

RESEARCH-ANIMAL

The effect of letrozole treatment on the fertility and embryos parameters in female mice: Histological and hormonal study

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Abstract

Objective: To evaluate how letrozole affects reproductive hormones and embryos in mature female mice.

Method: The investigative study was conducted from December 2021 to February 2022 at the Pathology Department of Kut Technical Institute, Al-Kut, Iraq, and comprised adult female albino mice weighing 23-25g. The mice were divided into control group A, those receiving 1.5mg/kg/day letrozole in group B, and those receiving 2.5mg/kg/day letrozole in group C. The intervention lasted 14 days. Blood samples were collected under anaesthesia by heart puncture for hormonal testing. Data was analysed using SPSS 15.

Results: Of the 30 mice, 10(33.3%) were in each of the 3 groups. Right and left ovaries of mice in groups B and C showed a significant rise in multi-follicular cells compared to group A ($p<0.05$). Follicle-stimulating hormone, oestrogen and progesterone concentrations as well as luteinizing hormone increased significantly in the intervention groups compared to the control group ($p<0.05$). The impact of letrozole on embryos was significant in groups B and C compared to group A.

Conclusions: Letrozole was found to have a positive effect on reproductive parameters and hormones in mice.

Keywords: Mice, Ovary, Letrozole, oestrogen, Progesterone, Luteinizing hormone.

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Introduction

Ovulation issues, also known as ovulatory dysfunction, are one of the most frequent reasons for infertility and subfertility in couples. In contrast, 8-10% of women have polycystic ovarian syndrome (PCOS), which may be a significant contributor to female infertility.¹ Women who have this syndrome exhibit hyperandrogenism, polycystic ovarian morphology alterations, and excessive gonadotropin production (hyperinsulinaemia).² Successful stimulation of ovulation frequently restores normal fertility. The best medication to treat PCOS is letrozole.³

In a 1986 in vivo experiment, Ciba-Geigy (later Novartis) investigated a novel chemical⁴ CGS 20267, a third-generation nonsteroidal aromatase inhibitor that is now sold as letrozole.⁵ Letrozole was found to be effective in a variety of breast cancer scenarios, which was the drug's sole registered use at the time.⁶ Letrozole was first employed in animal ovulation induction (OI) in 1993.⁷ Patients with PCOS had a high rate of ovulation, according to the initial pilot trial for the clinical utilisation of letrozole for OI in 2000.⁸ According to a 2004 study, letrozole is a more effective treatment for OI than clomiphene in PCOS-affected women. This implied that letrozole would be a superior first-line

treatment.⁹ Since that time, letrozole's usage in the treatment of infertility has gained significant popularity, and research into its clinical effects and mode of action has continued. Its use was also associated with a number of advantageous side-effects.¹⁰ Letrozole, a selective oestrogen receptor modulator, enhances the synthesis of gonadotropin by preventing the hypothalamus's negative feedback loop. It is a recently developed drug that is used in infertility of female, primarily for ovarian stimulation to treat oligo-ovulation or anovulation and for ovarian hyperstimulation, such as during an in vitro fertilisation (IVF) process.¹¹ Nonsteroidal aromatase inhibitors, which include letrozole, are a class of medicines. As an anti-oestrogen, it inhibits the body's production of oestrogen by competitively binding to oestrogen receptors in the hypothalamus and pituitary glands. There are times when human chorionic gonadotropin (hCG), human menopausal gonadotropin (hMG) and other gonadotropins are added to luteinizing hormone (LH) and follicle-stimulating hormone (FSH).¹² Letrozole has recently been discovered to sensitise pituitary gonadotropins to luteinizing hormone-releasing hormone (LHRH) activity, resulting in an excess of LH and FSH secretion in reaction to LHRH both in vivo and in vitro.¹³ Furthermore, numerous studies have shown that letrozole affects the ovarian tissues that are sensitive to oestrogens, such as the rodent ovary and the human endometrium, as well as the physiological activity of oestrogens.¹⁴ Additionally, there are a number of side-effects associated with letrozole that can be avoided, including ovarian hyperstimulation, stout mucus of the

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cervical, abdominal pain, loss of hair, as well as a physiological or corpora lutea cyst.¹⁵

The current study was planned to investigate how letrozole affects the histology and hormonal conditions of adult female mice.

Materials and Methods

The investigative study was conducted from December 2021 to February 2022 at the Pathology Department of Kut Technical Institute, Al-Kut, Iraq. After approval from the ethics review committee of the College of Medicine, Al-Nahrain University, Baghdad, Iraq, adult female albino mice weighing 23-25g were acquired from the Kut Technical Institute's animal housing unit. The mice were divided into control group A, those receiving 1.5mg/kg/day letrozole in group B, and those receiving 2.5mg/kg/day letrozole in group C. The intervention lasted 14 days.

Letrozole was dissolved in distilled water to make a stock solution, which was then divided into two concentrations of 1.5mg/ml and 2.5mg/ml. The mice were administered each concentration of letrozole orally by intragastric intubation.

Freshly sacrificed mice's ovaries were fixed with 10% formalin for 12h, dehydrated with increasing intensity of ethanol alcohol, cleaned with xylene for 30m, and exchanged with other paraffin overnight in the oven. From a paraffin block, serial sections were cut and stained with alum haematoxylin and eosin (H&E). Using a light microscope, the slides were examined 4X. All serial portions of the ovaries were enumerated in the light of literature.¹⁶ The parameters included the number of primary follicles, developing follicles, Graafian follicles, ovarian weight, as well as the number of single and twin embryos.

Blood samples of the mice were collected under anaesthesia by heart puncture, using a needle (22-19mm). The serum for the hormone testing was obtained by centrifugation at 3,000rpm for 10m and kept at -20°C, and the testing was done using a Mini-Vidas instrument.

Data was analysed using SPSS 15. Paired sample t-test was used in crude data analysis to compare treatment groups with the control group. The findings were expressed as mean and standard error of mean (SEM).¹⁷ Statistical significance was defined as $p < 0.05$.

Results

Of the 30 mice, 10(33.3%) were in each of the 3 groups. Right and left ovaries of mice in groups B and C showed a significant rise in multi-follicular cells compared to

group A ($p < 0.05$). FSH, oestrogen and progesterone concentrations as well as LH increased significantly in the intervention groups compared to the control group ($p < 0.05$). The impact of letrozole on embryos was significant in groups B and C compared to group A (Tables 1-2; Figures 1-2).

Transverse ovarian sections of group A showed normal histology and no obvious pathological traits (Figure 3). Groups B (Figure 4) and C (Figure 5) showed a clear improvement in all stages of follicle development.

Table-1: Letrozole's impact on the variety of ovarian follicles.

Treatment	primary follicles	growing follicles	graafian follicle
Control	5.766±0.273	3.467±0.230	3.547±0.116
1.5mg/kg Letrozole	*12.312±0.152	*10.040±0.200	*7.331±0.261
2.5 mg/kg Letrozole	*15.241±0.202	*11.140±0.135	*7.393±0.134

* $p < 0.05$.

Table-2: Effect of letrozole on the embryos.

Treatment	Number of embryos	Number of twin embryos
Control	*7.502±0.368	0.045±0.130
1.5 mg/kg Letrozole	*18.463±0.765	*5.131±0.413
2.5 mg/kg Letrozole	*17.433±0.871	*6.204±0.324

* $p < 0.05$.

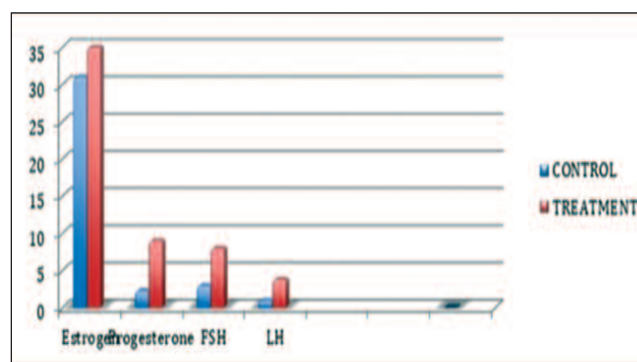


Figure 1: Effect of letrozole (1.5mg/kg) dose on hormones in group B female mice.

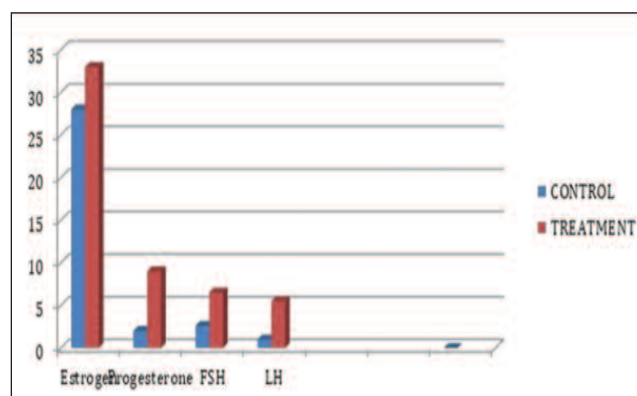


Figure 2: Effect of letrozole (2.5mg/kg) dose on hormones in group C female mice.

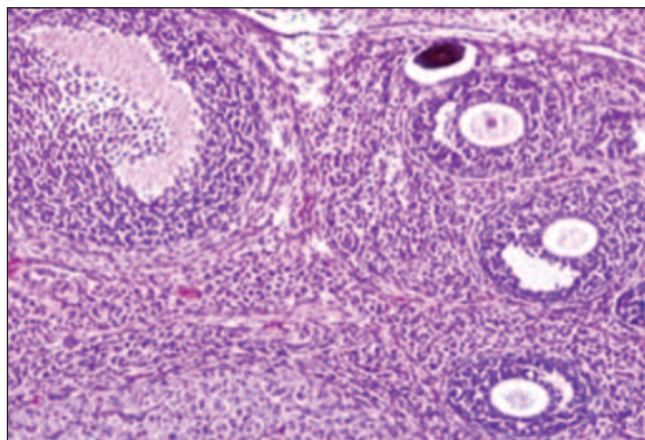


Figure 3: A transverse cut through the ovary of an adult female mouse from control group A, displaying the corpus leutum and the number of follicles (Haemotoxylin and Eosin [H&E] 4X).

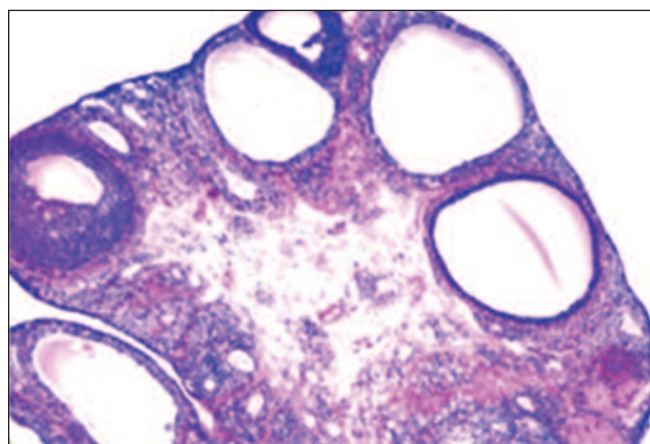


Figure 4: Transverse section of an ovary from a mature female mouse treated with 1.5mg/kg/day of letrozole in group B, displaying the corpus leutum (Haemotoxylin and Eosin [H&E] 4X) and the number of follicles (milt ovum follicles).

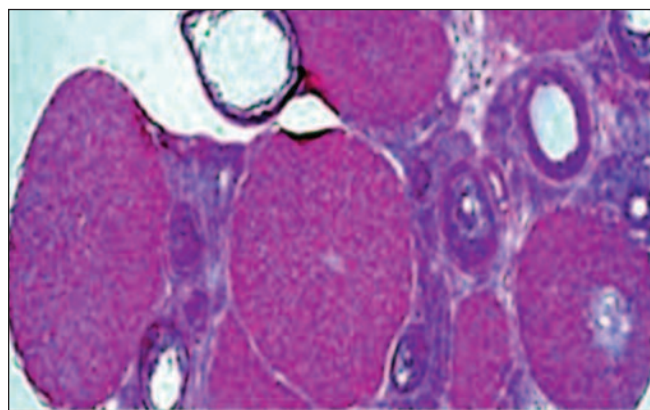


Figure 5: Transverse section of an ovary from a mature female mouse treated with 2.5mg/kg/day of letrozole in group C, displaying the corpus leutum (Haemotoxylin and Eosin [H&E] 4X) and the number of follicles (milt ovum follicles).

Discussion

Letrozole is the most successful medicine for treating female infertility because it is very good at promoting ovulation in females who have anovulation or oligo-ovulation.¹⁸ One or more dominant follicles develop and ripen with one oocyte in a successful regular cycle.¹⁹ In the current study, there were many follicles (plural ovum) visible in histological sections, demonstrating a significant increase in all follicles and embryos at various stages of evolution after treatment with letrozole doses 1.5mg/kg and 2.5mg/kg compared to the controls.

Letrozole is a nonsteroidal, highly-selected oral aromatase inhibitor that can block the conversion of androstenedione to estrone and testosterone to estradiol (E2) by reversibly binding to the rate-limiting enzyme P450 aromatase in the oestrogen biosynthesis pathway.²⁰ Letrozole is currently often used to increase follicle size in ovulatory women, and to prompt ovulation in anovulatory infertile patients because of the down-regulated oestrogen's effect on pituitary FSH output. Letrozole is also used in conjunction with intracytoplasmic sperm injection (ICSI) and IVF cycles.^{21,22} In female cancer patients with oestrogen-sensitive tumours, letrozole is also employed to preserve fertility. Studies have also demonstrated the efficiency of letrozole in endometrial preparation for frozen-thawed embryo transfer (FET). By examining the drug's mechanism of action and clinical outcomes for female infertility, evidence is available for the use of letrozole in a variety of infertility therapy settings.^{23,24}

In order to bolster this viewpoint, when oestrogen-negative feedback is eliminated, natural compensating mechanisms kick in, altering pulsatile hypothalamic gonadotropin-releasing hormone (GnRH) output and stimulating enhanced pituitary gonadotropin release, which drives ovarian follicular activity in ovulatory women. The frequency of GnRH pulses rises after letrozole therapy.²⁵ In anovulatory PCOS patients whose GnRH pulse hesitancy is unusually risen, letrozole medication increases throb amplitude, but not hesitancy.²⁶ Furthermore, plasma levels of oestrogen, progesterone, FSH and LH rose noticeably ($p < 0.05$) in both intervention groups in the current study. Letrozole increases both LH and FSH levels via inhibiting protein kinase C and reducing cyclic adenosine monophosphate (cAMP) levels in follicular fluid.^{27,28} In PCOS women, letrozole citrate-induced ovulation is accompanied by increased LH and FSH release as well as increased oestrogen secretion. The increased LH pulse amplitude and lower pituitary sensitivity to GnRH following letrozole suggest a hypothalamic influence.²⁹ Letrozole isomers exhibit the same patterns of oestrogenic and anti-oestrogenic activity depending on the species. Numerous

organs, tissues and cell kinds have been found to have these diverse characteristics. In healthy sheep, letrozole reduced ovulation, but enhanced estrous, indicating that letrozole affects many brain regions.^{30,31} However, letrozole blocks the negative feedback from oestrogen and progesterone in women by competing with circulating endogenous E2 for a binding site in the hypothalamus. Along with future FSH and LH secretions, this significantly increases GnRH secretion.³² The ewes, on the other hand, have their ovarian activity stopped, preventing the development of their follicles, ovulation, and corpora lutea.³³ When rats or mice were given letrozole, no gonadotropin-like or pituitary-stimulating activity was found.^{34,35} Letrozole increased the synthesis of growth hormone (GH), which increased the number of small-sized follicles, and accelerated LH secretion to levels that were enough to turn small-sized follicles into medium and large-sized follicles. In the current study, letrozole groups' response to various therapies during the first follicular wave were more successful than control group responses for super-ovulatory mechanisms. The results confirmed the hypothesis of an earlier study that superovulation yields could be improved by beginning treatment early in the luteal phase of the estrous cycle.²⁸

Conclusions

Letrozole was found to have a hyper-stimulatory effect on ovulation, resulting in multi-follicle ovum generation and boost in the digit of recruited follicles in albino female mice without impairing carpel steroidogenic activity. As such, letrozole may be considered the best first-line treatment for ovulatory problems.

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Conflict of Interest: None.

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References

1. Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil Steril* 2012;97:28-38.e25. doi: 10.1016/j.fertnstert.2011.09.024.
2. Teede HJ, Misso ML, Deeks AA, Moran LJ, Stuckey BG, Wong JL, et al. Assessment and management of polycystic ovary syndrome: summary of an evidence-based guideline. *Med J Aust* 2011;195:S65-112. doi: 10.5694/mja11.10915.
3. Yao K, Bian C, Zhao X. Association of polycystic ovary syndrome with metabolic syndrome and gestational diabetes: Aggravated complication of pregnancy. *Exp Ther Med* 2017;14:1271-6. doi: 10.3892/etm.2017.4642.
4. Abbott DH, Bacha F. Ontogeny of polycystic ovary syndrome and insulin resistance in utero and early childhood. *Fertil Steril* 2013;100:2-11. doi: 10.1016/j.fertnstert.2013.05.023.
5. Anderson AD, Solorzano CM, McCartney CR. Childhood obesity and its impact on the development of adolescent PCOS. *Semin Reprod Med* 2014;32:202-13. doi: 10.1055/s-0034-1371092.
6. Padmanabhan V, Veiga-Lopez A. Animal models of the polycystic ovary syndrome phenotype. *Steroids* 2013;78:734-40. doi: 10.1016/j.steroids.2013.05.004.
7. Walters KA, Bertoldo MJ, Handelsman DJ. Evidence from animal models on the pathogenesis of PCOS. *Best Pract Res Clin Endocrinol Metab* 2018;32:271-81. doi: 10.1016/j.beem.2018.03.008.
8. van Houten EL, Visser JA. Mouse models to study polycystic ovary syndrome: a possible link between metabolism and ovarian function? *Reprod Biol* 2014;14:32-43. doi: 10.1016/j.repbio.2013.09.007.
9. Scanlan N, Dufourny L, Skinner DC. Somatostatin-14 neurons in the ovine hypothalamus: colocalization with estrogen receptor alpha and somatostatin-28(1-12) immunoreactivity, and activation in response to estradiol. *Biol Reprod* 2003;69:1318-24. doi: 10.1095/biolreprod.103.017848.
10. Franks S. Animal models and the developmental origins of polycystic ovary syndrome: increasing evidence for the role of androgens in programming reproductive and metabolic dysfunction. *Endocrinology* 2012;153:2536-8. doi: 10.1210/en.2012-1366.
11. Moore AM, Prescott M, Marshall CJ, Yip SH, Campbell RE. Enhancement of a robust arcuate GABAergic input to gonadotropin-releasing hormone neurons in a model of polycystic ovarian syndrome. *Proc Natl Acad Sci U S A* 2015;112:596-601. doi: 10.1073/pnas.1415038112.
12. Chaube SK, Prasad PV, Tripathi V, Shrivastav TG. Clomiphene citrate inhibits gonadotropin-induced ovulation by reducing cyclic adenosine 3',5'-cyclic monophosphate and prostaglandin E2 levels in rat ovary. *Fertil Steril* 2006;86(Suppl 4):1106-11. doi: 10.1016/j.fertnstert.2006.03.027.
13. Moore AM, Prescott M, Campbell RE. Estradiol negative and positive feedback in a prenatal androgen-induced mouse model of polycystic ovarian syndrome. *Endocrinology* 2013;154:796-806. doi: 10.1210/en.2012-1954.
14. Roland AV, Nunemaker CS, Keller SR, Moenter SM. Prenatal androgen exposure programs metabolic dysfunction in female mice. *J Endocrinol* 2010;207:213-23. doi: 10.1677/JOE-10-0217.
15. Kauffman AS, Thackray VG, Ryan GE, Tolson KP, Glidewell-Kenney CA, Semaan SJ, et al. A Novel Letrozole Model Recapitulates Both the Reproductive and Metabolic Phenotypes of Polycystic Ovary Syndrome in Female Mice. *Biol Reprod* 2015;93:69. doi: 10.1095/biolreprod.115.131631.
16. Skarra DV, Hernández-Carretero A, Rivera AJ, Anvar AR, Thackray VG. Hyperandrogenemia Induced by Letrozole Treatment of Pubertal Female Mice Results in Hyperinsulinemia Prior to Weight Gain and Insulin Resistance. *Endocrinology* 2017;158:2988-3003. doi: 10.1210/en.2016-1898.
17. Kelley ST, Skarra DV, Rivera AJ, Thackray VG. The Gut Microbiome Is Altered in a Letrozole-Induced Mouse Model of Polycystic Ovary Syndrome. *PLoS One* 2016;11:e0146509. doi: 10.1371/journal.pone.0146509.
18. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013;8:e61217. doi: 10.1371/journal.pone.0061217.
19. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;15:550. doi: 10.1186/s13059-014-0550-8.
20. Torres PJ, Siakowska M, Banaszewska B, Pawelczyk L, Duleba AJ, Kelley ST, et al. Gut Microbial Diversity in Women With Polycystic

- Ovary Syndrome Correlates With Hyperandrogenism. *J Clin Endocrinol Metab* 2018;103:1502-11. doi: 10.1210/jc.2017-02153.
21. Torres PJ, Ho BS, Arroyo P, Sau L, Chen A, Kelley ST, et al. Exposure to a Healthy Gut Microbiome Protects Against Reproductive and Metabolic Dysregulation in a PCOS Mouse Model. *Endocrinology* 2019;160:1193-1204. doi: 10.1210/en.2019-00050.
 22. Liu R, Zhang C, Shi Y, Zhang F, Li L, Wang X, et al. Dysbiosis of Gut Microbiota Associated with Clinical Parameters in Polycystic Ovary Syndrome. *Front Microbiol* 2017;8:e324. doi: 10.3389/fmicb.2017.00324.
 23. Hu M, Richard JE, Maliqueo M, Kokosar M, Fornes R, Benrick A, et al. Maternal testosterone exposure increases anxiety-like behavior and impacts the limbic system in the offspring. *Proc Natl Acad Sci U S A* 2015;112:14348-53. doi: 10.1073/pnas.1507514112.
 24. Torres PJ, Skarra DV, Ho BS, Sau L, Anvar AR, Kelley ST, et al. Letrozole treatment of adult female mice results in a similar reproductive phenotype but distinct changes in metabolism and the gut microbiome compared to pubertal mice. *BMC Microbiol* 2019;19:57. doi: 10.1186/s12866-019-1425-7.
 25. Torres Fernandez ED, Adams KV, Syed M, Maranon RO, Romero DG, Yanes Cardozo LL. Long-Lasting Androgen-Induced Cardiometabolic Effects in Polycystic Ovary Syndrome. *J Endocr Soc* 2018;2:949-64. doi: 10.1210/js.2018-00131.
 26. Thaïss CA, Itav S, Rothschild D, Meijer MT, Levy M, Moresi C, et al. Persistent microbiome alterations modulate the rate of post-dieting weight regain. *Nature* 2016;540:544-51. doi: 10.1038/nature20796.
 27. Thackray VG. Sex, Microbes, and Polycystic Ovary Syndrome. *Trends Endocrinol Metab* 2019;30:54-65. doi: 10.1016/j.tem.2018.11.001.
 28. Rajan RK, M SS, Balaji B. Soy isoflavones exert beneficial effects on letrozole-induced rat polycystic ovary syndrome (PCOS) model through anti-androgenic mechanism. *Pharm Biol* 2017;55:242-51. doi: 10.1080/13880209.2016.1258425.
 29. Karateke A, Dokuyucu R, Dogan H, Ozgur T, Tas ZA, Tutuk O, et al. Investigation of Therapeutic Effects of Erdosteine on Polycystic Ovary Syndrome in a Rat Model. *Med Princ Pract* 2018;27:515-22. doi: 10.1159/000494300.
 30. Patel R, Shah G. Evaluation of ovarian and metabolic effects of GnRH modulators in two rat models of polycystic ovary syndrome. *Mol Reprod Dev* 2018;85:778-89. doi: 10.1002/mrd.23059.
 31. van Houten EL, Kramer P, McLuskey A, Karels B, Themmen AP, Visser JA. Reproductive and metabolic phenotype of a mouse model of PCOS. *Endocrinology* 2012;153:2861-9. doi: 10.1210/en.2011-1754.
 32. Chen D, Shen X, Fu Y, Ding C, Zhong Y, Zhou C. Pregnancy Outcomes Following Letrozole Use in Frozen-thawed Embryo Transfer Cycles: A Systematic Review and Meta-analysis. *Geburtshilfe Frauenheilkd* 2020;80:820-33. doi: 10.1055/a-1202-2059.
 33. Hu S, Yu Q, Wang Y, Wang M, Xia W, Zhu C. Letrozole versus clomiphene citrate in polycystic ovary syndrome: a meta-analysis of randomized controlled trials. *Arch Gynecol Obstet* 2018;297:1081-8. doi: 10.1007/s00404-018-4688-6.
 34. Gadalla MA, Norman RJ, Tay CT, Hiam DS, Melder A, Pundir J, et al. Medical and Surgical Treatment of Reproductive Outcomes in Polycystic Ovary Syndrome: An Overview of Systematic Reviews. *Int J Fertil Steril* 2020;13:257-70. doi: 10.22074/ijfs.2020.5608.
 35. Yu Q, Hu S, Wang Y, Cheng G, Xia W, Zhu C. Letrozole versus laparoscopic ovarian drilling in clomiphene citrate-resistant women with polycystic ovary syndrome: a systematic review and meta-analysis of randomized controlled trials. *Reprod Biol Endocrinol* 2019;17:17. doi: 10.1186/s12958-019-0461-3.
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