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

Sally Adnan Mousa AL-Rikabi, Noor Noori Abid Al Shemary

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

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

* p<0.05.

Table 2: Effect of letrozole on the embryos.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of embryos</th>
<th>Number of twin embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>*7.502 ±0.368</td>
<td>0.045 ±0.130</td>
</tr>
<tr>
<td>1.5 mg/kg Letrozole</td>
<td>*18.463 ±0.765</td>
<td>*5.131 ±0.413</td>
</tr>
<tr>
<td>2.5 mg/kg Letrozole</td>
<td>*17.433 ±0.871</td>
<td>*6.204 ±0.324</td>
</tr>
</tbody>
</table>

* p<0.05.
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. One or more dominant follicles develop and ripen with one oocyte in a successful regular cycle. 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. 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. 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.

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. In anovulatory PCOS patients whose GnRH pulse hesitancy is unusually risen, letrozole medication increases throb amplitude, but not hesitancy. 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. 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. 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. 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. The ewes, on the other hand, have their ovarian activity stopped, preventing the development of their follicles, ovulation, and corpora lutea. When rats or mice were given letrozole, no gonadotropin-like or pituitary-stimulating activity was found. 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 follicles, and accelerated LH secretion to levels that were sufficient to develop lutea. When rats or mice were given letrozole, no development of their follicles, ovulation, and corpora lutea. Letrozole was found to have a hyper-stimulatory effect on 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. 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. The ewes, on the other hand, have their ovarian activity stopped, preventing the development of their follicles, ovulation, and corpora lutea. When rats or mice were given letrozole, no gonadotropin-like or pituitary-stimulating activity was found. 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.

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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References


