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Hormonal, Histological and Comparative Study an Effect of Pure Ginseng on Testicular Function during and out of Breeding Season of Rams in Basrah

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Abstract –The study aimed to investigate the effect of *Panax ginseng* powdered supplemented 2and 5 gram as a diet on testosterone, SSH and ICSH levels, on the other hand, *Panax ginseng* effect on some physical parameters of sperms, histological testis traits, and testicular size in and out of season in adultrams.This study was conducted at the laboratories of Theriogenology, Department of Surgery and Obstetrics, College of Veterinary Medicine, Basrah University. The experimental study extended from May 2019 to the end of December 2020. Twenty adult rams (18-24 month old) were divided randomly into 3 groups; The 1st group 8 adult rams were subdivided into 4 rams fed 2 gram standard administered containing *Panax ginseng*, while the other 4 adult rams fed 5 gram standard administered containing *Panax ginseng* once a day for 90 days during the breeding season. The 2nd group 8 adult rams were subdivided also into 4 rams fed 2 gram standard administered containing *Panax ginseng*, while the other 4 rams fed 5 gram standard administered containing *Panax ginseng* once a day for 90 days out of season. The 3rd group 4 adult rams were used as the control group two times (in and out of season).The results illustrated the testosterone ,SSH and ICSH were high significant ($P<0.05$) values between treated groups of ginseng supplemented 2and 5 gram as a diet compared with control group in and out of season .The physical parameters of sperms evaluation (total sperms count, sperms motility, and progressive movement) of this study showed superiority improving of above parameters ,while the total immotile sperms andnon-progressive sperms movement showedlow significant ($P<0.05$) values in treated groups of ginseng supplemented 2 and 5 gram as a diet compared with control group in and out of season.The angiogenesis of the seminiferous tubules were increased and the incensement of the numbers of spermatogonia, primary and secondary spermatocytes were observed in the *Panax ginseng* groups through a microscopic examination during and out of season.Values of testicular diameter showed a highly significant difference ($P<0.05$) at (75 and 90) days in treated groups of ginseng supplemented 2 and 5 gram as a diet compared with control group in and out of season. From the above mentioned results, we conclusion that *Panax ginseng* may affect the morphology and motility of sperms, the important factor in male fertility, and its antioxidant status in rams.

KEY WORDS: Ram , *Panax ginseng*,Testis , Testosterone, SSH, ICSH.

Introduction

Sheep reproduction is one of the most important sources of livestock in Iraq ,which is about 50% ,the most challenges facing the sheep reproduction in the world is seasonality of production. In

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order to increase fertility, we must inter a very good fertile rams in the herds specially in breeding season.And if we gain a better results of fertility we must use medication of rams before and during season to increase male reproduction performance [1]. The organs and tissues of high capacity (testis) are exposed to free radicals at a higher rate [2].To treat tissues from injuries, antioxidants prevent or slow down the generation of free radicals that are generated by various vital activities in the body, so they act as a defensive line against the destructive activity of free radicals in terms of their generation or their chain of interactions [3]. Recent studied have tended to used medicinal herbs as food additives which help to increase growth and protect them from many diseases[4]. These additives include*Panaxginseng* and*Palm pollen* extracts that contribute to improve male and female fertility [5]. These herbs contain estrogen as well as other nutrients, proteins, essential and non-essential amino acids, carbohydrates, vitamins and minerals [4]. The major active ingredient of ginseng is the ginseng saponin, which is composed of various ginsenosides[6]. Currently, approximately 30 ginsenosides have been identified [7-8]. There are at least 9 known species of ginseng, including the Asian (*Panax ginseng*), American (*Panax quinquefolium*), and Japanese (*Panax japonicus*) ginseng. These varieties have shown effects such as antioxidant effects, enhancement of diverse physiologic effects including immune stimulatory effects [9,10].Effects on the neuronal system [11,12] control of the metabolic state [13-14]. In addition to the general properties of ginseng, which is considered as a general tonic and antioxidant.Therefore, the presentexperimentaimed to studyan effects of ginseng root powder on testicular morphology, (FSH,LH,Testosterone) blood levels, Histological evaluation and physiological characteristics of sperms in ram.

Materials and Methods

Experimental study :

This study was conducted at the animal farm of Veterinary Medicine College of BasrahUniversity from May to the end of December. The experiment was done on 20adult rams, their ages ranged between (18-24) month, and weights ranged between (30-40) kg.

Animals management :

For protective and clinical examinations of all the experimental animals, oral dose of 15 mg Albendazole /kg body weight, and 20 mg Ivermectin /kg body weight were injected by subcutaneous to eliminate internal and external parasites, as well as the animals were vaccinated against infectious diseases, especially (*Clostridiums* Spp), also included clinical examination of the external genitals to ensure that the testes were safe and at their normal position. Animals were trained for using of artificial vagina and then used for 3 months to collect semen twice a week for each animal by using female in estrus induction by injected with estradiol benzoate (4) mg /ewe intramuscularly. The primary collection of semen is important to the evaluation of volume, color, and physiological parameters of sperms.

Experimental design:

Rams were divided into three equal groups: The 1st group was served as the control group(n=4) , 2nd group (n=8) ,and 3rd group (n=8) were treated for90 days according to the following:

Rams were divided into three groups: The control group: 4 rams / treated without herb

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supplemented in and out of season.To evaluatehormonal profile (Testosterone, FSH, and LH), semen collection for seminal fluid analysis,testes measurementand then histological testicular examination after 3 months of treatment .Group 1: 4 rams / treated with supplemented ginseng (5gm) orally in season and 4 rams / treated with supplemented ginseng (2gm) orally in season, to evaluate hormonal profile (Testosterone, FSH, and LH), and semen collection for seminal fluid analysis , testes measurement and then histologically. To compare (during and after 90 days) with control group .

Group 2: 4 rams / treated with supplemented ginseng (5gm) orally out of season , and 4 rams / treated with supplemented ginseng (2gm) orally out of season, to evaluate hormonal profile (Testosterone, FSH, and LH), and semen collection for seminal fluid analysis , testes measurement ,then histologically. To compare (during and after 90 days) with control group .

Hormonal estimation :

The blood samples which collected from jugular vein were put in the blood collecting tube without anticoagulant and then put in a centrifuge at 3000 rpm for 10 minutes to estimate (testosterone,SSH and ICSH) levels by using special kits for each hormonal profile .

Semenevaluation:

Samples of semen were collected directly from rams by artificial vagina , semen transported to the laboratory of unit center researches in the College of Veterinary Medicine/Basrah University .The samples were evaluated by using computerized semen analyzer apparatus[15] to evaluate the following : total sperms count ,sperms motility and immotility ,progressive and non-progressive motility as showed in figure (1).

Testis morphometric :

During experimental study , testis was measured from lateral to medium border for all groups in and of season by using electronic digital caliper (a vernier scale ; which is visual aid to take an accurate measurement reading between two graduation markings on a linear scale by using mechanical interpolation ;thereby increasing resolution and reducing measurement) as showed in figure (2).

Histological study :

After 90 days of experiment, all rams of groups were castrated as showed infigure (3 and 4), the testes were placed in special containerscontaining10% formalin forhistological examination [16] .

Statistical analysis:

The data of this study were analysed by one way ANOVA model of SPSS (SPSS Science, Chicago, USA). Difference were compared by Tukeys multible comparison post hoc test. All data were presented as mean ± SEM and the differences were considered as significant at $P < 0.05$.

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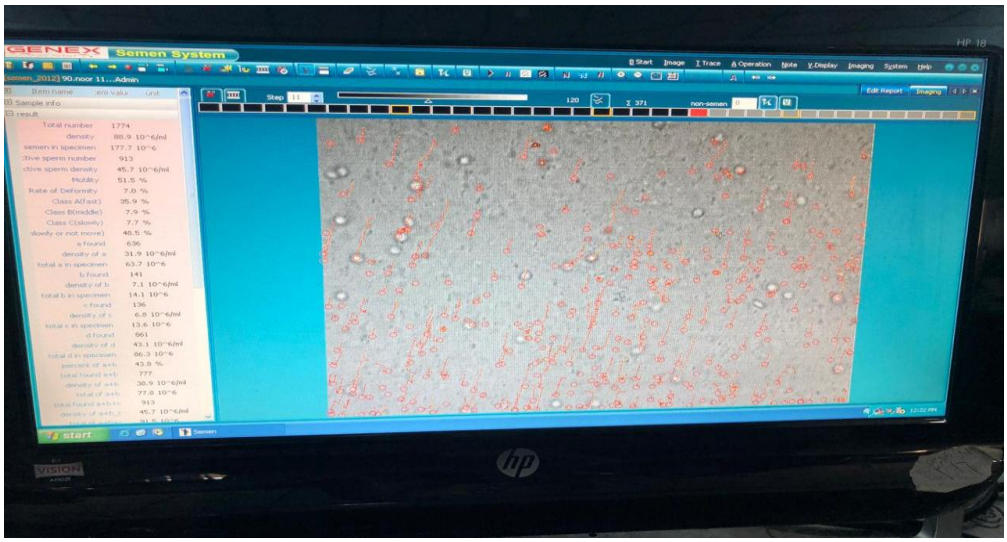
e 

Figure (1) : Computerized semen analyzer apparatus .

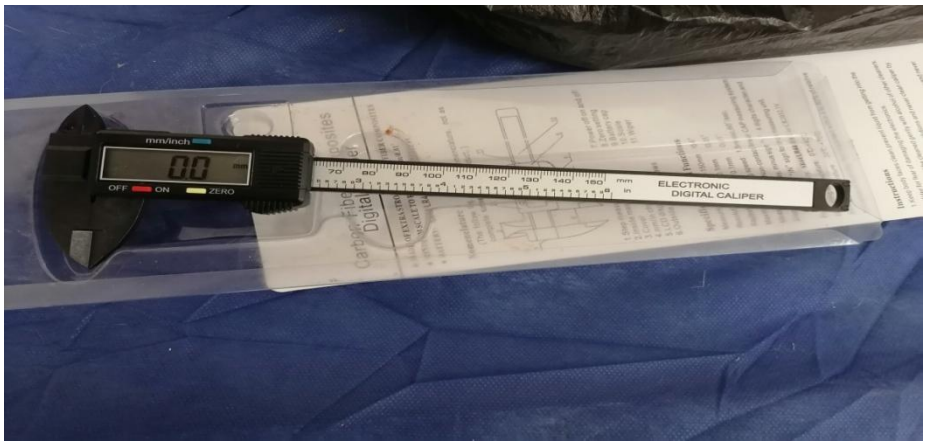


Figure (2) : Electronic digital caliper .

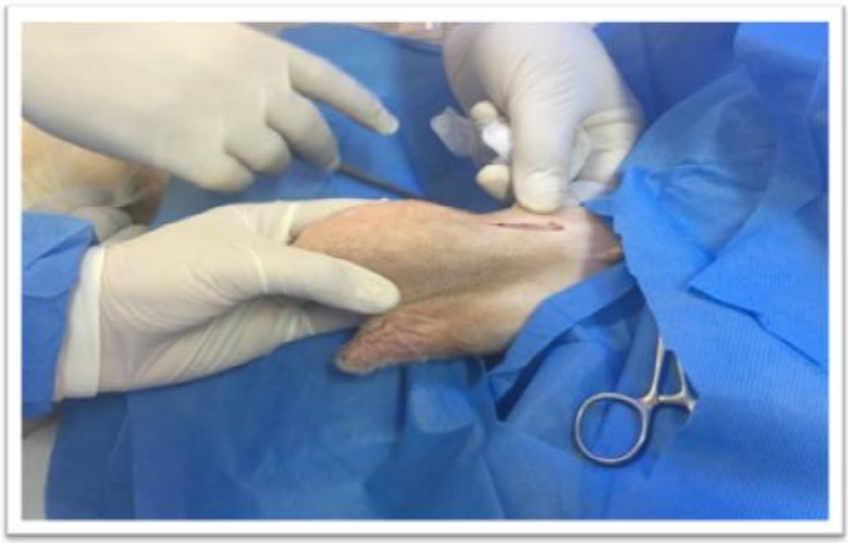


Figure (3): Showed site of incision in the testis.

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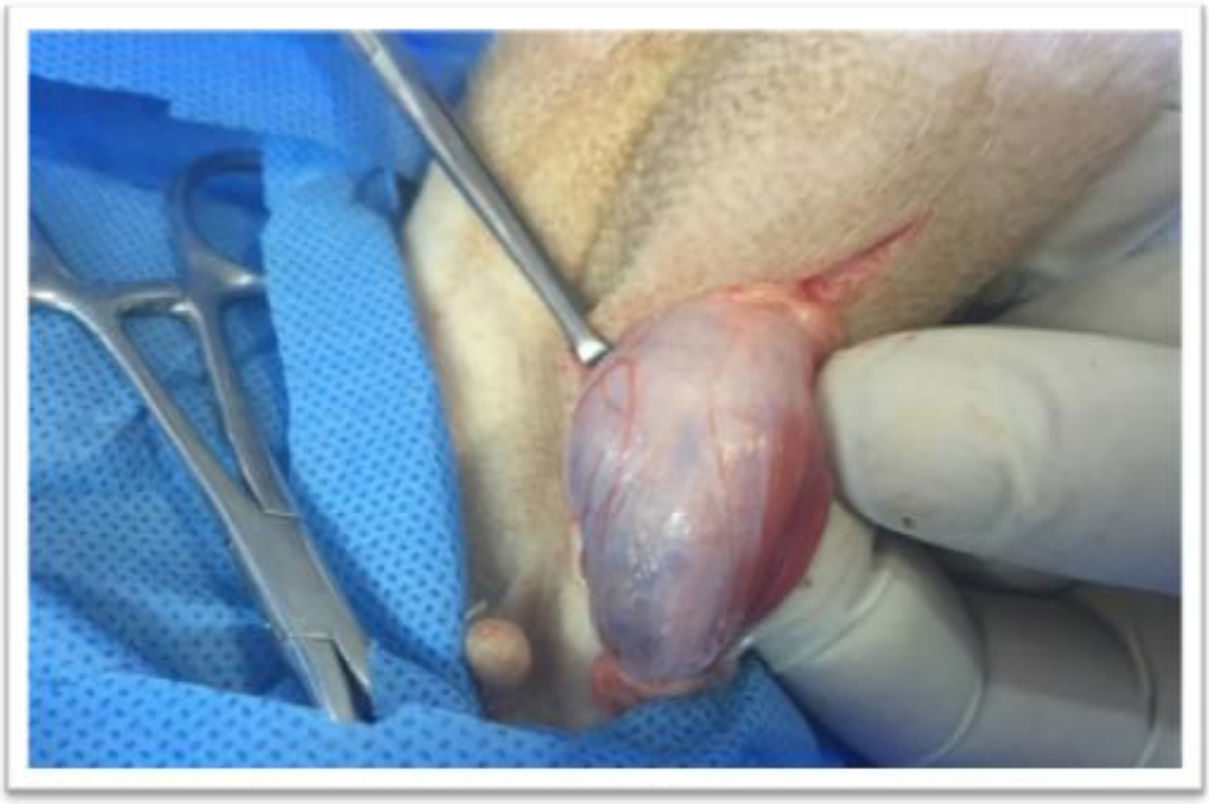

Figure (4): Showed spermatic cord .

Results:

Effect of ginseng as a diet supplement on rams reproductive hormones at a different periods in season:

The results illustrated that testosterone levelswerenon -significant in treated groups at (0,15 and 30) dayof ginseng supplemented at 2and 5 gram as a diet compared with control group.Moreover ,there werehigh significant difference($P<0.05$) valuesbetween treatedgroups on (45, 60, 75 and 90) day of ginseng supplemented 2 and 5 gram as a diet compared with control groupas showed in table (1) .

On the other hand , the study showed that there was no significance betweencontrol group and treated groups (2 and 5 gm) of SSH levels at (0, 15 and 30) day of ginseng supplemented as a diet.Moreover, the results also showed the high significant ($P<0.05$)values ofSSH levels of ginseng supplemented at 5 gram as a diet in treated group on(45 and 60) day compared with the control group and treated group of ginseng supplemented 2 gram(102.88 ± 8.60,113.96 ± 9.11) ng/ml respectively. While the results also appeared high significant ($P<0.05$) values of SSH levels of ginseng supplemented 2 and 5 gram as a diet in treated groups on(75 and90) days compared with the control group(107.59 ± 6.92, 120.29 ± 7.23,104.43 ± 7.11, 118.21 ± 7.32) ng/ml respectively.

The results indicated that total average of ICSHlevels werenon-significant values in treated groups of ginseng supplemented 2 and 5 gram as a diet on (0, 15, 30 and 45) day compared with control group. On the other hand,the results also illustrated the ICSHlevels were high significant ($P<0.05$) values between treated groups of ginseng supplemented 2 and 5 grams as a diet at (60)

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day compared with the control group (3.21 ± 0.11,3.81 ± 0.29,2.63 ± 0.31) ng/ml respectively. Also, the results showed thatthe ICSH levels were high significantly (P<0.05) values between the treated group of ginseng 5 gram as a diet on (75) day compared with the control group and treated group of ginsengsupplemented 2 gram as a diet (3.42 ± 0.26, 2.32 ± 0.27, 2.79 ± 0.23) ng/ml respectively.While the results of ICSHlevels were highsignificant (P<0.05) values between treated groups of ginseng supplemented 2 and 5 gram as a diet at (90) day compared with the control group (3.28 ± 0.43, 3.55 ± 0.17, 2.71 ± 0.19) ng/ml respectively as showed in table (1).

Table (1): Effect of (2 and 5) gm dose of ginseng as a diet supplement on rams reproductive hormones at a different periods in 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±8.21 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

Data represent levels of reproductive hormones ng/mlas (mean±SEM).

Different letters within each column in each hormone indicate significant difference (P<0.05).

G1, Ginseng 2 gram ; G2, Ginseng 5 gram.

Effect of ginseng as a diet supplement on rams reproductive hormones at a different periods out of season:

This study conducted that the protective effect of testosterone levels were non-significantly between treated groups and control group at (0 and 15) dayof ginseng supplemented 2and 5 gram. While there were high significant (P<0.05) values oftestosterone levels between the treated group at (30, 45 and 60) day of ginseng supplemented 5 gram as a diet compared with the control

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group and treated group of ginseng supplemented 2 gram as a diet (1.38 ± 0.31,1.97 ± 0.33, 2.45 ± 0.39) ng/ml respectively.The results also showed high significant (P<0.05) values of testosterone levels in treated groups at (75 and90) day of ginseng supplemented 2 and 5 gram as a diet compared with control group (1.91 ± 0.23,2.99 ± 0.47, 0.39 ± 0.05, 2.35 ± 0.42, 3.57± 0.41, 0.41± 0.05)ng/ml respectivelyas showed in table (2) .

The results also appeared non-significantly in SSH levels of ginseng between treated groups and control group on (0 and15) day of ginseng supplemented.The results also illustrated there were significantdifference (P<0.05) values inSSH levels between the treated group at (30) day of ginseng supplemented 5 gram as a diet compared with the control group and treated group of ginseng supplemented 2 gram as a diet (29.79 ± 3.32,15.09 ± 1.9,22.67 ± 5.21) ng/ml respectively. At (45) day ofginseng supplemented the results appearedthere werea significant difference (P<0.05) values of SSH levels between the treated groups of ginseng supplemented 2 and5 gram as a diet compared with 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 high significant (P<0.05) values of SSH levels in treated groups at (60, 75 and 90) day of ginseng supplemented 2 and 5 gram as a diet compared with control group .

ICSH levels at (0,15 and 30) day there were no significant between treated groups of ginseng supplemented 2 and 5 gram as a diet compared with control group. The present study showed high significant (P<0.05) values of ICSH levels in treated groups at (45 , 60 , 75 and 90) day of ginseng supplemented 2 and 5 gram as a diet compared with control group as showed in table (2).

Table (2): Effect of (2 and 5) gm dose of ginseng as a diet supplement on rams reproductive hormones at a different periods out 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

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	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**

Data represent levels of reproductive hormones ng/mlas (mean±SEM).

Different letters within each column in each hormone indicate significant difference (P<0.05) .

G1, Ginseng 2 gram; G2, Ginseng 5 gram.

Effect of ginseng as a diet supplement on rams physical parameters of sperms at a different periods in the season :

The physical parameters of sperms evaluation (total sperms count, sperms motility, sperms immotility, progressive and non-progressive movement) in season at (0, 15, 30, 45, 60, 75, and 90) day after treated groups and effect of ginseng supplemented 2 and 5 gram as a diet compared with the control group. General speaking, the results indicated a gradual increasing in sperms motility. At (0,15, 30,45 and 60) dayafter treated groups of ginseng supplemented 2 and 5 gram as a diet, the values oftotal sperms count were not different significantlycompared with the control group. While the study showed superiority in improving sperms concentration were high significant (P<0.05) values between treated groups at (75 and 90) day of ginseng supplemented 2 and 5 gram as a diet compared with 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)X10^9cell/ml respectively as showed in table (3) and figure(5).

Table (3): Effect of ginseng on total sperms count in 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±SEM) .

Different letters within each column indicate significant difference (P<0.05) .

G1, Ginseng 2 gram ; G2, Ginseng 5 gram.

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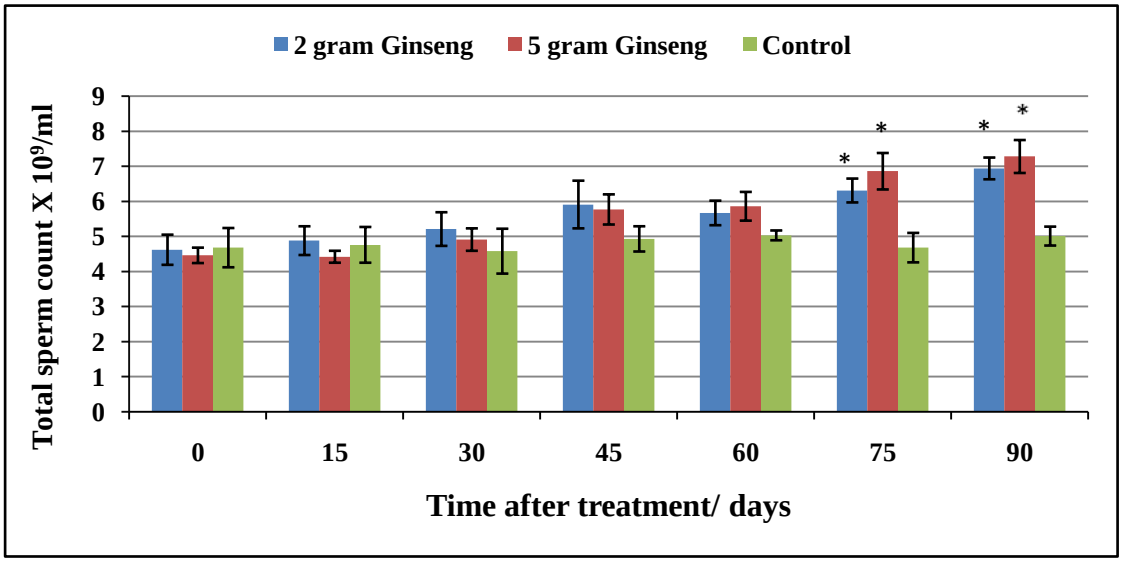

Figure(5): Effect of ginseng on totalsperms count in season .

The results of this study showed the total motile live sperms(0, 15, 30, and 45) day these values were not different significantly after treated groups of ginseng supplemented 2 and 5 gram as a diet compared with the control group. Whilethe total motile live sperms at (60) day recorded high significant ($P<0.05$) valuesin the treated group of ginseng supplemented 5 gram as a diet compare with 2 gram and control group (5.36 ± 0.32, 4.92 ± 0.32, 4.08 ± 0.34)$X10^9$cell/ml respectively. On the other hand, the results of this study showed at (75 and90) day high significant ($P<0.05$) valuesin treated groups of ginseng supplemented 2 and 5 gram as a diet compared with the control group (5.85 ±0.27, 6.44 ±0.41, 3.81 ±0.19, 3.36 ±0.37, 6.84 ±0.47, 4.12 ±0.28)$X10^9$cell/mlrespectively as showed in table (4) and figure(6).

Table (4): Effect of ginseng on spermsmotility in breeding season .

Groups	Time after treatment/ days						
	0	**15**	**30**	**45**	**60**	**75**	**90**
G1	3.6±0.32 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±SEM) .

Different letters within each column indicate significant difference ($P<0.05$) .

G1, Ginseng 2 gram ; G2, Ginseng 5 gram .

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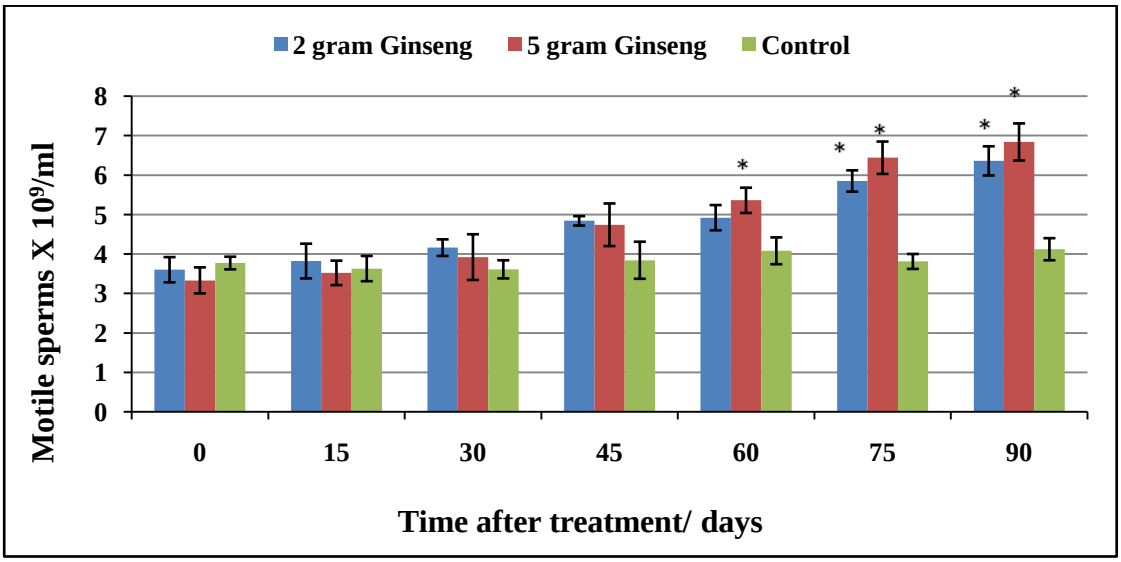

Figure(6): Effect of ginseng on sperms motility in season.

There was no significant differences in sperms immotility at (0,15, 30 and 45) day in treated groups of ginseng supplemented 2 and 5 gram as a diet compared with control group . Whilethe total immotile sperms at (60, 75 and90) day recorded low significant ($P<0.05$) values in treated group of ginseng supplemented 5 gram as a diet compared with 2 gram and control group as showed in table (5) and figure (7).

Table (5): Effect of ginseng on sperms immotility in 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±SEM) .

Different letters within each column indicate significant difference ($P<0.05$) .

G1, Ginseng 2 gram; G2, Ginseng 5 gram .

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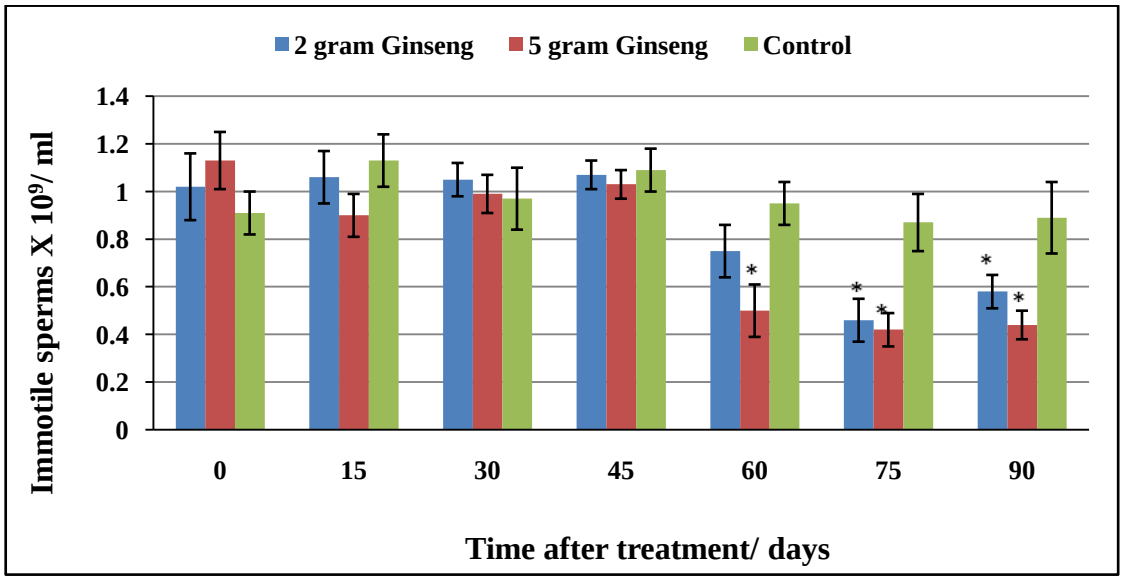

Figure(7): Effect of ginseng on sperms immotility in breedingseason.

On the other hand, the present study showed that there were no significant differences in progressive sperms motile at (0,15,30 and 45) day in treated groups of ginseng supplemented 2 and 5 gram as a diet compared with the control group. While this study also appeared there werea highly significant difference($P<0.05$) values in progressive sperms motile around (60) day in treated groupof ginseng supplemented 5 gram as a diet compared with 2 gram and control groups (4.65±0.12,4.09±0.22, 3.21±0.28)$X10^9$cell/ml respectively. The present study illustrated high significant difference ($P<0.05$) values in progressive sperms motile at (75 and 90) day in treated groups of ginseng supplemented 2 and 5 gram as a diet compared with 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) $X10^9$cell/ml respectively as showed in table (6) and figure(8).

Table (6): Effect of ginseng on progressive spermsmotility in 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±SEM).

Different letters within each column indicate significant difference ($P<0.05$) .

G1, Ginseng 2 gram ; G2, Ginseng 5 gram .

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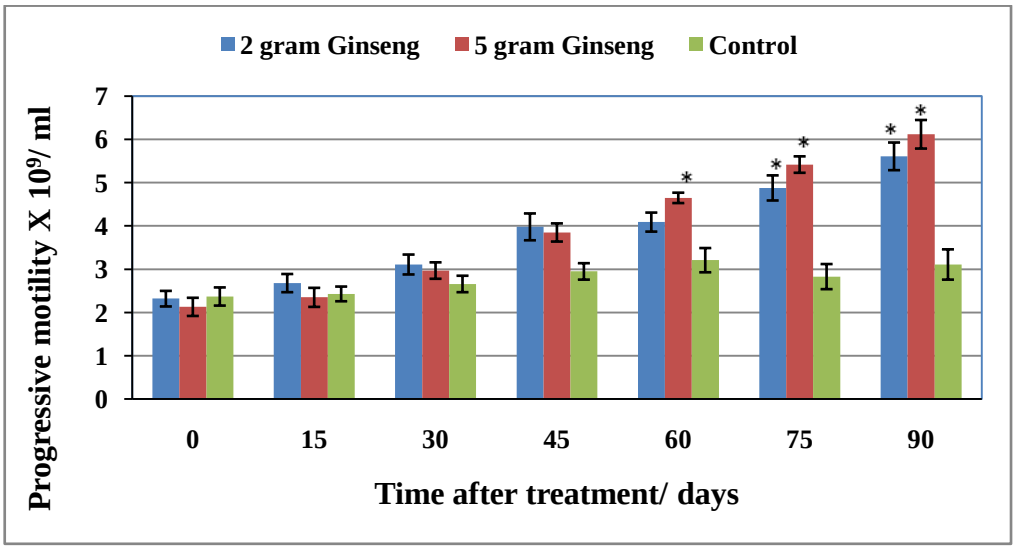

Figure(8): Effect of ginseng on progressive sperms motility inbreedingseason.

The present study also showed there were no significant differences in non- progressive sperms motility at (0,15, 30, 45, 60 and 75) day in treated groups of ginseng supplemented 2 and 5 gram compared with control group.While the study appeared high significant difference ($P<0.05$) values in non-progressive sperms motility at (90) day in treated groups of ginseng supplemented 2 and 5 gram as a diet compared with control group (0.75±0.06, 0.72±0.11, 1.01±0.05) $X10^9$cell/ml respectively as showed in table (7) and figure(9).

Table (7): Effect of ginseng onnon-progressivesperms motility in 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±SEM) .

Different letters within each column indicate significant difference ($P<0.05$) .

G1, Ginseng 2 gram; G2, Ginseng 5 gram .

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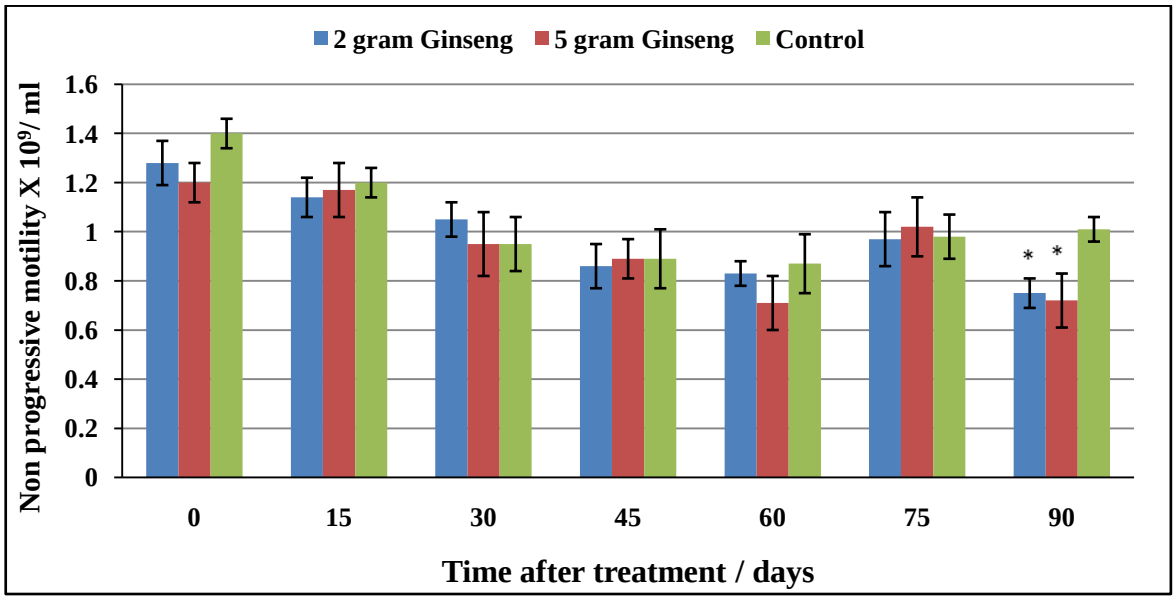

Figure(9): Effect of ginseng onnon-progressive sperms motility in season.

Effect of ginseng as a diet supplement on rams physicalparameters of sperms at a different periods out of season :

The present study showed there were no any significant differences in total sperms count at (0,15,30, and 45) day in treated groups of ginseng supplemented 2 and 5 gram as a diet compared with the control group. While the study appeared there werea highly significant difference (P<0.05) values in total sperms countat (60 and75) day in treated group of ginseng supplemented 5 gram as a diet compared with 2 gram andcontrol . On the other hand, the total sperms count werea highly significant difference (P<0.05) values at (90) dayof treated groups of ginseng supplemented 2 and 5 gram as a diet compared with the control group (7.42±0.31, 7.78±0.24, 5.27±0.35) X10^{9}cell/ml respectively as showed in table (8) and figure(10).

Table (8): Effect of ginseng on total sperms count out of breedingseason .

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

Data represent total sperm count X10^9 sperm/ml as (mean±SEM) .

Different letters within each column indicate significant difference (P<0.05) .

G1, Ginseng 2 gram; G2, Ginseng 5 gram .

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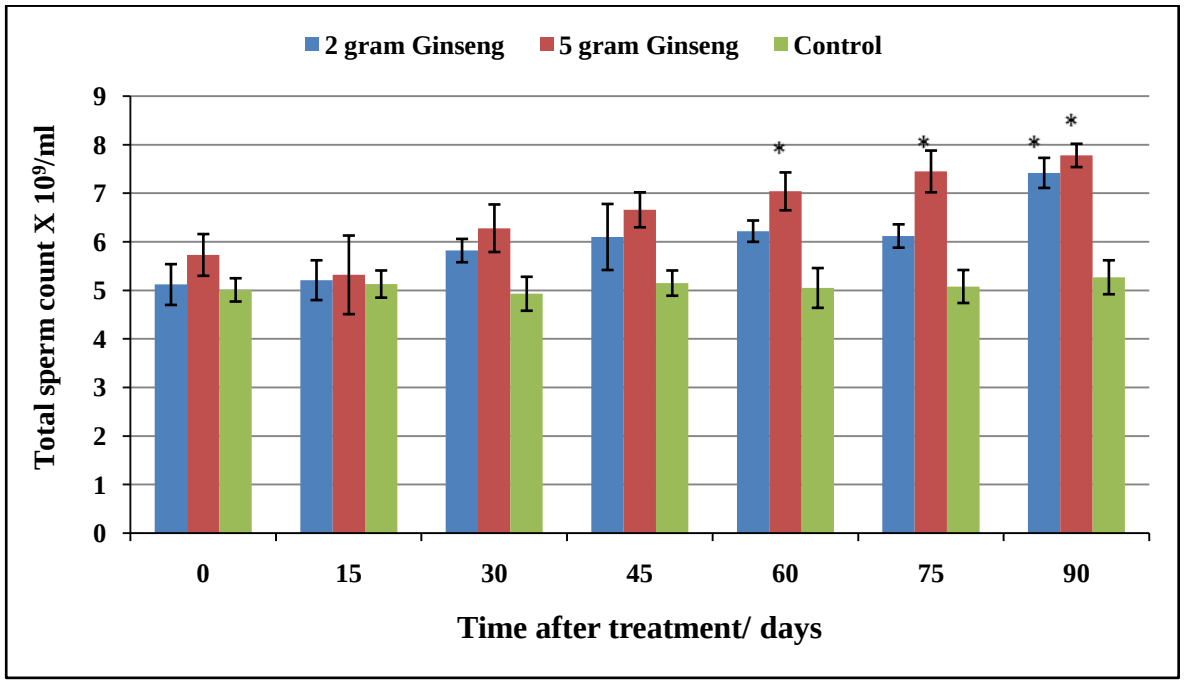

Figure(10): Effect of ginseng on totalsperms count out ofbreedingseason .

The study also illustrated the values of total motile live spermsat (0, 15, 30, and 45) day were not different significantly of treated groups of ginseng supplemented 2 and 5 gram as a diet compared with the control group.While the total motile live sperms at (60, 75, and90) day recorded high significant ($P<0.05$) values in the treated groups of ginseng supplemented 2 and 5 gram as a diet compared with the control group as shown in table (9) and figure (11).

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 spermsmotility $X10^9$ sperm/ml as (mean±SEM) .

Different letters within each column indicate significant difference ($P<0.05$) .

G1, Ginseng 2 gram ; G2, Ginseng 5 gram .

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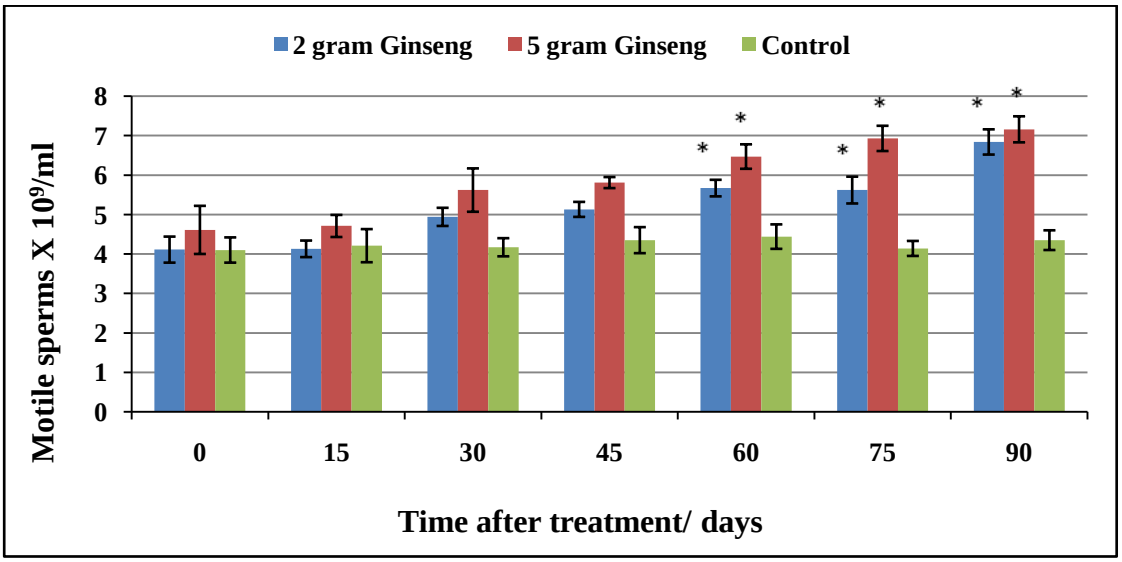

Figure(11): Effect of ginseng on sperms motility out ofbreedingseason .

On the other hand, the present study showed there were no any significant differences in sperms immotility at (0,15,30, 45 and 60) day in treated groups of ginseng supplemented 2 and 5 gram as a diet compared with control group.While the study appearedthe total immotile sperms at (75 and 90) day recorded low significant (P<0.05) valuesin treated groups of ginseng supplemented in 2 and 5 gram as a diet compared with control group as showed in table (10) and figure(12).

Table (10): Effect of ginseng on sperms im-motility out of breedingseason .

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±SEM).

Different letters within each column indicate significant difference (P<0.05) .

G1, Ginseng 2 gram ; G2, Ginseng 5 gram .

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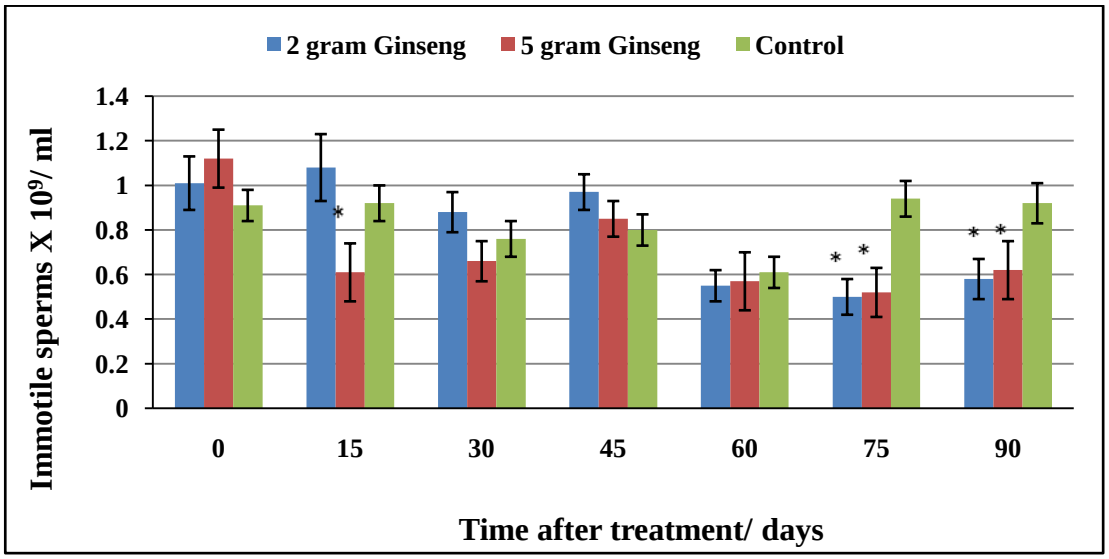

Figure (12): Effect of ginseng on sperms immotility out ofbreedingseason .

Totalprogressivesperms motilityshowed there were no significant differences at (0 and 15) day in treated groups of ginseng supplemented 2 and 5 gram as a diet compared with the control group. Moreover , the present study appearedthere were high significant (P<0.05) values at (30,45,60,75 and90) dayin treated groups of ginseng supplemented in 2 and 5 gram as a diet compared with control group as showed in table (11) and figure(13).

Table (11): Effect of ginseng on progressivesperms motilityout of breedingseason .

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±SEM) .

Different letters within each column indicate significant difference (P<0.05) .

G1, Ginseng 2 gram ; G2, Ginseng 5 gram .

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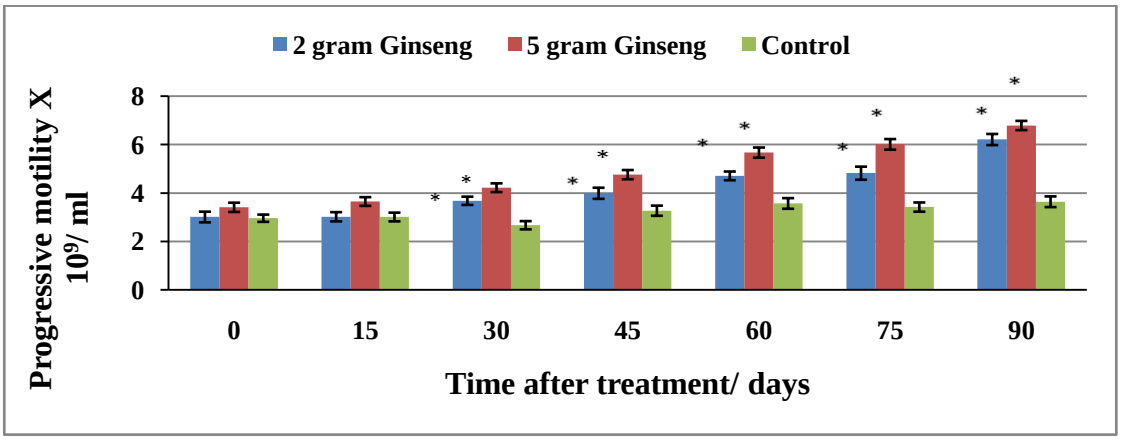

Figure(13): Effect of ginseng on progressive sperms motility out ofseason .

The present study also showed there were no significant differences in non-progressive sperms motility at (0,15,30, 45, 60 and 75) day in treated groups of ginseng supplemented 2 and 5 gram as a diet compared with control group.While the study appeared high significant difference ($P<0.05$) values in non-progressive sperms motility at (90) day in treated group of ginseng supplemented 5 gram as a diet compared with 2gram and control groups (0.37 ±0.11, 0.63 ±0.09, 0.71 ±0.07) $X10^9$cell/ml respectively as showed in table (12) and figure(14).

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 indicate significant difference ($P<0.05$) .

G1, Ginseng 2 gram ; G2, Ginseng 5 gram .

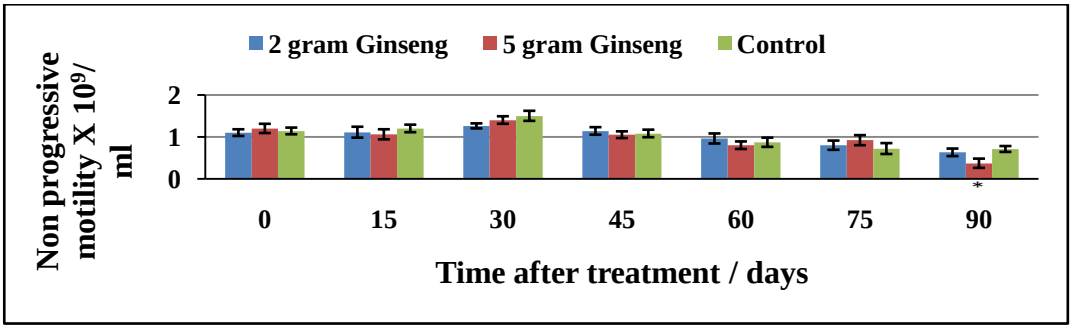

Figure (14): Effect of ginseng onnon-progressive sperms motility out of season.

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Effect of ginseng on testes diameter in and out of season:

Values of testicular diameter were recorded in season, there were no different significance in treated groups of ginseng supplemented 2 and 5 gram as a diet compared with control group at (0, 15,30, 45and 60) day.On the other hand the study appeared high significancein treated groups of ginseng supplemented 2 and 5 gram as a diet compared with the control group at (75 and 90) day.

Also the study showed the values of testiculardiameter during out of season were not different significantly in treated groups of ginsengsupplemented 2and 5 gram as a diet compared with the control group at (0, 15, 30, and 45) day. While the study illustrated there were high significance in treated groups of ginseng supplemented 2 and 5 gram as a diet compared with the control group at (60, 75and 90) dayas showed in table (13).

Table (13): Effect of 2 and 5 gram ginseng as a diet supplement on testis diameter (mm) of rams at different periods in and out of 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 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 period indicate significant difference ($P<0.05$) .

G1, Ginseng 2 gram ; G2, Ginseng 5 gram .

4.6: Histological evaluation:

The histological observations in control group showed that the testicle and the somniferous tubules lined by stratified epithelial cells and talling of Sertoli cells which were few in numbers and act as supporting cells which seated at the basement membrane of the tubules and extended

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into the lumen 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 figure(15 and 16).

The epithelial tissue of the seminiferous tubules that form of 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 control group as in figure (17). The treated groupsweredistinguished by a significant augment of primary, secondary spermatocytes and spermatids as compared with the control group, as well as an invasion of blood vessels. figure (18).

While the study showed superiority in improving that they were filled with a high numbers of sperm cells filled the seminiferous tubules lumen,with an increasing in size of interstitial cells concentration were recorded high significant values between treated groups of ginseng supplemented 2 and 5 gram as a diet compared with control group figure (19 and 20).

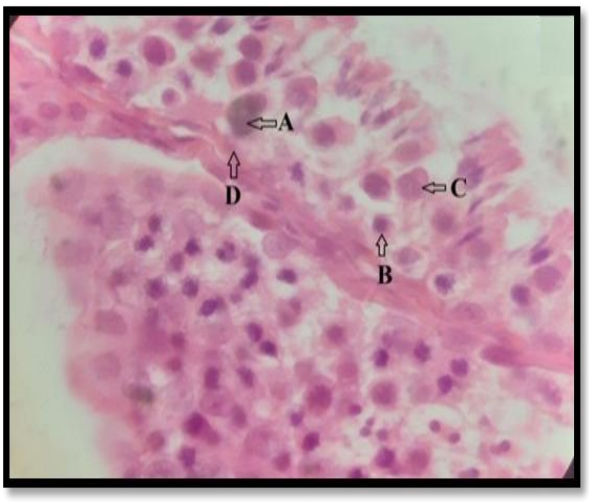

Figure (15): Normal seminiferous tubules of testis of control group out of season, Sertoli cell (A), Primary spermatocyte (B), Secondary spermatocyte (C), Basement membrane (D). H&E ,X 1000 .

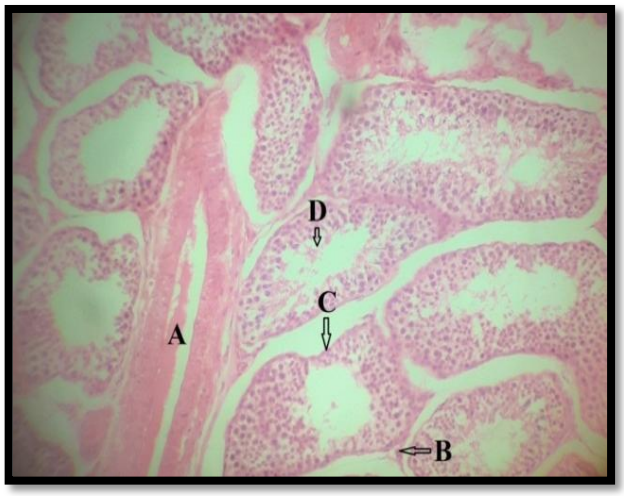

Figure (16): Cross section of testis of control group out of season: Blood vessels (A), Lydig cell (B),Seminiferous tubules (C),Spermatids (D). H&E ,X 200 .

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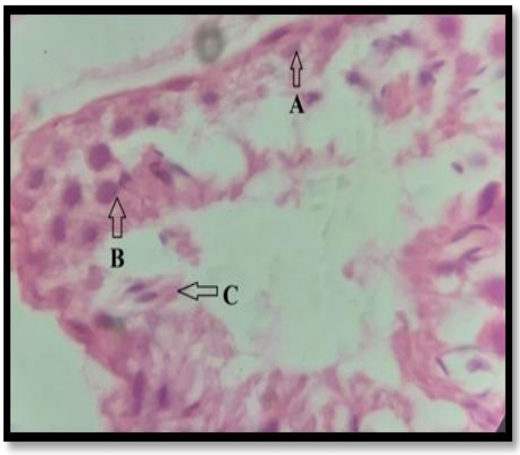

Figure (17): A and B: Seminiferous tubules of testis of control group out of season : Primary spermatogonia (A, B and C). H&E,X200 .

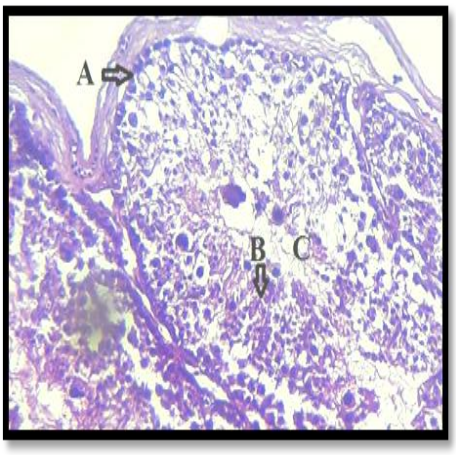

Figure (18):Aand B: Testis of treated group of ginseng supplemented 2 gram out of season showed: Primary spermatocytes(A), Secondary spermatocytes (B), Spermatids(C), H&E,X200.

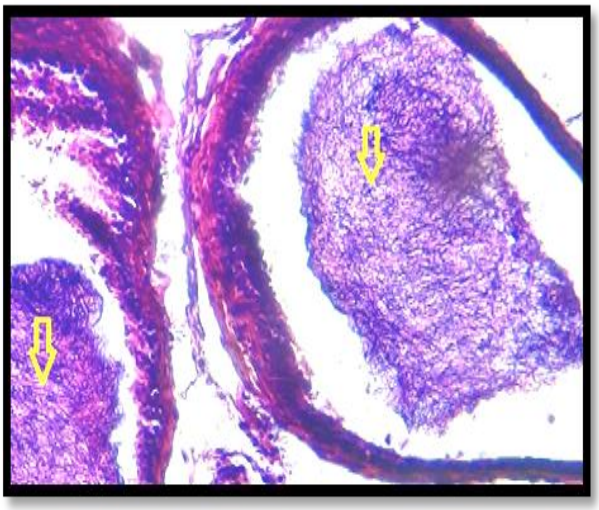

Figure (19): A and B: Testis of treated group of ginseng supplemented 5 gram during season showed the seminiferous tubules fill with spermatids. H&E, X 400 .

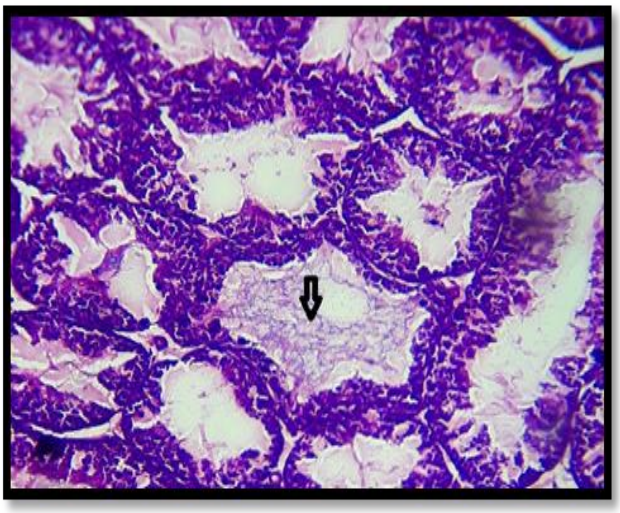

Figure (20): A and B: Testis of treated group of ginseng supplemented 5 gram out of season showed the seminiferous tubules fill with spermatids. H&E, X 200.

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Discussion

Effect of ginseng as a diet supplement on rams reproductive hormones at different periods in 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 has been conducted to investigate its effects on sexual performance. In terms of libido, enhancement of copulatory behavior after treatment with *Panax ginseng* and *Panax quinquefolium* has been demonstrated in rodent models[17]. 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, and polyacetylene, and polysaccharides [21]. As well as it contains other materials that enhance sexual activity [22]. [23] mentioned thatginseng root contains in its formula ginsucite, which is similar in composition to steroid hormones. The researchers note that ginseng root combines two types of characteristics that were important for the functioning of the male reproductive system , the first act was to provide protection and to support testicular tissue and cells from harmful substances that may be accidentally produced during vital processes, including effective free radicals oxygen (ROS) [24]. The researcher indicated the second feature which is to enhance the action of the sex hormones that are important for the performance of testicular functions [25]. [26] noticed that ginseng roots had the ability to enhance the androgen receptors inside the seminiferous tubules, as well as the ability of the ginseng root to enhance the production of proteins and protect DNA in testicular tissue.The results of the current study were in agreement of ginseng root to increases testosterone, FSH and LH levels. [26] pointed that the treatment with ginseng root improved the fertility of mice through its effect on the hypothalamic-pituitary axis of the testes, where 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 to stimulate the gene expression of the gene (CYP19, LH, and FSH) which is responsible for producing FSH and LH . On the other hand, the other previous study delineating the changes in the structure of the ovarian theca internal cells following (KRG) administration in a rat model suggested that KRG possibly stimulated steroid-producing cells [27]. The other studies which agrees with the current results; in vitro studies on neurotransmitters such as dopamine, acetylcholine, and gamma-aminobutyric acid (GABA) have also shown an association with ginsenoside [18,19]. Ginsenoside Rb1 treatment increased LH secretion from anterior pituitary gland of male rats [20]. Many researchers which dis-agree with the present study of ginseng treated have shown the advantage 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 [18, 19] .[14] mentioned increased significant serum testosterone level in treated groups of ginseng supplemented as a diet compared with control groups It is due to the increase in the number of receptors for the testosterone . Other studies conducted on ginseng have confirmed a role in increasing the LH , which may be suggested have increased the effectiveness of the testicles through the LH whereplays a vital role in distinguishing and motivate Leydig cells which release the testosterone , which leads to increase the effectiveness of testis [28].

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Effect of ginseng as a diet supplement on rams physical parameters of sperms at a different period in and out of season:

The present study showed a significant differences in physical parameters between the treated group and the control group in and out of season at different times. It was effective to be treated with ginseng that increases the motility and morphology of epididymal sperms, the results were in agreement with [29] who pointed out that sperms motility was related to fertility and motility of human sperms, was recognized as playing the most important role in fertility. [30] reported the morphology of sperms influences fertility, which may suggest the mechanism of ginsengon improving sperms quality was antioxidant property but there was no significant increasing in testicular catalase and peroxidase.Many researchers agree with the present study mentioned ginseng's effects on semen, various studies have shown that an improved semen quality in animal models, In rats, numerous ginsenosides have shown enhancing effects on sperm count and motility after treatment. Saponins from the cultured root of *wild P. ginseng* showed effects on spermatogenesis in male rats [31]. In one of the studies, the researchers administered *P. ginseng* powder orally after inducing oligospermia in rats using dioxin and examined the sperms count and testes histologically. The *P. ginseng* saponins revived spermatogenesis in their study, suggesting its possible role in reversing the damage. In previous studies, ginseng-treated rats have shown increased spermatogenesis by increasing the glial cell-derived neurotrophic factor (GDNF) expression in Sertoli cells [32]. Testicular cyclic adenosine monophosphate (cAMP)- responsive element modulator (CREM) [33].GDNF is known to be a possible regulating factor of the lifespan of spermatogonial cells, and CREM is an essential factor for spermatid maturation. An animal study using *P. ginseng* showed sperms hyperactivation at the genetic level, suggesting possible improvement of sperms quality [34]. 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 sperms motility while decreased apoptosis in testicular tissue and stimulating the yield of normal spermatozoa [35,36] in their studies performed in rats treated with cyclophosphamide and ginseng . [25] showed possible gonadoprotective effects of ginseng , they suggested that the possible protective role may be related to a ginseng-induced decrease in reactive oxygen species. The other study that agreed with the present study is a case-control study, treatment with *P. ginseng* was reported to improve the sperms quality and sex hormone profiles [17]. Moreover, one study mentioned, the beneficial effect of KRG on erection in men with erectile dysfunction has been identified in meta-analyses of randomized controlled trials[37,25]. In another study, performed in rats treated with cyclophosphamide and ginseng, more over study under disease conditions such as cancer and diabetes, and in the post-radiation period, ginseng showed improvement of sperms profiles after treatment of animals exposed to varying degrees of radiation [38] suggesting a future positive role for ginseng in this area.

Histological Evaluation:

In histological previous studies, ginseng-treated rats have shown an increased thickness of basement membrane seminiferous tubule, germinal layer and increased primary and secondary spermatogenesis thissignificant due to an increased androgen receptors of the seminiferous tubule in testis and higher protein production in testicular cells [25,39] suggested the relationship between the thickness of the germinal layer in the seminiferous tubule of testicular tissue and

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testosterone level in male Japanese quail.Through the results of our current study, it was found that the ginseng root effected the changing in measurements of testicles treated groups compared with the control group, these changes may be attributed to an increasing the production of testosterone according to results indicated , and this hormone has a significant effect on the process of producing sperms inside testicular seminiferous tubules [40]. The results also indicated that the treated groups by ginseng root led to a significant increasing in tubules diameters and thickness of seminal germ layer has been previously indicated by researchers, to 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 broilers. Therefore, the changes in testicle dimensions is only a reflection of the content of the seminal tubules that form most of their mass [23].

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