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Original Article

Hormonal, Histological, and Comparative Study of the Effect of Pure Ginseng on Testicular Function in the Breeding/Non-Breeding Season of Rams in Basrah

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Abstract

This study aimed to investigate the effect of the administration of powdered *Panax ginseng* as a dietary supplement on testosterone concentration, spermatogenesis stimulating hormone, interstitial cell-stimulating hormone levels, sperms morphological characteristics, testis histological traits, and testicular size in the breeding and non-breeding season in adult rams. In total, 20 adult rams were included and randomly divided into three groups. The first group of adult rams (n=8) was subdivided into two subgroups of four rams (Sub-G1-B and Sub-G2-B). TheSub-G1-B and Sub-G2-B were fed 2 and 5 g of *P. ginseng* once a day, respectively, for 90 days during the breeding season. The second group of adult rams (n=8) was subdivided into two subgroups of four rams (Sub-G1-NB and Sub-G2-NB). The Sub-G1-NB and Sub-G2-NB were fed 2 and 5 g of *P. ginseng* once a day, respectively, for 90 days during the non-breeding season. The third group of adult rams (n=4) was considered the control group two times (in and out of the season). The results showed that the testosterone concentration and gonad protective and interstitial cell-stimulating hormone levels increased significantly ($P<0.05$) in both the experimental groups that received ginseng supplementation, compared to the control group in and out of the breeding season. The evaluation of sperm morphometric parameters, such as total sperm count, total motility, and progressive motility, showed superiority in improving the above-mentioned parameters. However, the total immotile sperms and non-progressive sperms underwent a significant decrease ($P<0.05$) in both experimental groups of ginseng supplemented, compared to the control group in and out of season. The angiogenesis of the seminiferous tubules increased significantly ($P<0.05$) in both experimental groups. Through a microscopic examination, the recorded data showed a significant increase in the population of spermatogonial stem cells as well as primary and secondary spermatocytes in both experimental groups. Values of testicular diameter showed a significant increase ($P<0.05$) after a period of 75 and 90 days following the initiation of treatments in both experimental groups, compared to the control group in and out of the season. It can be concluded that *P. ginseng* has some beneficial effects on the antioxidant status of the semen, the morphometric parameters, and other critical traits of sperm and testicles which are the important factors in male fertility.

Keywords: ICSH, Panax ginseng, Ram, SSH, Testis, Testosterone

1. Introduction

Sheep reproduction is one of the most important sources of livestock in Iraq, which provides about 50% of the livestock of this country. Most of the challenges facing sheep reproduction in the world are about the seasonality of reproduction. To increase fertility, it is suggested to use superior fertile rams in herds, especially in the breeding season. On the other hand, applying some medications or herbal remedies may lead to better results in terms of fertility in rams during the breeding and non-breeding seasons (1). The organs

and tissues with high biological and metabolic activities, such as testis, are vulnerable due to exposure to free radicals, including reactive oxygen species (ROS) at higher concentrations (1).

To cure tissues from injuries, antioxidants are used to prevent or slow down the generation of free radicals that are generated by various biological and metabolic activities in living organisms. Therefore, the application of herbal medications and antioxidant acts as a defensive line against the destructive activity of free radicals in terms of their generation or their chain of interactions (2). Recent studies have tended to apply medicinal herbs as diet additives to help increase the growth rate and protect cells in the living organisms from adverse effects on the productivity of animals (3).

Some of the most famous additives with a high antioxidant capacity that were used as herbal medications to cure human infertility are as follows: *Panaxginseng* and *Palmpollen* extracts (4). These herbs contain estrogen as well as other nutrients, proteins, essential and non-essential amino acids, carbohydrates, vitamins, minerals, and antioxidants (3). The major active ingredient of ginseng is ginseng saponin, which is composed of various ginsenosides (5). Until now, approximately 30 ginsenosides have been identified (6, 7).

There are at least nine known species of ginseng, including the Asian (*Panax ginseng*), American (*Panaxquinquefolium*), and Japanese (*Panaxjaponicus*) ginseng. These varieties have had some beneficial effects on antioxidant activities, immune system (8, 9), neuronal system (10, 11), and metabolic state (12, 13). In addition to the general properties of ginseng, it is considered a general tonic and antioxidant. Therefore, the present study aimed to examine the possible effects of ginseng root powder on testicular morphology, (follicle-stimulating hormone [FSH], luteinizing hormone [LH], testosterone) blood levels, histological parameters, and physiological characteristics of sperms in ram.

2. Materials and Methods

2.1 Experimental Study

This study was conducted at the animal farm of Veterinary Medicine College of Basrah University, Basrah, Iraq from May to the end of December, 2020. The experiment was performed on 20 adult rams whose ages and weights were within the ranges of18-24 months and 30-40 kg, respectively.

2.2. Animals Management

For protective and clinical examinations of all the experimental animals, an oral dose of 15 mg Albendazole/kg body weight and 20 mg of Ivermectin/kg body weight was injected by subcutaneous to eliminate internal and external parasites. Moreover, the animals were vaccinated against infectious diseases, especially *Clostridium* species. Furthermore, their external genitals were clinically examined to ensure that the testes were safe and in their normal position. The animals were trained to use artificial vaginas and then used them for 3 months. Semen was collected twice a week from each animal by using females in estrus induction by intramuscular injection of estradiol benzoate (4 mg/ewe). The primary collection of semen is important to the evaluation of volume, color, and physiological parameters of sperms.

2.3. Experimental Design

This study was performed on three different groups. The first group of adult rams (n=8) was subdivided into two subgroups of four rams (Sub-G1-B and Sub-G2-B). The Sub-G1-B and Sub-G2-B were fed 2 and 5 g of *P.ginseng* once a day for 90 days during the breeding season, respectively. The second group of adult rams (n=8) was subdivided into two subgroups of four rams (Sub-G1-NB and Sub-G2-NB). The Sub-G1-NB and Sub-G2-NB were fed 2 and 5 g of *P.ginseng* once a day for 90 days during the non-breeding season, respectively. The third group of adult rams (n=4) was considered the control group two times (in and out of breeding season).

2.4. Hormonal and Antioxidant Assay

The blood samples which were collected from the

jugular vein were put in the blood-collecting tubes without anticoagulant and then centrifuged at 3000 rpm for 10 min. The separated serum was kept in the refrigerator at -20°C until the day of assays. Testosterone, spermatogenesis stimulating hormone (SSH), and interstitial cell-stimulating hormone (ICSH) levels were measured using special kits (Abnova, England) as described by Dadashpour Davachi, Bartlewski (14) with some modifications.

2.5. Semen Evaluation

Samples of semen were directly collected from rams using an artificial vagina. The semen samples were transported to the laboratory of the Research Center in the College of Veterinary Medicine, Basrah University. The samples were evaluated by using computerized semen analyzer apparatus (14) to evaluate the following parameters: total sperm count, sperm motility and non-motility, and progressive and non-progressive motility.

2.6. Testis Morphometric

During the experimental study, testis was measured from lateral to the median border for all groups in breeding and non-breeding season by using an electronic digital caliper (a vernier scale; which is a visual aid to take an accurate measurement by reading the distance between two graduation markings on a linear scale using mechanical interpolation, thereby increasing resolution and reducing measurement) as shown in figure 1.

Figure 1. Electronic digital caliper.

2.7. Histological Study

For the purposes of the study, 90 days after the initiation of the experiment, all rams in the experimental groups were castrated as shown in figures 2 and 3. The testes were placed in special containers containing 10% formalin for histological examination (15).

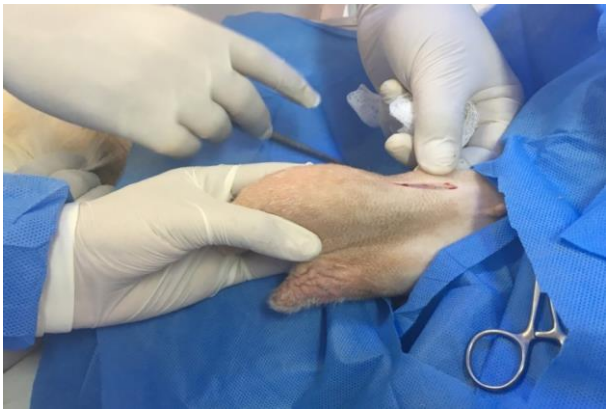

Figure 2. Site of incision in the testis.

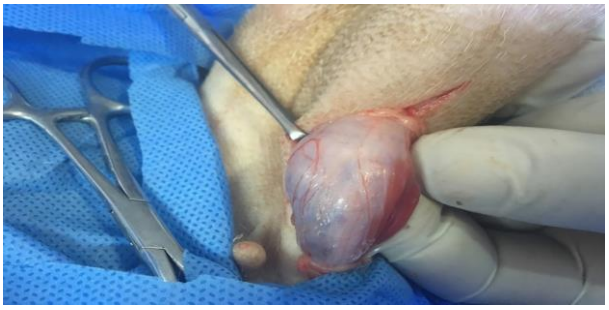

Figure 3. Spermatic cord.

2.8. Statistical Analysis

The data of this study were analyzed by one-way ANOVA model in SPSS software (SPSS Science, Chicago, USA). The difference was compared by Tukey's multiple comparison post-hoc test. All data were presented as mean±standard error of the mean (SEM) and the differences were considered significant at $P < 0.05$.

3. Results

3.1. Effect of Ginseng as a Dietary Supplement on Reproductive Hormones of Rams at Different Periods in the Breeding Season

The results showed that testosterone levels did not alter significantly in the experimental groups at 0, 15, and 30 days after ginseng administration as a dietary supplement, compared to the control group. However,

significant increases were recorded (P<0.05) in the experimental groups at 45, 60, 75, and 90 days after ginseng administration as a dietary supplement, compared to thec ontrol group as shown in table 1.

On the other hand, the recorded data of the present study showed that there were no significant differences between the control and experimental groups in terms of SSH levels at 0, 15, and 30 days after ginseng administration as a dietary supplement. Moreover, the results of the present study showed a significant (P<0.05) increase in the SSH concentrations after the administration of5 g of ginseng in experimental groups at 45 and 60 days after Ginseng administration as a dietary supplement, compared to the control and experimental groups that received 2 g of ginseng (102.88±8.60 and 113.96±9.11ng/ml, respectively). The results at 75 and 90 days after ginseng administration also indicated a significant (P<0.05) increase in the SSH levels in the experimental groups which received 2 and 5 g ginseng, compared to the control group (107.59±6.92, 120.29±7.23,104.43±7.11, and 118.21±7.32ng/ml), respectively.

The results also showed that the ICSH concentration did not change significantly in the experimental groups at0, 15, 30, and 45 days after Ginseng administration as a dietary supplement, compared to the control group. The results also indicated a significant increase in the ICSH levels (P<0.05) in the experimental groups as a dietary supplement at60 days after ginseng administration, compared to the control group (3.21±0.11,3.81±0.29,2.63±0.31 ng/ml, respectively).

Furthermore, the results showed that the ICSH levels underwent a significant increase(P<0.05) in the group that received 5 g ginseng as a dietary supplement at 75 days after ginseng administration, compared to the control group as well as the experimental group which received 2 g of ginseng (3.42±0.26, 2.32±0.27, 2.79±0.23 ng/ml, respectively).The recorded data of the ICSH levels significantly (P<0.05) increased in the experimental groups at 90 days after ginseng administration, compared to the control group (3.28±0.43, 3.55±0.17, 2.71±0.19 ng/ml, respectively) as shown in table 1.

Table 1. Effect of 2 and 5 g of Ginseng as a dietary supplement on the reproductive hormones of rams at different periods in the breeding season.

Groups		Time after treatment (days)						
		0	15	30	45	60	75	90
Testosterone	G1	3.10±0.49^a	3.17±0.51^a	3.60±0.39^a	3.98±0.34^a	4.22±0.32^a	4.91±0.62^a	5.36±0.53^a
	G2	3.37±0.44^a	3.59±0.64^a	3.65±0.51^a	4.52±0.62^a	4.86±0.42^a	4.48±0.39^a	6.73±0.48^a
	Control	2.81±0.51^a	2.94±0.32^a	3.28±0.42^a	3.13±0.29^b	3.37±0.27^b	3.31±0.43^b	3.52±0.37^b
SSH	G1	80.21±4.65^a	89.11±9.22^a	84.26±7.66^a	90.56±5.45^a	89.43±8.2^a	107.5±6.92^a	104.4±7.32^a
	G2	83.53±5.71^a	91.86±6.43^a	90.91±6.45^a	102.88±8.6^b	113.9±9.11^b	120.2±7.23^a	118.2±4.97^a
	Control	92.08±4.47^a	85.09±4.54^a	88.71±4.93^a	84.71±4.87^a	79.83±7.43^a	80.5 ±4.89^b	88.21±4.97^b
ICSH	G1	2.11±0.23^a	2.66±0.34^a	2.91±0.33^a	2.99±0.41^a	3.21±0.11^a	2.79±0.23^a	3.28±0.43^a
	G2	2.89±0.33^a	2.16±0.25^a	2.80±0.19^a	2.95±0.11^a	3.81±0.29^a	3.42±0.26^b	3.55±0.17^a
	Control	2.69±0.27^a	2.66±0.45^a	2.91±0.35^a	2.51±0.41^a	2.63±0.31^b	2.32±0.27^a	2.71±0.19^b

The data represent levels of reproductive hormones ng/mlas (mean±standard error of the mean).Different letters within each column in each hormone indicate a significant difference (P<0.05). G1: ginseng 2 gram, G2: ginseng 5 gram, SSH: spermatogenesis stimulating hormone, ICSH: interstitial cell-stimulating hormone

3.2. Effect of Ginseng as a Dietary Supplement on Rams Reproductive Hormones at Different Periods out of Breeding Season

The results showed that ginseng does not have any significant effects on the testosterone levels in the experimental groups, compared to the control group at0 and 15 days after ginseng administration as a dietary supplement. However, a significant (P<0.05) increase was recorded on the testosterone levels in the experimental group at 30, 45, and 60 days after using ginseng (5 g) as a dietary supplement, compared to the control group and the experimental group that was received 2 g of ginseng as a dietary supplement(1.38±0.31,1.97±0.33, 2.45±0.39 ng/ml, respectively).The recorded data showed a significant (P<0.05) increase in the case of testosterone level in the experimental groups at 75 and 90 days of ginseng administration (2 and 5 g) as a dietary supplement, compared to the control group(1.91±0.23,2.99±0.47, 0.39±0.05, 2.35±0.42, 3.57±0.41, and 0.41±0.05 ng/ml, respectively) (Table 2).

The results revealed that there were not any significant differences in the SSH levels in the experimental groups 1 and 2 and the control group at 0 and15 days after ginseng administration as a dietary supplement. At 30 days after ginseng administration, the results also indicated significant differences (P<0.05) between the SSH levels of the experimental group 2 which received 5 g ginseng as a dietary supplement, compared to the control group and experimental group 1 which received 2 g ginseng as a dietary supplement(29.79 ± 3.32,15.09 ± 1.9,22.67 ± 5.21 ng/ml, respectively).At 45 days of ginseng administration, the results showed a significant difference (P<0.05) in the concentration of SSH levels between the experimental groups 1 and 2 after ginseng administration as a dietary supplement, compared to the control group(40.8±5.31, 58.71±7.71, 12.47±2.05 ng/ml, respectively). On the other hand, the present study showed a significant (P<0.05) increase in the concentration of SSH in experimental groups 1 and 2 at 60, 75, and 90 days after ginseng administration as a dietary supplement, compared to the control group.

The recorded data showed that there were no significant differences in the ICSH levels at 0,15, and 30 days after ginseng administration as a dietary supplement between experimental groups 1 and 2, compared to the control group. Results of the present study showed a significant (P<0.05) increase in the ICSH levels in the experimentalgroups1 and 2 at 45, 60, 75, and 90 days after ginseng administration as a dietary supplement, compared to the control group (Table 2).

Table 2. Effect of 2 and 5 g of ginseng as a dietary supplement on the reproductive hormones of rams at different periods in the non-breeding season.

Groups		Time after treatment (days)						
		0	15	30	45	60	75	90
Testosterone	G1	0.57±0.11^{a}	0.51±0.18^{a}	0.64±0.09^{a}	0.66±0.13^{a}	0.98±0.12^{a}	1.91±0.23^{a}	2.35±0.42^{a}
	G2	0.61±0.13^{a}	0.78±0.07^{a}	1.38±0.31^{b}	1.97±0.33^{a}	2.45±0.39^{b}	2.99±0.47^{a}	3.57±0.41^{a}
	Control	0.79±0.09^{a}	0.68±0.07^{a}	0.61±0.11^{a}	0.59±0.08^{a}	0.63±0.07^{a}	0.39±0.05^{b}	0.41±0.05^{b}
SSH	G1	14.82±2.96^{a}	21.89±4.11^{a}	22.67±5.21^{a}	40.8±5.31^{a}	42.12±4.84^{a}	44.91±3.39^{a}	52.43±2.68^{a}
	G2	10.01±1.09^{a}	15.96±2.24^{a}	29.79±3.32^{b}	58.71±7.71^{a}	71.61±5.11^{b}	66.32±3.43^{b}	69.99±4.11^{b}
	Control	13.94±1.24^{a}	17.83±2.12^{a}	15.09±1.90^{a}	12.47±2.05^{b}	17.04±2.18^{c}	17.66±1.16^{c}	21.52±2.98^{c}
ICSH	G1	0.68±0.09^{a}	0.52±0.05^{a}	1.91±0.18^{a}	1.97±0.25^{a}	1.55±0.18^{a}	2.85±0.52^{a}	2.94±0.32^{a}
	G2	0.81±0.13^{a}	0.43±0.08^{a}	1.94±0.14^{a}	1.75±0.39^{a}	2.35±0.61^{a}	2.68±0.57^{a}	3.15±0.58^{a}
	Control	0.72±0.08^{a}	0.59±0.06^{a}	0.81±0.11^{a}	0.73±0.12^{b}	0.66±0.12^{b}	0.84±0.06^{b}	0.78±0.07^{b}

The data represent levels of reproductive hormones ng/mlas (mean±standard error of the mean).Different letters within each column in each hormone indicate a significant difference (P<0.05).G1: ginseng 2 g, G2: ginseng 5 g, SSH: spermatogenesis stimulating hormone, ICSH: interstitial cell-stimulating hormone

3.3. Effect of Ginseng as a Dietary Supplement on Morphometric Parameters of the Sperm of Rams at Different Times after Administration in the Breeding Season

The morphometric parameters of the sperm, including total sperm count, motility, immotility, progressive and non-progressive movement in the breeding season improved significantly at 0, 15, 30, 45, 60, 75, and 90 days after Ginseng administration as a dietary supplement in the experimental groups 1 and 2,compared to the control group. The results indicated a gradual increment in sperm motility. At 0, 15, 30, 45, and 60 days after Ginseng administration as a dietary supplement in the experimental groups 1 and 2, the values of total sperm count did not differ significantly, compared to the control group. The recorded data showed significant (P<0.05) improvement in terms of sperm concentration in experimental groups 1 and 2 at 75 and 90 days after Ginseng administration as a dietary supplement, compared to the control group (6.31±0.34, 6.86±0.52, 4.68±0.42, 6.94±0.31, 7.28±0.47, 5.01±0.27×10^9cell/ml, respectively) as shown in table 3 and figure 4.

The results of this study showed that the total motile sperms at 0, 15, 30, and 45 days after ginseng administration as a dietary supplement did not differ significantly in experimental groups 1 and 2,compared to the control group. However, the total motile live sperms at 60 days after ginseng administration as a dietary supplement underwent a significant (P<0.05) increase in the experimental group 2 (5 g), compared to group 1 (2 g) and control group (5.36±0.32, 4.92±0.32, 4.08±0.34×10^9cell/ml, respectively). On the other hand, the results of this study showed a significant (P<0.05) increase in the sperm motility in the breeding season in the experimental groups 1 and 2, compared to the control group at 75 and 90 days after ginseng administration (5.85±0.27, 6.44±0.41, 3.81±0.19, 3.36±0.37, 6.84±0.47, 4.12±0.28×10^9cell/ml, respectively) (Figure 5) (Table 4).

Table 3. Effect of Ginseng on total sperm count in the breeding season.

Groups	Time after treatment(days)						
	0	15	30	45	60	75	90
G1	4.62±0.43^a	4.88±0.41^a	5.21±0.48^a	5.91±0.68^a	5.67±0.35^a	6.31±0.34^b	6.94±0.31^b
G2	4.46±0.22^a	4.42±0.17^a	4.91±0.32^a	5.77±0.43^a	5.86±0.41^a	6.86±0.52^b	7.28±0.47^b
Control	4.68±0.56^a	4.76±0.51^a	4.58±0.64^a	4.93±0.36^a	5.03±0.14^a	4.68±0.42^a	5.01±0.27^a

Data represent total sperm count X10^9 sperm/ml as (mean±standard error of the mean).Different letters within each column in each hormone indicate a significant difference (P<0.05). G1: ginseng 2 gram, G2: ginseng 5 gram, SSH: spermatogenesis stimulating hormone, ICSH: interstitial cell-stimulating hormone

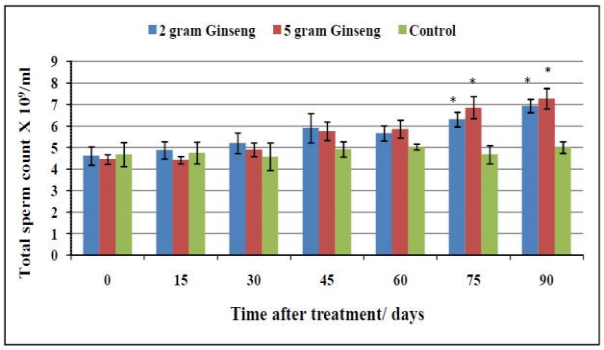

Figure 4. Effect of ginseng on total sperm count in the breeding season.

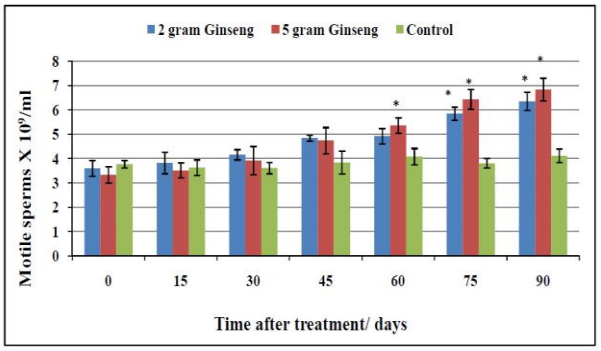

Figure 5. Effect of ginseng on sperms motility in the breeding season.

Table 4. Effect of ginseng on sperm motility in the breeding season.

Groups	Time after treatment(days)						
	0	15	30	45	60	75	90
G1	0.32±3.6[a]	3.82±0.44[a]	4.16 ±0.21[a]	4.84±0.12[a]	4.92±0.32[a]	5.85±0.27[b]	3.36±0.37[b]
G2	3.33±0.33[a]	3.52±0.31[a]	3.92±0.58[a]	4.74±0.54[a]	5.36±0.32[b]	6.44±0.41[b]	6.84±0.47[b]
Control	3.77±0.16[a]	3.63±0.32[a]	3.61±0.23[a]	3.84±0.74[a]	4.08±0.34[a]	3.81±0.19[a]	4.12±0.28[a]

Data represent sperms motility $X10^9$ sperm/ml as (mean±standard error of the mean).Different letters within each column in each hormone indicate a significant difference (P<0.05). G1: ginseng 2 g, G2: ginseng 5 g, SSH: spermatogenesis stimulating hormone, ICSH: interstitial cell-stimulating hormone

There was no significant difference in sperm immotility at 0, 15, 30, and 45 days after ginseng administration as a dietary supplement in experimental groups 1 and 2, compared to the control group. However, the total immotile sperms at 60, 75, and 90 days after ginseng administration were the lowest (P<0.05) values in the experimental group that received 5 g ginseng, compared to one that received 2 g ginseng and control group as shown in table 5 and figure 6.

Moreover, the results showed no significant differences in progressive motility at 0, 15, 30, and 45 days in experimental groups, compared to the control group. The findings also indicated a significant difference (P<0.05) in progressive motility around 60 days in the experimental group with 5 g ginseng, compared to the experimental group 1 and control group (4.65±0.12, 4.09±0.22, 3.21±0.28×10^9cell/ml, respectively). In addition, the results showed a significant increase (P<0.05) in progressive motility 75 and 90 days after ginseng administration in the experimental groups 1 and 2, compared to the control group (4.88±0.29, 5.42±0.19, 2.83±0.29, 5.61±0.32, 6.12±0.33, 3.11±0.35×10^9cell/ml, respectively), as shown in figure 7 and table 6.

Table 5. Effect of ginseng on sperm immotility in the breeding season.

Groups	Time after treatment(days)						
	0	15	30	45	60	75	90
G1	1.02±0.14[a]	1.06 ±0.11[a]	1.05 ±0.07[a]	1.07±0.06[a]	0.75±0.11[a]	0.46±0.29[b]	0.58±0.07[b]
G2	1.13±0.33[a]	0.9±0.09[a]	0.99±0.08[a]	1.03±0.06[a]	0.5±0.11[b]	0.42±0.19[b]	0.44±0.06[b]
Control	0.91±0.09[a]	1.13±0.11[a]	0.97±0.13[a]	1.09±0.09[a]	0.95±0.09[a]	0.87±0.12[a]	0.89±0.15[a]

Data represent sperms immotility $X10^9$ sperm/ml as (mean±standard error of the mean).Different letters within each column in each hormone indicate a significant difference (P<0.05). G1: ginseng 2 g, G2: ginseng 5 g, SSH: spermatogenesis stimulating hormone, ICSH: interstitial cell-stimulating hormone

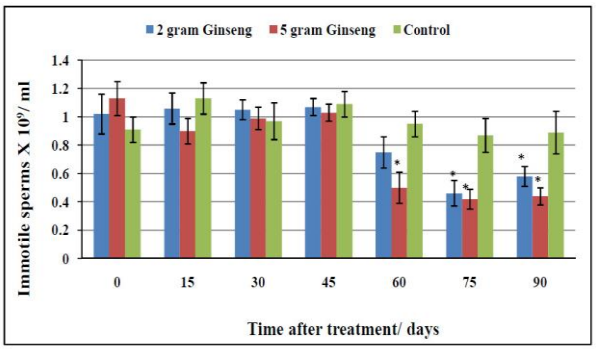

Figure 6. Effect of ginseng on sperm immotility in the breeding season.

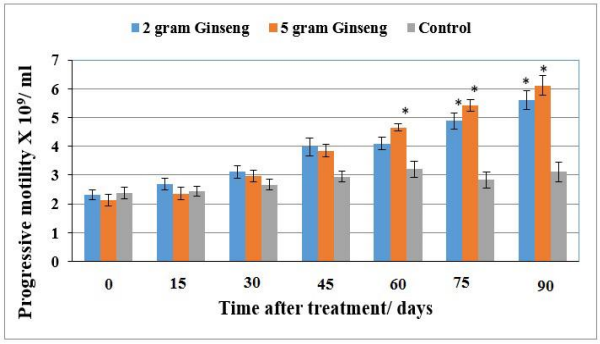

Figure 7. Effect of ginseng on progressive sperm motility in the breeding season.

Table 6. Effect of ginseng on progressive sperm motility in the breeding season.

Groups	Time after treatment/days						
	0	15	30	45	60	75	90
G1	2.32±0.18[a]	2.68±0.21[a]	3.11±0.23[a]	3.98±0.31[a]	4.09±0.22[a]	4.88±0.29[b]	5.61±0.32[b]
G2	2.13±0.21[a]	2.35±0.22[a]	2.97±0.19[a]	3.85±0.21[a]	4.65±0.12[b]	5.42±0.19[b]	6.12±0.33[b]
Control	2.37± 0.21[a]	2.43±0.17[a]	2.66±0.19[a]	2.95±0.19[a]	3.21±0.28[a]	2.83±0.29[a]	3.11±0.35[a]

Data represent progressive sperms motility $X10^9$ sperm/ml as (mean±standard error of the mean). Different letters within each column in each hormone indicate a significant difference (P<0.05). G1: ginseng 2 g, G2: ginseng 5 g, SSH: spermatogenesis stimulating hormone, ICSH: interstitial cell-stimulating hormone

The results also showed no significant differences in non-progressive motile sperm at 0, 15, 30, 45, 60, and 75 days after ginseng administration in experimental groups 1 and 2, compared to the control group. Furthermore, the findings indicated a significant increase (P<0.05) in non-progressive motility at 90 days after ginseng administration in experimental groups 1 and 2, compared to the control group (0.75±0.06, 0.72±0.11, 1.01±0.05×10^9 cell/ml, respectively (Table 7 and Figure 8).

3.4. Effect of Ginseng as a Dietary Supplement on Morphometric Parameters of Sperm of the Rams at Different Periods during the Non-Breeding Season

The present study showed no significant difference in total sperm count at 0, 15, 30, and 45 days after ginseng administration in the experimentalgroups1 and 2, compared to the control group. Moreover, the recorded data showed significant increases (P<0.05) in total sperm count at 60 and 75 days after ginseng administration in the experimental group 2 (5 g), compared to group1 (2 g) and control group. On the other hand, the total sperm count significantly increased (P<0.05) at 90 days after ginseng administration in the experimental groups 1 and 2, compared to the control group (7.42±0.31, 7.78±0.24, 5.27±0.35×10^9cell/ml, respectively), as shown in figure 9 and table 8.

Table 7. Effect of ginseng on non-progressive sperm motility in the breeding season.

Groups	Time after treatment (days)						
	0	15	30	45	60	75	90
G1	1.28±0.09[a]	1.14±0.08[a]	1.05 ±0.07[a]	0.86±0.09[a]	0.83±0.05[a]	0.97±0.11[a]	0.75±0.06[a]
G2	1.2 ±0.08[a]	1.17±0.11[a]	0.95±0.13[a]	0.89±0.08[a]	0.71±0.11[a]	1.02±0.12[a]	0.72±0.11[b]
Control	1.4± 0.06[a]	1.2±0.6[a]	0.95±0.11[a]	0.89±0.12[a]	0.87±0.12[a]	0.98±0.09[a]	1.01±0.05[a]

Data represent non-progressive sperms motility $X10^9$ sperm/ml as (mean±standard error of the mean). Different letters within each column in each hormone indicate a significant difference (P<0.05). G1: ginseng 2 g, G2: ginseng 5 g, SSH: spermatogenesis stimulating hormone, ICSH: interstitial cell-stimulating hormone

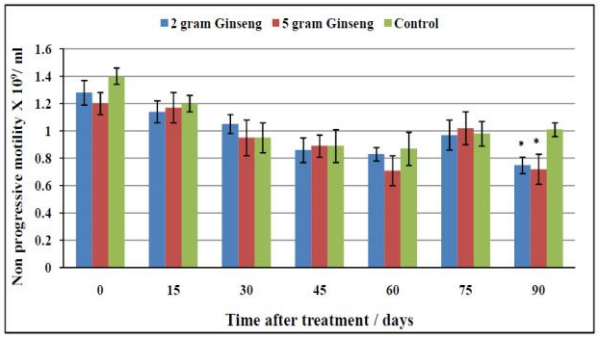

Figure 8. Effect of ginseng on non-progressive sperms motility in season

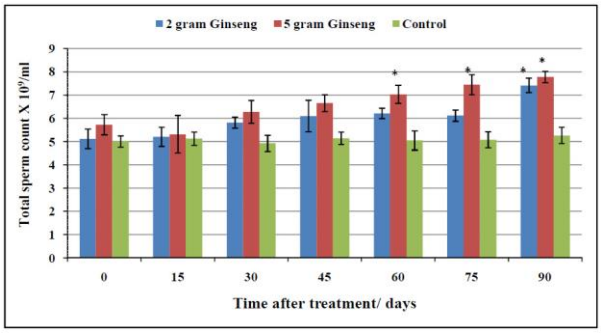

Figure 9. Effect of ginseng on total sperms count out of breeding season

Table 8. Effect of ginseng on total sperm count out of breeding season.

Groups	Time after treatment(days)						
	0	**15**	**30**	**45**	**60**	**75**	**90**
G1	0.42± 5.12[a]	0.41±5.21[a]	0.24±5.82[a]	0.68 ±6.1[a]	0.22±6.22[a]	0.24±6.12[a]	0.31±7.42[b]
G2	0.43±5.73[a]	0.81±5.32[a]	0.49±6.28[a]	0.36±6.66[a]	0.39±7.04[b]	0.43±7.45[b]	0.24±7.78[b]
Control	0.43±5.01[a]	0.28±5.13[a]	0.35±4.93[a]	0.26±5.15[a]	0.41±5.05[a]	0.34±5.08[a]	0.35±5.27[a]

Data represent total sperm count $X10^9$ sperm/ml as (mean±standard error of the mean). Different letters within each column in each hormone indicate a significant difference (P<0.05). G1: ginseng 2 g, G2: ginseng 5 g, SSH: spermatogenesis stimulating hormone, ICSH: interstitial cell-stimulating hormone

The results of the present study showed that the values of total motile live sperms at days 0, 15, 30, and 45 in the experimental groups 1 and 2 did not have a significant difference with those of the control group. Moreover, the total motile live sperms at 60, 75, and 90 days after ginseng administration underwent a significant increase (P<0.05) in the experimental groups 1 and 2, compared to the control group as shown in table 9 and figure 10.

On the other hand, the present study showed no significant differences in immotility at 0, 15, 30, 45, and 60 days after ginseng administration in the experimental groups 1 and 2, compared to the control group. The results also showed that the total immotile sperms at 75 and 90 days after ginseng administration underwent a significant (P<0.05) decrease in the experimental groups 1 and 2, compared to the control group as shown in figure 11 and table 10.

Table 9. Effect of ginseng on sperms motility out of breeding season

Groups	Time after treatment/ days						
	0	**15**	**30**	**45**	**60**	**75**	**90**
G1	4.11±0.33[a]	4.13±0.21[a]	4.94±0.23[a]	5.13±0.19[a]	5.67±0.21[b]	5.62±0.34[b]	6.84±0.32[b]
G2	4.61±0.61[a]	4.71±0.82[a]	5.62±0.55[a]	5.81±0.14[a]	6.47±0.31[b]	6.93±0.32[b]	7.16±0.33[b]
Control	4.1±0.32[a]	4.21±0.42[a]	4.17±0.23[a]	4.35±0.33[a]	4.44±0.31[a]	4.14±0.19[a]	4.35±0.285[a]

Data represent sperms motility $X10^9$ sperm/ml as (mean±standard error of the mean). Different letters within each column in each hormone indicate a significant difference (P<0.05). G1: ginseng 2 g, G2: ginseng 5 g, SSH: spermatogenesis stimulating hormone, ICSH: interstitial cell-stimulating hormone

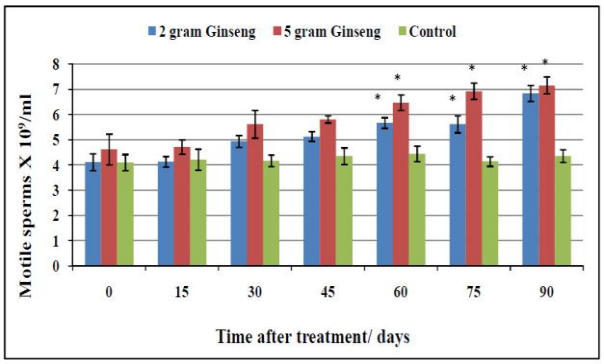

Figure 10. Effect of ginseng on sperms motility out of breeding season

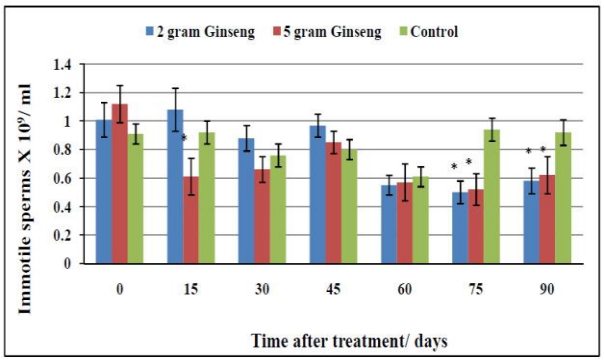

Figure 11. Effect of ginseng on sperms immotility out of breeding season

Table 10. Effect of ginseng on sperms immotility out of breeding season

Groups	Time after treatment/days						
	0	15	30	45	60	75	90
G1	1.01±0.12[a]	1.08±0.15[a]	0.88±0.09[a]	0.97±0.08[a]	0.55±0.07[a]	0.46±0.09[b]	0.58±0.07[b]
G2	1.12±0.13[a]	0.61±0.13[b]	0.66±0.09[a]	0.85±0.08[a]	0.57±0.31[a]	0.42±0.07[b]	0.44±0.06[b]
Control	0.91±0.07[a]	0.92±0.08[a]	0.76±0.08[a]	0.8± 0.07[a]	0.61±0.07[a]	0.87±0.12[a]	0.89±0.15[a]

Data represent sperms im-motility $X10^9$ sperm/ml as (mean±standard error of the mean). Different letters within each column in each hormone indicate a significant difference (P<0.05). G1: ginseng 2 g, G2: ginseng 5 g, SSH: spermatogenesis stimulating hormone, ICSH: interstitial cell-stimulating hormone

The results of the current study showed no significant differences in the total progressive motility at 0 and 15 days after ginseng administration in experimental groups 1 and 2, compared to the control group. Moreover, the present study showed a significant (P<0.05) increase in the total progressive motility at 30,45, 60, 75, and 90 days after administration in the experimental groups 1 and 2, compared to the control group as shown in table 11 and figure 12.

The present study also showed no significant differences in non-progressive motility at 0, 15, 30, 45, 60, and 75 days after ginseng administration in the experimental groups 1 and 2, compared to the control group. While the results showed a significant (P<0.05) increase in non-progressive motility at 90 days in group 2 (5 g), compared to group 1 (2 g) and control group (0.37±0.11, 0.63±0.09, 0.71±0.07×10^9cell/ml), respectively, as shown in table 12 and figure 13.

Table 11. Effect of ginseng on progressive sperms motility out of breeding season

Groups	Time after treatment/days						
	0	15	30	45	60	75	90
G1	3.01±0.22[a]	3.02±0.19[a]	3.68±0.17[b]	3.99±0.23[b]	4.71±0.18[b]	4.82±0.27[b]	6.21±0.23[b]
G2	3.41±0.19[a]	3.65±0.18[a]	4.22±0.18[b]	4.76±0.19[b]	5.67±0.21[b]	6.01±0.22[b]	6.79±0.19[b]
Control	2.96±0.15[a]	3.01±0.18[a]	2.67±0.17[a]	3.27±0.21[a]	3.57±0.22[a]	3.42±0.19[a]	3.64±0.22[a]

Data represent progressive sperms motility $X10^9$ sperm/ml as (mean±standard error of the mean). Different letters within each column in each hormone indicate a significant difference (P<0.05). G1: ginseng 2 g, G2: ginseng 5 g, SSH: spermatogenesis stimulating hormone, ICSH: interstitial cell-stimulating hormone

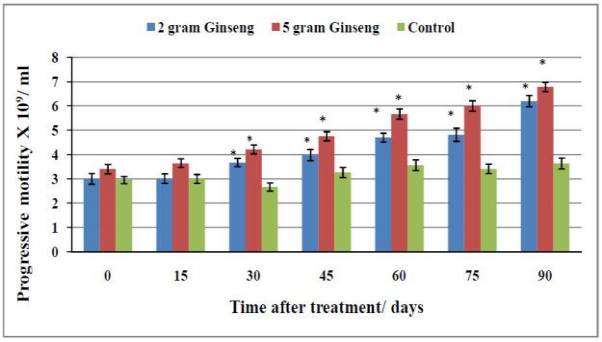

Figure 12. Effect of ginseng on progressive sperms motility out of season

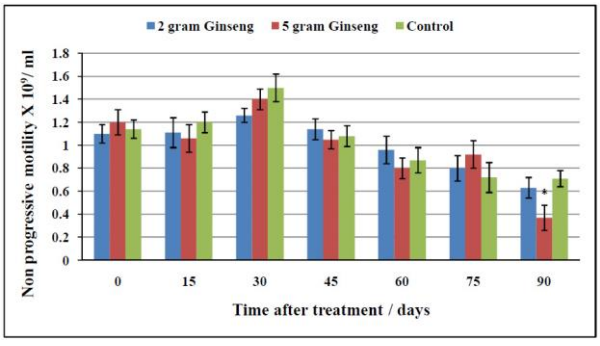

Figure 13. Effect of ginseng on non-progressive sperms motility out of season

Table 12. Effect of ginseng on non-progressive sperms motility out of season.

Groups	Time after treatment/days						
	0	**15**	**30**	**45**	**60**	**75**	**90**
G1	1.10±0.08[a]	1.11±0.13[a]	1.26±0.06[a]	1.14±0.09[a]	0.96±0.12[a]	0.8±0.0.11[a]	0.63±0.09[a]
G2	1.2 ±0.11[a]	1.06±0.12[a]	1.4 ±0.09[a]	1.05±0.08[a]	0.8 ±0.09[a]	0.92 ±0.12[a]	0.37±0.11[b]
Control	1.14±0.08[a]	1.2± 0.09[a]	1.5 ±0.12[a]	1.08±0.09[a]	0.87±0.11[a]	0.72 ±0.13[a]	0.71±0.07[a]

Data represent non-progressive sperms motility $X10^9$ sperm/ml as (mean±SEM). Different letters within each column in each hormone indicate a significant difference (P<0.05). G1: ginseng 2 g, G2: ginseng 5 g, SSH: spermatogenesis stimulating hormone, ICSH: interstitial cell-stimulating hormone

3.5. Effect of Ginseng on Testes Diameter in and out of Season

Values of testicular diameter were recorded in the breeding season, and no significant differences were found in the experimental groups 1 and 2,compared to the control group at 0, 15,30, 45, and 60 days after ginseng administration. The results also showed that the values of testicular diameter during the non-breeding season were not different significantly in the experimental groups 1 and 2,compared to the control group at 0, 15, 30, and 45 days after ginseng administration (Table 13).

3.6. Histological Evaluation

The histological observations in the control group showed that the testicle and the somniferous tubules lined by stratified epithelial cells and Sertoli cells which were few in numbers and acted as supporting cells that seated at the basement membrane of the tubules. The interstitial thin tissue between the tubules interpose by blood vessels and Leydig cells, the outer connective tissue capsule is surrounding the organ as showed in figures 14 and 15.

Table 13. Effect of 2 and 5 g ginseng as a dietary supplement on testis diameter (mm) of rams at different periods in and out of the breeding season.

Groups		Time after treatment/days						
		0	**15**	**30**	**45**	**60**	**75**	**90**
In season	**G1**	50.35±1.28[a]	50.37±1.32[a]	50.41±1.27[a]	51.22±1.13[a]	52.42±1.52[a]	55.18±0.56[b]	57.32±1.28[b]
	G2	49.73±1.61[a]	50.24±1.48[a]	50.62±1.34[a]	50.66±1.22[a]	52.78±1.18[a]	54.42±1.33[b]	57.12±1.27[b]
	Control	50.92±1.37[a]	51.16±1.43[a]	51.81±1.18[a]	51.37±2.09[a]	51.42±2.11[a]	52.04±1.94[a]	52.23±1.53[a]
Out of season	**G1**	41.82±2.14[a]	41.39±1.51[a]	41.63±1.42[a]	41.82±2.06[a]	46.12±2.28[a]	48.43±2.04[a]	52.43±2.68[a]
	G2	43.54±2.34[a]	43.26±1.92[a]	43.71±1.82[a]	44.22±1.81[a]	47.31±1.21[a]	48.78±2.53[a]	55.07±2.71[a]
	Control	44.52±1.76[a]	43.33±1.16[a]	43.62±1.31[a]	44.41±1.07[a]	43.04±2.28[b]	43.81±2.12[b]	44.13±2.42[b]

Data represent testis diameter (mm) as (mean±SEM). Different letters within each column in each hormone indicate a significant difference (P<0.05). G1: ginseng 2 g, G2: ginseng 5 g, SSH: spermatogenesis stimulating hormone, ICSH: interstitial cell-stimulating hormone

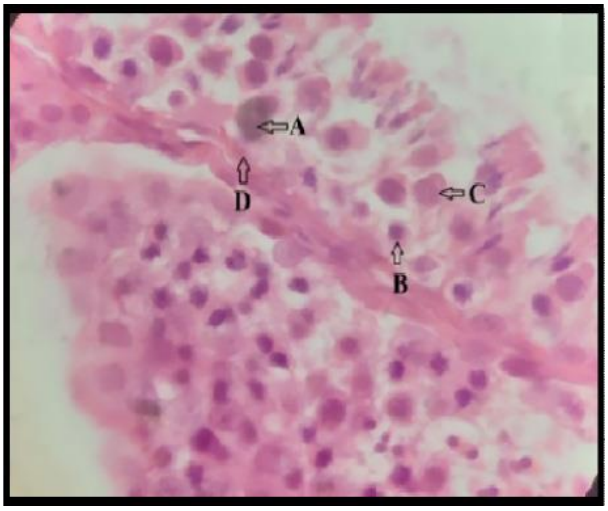

Figure 14. Normal seminiferous tubules of testis of the control group out of breeding season, Sertoli cell. A) Primary spermatocyte, B) Secondary spermatocyte, C) Basement membrane (D). H&E,X 1000

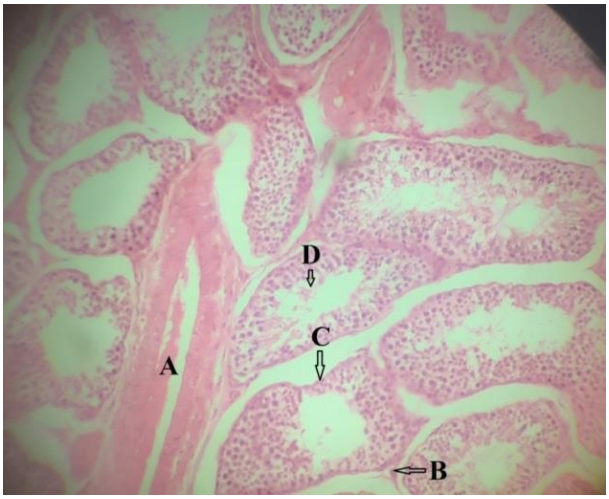

Figure 15. Cross section of testis of control group out of breeding season: A) Blood vessels, B) Lydig cell, C) Seminiferous tubules, and D) Spermatids. H&E,X 200

The epithelial tissue of the seminiferous tubules that form different stages of spermatogenic cells, the primary and secondary spermatocytes, with the spermatids that were found in a cluster at the lumen of the tubules in the control group are shown in figure 16. The experimental groups were distinguished by a significant augment of primary and secondary spermatocytes as well as spermatids, compared to the control group, as well as an invasion of blood vessels (Figure 17).

The results showed superiority in improving as they were filled with high numbers of sperm cells filling the seminiferous tubules lumen. Moreover, there was an increase in the size of interstitial cells concentration and high significant values were recorded between experimental groups 1 and 2, compared to the control group (Figures 18 and 19).

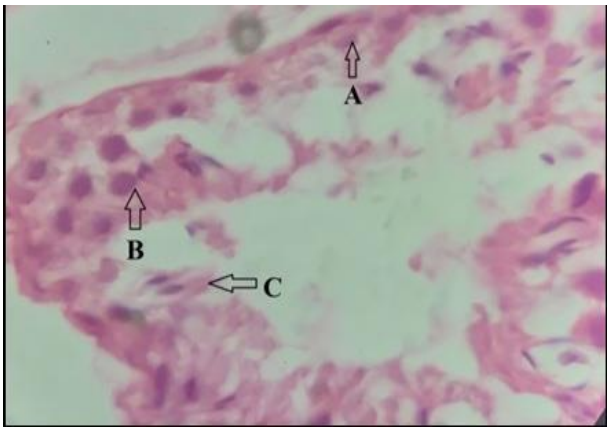

Figure 16. A and B) Seminiferous tubules of testis of control group out of season: primary spermatogonia (A, B, and C). H&E,X200

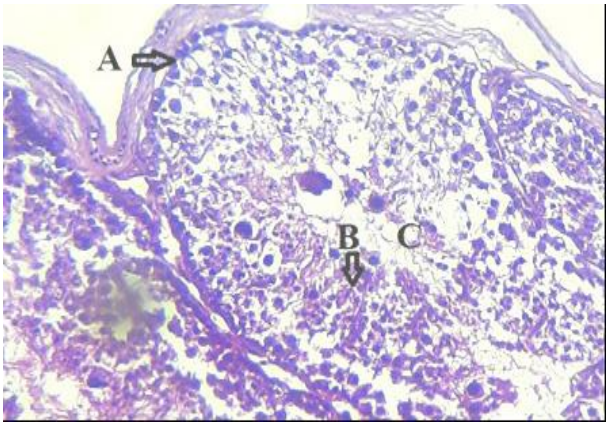

Figure 17. A and B: Testis of experimental groupreceiving2 g ginseng supplement out of breeding season showed: A) primary spermatocytes, B) secondary spermatocytes, and C) spermatids, H&E,X200

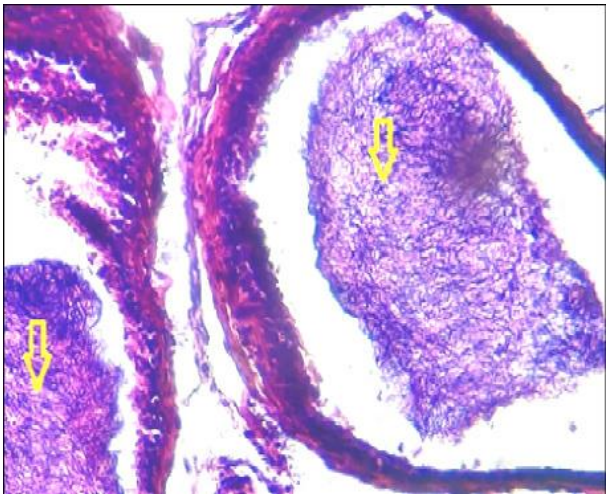

Figure 18. A and B) Testis of experimental group receiving 5 g of ginseng supplement during breeding season showed that the seminiferous tubules are filled with spermatids. H&E, X 400

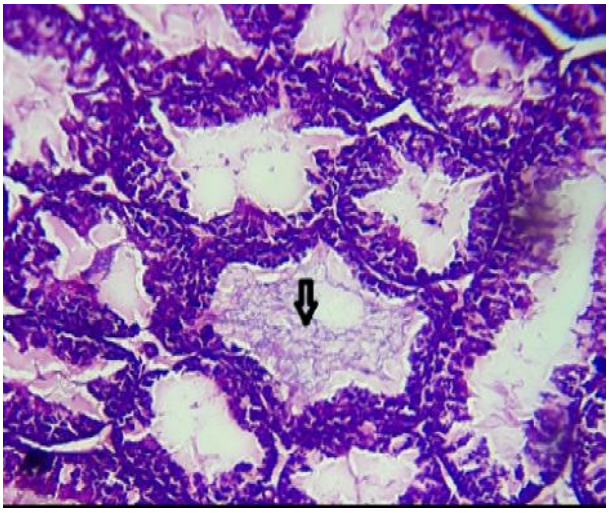

Figure 19. A and B) Testis of experimental group receiving 5 g of ginseng supplement out of breeding season showed that the seminiferous tubules are filled with spermatids. H&E, X 200

4. Discussion

4.1. Effect of Ginseng as a Dietary Supplement on Reproductive Hormones of Rams at Different Periods in the Breeding Season

The relationship between ginsenosides and male reproduction has been evaluated for over 20 years. However, few studies have been performed on humans, and most have used animal models. Ginseng has been considered a tonic, and therefore, a considerable number of experiments have been conducted to investigate its effects on sexual performance. In terms of libido, enhancement of copulatory behavior after treatment with *P. ginseng* and *P. quinquefolium* has been demonstrated in rodent models (16). Not all varieties of ginseng have shown the advantages of increasing the sex drive in studies, which may have been caused by different components that may or may not increase serum testosterone and LH levels.

The ginseng root has a high content of active substances, such as saponins, phenolic compounds, alkaloids, polyacetylene, and polysaccharides (17). It also contains other materials that enhance sexual activity (18). Solakidi, Psarra (19) have mentioned that ginseng root contains ginsucitein its formula, which is similar in composition to steroid hormones. The researchers have noted that ginseng root consists of two types of characteristics that are important for the performance of the male reproductive system.

The first act is to provide protection and support testicular tissue and cells from harmful substances that may be accidentally produced during vital processes, including effective free radicals oxygen (20). The researchers have indicated that the second feature is to enhance the action of the sex hormones that are important for the performance of testicular functions (21). Linjawi (22) noticed that ginseng roots had the ability to enhance the androgen receptors inside the seminiferous tubules, improve the production of proteins, and protect the DNA in testicular tissue.

The results of the present study indicated that ginseng root increases testosterone, FSH, and LH levels. The findings of another previous study (22) pointed that the treatment with ginseng root improved the fertility of mice through its effect on the hypothalamic-pituitary axis of the testicles which led to a significant increase in the levels of testosterone, FSH, and LH. In addition, it has been found that the ginseng root has the ability to inhibit damage in DNA and stimulate the gene expression of the gene (CYP19, LH, and FSH) which is responsible for producing FSH and LH. Besides,

another previous study delineating the changes in the structure of the ovarian theca internal cells following administration of Korean red ginseng (KRG) in a rat model suggested that KRG possibly stimulated steroid-producing cells (23).

There are other studies whose results are in line with those of the present study. For instance, the *in vitro* studies on neurotransmitters, such as dopamine, acetylcholine, and gamma-aminobutyric acid, have also shown an association with ginsenoside (24, 25). Ginsenoside Rb1 treatment increased LH secretion from the anterior pituitary gland of male rats (26). Many researchers who disagree with the results of the present study regarding ginseng treatment have shown the advantage of increasing the sex drive in their studies, which may have been caused by different components that may or may not increase serum testosterone and LH levels (24, 25)

Zhang andGao De Souza, Jenkins (12) reported a significant increase in serum testosterone level in experimental groups of ginseng supplemented as a diet, compared to the control groups. It is due to the increase in the number of receptors for testosterone. Other studies conducted on ginseng have confirmed its role in increasing the LH level, which may be suggested to have increased the effectiveness of the testicles through the increase of LH. The LH plays a vital role in distinguishing and motivating Leydig cells which release the testosterone and increase the effectiveness of testis (27).

4.2. Effect of Ginseng as a Dietary Supplement on Physical Parameters of the Sperms of Rams at a Different Period in and out of Season

Results of the present study showed significant differences in physical parameters between the experimental group and the control group in and out of breeding season at different times. Treatment with ginseng was effective and increased the motility and morphology of epididymal sperms. This is in line with the results of the study performed by Dahlberg Dahlberg (28) who pointed out that sperm motility played the most important role in fertility.

Morgentaler, Fung (29) reported that the morphology of sperms influences fertility, which may suggest the mechanism of ginseng which improved the quality of sperms was its antioxidant property; however, there was no significant increase in testicular catalase and peroxidase. The findings of many studies are consistent with those of the present study in terms of the effect of ginseng on semen. Moreover, various studies have shown an improved semen quality in animal models. In rats, numerous ginsenosides have been shown to enhance sperm count and motility after treatment. Saponins from the cultured root of wild *P. ginseng* were effective on spermatogenesis in the male rats (30).

In one of the studies (31), the researchers administered *P. ginseng* powder orally after inducing oligospermia in rats using dioxin and examined the sperm count and testes histologically. The *P. ginseng* saponins revived spermatogenesis in their study, suggesting its possible role in reversing the damage (31). In previous studies, ginseng-treated rats experienced increased spermatogenesis by increasing the glial cell-derived neurotrophic factor expression in Sertoli cells (31).Testicular cyclic adenosine monophosphate-responsive element modulator (32).

The glial cell-derived neurotrophic factor is known as a possible regulating factor of the lifespan of spermatogonial cells, and cyclic adenosine monophosphate-responsive element modulator is an essential factor for spermatid maturation. An animal study using *P. ginseng* showed sperms hyperactivation at the genetic level, suggesting possible improvement of sperm quality (33).In rat models of induced genito-urinary inflammation, such as epididymo-orchitis and prostatitis, KRG enhanced the anti-infective effects when it was administered with antibiotics.

In addition, it increased sperm motility, decreased apoptosis in testicular tissue, and stimulated the yield of normal spermatozoa (32, 34) in the studies performed on rats treated with cyclophosphamide and ginseng. Hong, Ji (21) reported possible gonadoprotective effects of ginseng and suggested that the possible protective role may be related to a ginseng-induced

decrease in reactive oxygen species. Another study whose results were in line with those of the present research is a case-control study that reported treatment with *P. ginseng* was able to improve the sperm quality and sex hormone profiles (16).

Moreover, according to the findings of a study, the beneficial effect of KRG on erection in men with erectile dysfunction has been identified in meta-analyses of randomized controlled trials (21, 35). Another research was performed on rats under disease conditions, such as cancer and diabetes, after the radiation period treated with cyclophosphamide and ginseng. The administration of ginseng showed improvement of sperm profiles after treatment of animals exposed to varying degrees of radiation (36) suggesting a future positive role for ginseng in this area.

4.3. Histological Evaluation

In previous histological studies, ginseng-treated rats have experienced an increase in the thickness of basement membrane seminiferous tubule, germinal layer, and primary and secondary spermatogenesis. This is significant due to an increase in the androgen receptors of the seminiferous tubule in the testis and higher protein production in testicular cells (21, 37). This suggests a relationship between the thickness of the germinal layer in the seminiferous tubule of testicular tissue and testosterone level in male Japanese quail.

Through the results of the current study, it was found that the ginseng root changed the measurements of testicles of the experimental groups, compared to the control group. These changes may be due to an increase in the production of testosterone according to the results, and this hormone has a significant effect on the process of producing sperms inside testicular seminiferous tubules (38).

The results also indicated that the administration of ginseng root in the experimental groups led to a significant increase in the tubules diameters and thickness of the seminal germ layer. The existence of a highly significant positive correlation coefficient between the thickness of the germ layer and the level of the testosterone in the roosters of broilershadbeen previously indicated by researchers. Therefore, the changes in testicle dimensions are only a reflection of the content of the seminal tubules that form most of their mass (19).

Authors' Contribution

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

Ethics

All the procedures were approved by the Ethics Committee at the University of Basrah, Basrah, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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