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EFFECT OF ADDING ANASTROZOLE AS AN AROMATASE INHIBITOR ON THE PRODUCTIVE AND REPRODUCTIVE TRAITS FOR BROWN LOHMANN ROOSTER

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Abstract

This study was conducted in the poultry field of the Department of Animal Production, College of Agriculture, Al-Qasim Green University for the period from 09/28/2019 to 11/15 2019. To study the effect of adding anastrozole as an aromatase inhibitor on the productive and reproductive traits of Lehman Brown roosters. In this study, 26 roosters were used with 4 treatments, the first and second treatments included 6 replicates, the third and fourth treatments included 7 replicates by one rooster per replicate and for all experiment treatments. While the first treatment (comparison treatment) (T1) it was not given the aromatase inhibitor (anastrozole), As for the second, third and fourth treatments (T2, T3 and T4), the aromatase inhibitor inestrozole was given as capsules in concentrations (0.2, 0.4 and 0.6) mg, respectively, by one capsule per day, and the results of the study showed the following: Significant excelled of all experiment weeks in the average body weight of the fourth treatment on the rest of the experiment's treatments, while the results of the study showed a significant excelled in the semen volume, sperm concentration and salt resistance for the fourth treatment on the rest of the experiment's treatments, while the percentage of distortions in the first treatment increased compared to the rest of the experiment treatments, The results of histological segmentation of the testicle tissue showed an improvement in the process of biosynthesis of sperm and an increase in the diameter of the spermatic tubule in the fourth treatment compared to the rest of the experiment treatments.
Keywords: Anastrozole, aromatase inhibitor, brown Lohmann rooster

Introduction

The testicle is one of the organs with biological activity in the body and it is located under the influence of tissue changes that represent a complete cessation of sperm production and the disintegration of Epithelial spore cells and thus the effect on fertility. Where the rooster's fertility begins to decreases when reaching the age of 45 weeks and this is evidenced by the decrease in the effectiveness of the gonads (Röhss, and Silverin, 1983). Therefore, researchers increased their interest in the reproductive function of roosters and factors affecting the quality of semen and the ability to fertilization (Al-Daraji, 2008; Alrawi *et al*., 2011). Several studies have been conducted on aged roosters, which showed a significant decrease in hypothalamus secretions (Vizcarra, *et al*., 2010). This resulted in a decrease in the synthesis and release of both Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland (Röhss, Silverin, 1983). Sexton, *et al*., 1989) indicated that rooster's fertility peaked at age 37-40 weeks under controlled conditions and then began to decline thereafter. Fertility at the age of 70 weeks is low and as a result, the roosters are excluded from the herd, where this rapid decline in fertility is associated with a decrease in sperm concentration in the ejaculation as a result of the survival of the sperm trapped inside the testicle by Sertoli cells (Rosenstrauch *et al*., 1994). The decrease in fertility occurs due to the conversion of testosterone to estradiol by the aromatase enzyme, and this leads to the prevention of reproductive hormone secretions, affecting the secretion of testosterone inside the testicle (Thibier and Gagner, 2002). Here the role of the aromatase inhibitor (Anastrozole) is highlighted, as this substance works to inhibit the activity of the aromatase enzyme by binding to the iron group present in the enzyme (Schieweck, *et al*., 1993). Weil *et al*. (1999) have indicated the possibility of utilizing Aromatase inhibitors by reducing the conversion of testosterone to Estradiol and thus preventing a decrease in the level of testosterone in addition to a decrease in the level of Estradiol and thus stimulating hormonal production (LH and FSH) and this leads to improved fertility layer breeder males, and due to the lack of a study on the use of (anastrozole) as an Aromatase inhibitor in the roosters, this study came to determine the best concentrations of (anastrozole) that can be given to the roosters, which we can recommend and study the extent of their effect on some of the productive and reproductive traits of roosters.

Material and Methods

This experiment was conducted in the poultry field of the Department of Animal Production in the College of Agriculture, Al-Qasim Green University, for a period of 7 weeks for the period from 9/28/2019 to 11/15/2019. In this experiment, 26 roosters of the Lehmann-Brawan breed were used. The roosters were prepared from the Haj Abu Ali Jaflawy National Fields in Babylon province, at an average weight of 2.5 kg. At the age of (45) weeks, 170 g of the diet were provided per roosters per day using plastic plates, and the inverted manholes were used to provide water. The lighting system (16 hours/day) was followed for the period of the experiment with daily giving 8 hours of darkness, and the traits that were studied are the productive traits (weight gain), reproductive traits (semen volume, sperm concentration, deformities and salt resistance) in addition to histological segmentation of the testicle. , The Statistical Analysis System -AS (2012) was used in data analysis to study the effect of different treatments on the traits studied according to completely randomized design (CRD), and The significant differences between the averages were compared to the Duncan (1955) polynomial test. Experiment treatments :

Roosters were randomly distributed with 4 treatments, and the first and second treatments included 6 replicates per treatments with one rooster per replicate and the third and fourth treatments 7 replicates per treatments and by one rooster per treatment, And exposed to the same conditions of nutrition, temperature and lighting, and gave the substance Alanestrozole to roosters in the form of capsules that are dosed for roosters as shown below:

1- T1: control.

2- T2: Anastrozole was given at a concentration of 0.2 mg.

3- T3: Anastrozole was given at a concentration of 0.4 mg

4- T4: Anastrozole was given at a concentration of 0.6 mg.

The diet used in the study:

Roosters were fed on a commercial diet for male laying hens, where they were prepared in the Ghadeer Babel Feed Factory, Feed and capsules were given at fixed times throughout the experiment, and Table 1 shows the components of the diet.

Table 1: The diets components used for feeding roosters

Diets	**%**
yellow corn	40
wheat	18
Soybean meal	24
bran	6
Premix	2.5
oil	0.9
Lime	6
A mixture of vitamins and minerals	2.6
The calculated chemical composition	
Crude protein	17.7
Representative energy (kilograms / kg feed)	2804
Methionine + cysteine%	0.64
Calcium%	3.72
Available phosphorus%	3.4

•According to the chemical analysis of the diet according to NRC (1994).

Birds and semen collection:

The process of collecting semen was conducted following the method of abdominal massaging of roosters, one time per week for each rooster, according to the method (Quinn and Burrows, 1937) which is between (Al-Daraji *et al.*, 2000), where this method requires the two persons to do the collection process, the first of which is to catch the rooster where Makes the head back and the collector forward ,The second person performs a massage for the dorsal-ventral region (starting from the back of the rooster to the base of the tail), where 1.5 ml tubes were used for the purpose of collection and were then placed in an incubator with a temperature of 37 °C, and then tests were performed using the optical table with a thermal table.

Results and Discussion

The effect of adding Anastrozole as an Aromatase inhibitors on the productive traits:

Live body weight:

Table 2 shows the effect of adding Anastrozole as an Aromatase inhibitors on the average body weight of roosters for 6-week from experiment weeks, where we notice the fourth and third treatment significantly ($p < 0.01$) in the first week on the second and first treatments where they recorded the highest body weight (2621.43, 2606.67 g/rooster)) Respectively, As for the second treatment, there were no significant differences between it and the first treatment on the one hand, and the third and fourth treatments on the other hand, it recorded a body weight (2497.14 g/rooster) compared to the first treatment that slowed down the lowest body weight (2386.67 g/rooster). During the second week, the fourth and third treatment continued significantly excelled ($p<0.05$) by recording the highest body weight (2719.14, 2648.33 g/rooster) respectively, while the second treatment was not significant differences between it and the first treatment on the one hand and the third and fourth treatments on the other hand It recorded a body weight (2567.85 g/rooster), respectively, compared to the first treatment that recorded the lowest body weight (2456.67 g/rooster).

As for the third and fourth weeks, the fourth and third treatments ($p < 0.01$) were significantly excelled, registering the highest body weight (2800, 2847.14 g/rooster) respectively for the fourth treatment and (2685, 2720 g / rooster), respectively, for the third treatment, As for the second treatment, there were no significant differences between it and the first treatment on the one hand, and the fourth and third treatment on the other hand, it recorded a body weight (2637.14 and 2695 g / rooster) respectively, compared to the first treatment that recorded the lowest body weight (2494.17, 2582.33 g / rooster), But in the fifth week, the fourth treatment was significantly excelled ($p < 0.01$) to the rest of the treatments, where it recorded a live body weight (2877.86 g / rooster).As for the third and second treatments, they significantly increased ($p < 0.01$) on the first treatment, where they recorded a body weight (2755, 2695 g / rooster), while the first treatment recorded the lowest body weight (2573.33 g / rooster). As for the sixth week, we notice that the fourth and third treatments significantly increased ($p < 0.01$) on the second and first treatments, where they recorded the highest body weight (2904.29, 2798.33 g / rooster). As for the second treatment, there were no significant differences between them and the first treatment on the one hand and the third and fourth treatments from, on the other hand, it recorded a body weight (2727.86 g / rooster) compared to the first treatment that recorded the lowest body weight (2617.50 g / rooster). The significant improvement in body weight average may be due to the role of high testosterone in affecting the actin and myosin proteins in the muscles and thus an increase in muscle growth occurs, where (Robert, *et al*., 1989) indicated the role of testosterone in increasing muscle mass by increasing The synthesis of muscle proteins, as confirmed by (Upendram *et al*., 2010), where they indicated in the results of their experiment the role of testosterone in improving muscle strength and improving body formation in addition to improving physical functions.

Table 2: Effect of adding Anastrozole as an aromatase inhibitor on the average of live body weight

Average ± standard error (g)						Treatments
sixth week	fifth week,	fourth week	third week	second week	First week	
2617.50 55.10±b	63.96± 2573.33 c	66.50± 2528.33 b	65.75± 2494.17 b	63.70 2456.67 b	58.11± 2386.67 b	T1
2727.86 67.51±ab	65.10± 2695.00 bc	66.01± 2695.00 ab	66.39± 2637.14 ab	59.36± 2567.86 ab	39.08± 2497.14 ab	T2
2798.33 26.67±a	24.04± 2755.00 ab	29.77± 2720.00 a	30.63± 2685.00 a	37.36± 2648.33 a	39.29± 2606.67 a	T3
2904.29 64.32±a	63.16± 2877.86 a	60.69± 2847.14 a	68.84± 2800.00 a	62.70± 2713.14 a	67.13± 2621.43 a	T4
**	**	**	**	*	**	Level of significance
The averages that have different letters within one column differ significantly among themselves *(P <0.05), ** (P <0.01)						

The effect of adding Anastrozole as an aromatase inhibitor on the properties of semen

The results of the statistical analysis of Table 3 indicated the effect of the addition of Anastrozole as an aromatase inhibitor on the traits of the semen to a significant difference (p <0.01) in the average of semen volume, sperm concentration and salt resistance for the fourth treatment where it recorded 0.627 ml, 14.57 x 109 / ml and 96.19%, respectively, Compared to the third, second and first treatments, The third and second treatments recorded a significant increase (p <0.01) on the first treatment (control), where they recorded 0.475 ml and 0.416 ml respectively in the average semen volume and 13.84 x 109 / ml and 11.48 x 109 / ml respectively in the sperm concentration in addition To 94.11% and 90.03%, respectively, in salt resistance compared to the first treatment (control), which recorded a significant decrease (p <0.01), where the average of semen volume, sperm concentration and salt resistance was 0.336 ml, 8.96 x 109 / ml and 86.62% Respectively . As for distortions, the first treatment (control) showed a significant excelled (p <0.01) on the second, third, and fourth treatments in the distortion ratio, which was 12.53%.The second and third treatments also excelled the fourth treatment, recorded 9.03% and 4.53%, respectively, compared to the fourth treatment, which recorded a significant decrease in the percentage of distortions, where it was 3.07%. Testosterone is the hormone responsible for normal testicular development and maintenance of secondary sexual treatments (Sinclair, *et al*., 2015; Niederberger, 2016), and that the significant improvement in semen traits may be due to an increased level of testosterone due to aromatase inhibitors, This is consistent with Weinbauer and Nieschlag (1991) explained that the increase in the percentage of testosterone causes an increase in male sexual desire, in addition to increasing the semen volume and the improvement of semen quality through the effect of testosterone directly on the process of sperm production. The results also agreed with Al-Darraji *et al.* (2000); Hassan, (2001), indicating that there is a significant positive correlation between the semen volume and the sperm concentration. In addition to the fact that the high FSH hormone has a role in improving semen traits.

This is in agree with (Sharpe, 1999), where FSH has a role in the growth, differentiation, activity and creation of sperm. The results were also consistent with his study (Başar and Tuğlu, 2009) in which they used anastrozole, as an aromatase inhibitor where the semen traits improved and the number of semen increased after treatment ended. The significant increase in the weight of the testicle can positively affect the semen traits, and this is consistent with both AL-Darraj, (2001 a, b); AL-Darraj *et al.* (2001) where they indicated that there is a significantly positive relationship between the weight of the testicles and each of Sperm concentration and normal sperm percentage, as testosterone can maintain or improve testicular weight Rommerts, (1990); Al-Darraji, (2013b).

Table 3: The effect of adding anestrozole as an aromatase inhibitor on semen treatments

Average ± standard error				**Treatments**
(%) **Salt resistance**	**deformities** (%)	**sperm concentration in (semen 10^9ml)**	**semen volume (ml)**	
d 0.21± 86.62	a 0.14± 12.53	d 0.02± 8.96	d 0.02± 0.336	T1
c 0.01± 90.03	b 0.01± 9.03	c 0.02± 11.48	c 0.02± 0.416	T2
b 0.02± 94.11	c 0.01± 4.53	b 0.01± 13.84	b 0.01± 0.475	T3
a 0.14± 96.19	d 0.02± 3.07	a 0.01± 14.57	a 0.01± 0.627	T4
**	**	**	**	Level of significance
The averages that have different letters within one column differ significantly among themselves *(P <0.05), ** (P <0.01)				

Histological segmentation:

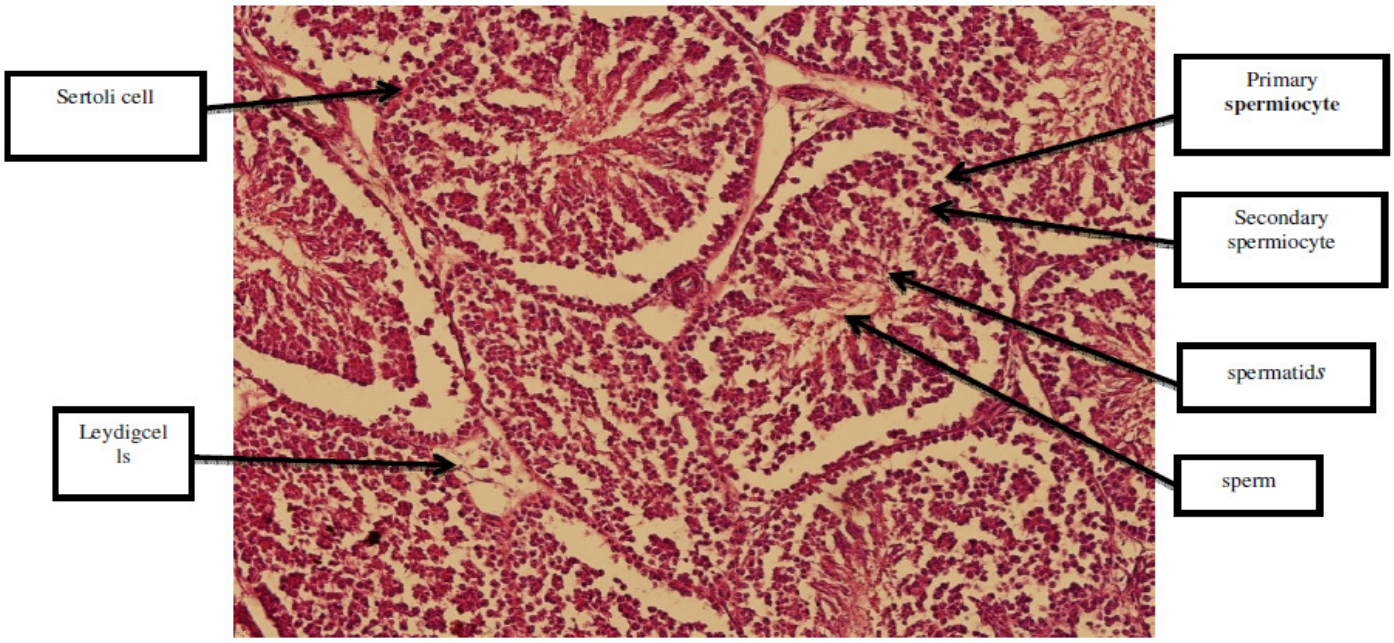
Testis:

The shape of the Histological sections of the first treatment (control) showed that the percentage of spermatic tubules that have reached the stage of completion in the process of biosynthesis of sperm up to 40% of the proportion of total tubules, in addition to that it is possible to distinguish the tubules that did not complete the biosynthesis of sperm, In addition to the proliferation of cells in your hands and Sertoli cells naturally, it is possible to notice the appearance of all stages of the vital synthesis of sperm from the initial sperm cells to the sperm, as the wall of the seminal tubule appears healthy without any thickening or fibrosis (Picture 1).

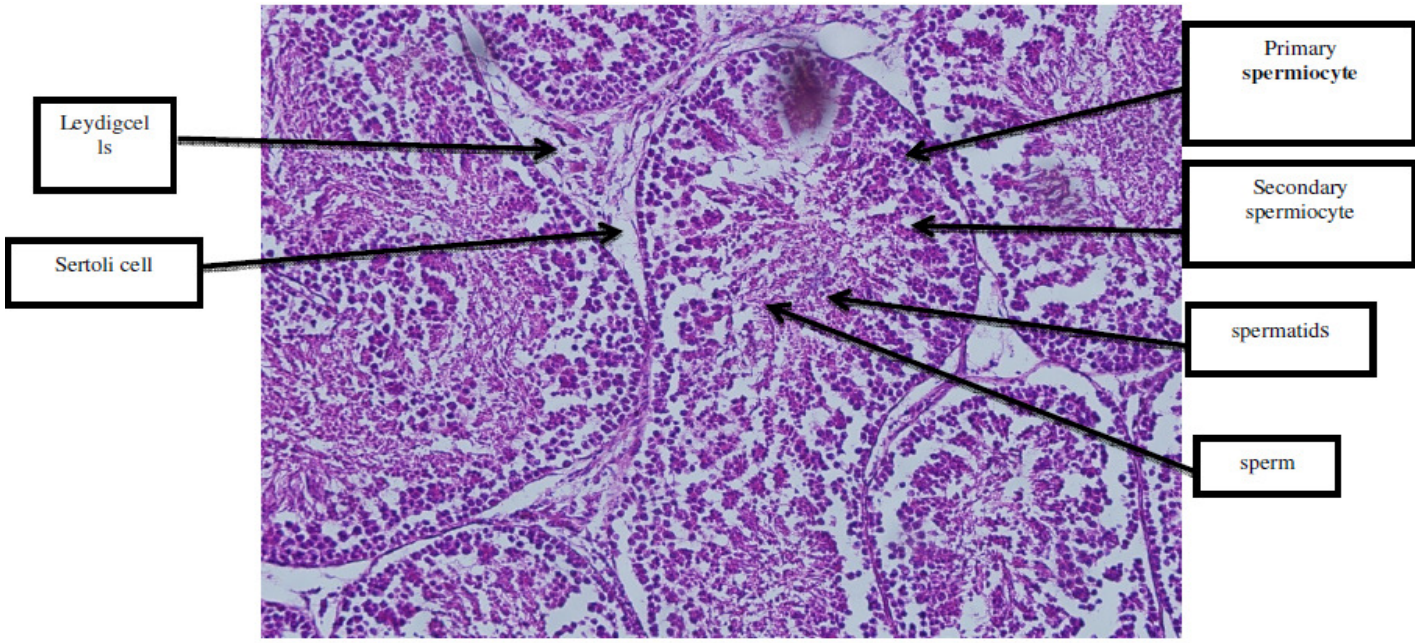
Also, the shape of the Histological sections the second treatment showed that the percentage of tubules that have reached the stage of completion in the process of biosynthesis of sperm is up to 70% of the proportion of the total tubules, while noting the presence of LIDIC cells and Sertoli cells naturally, And that the diameter of the spermatic tube appears slightly larger compared to the first treatment, and that its wall appears healthy and free from any fibrosis or thickening (Picture 2).

While the shape of the Histological sections of the third treatment showed that the percentage of tubules that have reached the stage of completion in the process of biosynthesis of sperm is up to 80% of the percentage of total tubules, Noting that your hands and Sertoli cells are naturally present, it is also clear that the sperm percentage inside one sperm has reached 50-45% and the remaining percentage represents the rest of the stages of the synthesis process with the possibility of distinguishing the increase in the diameter of the tubule in comparison with the first and second treatments in addition to the integrity of its wall From any fibres or thickening (Picture 3).

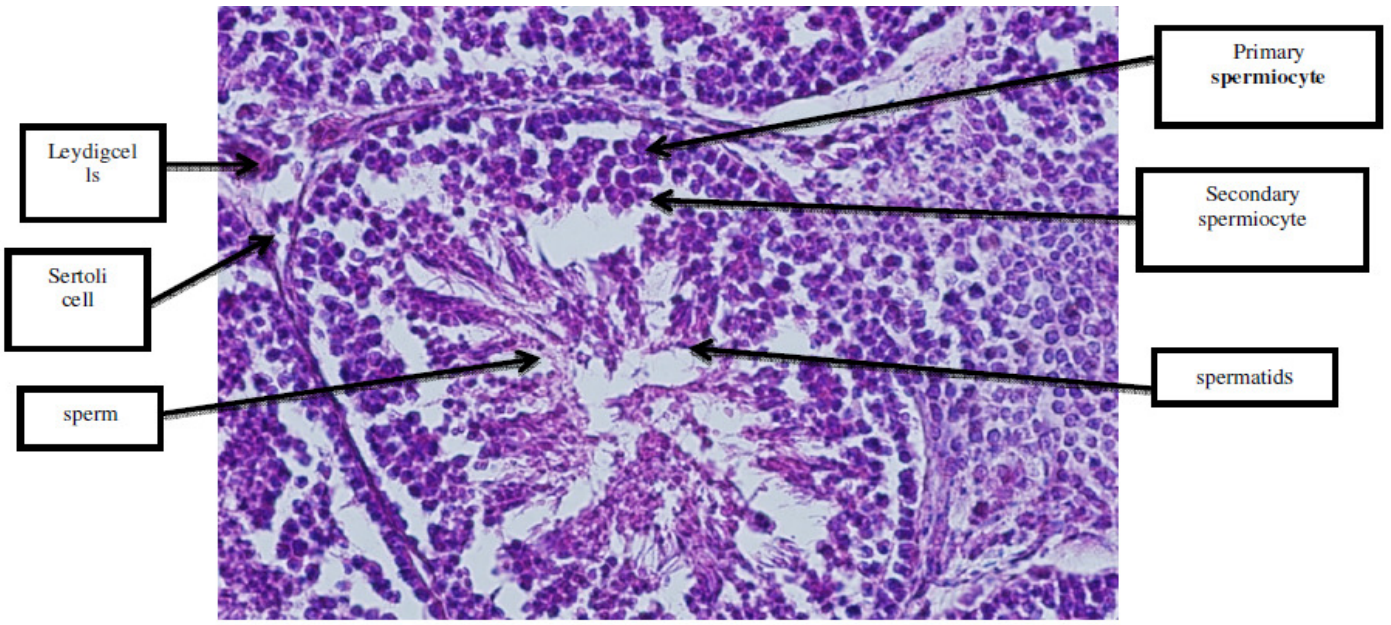
As for the fourth treatment, it appears that the percentage of spermatic tubules that have reached the stage of completion in the process of biosynthesis of sperm is up to 90% of the total percentage of tubules, in addition to that the sperm occupies about 70% of the seminal tubule compared with the rest of the treatments and the increase in the diameter of the tubule can be observed. Semen in this treatment with the integrity of a membrane from any fibrosis or thickening, in addition to that, your hands and Sertoli cells appear naturally, and the different stages of the biosynthesis process can be distinguished (Fig. 4).



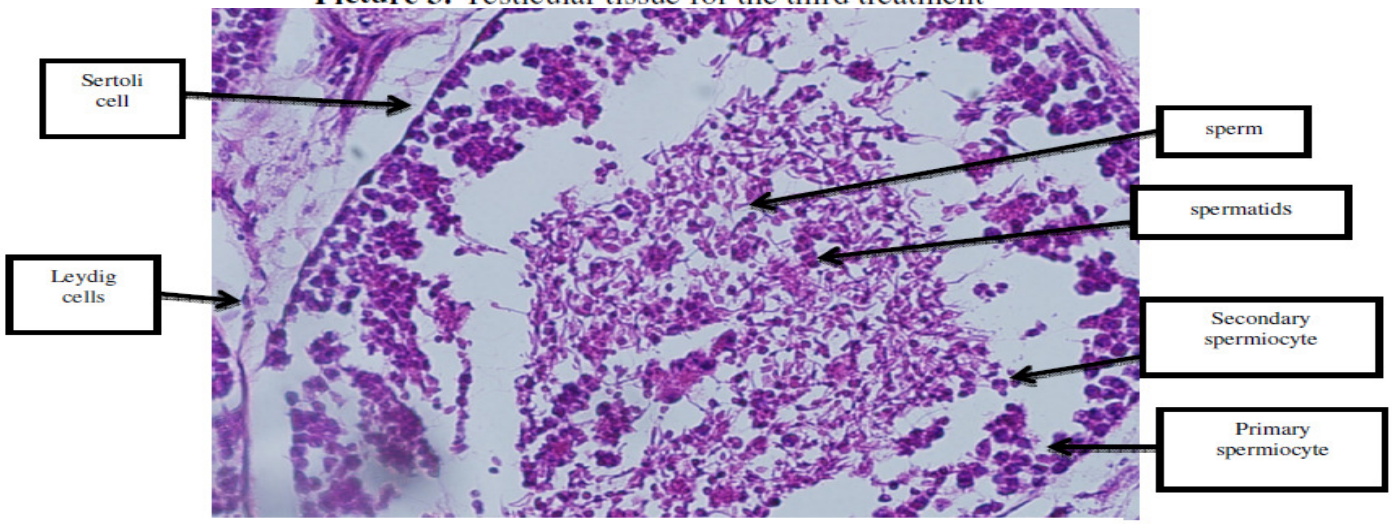
Picture 1: Testicular tissue for the first treatment



Picture 2: Testicular tissue for the second treatment



Picture 3: Testicular tissue for the third treatment



Picture 4: Testicular tissue for the Fourth treatment

High levels of estradiol hormone can adversely affect the secretion of GN-RH and consequently a decrease in the level of FSH in the blood and thus negatively affect the biosynthesis of sperm (Thibier, and Wagner, 2002), while inhibition Aromatase leads to a decrease in estradiol level and thus an increase in the level of FSH and this may stimulate sperm production (T'Sjoen *et al*., 2005), and this is consistent with (Sharp, 1999) as FSH has a key role in the synthesis process. Biosynthesis for sperm within the testicle,

This explains the improvement of the biosynthesis process for sperm in the fourth, third and second treatments, respectively. (Thompson and Berndtson, 1990) showed that after each stage of biosynthesis, cells move toward the center of the seminal tube to be excreted as a mature sperm.

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