Biochem. Cell. Arch. Vol. 21, No. 2, pp. 3635-3640, 2021	www.connectjournals.com/bca	*ISSN 0972-5075*
DocID: https://connectjournals.com/03896.2021.21.3635		*eISSN 0976-1772*

THE EFFECT OF PALM POLLEN SUPPLEMENT ON SPERM DEVELOPMENT IN IRAQI RAMS

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(Received 21 February 2021, Revised 12 April 2021, Accepted 23 April 2021)

ABSTRACT : The study was carried out to evaluated the effected of Palm pollen **on development on sperm rate, Palm pollen extracts that contribute to improve male fertility. It contains estrogenic substances and estrone in addition to other nutrients, as proteins, essential, non-essential amino acids. The period studied extended from Januray 2021 to the end of March 2021. This study was conducted on 15 intact straw male adult rams , age range between (18-20 months), and weights range between (20-25 kg). Rams 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. Rams was divided into three equals groups (5 rams in each group). The 1st group was served as the control group(n=5) , 2nd group, 3rd group were treated by *P. pollen* supplemented (3 gm and 7 gm) treated by capsule daily for 90 days. The present studied physical parameters of sperms evaluation (total sperms count, sperms motility and dead sperms) at (0, 15, 30, 45, 60, 75 and 90) day after treated groups of Palm pollen supplemented in group (B and C) compared with the group (A). After 90 days of experiment, all groups were castrated of testis by open surgery, to evaluation histological structure of the testis after treated. The results indicated a gradual increasing in sperms concentration in treated groups camper with control group. On the other hand, the study was showed the total sperms live at (45) days in group C recorded high significant (P<0.05) values in treated group C of Palm pollen supplemented as a diet compare with group B and control group. On the other hand, the results of this study showed at (60 ,75 and 90) day high significant (P<0.05) values in treated groups of Palm pollen supplemented group (B and C) compared with the group (A). Moreover study showed the total dead sperms at 30 days recorded low significant (P<0.05) values in treated group C of Palm pollen supplemented as a diet compared with group (B and A). On the other hand total dead sperms at 45, 60, 75 and 90 days low significant (P<0.05) values in treated group (B and C) of Palm pollen supplemented as a diet compared with group A. The study appear superiority enhanced of filled with a high numbers of spermatozoa cells were filled the cavity of seminiferous tubule, with an increase size of interstitial cells concentration were recorded high significant values between groups B and C of Palm pollen supplemented as a diet compared with group A. Thus, it has been concluded that Palm pollen supplement could be enhance sperm concentration, increase the total sperms live and decrease total dead sperms.**

Key words : Ram, palm pollen, sperm.

How to cite : Abeer A. Yassen, Rajaa A.Alzahra Ali, Ahzan K. Abdulameer and Ihsan A. Habeeb (2021) The effect of palm pollen supplement on sperm development in Iraqi rams. *Biochem. Cell. Arch.* **21**, 3635-3640. DocID: https://connectjournals.com/03896.2021.21.3635

INTRODUCTION

Sheep are seasonality reproduction and multiple sexual cycles within the breeding season that begins with the onset of ovarian activity during a short light day (Salamon *et al*, 1996). Sheep are animals that should be taken care of, breed, and raise for their high economic quality, easy to breed, and the low cost of breeding (Anwar *et al*, 1994). Sheep reproduction is one of the most important sources of livestock in Iraq, which is about 50%, the challenge facing the sheep reproduction in the world is seasonality of production. In 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 (Al-Saadi, 1989). The organs and tissues of high capacity (testis) are exposed to free radicals at a higher rate (Bartosikova *et al*, 2003). 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 (Linjawi, 2015). Recent studied have tended to used medicinal herbs as

food additives which help to increase growth and protect them from many diseases (Hassan, 2011). These additives such as Panax ginseng, Palm pollen and Pomegranate (*Punica granatum* L.) extracts that contribute to improve male and female fertility (Yesilbag *et al*, 2013). Palm pollen contributes to improving male fertility, it contains estrogenic substances and estrone in addition to other nutrients, as proteins, essential non-essential amino acids as well as it contain sugars, vitamins and minerals, esterone blindness, estradiol, beta-sitosterol, mino-esterol, and cholestero in addition to the presence of five types of flavonoids (Abbas and Ateya, 2011). Most important functions of palm pollen is a good source of antioxidants. It act to improve the level of the hormones testosterone and estrogen. It also acts to stimulate the ovaries it contain estrogen hormone that effects on ovulation as a role, which stimulate follicle-stimulating hormone and luteinizing hormone (Hammed *et al*, 2012).

Collected and isolated *P. pollen*

Palm pollen is collected from a local market shop in Basrah center. Plant powder is kept in a sterile black glass in the lab of Department of Surgery and Obstetric, College of Veterinary Medicine, University of Basrah. Stand by as used in the refrigerator at 4^0C.

Animals management and training

For protective and clinical examinations of all the experimental animals, oral dose of 10 ml Leavozain/ 25kg body weight and 20 mg Ivermectin/kg body weight were injected subcutaneously to eliminate internal and external parasites. 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. Rams 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 physiological parameters of sperms.

MATERIALS AND METHODS

Experimental study

This study was conducted on 15 intact straw male adult rams, age range between (18-20 months) and weights range between (20-25 kg). The study was investigated at the laboratories of Theriogenology, Department of Surgery and Obstetrics, College of Veterinary Medicine, Basrah University, during the period extended from 15/1/2021 to the end of 15/4/2021. The male rams were kept in special filed and their food was dried pellets and green plants. The rams divided into three equals groups (5 rams in each group). The 1st group was served as the control group(n=5), 2nd group, 3rd group were treated by *P. pollen* for 90 days according to the following:

Group A : 5 rams/treated without supplemented (control group).

Group B : 5 rams/supplemented *P. pollen* (3gm) treated by capsule daily.

Group C : 5 rams/supplemented *P. pollen* (7gm) treated by capsule daily.

Studied qualities

After 90 days of experiment, all groups were castrated of testis by open surgery, to evaluation histological structure of the testis after treated.

Semen evaluation

Semen were collected directly from rams by artificial vagina, semen transported to the laboratory of unit center research in the College of Veterinary Medicine, Basrah University. The samples were evaluated by using computerized semen analyzer apparatus at the degree (10 and 40 X) to evaluate Some Sperm Properties.

Histological study

After 90 days of the experiment, all rams of groups were castrated, the testes were placed in special containers containing 10% formalin for histological examination as Figs. 1, 2.

Fig. 1 : Ligation spermatic cord of testis.

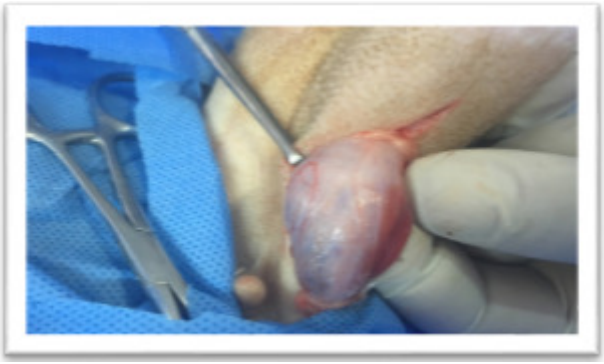

Fig. 2 : Castrated testis.

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 multiple comparison post hoc test. All data were presented as mean ± SEM and the differences were considered as significant at $P < 0.05$.

RESULTS

Effect of Palm pollen as a diet supplement on rams physical parameters of semen

The physical parameters of sperms evaluation (total sperms count, sperms motility, sperms immotility) at (0, 15, 30, 45, 60, 75 and 90) day after treated groups of Palm pollen supplemented in group (B and C) compared with group (A). The results indicated a gradual increasing in sperms concentration. At (0, 15, 30, 45 and 60) day after treated groups of Palm pollen supplemented in group (Band C) as a diet, the values of total sperms concentration were not different significantly compared with the group (A) in (0, 15, 30, 45 and 60) days. 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 Palm pollen supplemented in group (Band C) as a diet compared with the group (A) as shown in Table 1.

Effect of Palm pollen on sperms motility in rams

The results of this study appear the total live sperms (0, 15 and 30) day these values was non-significantly after treated groups of Palm pollen supplemented in group B and C compared with group A. While, the total sperms live at (45) days in group C recorded high significant ($P<0.05$) values compare with group B and A. On the other hand, the results of this study showed at (60, 75 and 90) day high significant ($P<0.05$) values in treated groups of Palm pollen supplemented in group (B and C) compared with the group (A) as shown in Table 2.

Effect of Palm pollen on dead sperms in rams

There was no significant differences in dead sperms motility at (0 and 15), day in treated groups of Palm pollen supplemented between group (B and C) as a diet

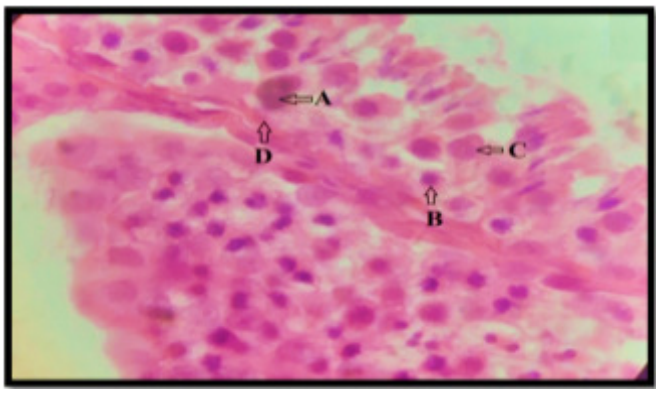

Fig. 3 : Normal seminiferous tubules of testis of group A, Sertoli cell (A), Primary spermatocyte (B), Secondary spermatocyte (C), Basement membrane (D). H&E, X 1000.

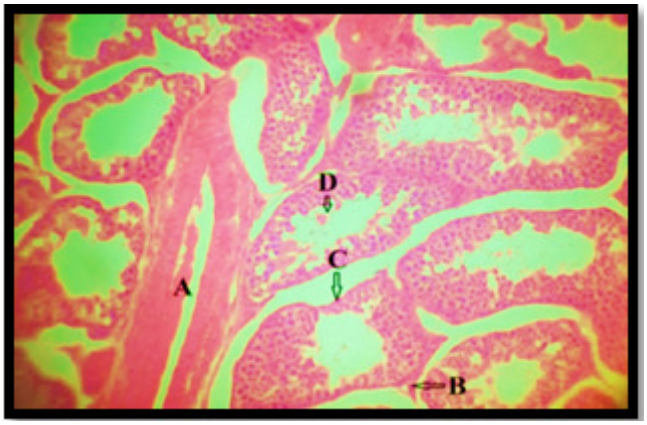

Fig. 4 : Cross section of testis of group A: Blood vessels (A), Lydig cell (B), Seminiferous tubules (C), Spermatids (D). H&E ,X 200.

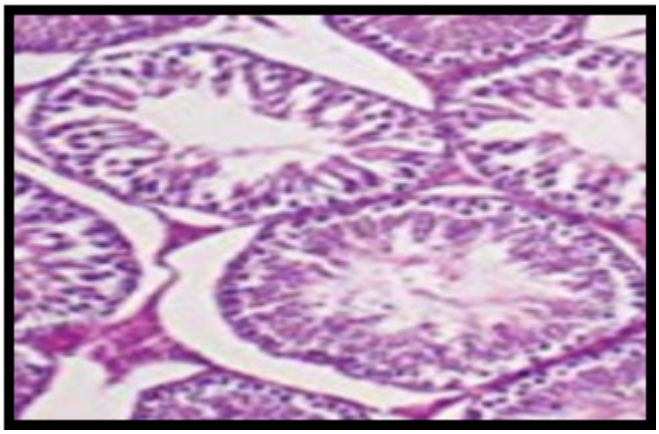

Fig. 5 : A and B: Seminiferous tubules of testis of group A: Primary spermatogonia. H&E,X200.

Table 1 : Effect of Palm pollen on total sperms count in rams.

Groups	Time after treatment/ days						
	0	15	30	45	60	75	90
GA	4.52±0.42a	4.61±0.21a	4.21±0.58a	4.98±0.91a	4.51±0.15a	4.25±0.32a	4.71±0.24a
GB	4.36±0.42a	4.52±0.19a	4.71±0.42a	5.78±0.33a	5.89±0.51a	6.11±0.42b	6.95±0.41b
GC	4.48±0.26a	4.76±0.31a	5.58±0.34a	5.85±0.19a	5.96±0.45a	6.39±0.74b	7.22±0.45b

Data represent total sperm count $X10^9$ sperm/ml as (mean ± SEM). Different letters within each column indicate significant difference ($P<0.05$). GA: Control group; GB Palm pollen supplemented (3 grams); GC, Palm pollen supplemented (7 grams).

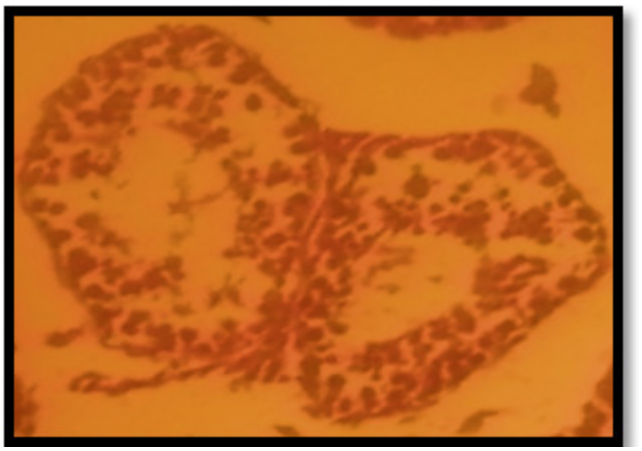

Fig. 6 : Palm pollen supplemented showed: Primary spermatocytes (Secondary spermatocytes, Spermatids) H&E, X200.

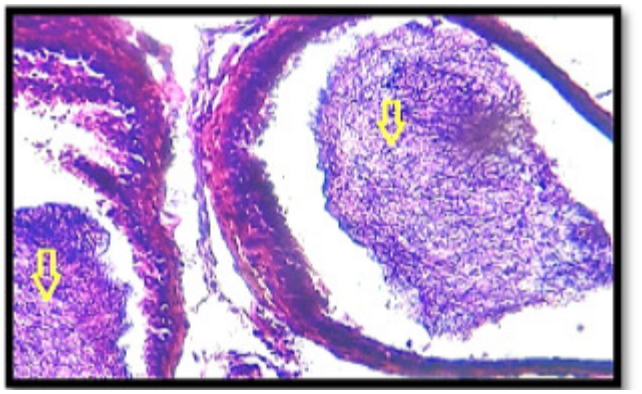

Fig. 7 : Testis of group B and C of Palm pollen supplemented showed: The seminiferous tubules fill with spermatids. H&E, X 400.

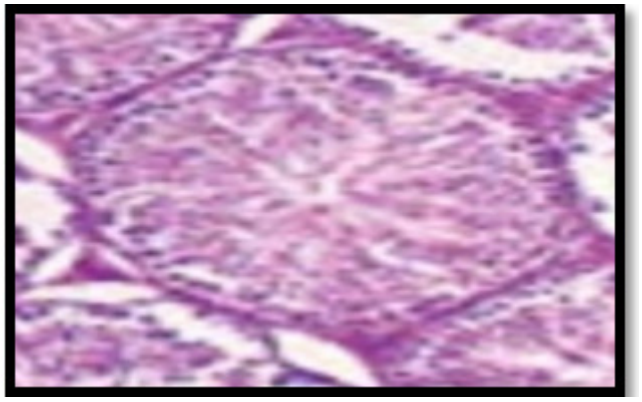

Fig. 8 : Testis of group B and C of palm pollen supplemented showed: The seminiferous tubules fill with sperm. H&E, X 200.

(P<0.05) values in treated group (B and C) of Palm pollen supplemented as a diet compared with group A as shown in Table 3.

Histological evaluation

The histological observations in group (A) showed that the testes and somniferous tubules lineation by stratified epithelial cells and talling of Sertoli cells, which were low in numbers and act as supported cells, which seated at the basement membrane of the tubule and extension into the cavity of the tubule. On the other hand, the interstitial thin tissue between the tubule interpose by blood vessels and Leydig cells, the outer connective tissue capsule is surrounded the organs as shown in Figs. 3 and 4.

Table 2 : Effect of Palm pollen on sperms motility in rams.

Groups	Time after treatment/ days						
	0	15	30	45	60	75	90
GA	0.49±3.09a	3.42±0.24a	3.12 ±0.11a	3.64±0.43a	3.72±0.52a	3.88±0.42a	3.38±0.38a
GB	3.21±0.36a	3.57±0.51a	3.52±0.28a	3.84±0.54a	4.19±0.82b	5.14±0.61b	5.71±0.47b
GC	3.29±0.19a	3.83±0.72a	4.65±0.15a	4.84±0.74b	5.27±0.36b	5.81±0.22b	7.17±0.57b

Data represent sperms live $X10^9$ sperm/ml as (mean ± SEM). Different letters within each column indicate significant difference (P<0.05). GA: Control group; GB Palm pollen supplemented (3 grams); GC, Palm pollen supplemented (7 grams).

Table 3 : Effect of Palm pollen on dead sperms in ram.

Groups	Time after treatment/ days						
	0	15	30	45	60	75	90
GA	3.01±0.32a	3.26 ±0.11a	3.47 ±0.07a	3.67±0.05a	3.65±0.21a	3.93±0.40a	3.98±0.26a
GB	3.11±0.36a	2.72 ± 0.19a	2.69±0.68a	1.93±0.11b	1.14±0.05b	1.12±0.18b	1.04±0.16b
GC	3.41± 0.69a	2.27±0.21a	1.97±0.96b	1.32±0.13b	0.95±0.09b	0.64±0.03b	0.59±0.11b

Data represent sperms live $X10^9$ sperm/ml as (mean ± SEM). Different letters within each column indicate significant difference (P<0.05). GA: Control group; GB Palm pollen supplemented (3 grams); GC, Palm pollen supplemented (7 grams).

compared with group A. While the total dead sperms at 30 days recorded low significant (P<0.05) values in treated group C of Palm pollen supplemented as a diet compared with group (B and A). On the other hand total dead sperms at 45, 60, 75 and 90 days low significant

The seminiferous tubules of epithelial tissue was different stages of spermatogenesis cells, the spermatocytogenesis (primary and secondary) with the spermogenesis (spermatids) were found in a cluster at the cavity of the tubule in control group as in Fig. 5. In

groups (B and B) were distinguished by a significant augment spermatocytogenesis (primary and secondary) with the spermogenesis (spermatids) as compared with the group A. Moreover, an increase in blood vessels was found as shown in Fig. 6.

The study appear superiority enhanced of filled with a high numbers of spermatozoa cells were filled the cavity of seminiferous tubule, with an increase size of interstitial cells concentration were recorded high significant values between groups B and C of Palm pollen supplemented as a diet compared with group A as in Figs. 7 and 8.

DISCUSSION

Modern studies have aimed to using medicinal herbs as food additives that help increase growth and treat many diseases, this herbs such as palm pollen additives that contribute to improving male fertility which contain estrogenic substances, estrone and some other nutrients, Proteins, essential and nonessential amino acids, carbohydrates, vitamins and minerals (Hassan, 2011). Shanoon *et al* (2015) mentioned that using palm pollen as an additive in diets leads to enhancing weak sperm and sexual dysfunction in general, and it improving the level of testosterone and estrogen hormones, because this herbs, which contains in its substance similar in composition to steroid hormones. As well as the palm pollen contains important nutrients such as sugars, estrone, esteriol, beta-sitosterol, minoosterol and cholesterol, in addition to the presence of five types of flavonoids which affects the process of increasing sexual libido in males. The researchers note that palm pollen combines two types of characteristics that were important for the functioning of the male reproductive system the first act to provide protection and support for testicular tissue and cells from harmful substances that may be accidentally produced during vital processes, including effective free radicals oxygen (ROS) (Liu *et al*, 2003). The researcher indicated the second feature is to enhance the action of the sex hormones that are important for the performance of testicular functions (Hong *et al*, 2002). Linjawi (2015) noticed that palm pollen had the ability to enhance the androgen receptor inside the seminiferous tubules, as well as the ability of the palm pollen to enhance the production of proteins and protect DNA in testicular tissue. Linjawi (2015) also mentioned that the adding of palm pollen in feeding lead to an increase the level of the testosterone hormone in serum, because may be to an increase the numbers of testosterone receptors in the testis, which in turn increases reproductive efficiency as well as increases sperm concentration, the number of live sperms as well as improves sperm motility. Studies conducted on palm pollen have confirmed its role an increasing the Luteinizing Hormone (LH), which acts to increase the effectiveness of testicles through an effect on Leydig Cells (Mahran *et al*, 1976). A study conducted on palm pollen have confirmed its role of an increasing the diameter of the seminal tubule, thickness of the germinal layer and diameter of the lumen of seminiferous tubule for testicles (Al-Rawi *et al*, 2012). Increasing the levels of FSH and testosterone when adding palm pollen lead to increase the weight of the testicle, size of the testis because increase the diameter of the seminal tubule (Arslan *et al*, 1993). On the other hand, an increase in FSH levels may cause maturation and an increase in the sperm generation process in the seminiferous tubules, thus leading to an increase in the thickness of the germ layer (Baines *et al*, 2008).

REFERENCES

Abbas A F and Ateya A M (2011) Estradiol, esteriol, estrone and novel flavonoids from date palm pollen. *Aust. J. Basic Appl. Sci*. **5**(8), 606-614.

Al-Rawi A J, Al-Daraji H J and Al-Baghdadi M F (2012) Influence of egg spermpenetration on histological testes traits in white leghorn cocks. *Anbar J. Vet. Sci.* **5**, 65-72.

Al-Saadi H A (1989) Artificial reproduction. Part 1. Dar Al Kutub For Printing & Publishing, University of Al Mosul, pp. 49-105.

Anwar A, Walsh G, Cromwell O, Kay A and Wardlaw A (1994) Adhesion to fibronectin primes eosinophils via alpha 4 beta 1 (VLA-4). *Immunology* **82**(2), 222.

Arslan M, Weinbauer G F, Schlatt S, Shahab M and Nieschlag E (1993) FSH and Testosterone, alone or in combination, initiate testicular growth and increase the number of spermatogonia and Sertoli cells in a juvenile non-human primate (*Macaca mulatta*). *J. Endoc.* **136**, 235-239.

Baines H, Nwagwu M O, Hastie G R, Wiles A R, Mayhew M T and Ebling F J P (2008) Effects of estradiol and FSH on maturation of the testis in the hypogonadal (hpg) mouse. *Rep. Biol and Endocrinol*. **6**(4), 1 -10.

Bartosikova L, Necas J, Kubinova R, llik J, Saplachate J, Florian M, Frydruch T, Frana P, Frana L and Dzurova J (2003) Antioxidative effect of morine in Ischemia reperfusion of kidney in the laboratory rate. *Acta Vet. Br*. **72**, 87-94.

Hammed M S, Arrak J K, AL-kafaji N J and Hassan A A (2012) Effect to of date palm pollen suspention on ovarian function and fertility in adult female rats exposed to lead acetate. *Diyala J. Med*. **3**(1), 90- 96.

Hassan H M (2011) Chemical composition and nutritional value of palm pollen grains. *Global J. Biotech. Biochem.* **6** (1), 1-7.

Hong B, Ji Y H, Hong J H, Nam K Y and Ahn T Y (2002) A double-blind crossover study evaluating the efficacy of korean red ginseng in patients with erectile dysfunction: A preliminary report. *J. Urol.* **168**(5), 2070- 2073.

Linjawi S A (2015) Evaluation of the protective effect of Panax ginseng nanoparticles against nicotineinduced reproductive disorders in male rats. *Int. J. Pharm. Sci. Rev. Res.* **32**(1), 38-45.

Liu Z Q, Wang Z C and Sun Y X (2003) *In vitro* study of the relationship between the structure of ginsenoside and its

antioxidative or prooxidative activity in free radical induced hemolysis of human erythrocytes. *J. Agric. Food Chem.* **51**, 2555-2558.

Mahran G H, Abdel – Wahab S M and Attia A M (1976) A phyto chemical study of date palm pollen plant. *Med*. **29**(2), 25-48.

Salamon S, Gillan L, Evans G and Maxwell W (1996) Fertility of ram semen after long-term frozen storage. *Proc. 13th Int. Congr. Anim. Reprod*., *Sydney* **3**(24), 25.

Shanoon A Q, Jassim M S, Mohamed A H, Latef M S, Abad A M and Raheem A L (2015) The effect of using different levels from date palm pollen in diet on productive performance and some eggs quality measurements for layer hens lohman. *Anim. and Vet. Sci*. Special Issue: *Recent Trends in Animal Production and Healthcare* **3**(6-1), 1-4.

Yesilbag D, Gezen S, Birick H and Meral Y (2013) Effects of dietary rosemaryand oregano volatile oil mixture on quail performance, egg traits and egg oxidative stability. *Br. Poult. Sci*. **54**(2), 231-