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*Original Article*

# Effect of Letrozole Administration as a Selective Aromatase Inhibitor on Male Rat's Reproductive Performance

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**Abstract**

This study was designed to investigate the effect of administrating a selective aromatase inhibitor, letrozole, on the parameters of the rat's sperm and testicular histomorphology. A total of 40 male Wistar rats with an age of about 5 months old and an average weight of 190±10 g were divided randomly into 4 groups (n=10 each) and treated for 6 weeks. The first group (C) was given normal saline only as a control group. The second (T1), third (T2), and fourth (T3) groups received doses of 0.5, 1, and 1.5 mg/animal letrozole, respectively. At the end of the experiment (42 days), all animals were euthanized and the samples were obtained for more evolutions. The results of this study showed a significant increase in sperms quality ($P$-value≤0.05) while indicating a significant decrease in sperm abnormality in the T1 group, compared to the C group. Moreover, there was a significant difference represented by an increase in sperm quality, and a significant difference represented by a decrease in sperms abnormality in the T2 group, compared to the C group. On the other hand, there was a significant difference represented by an increase in sperm quality, and a significant difference represented by a decrease in sperm abnormality in the T3 group, compared to the T2 group. Normal somniferous tubules with high spermatoscopy counts were shown in histologic sections in the control group, primary and secondary spermatocytes with spermatozoa. In the T1 group, there was adequate spermatogenesis with a thick basement membrane. In the T2 group, there was normal testicular tissue with a significant increase in spermatogenic activity and number. In the T3 group, there was no significant atrophy or other pathology. The results of the current study suggested that letrozole improved spermatogenesis while causing no visible pathological alterations in testis tissue.

**Keywords:** Aromatase inhibitors, Histological section, Letrozole, Rat testicles, Sperm parameters

## 1. Introduction

The testis performs two essential functions, namely the production of spermatozoa in the spermatogenesis process and the synthesis of steroids in interstitial Leydig cells involved in the secretion of testosterone and other steroids, including estrogens (1). The testes descend via the inguinal canal throughout adolescence, with the cremaster muscles connecting them to the internal inguinal ring. They descend in a young rat between the ages of 4 and 6 weeks. Because the rat has an open inguinal canal, the testes can travel up into the abdominal cavity throughout its life (2). The testicular parenchyma is made up of seminiferous tubules that are separated by interstitial tissue and surrounded by a three-layered capsule, namely the tunica vaginalis, tunica albuginea, and tunica vasculosa. The seminiferous tubules are lengthy and convoluted tubes that empty into the rete testis on both ends.

Spermatogenesis (formation of sperm) is the process through which primitive spermatogonia stem cells grow into highly specialized spermatozoa (3). Spermatogenesis occurs in the epithelial lining of the

seminiferous tubes found within the testes. Another role of the testes involves the secretion of steroidal hormones, such as testosterone, estrogen, and progesterone (2). The primary enzyme in estrogen production is aromatase, also known as estrogen synthetase (*CYP19A1*). The *CYP19A1* gene codes for an enzyme that is found in the endoplasmic reticulum of estrogen-producing cells. This gene belongs to the CYP gene family, which codes for enzymes that hydroxylate both endogenous and exogenous drugs. The CYP19A1 gene is localized on chromosome 15 and comprises nine exons; the start codon for translation is located on exon 2 (4).

The chemical structure of letrozole (4,4-(1H-1,2,4-triazol-1-yl-methylene)-bis-benzonitrile) is a type II, very potent non-steroidal aromatase inhibitor. Its chemical structure contains a triazole group that selectively interacts with the heme group of the P450arom enzyme, thereby reversibly inhibiting the bioactivity of the enzyme (5). Letrozole is a highly effective aromatase inhibitor. The relative potential for letrozole, anastrozole, and fadrozole has been assessed in a range of cellular endocrine and tumor systems containing aromatase (6). Aromatase inhibitors have been used to prevent androgens from being converted to estrogen, thereby increasing testosterone in the hopes of improving male infertility. Furthermore, aromatase inhibitors reduce the quantity of testosterone transformed into a more strong inhibitory signal, and estrogen, and therefore, impede the inhibitory feedback of testosterone on the hypothalamus pituitary gonadal axis (7). Aromatase inhibitors have been used to prevent androgens from being converted to estrogen, and consequently, increase testosterone in the hopes of improving male infertility. Letrozole is a class II non-steroidal aromatase inhibitor with high potency. It has a triazole group in its chemical structure that specifically binds with the heme group of the P450arom enzyme, reversibly reducing the bioactivity of the enzyme (8).

This study was designed to investigate the effect of the administration of a selective aromatase inhibitor, Letrozole, on rat sperm parameters and testicular histomorphological parameters.

## 2. Materials and Methods

### 2.1. Experimental Animals

In this study, 40 male Wistar rats weighing 180-250 g and aging less than 20 weeks were used. The rats were placed in well-ventilated wire-plastic cages and allowed to acclimate for 10 days before being grown under the controlled condition of a 12 light/12 dark cycle and at a temperature of 18-20°C. Water and sufficient food were provided to the animals.

### 2.2. Experimental Design

For the following 6 weeks, 40 adult male Wistar rats were randomly assigned into 4 groups (n=10 each) and received the treatments orally by gavage. The first group (C) was merely supplied as a control group with normal saline. The second (T1), third (T2), and fourth (T3) groups received 0.5, 1, and 1.5 letrozole mg/animal, respectively. All animals were killed, and the samples were obtained for histology studies during the last experiment.

### 2.3. Sperm Parameters

#### 2.3.1. Sperm Concentration

Sperms were calculated according to Comhaire, Huysse (9).

#### 2.3.2. Abnormal Sperm Percentage

The abnormal sperms were detected according to Van der Ven, Montag (10).

#### 2.3.3. Percentage of Sperm Viability

The percentage of sperm viability was detected according to Graham, Kunze (11).

#### 2.3.4. Total Sperm Motility

The total sperm motility was detected according to Bearden and Fuquay (12).

### 2.4. Histological Studies of Testes

At the end of all treatments, the bodyweight of each rat was measured, all animals were anesthetized by the mixture of Ketamine + xylazin (9 mg/kg/b.w, 10 mg/kg/b.w, respectively/IP). Afterward, the rat was anatomized to extract testis and epididymis from

subjects and reserved in formalin 10% for histological studies. (13).

## 3. Results and Discussion

### 3.1. Effect of Letrozole on Sperm Parameters of Male Rats

#### 3.1.1. Sperm Concentration

Table 1 shows the effect of letrozole on sperm concentration in epididymis and testes. The concentrations of sperms in epididymis were obtained at 15.000±0.57, 15.000±2.88, and 15.666±6.33 in the T1, T2, and T3 groups, receiving 0.5, 1, and 1.5 mg/animal letrozole, respectively, while it was estimated at 2.66±0.66 in the C group that was only given normal saline. The results indicated that there was no significant difference (*P*-value=0.9) between the T1 and T2 groups, whereas there was a significant increase in the T3 group (*P*-value=0.04), compared to other groups. It was found that the C, T1, and T2 groups did not differ significantly (*P*-value=1). The recorded data showed that the concentrations of sperms in T1, T2, and T3 groups were 8.33±2.02, 50.66±20.17, and 75.00±2.88, respectively, while in the C group, it was significantly lower (4.00±0.57) than in the T2 and T3 groups. The results revealed that there was no significant difference (*P*-value=0.1) between the T1 and C groups in the case of sperm cells concentration, whereas the recorded data showed a significant difference (*P*-value=0.01) between the T2 and T3 groups in this regard.

**Table 1.** Effect of letrozole on sperm parameters

| Groups Parameters | C | T1 | T2 | T3 |
|---|---|---|---|---|
| Sperm concentration in epididymis | 2.66±0.66 | 15.00±0.57 | 15.00±2.88 | 15.66±6.33 |
| Sperm concentration in testes | 4.00±0.57 | 8.33±2.02 | 50.66±20.17 | 75.00±2.88 |
| Percentage of total motility (%) | 6.33±0.88 | 7.66±1.45 | 28.33±1.66 | 35.00±2.88 |
| Percentage of abnormal sperms (%) | 35.00±2.88 | 30.00±5.77 | 23.33±3.33 | 20.00±2.88 |
| Percentage of sperm viability (%) | 55.00±2.88 | 65.00±2.88 | 75.00±2.88 | 85.00±2.88 |

#### 3.1.2. Percentage of Total Sperm Motility

Table 1 presents the effect of letrozole on sperm motility, abnormal sperm, and sperm viability. The percentages of sperm motility in T1, T2, and T3 groups were 7.66±1.45, 28.33±1.66, and 35.00±2.88, respectively, while in the C group, it was calculated at 6.33±0.88. Based on the findings, there was a significant difference between the T1 and T2 groups (*P*-value=0.04). It was also found that there was a significant difference represented by an increase in the T3 group (*P*-value=0.08), compared to the other groups. Nevertheless, no significant difference was observed between the C and T1 groups (*P*-value=1).

#### 3.1.3. Percentage of Sperm Viability

Table 1 shows that the difference was significant with an increase in the T3 group (85.00±2.88), in comparison to the other groups. The T1 group (65.00±2.88) and T2 group (75.00±2.88) were not significantly different. There was also a major disparity in abnormal sperm percentage. The difference in the T3 group was significant (20.000±2.88), compared to the other groups shown in table 1. The difference in the T2 group was significant (23.333±3.333), in comparison to the C group (35.0000±2.88). Although there was no significant difference between C (35,000±2.88) and T1 (30.000±5.77) groups.

In numerous spermatogenic situations, letrozole therapy improves motile sperm counts by boosting serum gonadotropin and testosterone levels (14). The results of a study conducted by Shuling, Kuei (15) indicated that after treatment with letrozole, the concentration and the total number of sperms increased 5.5 and 4.3 times, respectively, with no significant differences in sperm motility or semen volume. Based on the findings of a study carried out by Cavallini, Beretta (16), sperm concentration was 100 before

letrozole therapy and ranged from 40,000 to 90,000 ml-1 after treatment. Zhao, Pan (17) claimed that the testicular function of letrozole might be boosted by increasing serum and T levels, stimulating germ cells of the testicles, and mostly raising the number of motive sperms. Gregoriou, Bakas (18) found that all the sperm parameters would be increased after letrozole treatment due to an increase in gonadotrophin and testosterone levels. Letrozole function at the testicular level has been associated with stimulating sperm motility, concentration, and viability (19).

### 3.2. Testis Histological Study

There were normal seminiferous tubules with a high number of spermatogonia, primary and secondary spermatocytes, Sertoli cells, and spermatozoa in testes sections of the rats in the control group. The Leydig cells existed in the interstitial tissue (Figure 1). Testicular sections of rats in the T1 group showed that there was adequate spermatogenesis with a thick basement membrane (Figures 2 and 3). Figure 1 showed normal seminiferous tubules characterized by a high number of spermatogonia, primary and secondary spermatocytes, and spermatozoa (red arrow; 10× H&E). Figure 2 depicted normal seminiferous tubules characterized by adequate numbers of spermatogenesis (red arrow; 40× H&E). Figure 3 illustrates normal seminiferous tubules with relatively thick basement membrane (red arrow; 40× H&E).

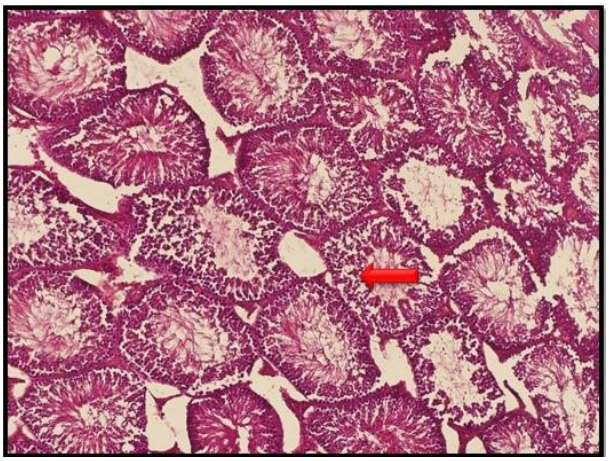

**Figure 1.** Cross-section of rat testis in the control group

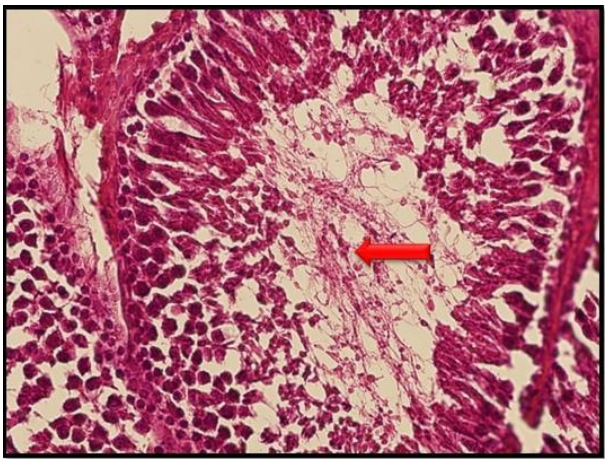

**Figure 2.** Cross-section of rat testis in the T1 group

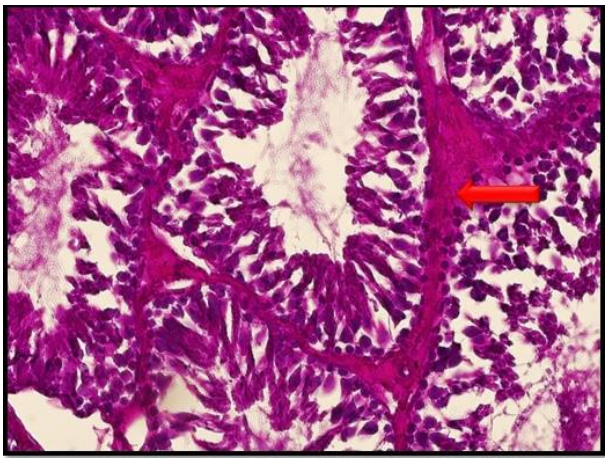

**Figure 3.** PAS of rat testis in the T1 group

This result led to the conclusion that the low dose of letrozole did not affect the testis tissues, which was consistent with that of a previously published work (20). The results of the present study were in agreement with that of the studies conducted by Janni and Hepp (21) and Kondarewicz, Kolasa (22), who reported that the low dose of letrozole did not significantly affect the testicular or epididymal tissues.

Testicular sections in the T2 group indicated normal tissue histology and an improvement in sperm quantity and quality (Figures 4 and 5). Figure 4 showed normal testicular tissue with a significant increase in spermatogenic activity and number (red arrow; 40× H&E). Figure 5 demonstrated normal

testicular tissue with mild thickening in the basement membrane (red arrow; 40× H&E).

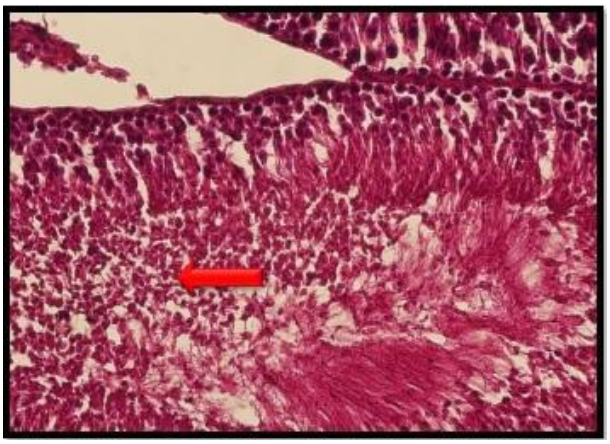

**Figure 4.** Cross-section of rat testis in the T2 group

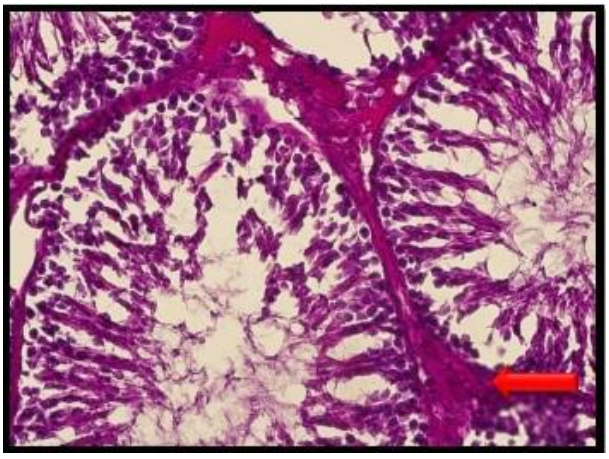

**Figure 5.** PAS of rat testis in the T2 group

Based on this finding, letrozole causes a rise in testosterone levels, which is required for spermatogenesis (23). Letrozole inhibits the aromatase enzyme reversibly and hinders the transformation of androgen precursors to estradiol in adipose tissue, according to the findings of studies conducted by Ribeiro, Gameiro (24), Uzun, Atli (25), and Shoshany, Abhyankar (26) who found that letrozole inhibits the aromatase enzyme reversibly and avoids the transformation of androgen precursors to estradiol in the fiber tissue. This leads to increased gonadotrophin production, and as a result, increased peripheral androgen levels, which stimulates spermatogenesis.

There was no substantial atrophy or other diseases in testicular sections of rats in the T3 group receiving letrozole (1.5mg/day) (Figure 6).

Based on figure 6, there was no significant atrophy or other pathology.

This finding suggests that letrozole improves spermatogenesis while causing no visible pathological alterations in testis tissue similar to the previously published study (27). The results of the current study revealed that letrozole had a direct influence on testicular steroidogenesis. Letrozole treatment resulted in a substantial reduction in E2 production in the testes. A lower E2 level is mostly owing to a considerable drop in the expression of the aromatase enzyme in the testes caused by letrozole; nevertheless, treatment with letrozole resulted in an increase in testosterone, presumably due to accumulation since testosterone was not converted to estradiol. E2 as a gonadotropin-releasing hormone feedback modulator was earlier found to boost the release of luteinizing hormone and follicle-stimulating hormone, and subsequently, increase testosterone synthesis.

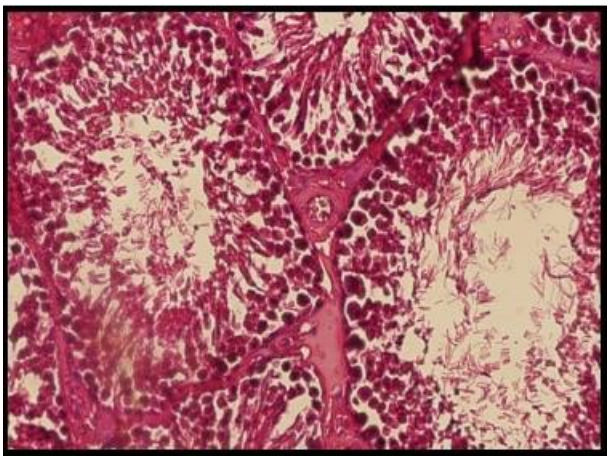

**Figure 6.** Cross-section of rat testis in the T3 group

## Authors' Contribution

Study concept and design: A. H. J.
Acquisition of data: A. S. A.
Analysis and interpretation of data: A. H. J.
Drafting of the manuscript: A. H. E.
Critical revision of the manuscript for important intellectual content: A. S. A.
Statistical analysis: H. K. J.
Administrative, technical, and material support: A. H. J., A. S. A., A. H. J. and A. H. E.

## Ethics

Ethical approval for the study was obtained from the Hilla University College, Babylon, Iraq and the Local Research Ethics Committees.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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