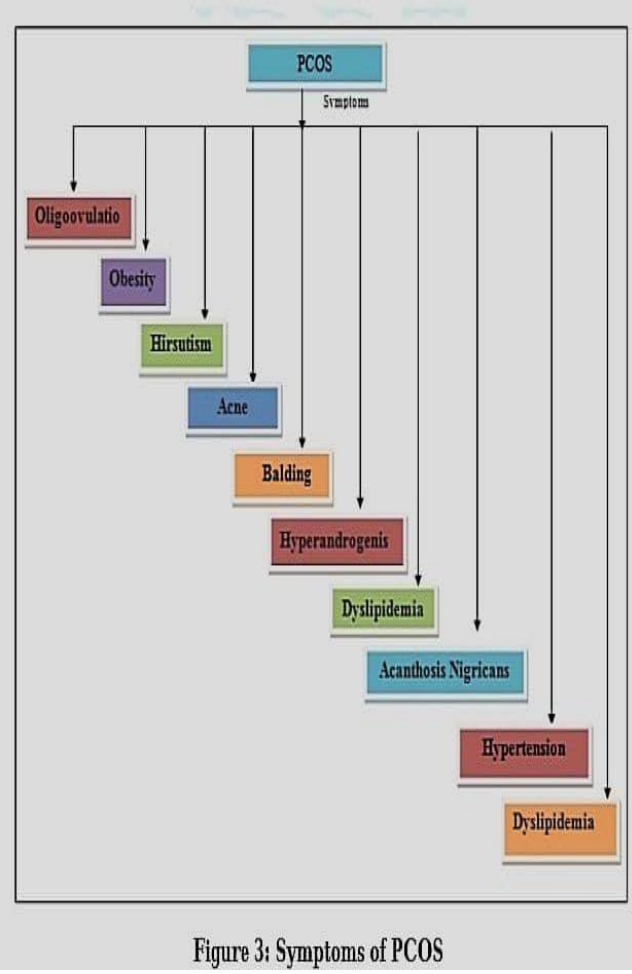


Figure 3: Symptoms of PCOS

**Abhishek Soni, Dr. Shivali Singla , Dr. Sachin Goyal Polycystic Ovary Syndrome .*

1.3- Definitions and Prevalence :

Polycystic ovary syndrome (PCOS) in women is a complex disorder of unknown etiology; however, PCOS rodent models highlight insight and opportunities to unravel the pathogenesis of this disorder.

According to the National Institutes of Health Office of Disease Prevention, in the U.S PCOS syndrome affects approximately 5 million women of reproductive age . Some research suggests that (5% to 10%) of women at 18 to 44 years of age are affected by PCOS, making it the most common endocrine disorder among women of childbearing age in the U.S[5] .The Prevalence of PCOS in India ranges from 3.7 to 22.5 per cent depending on the population studied and the criteria used for diagnosis [6] , in Chinese women aged (19 - 45) years is 5.6% [7], while other rates 23.5% in Spain and 23.9% in Portugal [8].The overall prevalence among Iranian women with PCOS was 26.6% . [9], while the Prevalence of PCOS among infertile Kurdish women, attending infertility Care and In vitro fertilization (IVF) Center in Erbil was 33% [10] .

1.4- Ovarian anatomy :

The word "ovary" is derived from the Latin word "ovum," meaning egg. The mammalian ovary is not merely the female gonad containing the supply of germ cells to produce the next generation, but also the female reproductive gland, controlling many aspects of female development and physiology .

Two ovaries are located in the pelvic cavity of female, each suspended between the peritoneum and the uterus by ligaments. The body of ovary is largely composed of stromal tissue, stratified into an inner medulla and outer cortex, which is encapsulated by the ovarian surface epithelium and an outermost covering of connective tissue(tunica albuginea) .

The cortical region of each ovary contains immature germ cells(oocytes), each surrounded by a single layer of squamous granulosa cells(GCs), collectively, this forms a structure called a primordial follicle (Findlay *at. al*., 2009). The basement

membrane separates the epithelial granulosa cells from the mesenchymal thecal and stromal cells.

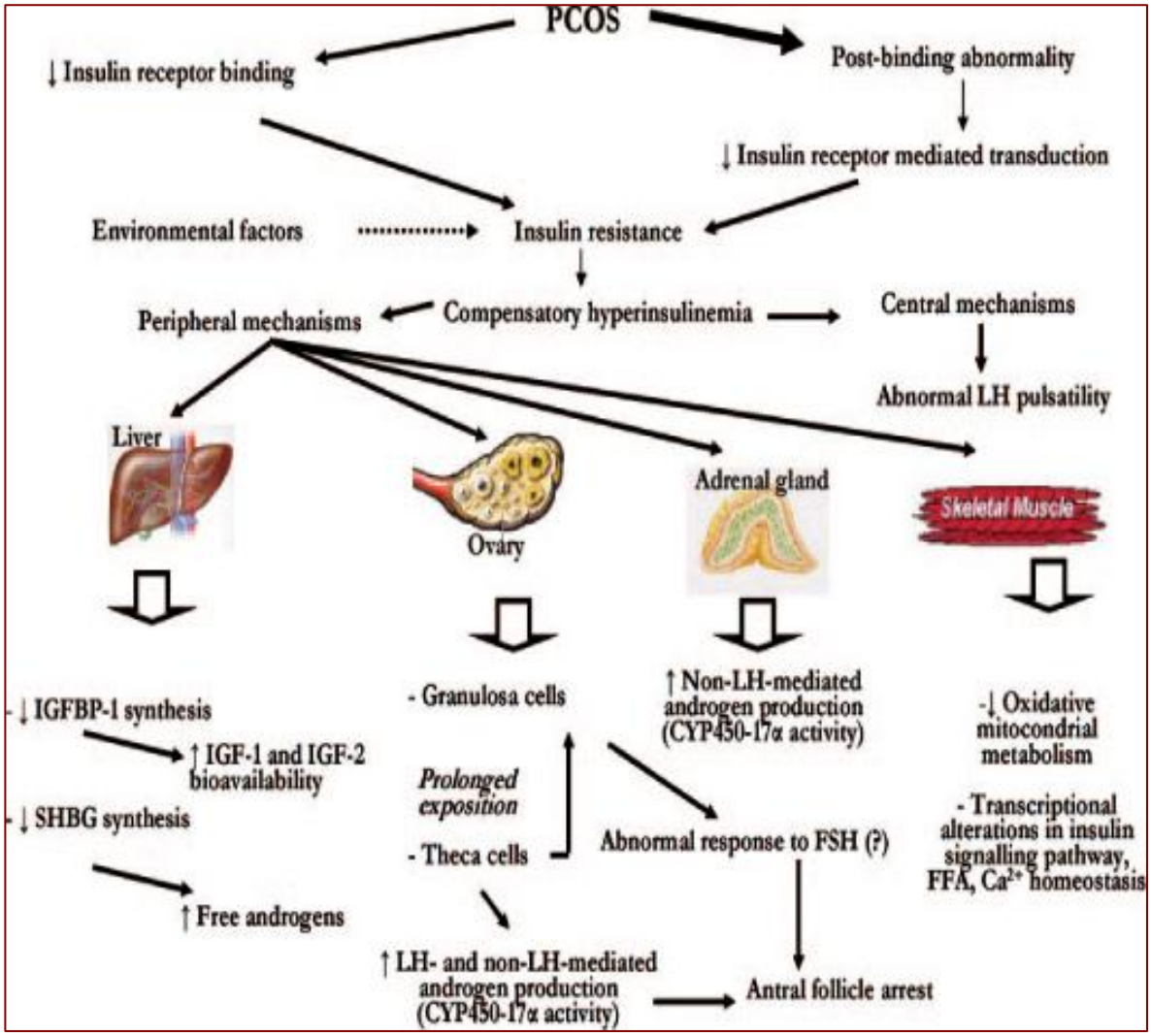
1.5- Pathophysiology of polycystic ovary syndrome(PCOS) :

Many researchers mentioned that the pathophysiology of PCOS is unclear and there is still missing a lot of reasons to reach an accurate diagnosis, however, it is believed that the genetic factors and environmental conditions may cause the hormonal imbalance in patients with PCOS. Also, some studies found that significantly higher than the 6-7% have increased androgens, insulin resistance, also, appear to be under significant genetic control, as established family studies.

Many reported indicated that the pathogenesis, although the etiology of PCOS is unknown and it's a complex and multifactorial causes but is poorly understood.

The folliculogenesis and follicles maturation are complicated developmental processes through which a mature follicle is differentiated from primordial follicles, yielding a one mature follicle that is eventually selected to ovulate, releasing a mature oocyte. This developmental process can be disrupted by abnormal extra-ovarian endocrine factor responsible for the pathogenesis of PCOS, consequently increasing the risks of impaired oocytes miscarriage, LH, FSH, Insulin and androgen…other .

In addition to that the intra-ovarian factors balance and regulate the folliculogenesis: Epidermal growth factors(EGF), Fibroblast growth factor (FGF), Intraovarain factor (IGF), Nurotrophin growth factor (NGF), Transforming growth factor-ß (TGF-ß), Vascular endothelial growth factor (VEGF), Cytokines and Resistin, and Other microvironment factors. Androgens and insulin are two key endocrine mediators. Several theories have been proposed to explain the pathogenesis of PCOS. However hyperinsulinemia may effects in female with PCOS through its action at non ovarian sites including the liver, adrenal glands, fatty acids and pituitary gland. [11]



this figure illustrates the complex interactions underlying the pathophysiology of PCOS (Nestler, 2008).

1.6- Non pharmacological Approaches :

Because the primary cause of PCOS is unknown, treatment is directed at the symptoms. Few treatment approaches improve all aspects of the syndrome, and the patient's desire for fertility may prevent her from seeking treatment despite the presence of symptoms. Treatment goals should include correcting anovulation, inhibiting the action of androgens on target tissues, and reducing insulin resistance .

Non pharmacological methods as :

1- Weight reduction for obese patients with PCOS : is beneficial in many ways. Weight loss helps to decrease androgen, luteinizing hormone (LH), and insulin levels. It also helps to regulate ovulation, thereby improving the potential for pregnancy. [12]

2- Laparoscopic ovarian drilling : is an outpatient surgical intervention in which multiple perforations are created in the ovarian surface and stroma.It is thought that this intervention destroys androgen-producing tissue, which should lead to decreased androgen levels. It has been found to be as effective
as medical interventions without increasing the risk of multiple pregnancies.[13]

1.7- Suggested Therapy For PCOS:

Multiple concomitant therapies have been applied in PCOS to address the variety of symptoms and to achieve better results. Medications should only be described as an adjunct to diet and exercise, 1)Oral contraceptive pills(OCPs), depending on the estrogen dose, can inhibit androgen, LH and FSH production and stimulate regular menstrual cycles, 2)Insulin-sensitizing drugs(ISD) metformin, rosiglitazone, pioglitazone, D-chiro-inositol 3)androgen-blocking drugs, spironolactone, flutamide, cyproterone, finsteride 4)Fertility drugs, Clomiphene,(Clomid) is generally the first fertility medication, Human Chorionic Gonadotropin (HCG) or Pergonal are the three groups of medications used for PCOS treatment.

1.7.1- Clomiphene :

The drug of choice for inducing ovulation in PCOS is clomiphene citrate (Clomid, Sanofi) .Clomiphene results in successful pregnancies approximately 30% of the time . Initially, a dose of 50 mg/day for 5 days is given. If ovulation occurs but no pregnancy results, 50 mg/day for 5 days is continued for the subsequent cycles. However, if ovulation does not occur after the first cycle, the dose may be increased to 100 mg daily for 5 days at least 30 days after the previous course of therapy [9].

Clomifene has both estrogenic and anti-estrogenic properties (selective estrogen receptor modulator) , but its precise mechanism of action has not been determined. Clomifene appears to stumulate the release of gonadotropins, follicle-stimulating hormone (FSH), and leuteinizing hormone (LH), which leads to the development and maturation of ovarian follicle, ovulation, and subsequent development and function of the coprus luteum, thus resulting in pregnancy. Gonadotropin release may result from direct stimulation of the hypothalamic-pituitary axis or from a decreased inhibitory influence of estrogens on the hypothalamic-pituitary axis by competing with the endogenous estrogens of the uterus, pituitary, or hypothalamus. [14]

1.7.2- Metformin :

Antidiabetic agents can be used to increase fertility, reduce insulin resistance, and decrease circulating androgen levels. The role of metformin in the treatment of infertility in patients with PCOS was compared with placebo in a study that involved 320 women, where After 3 months of treatment with no resulting pregnancies, an appropriate infertility drugs allowed to be added to the regimen for either group. When compare Metformin with placebo, there is a significantly higher rate of pregnancy (53.6% vs. 40.4%, respectively) and live birth rates (41.9% vs. 28.8%; respectively) compared with placebo. In a meta-analysis for the efficacy of metformin was evaluated in improving fertility for women with PCOS, there was no evidence of improved rates of live births with metformin alone or in combination with other drugs. [15]

1.7.3- Spironolactone :

Spironolactone (Aldactone, Pfizer) is antiandrogen that work in PCOS by decreasing androgen level, thereby reducing the signs of hirsutism and acne. This antiandrogen may also improve lipid levels, which can be elevated in patients with PCOS. The effects of spironolactone 100 mg daily were compared in 40 women with hirsutism for 6 months. The drug found to be efficacious. Spironolactone, at a dose of 25 to 100

mg twice daily, is the most commonly used antiandrogen because of its safety, availability, and low cost. Because of the increased risk of teratogenicity to the male fetus , contraception is recommended when patients are using antiandrogens for the treatment of PCOS. [16]

2- Materials and Method :

The study comprises two experiments a sixteen virgin adult cycling female rats 12 weeks old, weighing 200 ± 25 g were used.

Female rats were kept for adaptation period of one month at the animal house of College of pharmacy / University of Basra.

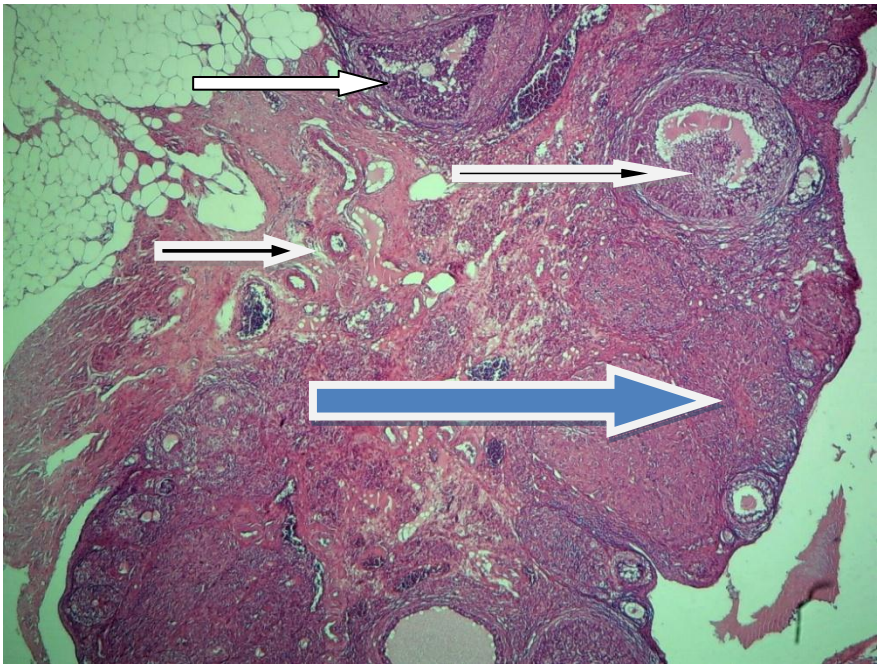
The animals were housed as four rats to each cage under optimum conditions (12/12 light, dark cycle, 25 ± 2 C˚).

Sixteen female were divided into two groups, including a control of eight rats that received normal slain only once daily orally, and induction group of eight rats which administered letrozole 1 mg/kg dissolved in D.W . [17] The treatment period was 28 days. On the day subsequent to last letrozole dose administration, rats were killed; uteri and ovaries were then excised and weighed. Histologic changes in ovaries were examined.

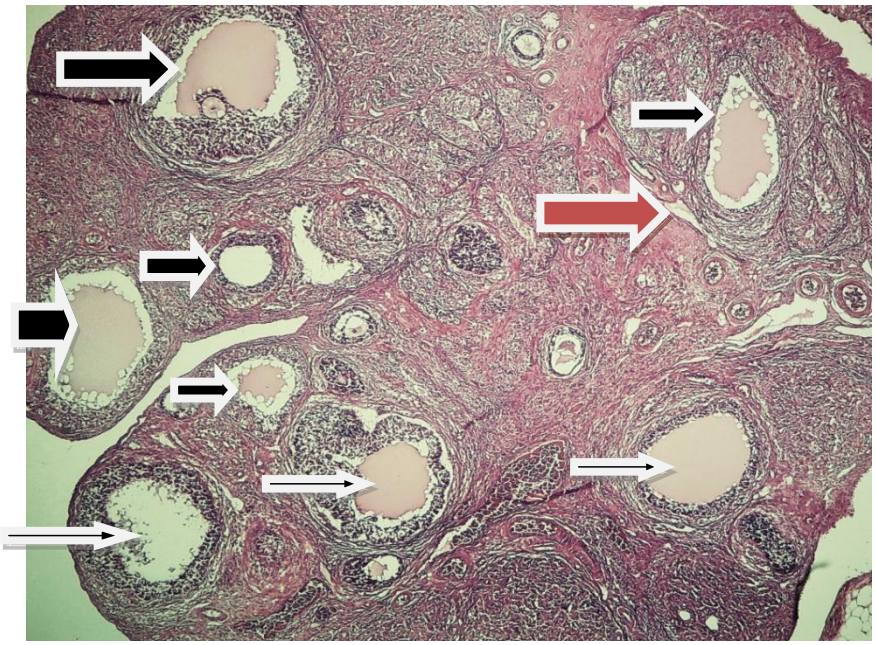
3- Result and Discussion :

The ovary of control female rats shows normal ovarian cellular tissue with normal Graafine follicles. Also, there are normal primary follicles and secondary follicles, in addition to post ovulatory corpora lutea, figure (6), while, figure (7), shows the ovary of letrazole treated female rats that contain both multi luteum cysts and follicular cysts. The follicular cyst varies in size, some of them are encapsulated with thick fibrous tissue capsules, the shell is still having ovum, the large, having degenerated

tissues. Figure (7) shows that the largest ovarian cyst with big antrum, thick of theca (hyperthecosis) and granulosa cells.



Figure(6) micrograph of ovary female rat of control, Shows normal ovarian cellular tissue with normal follicles (normal follicles (black arrow), corpus luteum (blue arrow), normal Graafine follicles (white arrow). Stain(H&E) 40X.



Figure(7): micrograph of ovary female rat of induced PCOS, Shows follicular cysts with colloid (black arrow); thickness in granulosa cell(red arrow). Stains (H&E) 400X.

In this study, the rat model of letrozole-induced PCOS was established to explore the changes of ovarian morphological. This study revealed that the rats in the control group had normal ovarian morphology and ovary color. However, in the model group, multiple transparent cystic of dilated follicles were found to be densely distributed on the ovarian surface, and the ovarian morphology was polycystic and the ovary color was pale. H&E staining found that the pathological changes of ovaries in the rat with letrozole-induced PCOS were structural abnormalities: immature follicles and atresia follicles significantly increased, multiple cystic dilated follicles appeared on the surface of the ovaries, and follicles and corpus luteum in development stage were significantly reduced. Microscopy (40×) showed that the structure of the follicles in the control group was intact with 8 to 9 layers of granulosa cells. However, in the model group, the granular cell layer and radioactive crowns in the follicles were significantly decreased or disappeared (Figure 7). As shown in Figure 7, the number of cystic dilated follicles on the ovary surface of rats in all the model groups was significantly increased ($P<0.05$).

4- Conclusion :

Polycystic ovary syndrome is a complex endocrine disorder . the suggested method to develop a new animal model for the study of polycystic ovaries by using the non-steroidal aromatase inhibitor, letrozole as inducer for PCOS with dose 1 mg/kg (dissolved in D.W) per day for 28 days . multiple treatment approaches for PCOS are required, depending on the reason a patient seeks treatment . Long-term consequences of PCOS, which include type-2 diabetes and cardiovascular disease, can be treated with Antidiabetic drugs and statins or other treatment according to the reason of cardiovascular disease.

Reference :

(1) FDA Approved Drug Products: Kisquali Femara Co-Pack Letrozole and Ribociclib Succinate Oral Tablets.

(2) Corbin C, Trant J, Walters K, Conley AJ. Changes in Testosterone Metabolism Associated with the Evolution of Placental and Gonadal Isozymes of Porcine Aromatase Cytochrome P450 1. Endocrinology. 1999;140(11):5202–10.

(3) Ryan KJ (1959) Biological aromatization of steroids. J Biol Chem 234:268–272

(4) Longcope C, Pratt JH, Schneider SH, Fineberg SE (1978) Aromatization of androgens by muscle and adipose tissue in vivo. J Clin Endocrinol Metab 46:146 – 152.

(5) National Institutes of Health Department of Health and Human Services. Beyond Infertility: Polycystic Ovary Syndrome (PCOS) NIH Pub. No. 08-5863, April 2008.

(6) Joshi B, Mukherjee S, Patil A, Purandare A, Chauhan S, Vaidya R. A cross-sectional study of polycystic ovarian syndrome among adolescent and young girls in Mumbai, India. Indian J Endocrinol Metab. 2014;18:317–24.

(7) Asuncion et al., 2000; Teimuraz et al.,2005; March et al., 2010; Franks, 2011.

(8) A.C. Santos, C. Lopes, H. Barros Prevalence of metabolic syndrome in the city of Porto Rev Port Cardiol (Lisboa), 23 (2004), pp. 45-52 .

(9) https://pubmed.ncbi .nlm.nih.gov/31235114 .

(10) Bayan H ،Shahla A. Prevalence and characteristics of polycystic ovarian syndrome in a sample of infertile Kurdish women attending IVF infertility center in maternity teaching hospital of Erbil City. No.7(2013), Article ID:37028,9 pages DOI:10.4236/ojog.2013.37104 .

(11) Nestler, JE. (2008). Metformin in the treatment of infertility in polycystic ovary syndrome. Fertil Steril, 90:Pp:14-16 .

(12) Guzick DS. Polycystic ovary syndrome. Obstet Gynecol. 2004;103(1):181–193.

(13) Farquhar C, Brown J, Marjoribanks J. Laparoscopic drilling by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. Cochrane Database Syst Rev. 2012 Jun.

(14) https://go.drugbank.com/drugs/DB00882

(15) Tang T, Lord JM, Norman RJ, et al. Insulin-sensitising drugs (metformin, rosiglitazone,pioglitazone, d-chiro-inositol) for women with polycystic ovary syndrome, oligoamenorrhoea, and subfertility.Cochrane Database Syst.

(16) Studen KB, Sebestjen M, Pfeifer M, et al. Influence of spironolactone treatment on endothelial function in non-obese women with polycystic ovary syndrome. Eur J Endocrinol. 2011;164(3):389–395.

(17) Kafali H, Iriadam M, Ozardali I, Demir N. Letrozole-induced polycystic ovaries in the rat: a new model for cystic ovarian disease. Arch Med Res 2004; 35: 103-108 .