PRODUCTIVE PERFORMANCE AND HISTOLOGICAL RESPONSES OF JAPANESE QUAIL BREEDERS TO AGE AT MATING AND SILVER NANOPARTICLES ADMINISTRATION

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SUMMARY

A total of 64 males and 192 females of Japanese quail breeders (*Coturnix coturnix japonica*) were divided into two groups of different ages (old birds; 52 weeks of age and young birds; 10 weeks of age). These subgroups were used in a series of four natural mating groups in a regular and reciprocal manner. In the 1st and 2nd mating groups; old males were paired with young and old females, respectively. While the 3rd and the 4th groups; young males were paired with young and old females, respectively. The first and the second subgroups of both ages (young and old) were subjected to a treatment of 20 ppm silver nanoparticles (AgNps) in drinking water. The third and fourth ones were used as a control treatment given fresh water with no supplement. Egg production parameters, feed consumption and feed conversion ratio were recorded biweekly. At the last week of the experimental period (8th wk), birds were weighed and slaughtered, autopsied and some organs were weighed. Histological observation was done for liver, ileum, testis and sperm storage tubules of hens. The results showed that older age of quail had significantly higher egg weights compared to those produced from the younger ones. Also, administration of AgNps improved live body weight (LBW) and egg weight of female Japanese quail breeder hens. Insignificant interaction between both age at mating and silver nanoparticles was found on feed consumption (FC), feed conversion ratio (FCR) and body weight (BW). Histological examinations showed that AgNps may have minor negative impacts on the liver and ileum tissues of laying quails. It could be concluded from the present study that; it is possible to improve the productive performance and healthy status of aged Japanese quail breeder stocks if the old age female breeders are mated with younger male ones. The magnitude of this effect could be achieved through the administration of AgNps in their drinking water.

Keywords: age at mating, egg production, silver nanoparticles, histological response, Japanese quail breeder.

INTRODUCTION

Quails have an economical importance as they provide an alternative to the commonly used chickens. Quail production in Egypt is rapidly growing, primarily because these birds do not require much space, easy to manage and reproductively mature in six weeks. Presently, Japanese quail (*Coturnix coturnix japonica*) are produced mainly for their meat and eggs, that have become highly popular to the consumers.

For improving the reproduction of breeder flocks, manipulating the physiological and environmental factors is necessary. Among the physiological factors that necessary for production and reproduction are age, body weight, hormones and sex ratio. The quail breeder age and age at mating are important factors affecting egg weight and egg production (El-Wardany *et al.*, 2016). The egg production can be affected by several factors such as feed consumption (quality and quantity), drinking water, intensity and duration of light, management, environment, stresses, immunosuppression, parasite infestation, exposure to disease and toxins, which all can result in economic loss. Nanotechnology is the promising and emerging technology that has tremendous potential to revolutionize agriculture and livestock sectors globally (Gopi *et al.*, 2017). Silver nanoparticles (AgNPs) are of interest due to their antimicrobial activity and are seen as potential candidates to replace antibiotics in animal husbandry (Sawosz *et al.*, 2012).

Supplementation of silver nanoparticles (AgNPs) improved the stomach internal environment for aged birds (Xia *et al.*, 2017). Approximately 80% of the silver in AgNPs was in the metallic form and the remaining silver was in ionic form. Ionic silver become silver chloride in the stomach or blood stream. However, only metallic particles can resist high acidity stomach of birds and remain effective in the blood stream. Silver has been known for its medicinal properties, especially as an antimicrobial agent, but it may be toxic when it is in ionic state. However, the toxicity of Ag can be eliminated, when used in nanoparticle form. Moreover, AgNPs affects performance, intestinal microflora and morphology of enterocytes of duodenal villi of quail, (Farzinpour and Karashi, 2013; El-Wardany *et al.*, 2016 and Rezaei *et al.*, 2018).

Therefore, the purpose of the present study was to elucidate the interactions between AgNPs in drinking water and age of quail breeder males and females at mating on: body weight, feed intake, feed conversion and egg production of Japanese quail breeder hens and the histological changes of uterovaginal junction of breeder females, as well as their liver and ileum.

MATERIALS AND METHODS

Experimental design and management:

A total of 64 males and 192 females of Japanese quail breeders flock (*Coturnix coturnix japonica*) represent two equal groups of different ages (old birds; 52 weeks of age and young birds; 10 weeks of age) were used in this study. Breeders of each age were randomly subdivided into 4 subgroups resulting in 8 subgroups each of 8 males and 24 females. Birds were kept in an individual wire cage system (18x18 cm) with a sloping floor for egg collection. These 8 subgroups were used in a series of four natural mating groups, each of 2 replicates, in a regular and reciprocal manner. In the 1st and 2nd mating groups, old males were paired with young and old females, respectively. While for the 3rd and the 4th groups, young males were paired with young and old females, respectively. For all mating groups, the sex ratio was 1 ♂: 3 ♀, thus each male was caged with each female for a period of 24 h.

Birds of one replicate within each of the four mating groups were offered drinking water supplemented with 20 ppm silver nanoparticles (AgNPs). Silver nanoparticles were obtained from International Care American Company. Silver nanoparticles concentration used in this study was based on the literature review. The experiment was extended for 8 weeks. While the other replicate within each mating was used as control treatments given fresh water with no AgNPs supplement. All birds were kept in a windowed house, fed *ad-libitum* on a diet which was formulated to meet the nutrients requirement of Japanese quail breeders according to NRC (1994). Drinking water was available all the time. Birds were exposed to a light cycle of 16 h light and 8 h dark throughout the experimental period.

Productive performance:

Live body weights (LBW) of female and male breeders were recorded at biweekly intervals. Individual egg weight and egg number were recorded daily through 8 wks of experimental period. Average egg weight and egg number were calculated for each replicate and treatment. Egg mass was calculated as egg production multiplied by average egg weight. Feed consumption was recorded weekly for each replicate and the average feed consumption/bird/day was calculated. Feed conversion ratio (FCR) was calculated as gram feed consumed per gram egg produced (g feed/g egg mass).

Slaughter traits:

At the end of the experimental period, three females and three males from each treatment were randomly selected and weighed using digital electronic balance, (Precisa digital electronic balance, PJ 57604, Switzerland). Their weights were recorded to the nearest 0.1 gram. Birds were slaughtered by severing the carotid arteries and jugular veins, after complete exsanguination they were autopsied. At autopsy, the abdominal cavity was opened. sex organs; (ovary, oviduct and testes) were removed and weighed. Their weights were recorded to the nearest 0.1 mg, and proportionated to the live body weights.

Histological study:

Tissue samples from liver, ileum and reproductive organs i.e. uterovaginal junction and testis were taken during the slaughtering time. Tissues were fixed in 10% formalin saline solution. Tissues were dehydrated in ascending grades of ethanol, and then cleared in xylene. Then embedded in paraffin wax. The paraffin

technique has been done according to Abd El-Hamid (1981). Transverse sections (4-5 µm thick) were done using a rotary microtome. All sections were stained with hematoxylin and eosin (H&E) stains. Sections were examined under light microscope and photographed by using computerized digital camera (iVU 3000) endorsed with software (ProgRes® CapturePro 2.707, 2010). The histological technique of tissues samples was conducted in the Pathology Laboratory, Faculty of Veterinary Medicine, Cairo University, Egypt.

Statistical analysis:

Data of the present study were subjected to a two-way analysis of variance with age at mating and drinking water silver nanoparticles treatment (N) as the main effects using the General Linear Models (GLM) procedure of SAS (1998). The following model was used:

$$Y_{ijk} = \mu + M_i + N_j + MN_{ij} + \epsilon_{ijk}$$

Where: Y_{ijk} = the observation, μ = an effect of overall mean, M_i = a fixed effect of i (age at mating), N_j = a fixed effect of j (silver nano-particle treatment), MN_{ij} = the interaction of i age at mating and the j silver nano-particle treatment, and ϵ_{ijk} = a random experimental error.

Duncan's multiple range test (Duncan, 1955) was used to separate difference among treatment means when separation was relevant. All Percentage data were subjected to arcsine transformation of the square root before statistically reanalyzed however, the actual percentage means are presented. Difference was considered significant at ($P < 0.05$).

RESULTS AND DISCUSSION

Egg production traits:

The parental age at mating had a significant effect on egg weight as shown in Table (1). It is clear that after two weeks of treatments, the Japanese quail female (treatment 2, 4) had significantly higher egg weight ($P < 0.01$) compared to those produced from the younger ones regardless of males age. A similar trend was also observed for the whole laying period (0-8wk). However, the age at mating had a remarkable significant improvement in the egg weight, but not the administration of AgNps. The increased egg weight is due mainly to age of females and to the changes in ovarian hormones secretion rates with age. This may be due to effect of estrogens which increases at the peak of egg production (Hansen *et al.*, 2003). Also, the synthesis and secretion of egg albumen by the oviduct is under estrogenic control (Christians and Williams, 1999).

These results are similar to the findings of Silversides and Scott (2001) and Erensayin *et al.* (2002). They reported a linear relationship between the egg weight and the age of female quail. Moreover, Ipek *et al.* (2004) revealed that egg weight was increased significantly with increasing Japanese breeder age. In this connection, Bahie El-Deen *et al.* (2008 and 2009) found that the mean egg weight of quail was 10.96 g during the first 45 days after sexual maturity, and reached to 11.07 g during 90 days of laying. The differences in egg weight refer to variation in body weight and age of quails. It has been reported that the mean egg weight in Japanese quails was 10 g, and generally exhibited an increase up to five months of breeder age (Nazlıgül *et al.*, 2001). It is well known that, eggs production depends on many factors including the length of the laying cycle, some physiological and nutritional effects and also is related to body weight of the bird at sexual maturity (Meky 2007; Bahie El-Deen *et al.*, 2008 and 2009). The present results showed that live body weight of laying quails was significantly heavier ($P < 0.05$) in AgNps treatment groups than the non-administered groups. This coincident with the higher egg weight, especially for M2 (old ♂ x old ♀) group. This could be explained by the highly significant effect of the interaction between age at mating and AgNps treatment. It may be that the effect of AgNps was more obvious in older groups than younger ones, probably by improving their physiological status. This was also reported by Sawosz *et al.* (2007).

It appears that AgNps could improve egg weight via its effect as antimicrobial drug and or by enhancing feed utilization by reducing intestine pH which in turn influences the development of lactic acid bacteria and *E. coli* colonies. In this respect, Sawosz *et al.* (2009) reported that 25 ppm of AgNps did not adversely affect intestinal villi morphology of quails but increased significantly the populations of lactic acid bacteria. Our results are in close agreement with those reported by Fondevila *et al.* (2009) who found a significant increase in egg weight of laying quails after AgNps administration at a level of 20 ppm.

Table (1): Effect of age at mating and silver nanoparticles administration on egg production traits of Japanese quail breeder hens.

Period (wk)	Nanosilver (N)	Mating system (M)				Overall mean	Significance		
		1	2	3	4		M	N	M*N
(0-8)		Average egg weight (g)							
	N ₀	11.4±0.12	12.4±0.10	11.8±0.24	11.6±0.05	11.80			
	N ₁	11.8±0.05	12.3±0.03	11.7±0.09	12.6±0.32	12.10			
	Overall mean	11.60 ^c	12.35 ^a	11.75 ^{bc}	12.10 ^{ab}		**	NS	*
(0-8)		Egg number (number/ hen/wk)							
	N ₀	5.77±0.12	4.65±0.14	5.63±0.31	4.97±0.24	5.26			
	N ₁	4.56±0.16	5.61±0.04	5.46±0.15	4.44±0.12	5.02			
	Overall mean	5.17 ^{ab}	5.13 ^b	5.55 ^a	4.70 ^c		**	NS	**
(0-8)		Egg mass (g/hen/wk)							
	N ₀	66.0±0.62	57.4±1.23	66.7±4.98	57.8±3.02	61.98			
	N ₁	53.6±2.05	68.7±0.89	63.7±2.17	55.8±0.09	60.45			
	Overall mean	59.80 ^{ab}	63.05 ^a	65.20 ^a	56.80 ^b		*	NS	*
(0-8)		Feed consumption (g/hen/wk)							
	N ₀	178.9±1.6	185.0± 1.1	181.3±3.9	179.1± 1.4	181.1			
	N ₁	177.0±1.2	186.4± 1.2	177.9±1.8	183.1± 2.1	181.1			
	Overall mean	177.9	185.7	179.6	181.1		NS	NS	NS
(0-8)		Feed conversion ratio (g feed: g egg mass)							
	N ₀	2.71±0.05	3.22±0.09	2.72±0.15	3.10±0.02	2.94			
	N ₁	3.30±0.05	2.71±0.16	2.79±0.07	3.28±1.03	3.02			
	Overall mean	3.01 ^b	2.97 ^b	2.76 ^c	3.19 ^a		**	NS	NS

1: (old ♂ x young ♀), 2: (old ♂ x old ♀), 3: (young ♂ x young ♀), 4: (young ♂ x old ♀).

N0: (Zero nanosilver), N1: (20ppm nanosilver). * $P \leq 0.05$ ** $P \leq 0.01$ NS: non-significant.

Mean within a column (having capital letters) or row (having small letters) are significantly different.

As shown in Table (1), the results indicate that, the highest values for egg number have been recorded for younger hens whether mated with young or old males, compared to older ones. Additionally, no significant effects due to AgNps administration on egg number were observed. The achieved improvement in egg production for the young hens is associated with higher circulating estrogen hormone level comparable to the old ones. Khalifa *et al.* (1983) referred the increase in egg production number to the physiological effect of estrogen upon the ovary and oviduct causing enhancing their ovulatory process, consequently increased the clutch size. Egg mass was generally higher for M3 (young ♂ x young ♀) group followed by M1 (old ♂ x young ♀) mating groups, respectively. While, the lower values were recorded for M4 (young ♂ x old ♀) and M2 (old ♂ x old ♀) groups (Table 1). Increasing egg mass may reflect an improvement in egg weight and /or egg number, which was achieved for M3 and M1 mating groups. These results are in close agreement with Bahie El-Deen *et al.* (2008 and 2009) who reported similar values for egg mass during the first 45 days after sexual maturity, and lower values during a period of 90 days, as birds become older.

The results indicated that, over the whole period of the study (0-8wk) there were no significant differences between all groups of mating treatments, as well as the administration of AgNps and the interaction with respect to feed consumption (Table 1). The present finding is coincided with those reported by Ahmadi (2012), who found that dietary supplementation of AgNps between 20 and 60 ppm had no significant effect on feed consumption of broiler chicks compared with un-supplemented chicks. Feed

conversion ratio, as one of the most important factors of economic productivity, was significantly improved ($P < 0.01$) at 2 wks, 4 wks and for the whole period (0-8 wks) with the younger age group, especially for M3 (young ♂x young ♀) compared to the other treatments. However, the interaction between both age at mating and AgNps administration was not significant for feed conversion ratio (Table1).

Live body weight (LBW) and reproductive organs:

Table (2), illustrates the effect of AgNps administration in drinking water and different age at mating on LBW of female Japanese quail breeders' hens. There was non-significant increase associated with age in LBW from the beginning till the end of treatment. This trend is in fact due to the differences in the initial LBW of different mating groups with the young females having lower body weight (M1 and M3) than (M2 and M4). The effect of AgNps on LBW of females was significant ($P < 0.01$) at 8 wks post-treatment which may reflect a positive response of laying quails to AgNps administration. This could be account for the beneficial effect of AgNps administration on intestinal microbial population, as an antipathogenic material. Thus, AgNps affect bacterial membrane leading to structural changes and consequently death of the pathogenic microorganisms (Sondi and Salopek-Sondi, 2004 and Maranbio- Jones and Hoek, 2010).

Table (2): Effect of age at mating and silver nanoparticles administration on average body weight and reproductive organs relative weights of Japanese quail breeder hens.

Nanosilver (N)	Mating system (M)				Overall mean	Significance		
	1	2	3	4		M	N	M*N
Average body weight (g/hen)								
N ₀	238±4.2	249±3.7	242±4.4	240±6.4	242 ^b			
N ₁	244±5.7	255±5.2	247±2.8	262±6.3	252 ^a			
Overall mean	241	252	245	251		NS	**	NS
Ovary (%)								
N ₀	1.34±0.08	1.61±0.16	2.56±0.09	1.53±0.07	1.76 ^b			
N ₁	2.57±0.31	1.75±0.04	3.04±0.29	1.61±0.37	2.24 ^a			
Overall mean	1.96 ^b	1.68 ^b	2.80 ^a	1.57 ^b		**	**	**
Oviduct (%)								
N ₀	3.18±0.03	3.13±0.16	3.69±0.19	2.81±0.28	3.20			
N ₁	3.31±0.16	2.79±0.22	4.22±0.35	2.64±0.08	3.24			
Overall mean	3.25 ^b	2.96 ^{bc}	3.96 ^a	2.73 ^c		**	NS	NS
Testis (%)								
N ₀	2.63±0.38	3.64±0.30	2.52±0.32	2.62±0.13	2.85			
N ₁	2.77±0.18	2.06±0.58	2.71±0.15	2.45±0.14	2.50			
Overall mean	2.70	2.85	2.62	2.54		NS	NS	NS

1: (old ♂x young ♀), 2: (old ♂x old ♀), 3: (young ♂ x young ♀), 4: (young ♂x old ♀).

N0: (Zero nanosilver), N1: (20ppm nanosilver). * $P \leq 0.05$ ** $P \leq 0.01$ NS: non-significant.

Mean within a column (having capital letters) or row (having small letters) are significantly different.

In general, the results of productive performance in the present study are in close agreement with the findings by Ayorinde and Oke (1995) who reported that the variation in body weight within a flock might be due to genetic variation and environmental factors which in turn affect the profitability of egg production. However, there was a tendency for egg number, egg weight, yolk weight and albumen weight to increase as body weight and age increased. Agaviezor *et al.* (2008) stated that body weight has been shown to

significantly determine the performance and the economic value of the hen. Furthermore, the present results indicated that age at mating, AgNps and their interaction were significantly ($P < 0.01$) increased ovary relative weight, especially in young female group (M3) followed by M1 group. A similar trend was also observed for the relative weight of the oviduct in respect of age at mating, however, no significant influence of AgNps nor the interaction were observed. The testis relative weight was not significantly changed with either age at mating or AgNps administration and also their interaction. However, there was numerical increase in testis percentage of old males (M1, M2) compared with young males (M3, M4). From the present results, it appears that the increase in ovary and oviduct relative weights in young females may be due to age-related changes in the ovarian function of laying quails.

It is well known that there are four to six large yolk-filled follicles accompanied a great number of small yellow follicles in which yellow yolk deposition has been initiated, and numerous small white follicles (Johnson, 2000). Regardless, the large follicles, it is clear that the number and weight of the other small follicles and the ovarian stroma weight may determine the ovary weight. In this concern, the number of atretic follicles and the rate of follicular atresia may influence the ovary weight in aged quail. This atresia occurs with high incidence in the prehierarchical follicles as a natural physiological phenomenon which is regulated by many factors including, age, nutrition and environment (Gilbert *et al.*, 1983 and Yoshimura *et al.*, 1994). These findings confirm our results with respect to the ovary weight. Moreover, the increase in oviduct percentage of young females might be attributed to the increase in plasma levels of sex steroid hormones that are responsible for the development of the oviduct in terms of hyperplasia of the epithelium lining of all segments, submucosal development of tubular glands coincident with the increase in the synthesis and accumulation of the specific water-soluble protein. This is in close agreement with the findings of many workers who stated that estrogen (either endogenous or exogenous) promotes the formation of tubular secretory glands and induces the synthesis of ovalbumin, conalbumin, ovomucoid and avidin (Pageaux *et al.*, 1984; Lien *et al.*, 1985; Abdel-Fattah, 1999; El-Affi and Abo-Taleb, 2002; Holmes *et al.*, 2003., Ottinger *et al.*, 2004). The lack of the significant influence of males age on testes relative weight in the present study could be explained by the fact that male quails undergo gradual reproductive decline, in contrast, to females. This may be related to the loss in plasma testosterone levels, which is coincided with the reduced baseline GnRH-I release from hypothalamic centers. This was also reported and reviewed by Ottinger *et al.* (2004).

Histological observations:

Liver histology:

Histological examination of liver sections for Japanese quail breeder hens as influenced by age and AgNps administration are shown in Figures 1-a to 1-h. It is clear from Fig.1-a and 1-b (old hens) that the hepatocytes of these sections showed normal appearance and structure, however some unfilterable fluids and fatty cirrhotic areas could be seen. The portal vein is greatly dilated which may reflect hyperactive response of liver tissues to the progressive demands for egg yolk formation. This was also observed liver sections of old hens in Fig. 1-c and 1-d. However, many congested liver areas are detected with some necrotic areas and an extended portal vein filled with numerous erythrocytes. Many dark stained areas with hypertrophic liver cells are also present, especially in Fig. 1-d.

In Fig. 1-e and 1-f, where young females were mated with old males, the microscopic examination of liver section showed normal histological structure without any degenerative changes in the hepatocytes and the portal vein, except few unfilterable fluid areas is observed. This may indicate the impact of hen age on the physiological status of all vital organs in the body. This concept was not observed in the liver tissues of hens with similar age, but 20 ppm AgNps was added to their drinking water (Fig. 1-g). In this section many congested sinusoids could be seen along with pale-stained areas indicative of some side effects of AgNps on liver tissues. A similar observation could be seen in Fig. 1-h where AgNps administration caused many necrotic areas with mild infiltration of inflammatory cells in the liver parenchyma. The above-mentioned changes in the liver sections are due mainly to AgNps administration with a minor effect of males age. The deleterious structural changes which had been detected in old hens is due to hen age along with AgNps administration. It is likely that AgNps as antimicrobial and antifungal drug may have negative impact on the liver tissue of laying quails. This effect was more obvious in old females than young ones. These results disagree the findings by Ahmadi *et al.* (2009) who reported that different levels of AgNps (0 to 900 ppm) had no significant effect on liver histology of broiler chickens. However, Loghman *et al.* (2012) and Rezaei *et al.* (2018) have found that administration of 0-12 ppm AgNps causes a dose-dependent changes in liver

cells of broiler chicks and laying quails, respectively. In the present study, only one dose was applied (20 ppm) for laying quails which may explain the differences in this respect.

Fig. (1-a) T.S. in the liver of hens from the mating group 2, without AgNps administration (H&E x 200).

Fig. (1-b) T.S. in the liver of hens from the mating group 4, without AgNps administration (H&E x 200).

Fig. (1-c) T. S. in the liver of hens from the mating group 2, with AgNps administration (H&E x 200).

Fig. (1-d) T.S. in the liver of hens from the mating group 4, with AgNps administration (H&E x 200).

Fig. (1-e) T.S. in the liver of hens from the mating group 1, without AgNps administration (H&E x 200).

Fig. (1-f) T.S. in the liver of hens from the mating group 3, without AgNps administration (H&E x 200).

Fig. (1-g) T. S. in the liver of hens from the mating group 1, with AgNps administration (H&E x 200).

Fig. (1-h) T.S. in the liver of hens from the mating group 3, with AgNps administration (H&E x 200).

PV: portal vein, h: hepatocytes, s: blood sinusoids, fa: fatty cirrhosis area, c: congested area, If: infiltrable fluids, nc: necrotic area and bd: bile duct

Ileum histology:

The microscopical examination of the small intestine sections (ileum) as influenced by AgNps administration and age at mating are illustrated in Figures 2-a to 2-h. It is clear from sections in Fig. 2-a and 2-b that the villi height was greatly increased in old laying quails, regardless male's age. This increase was more obvious in Fig. 2-b which may reflect the productive status hens from of this group. Many goblet cells are present in the epithelial lining of the villi. Also, the muscular mucosa layer was well arranged, but its diameter is greater in Fig. 2-b than in Fig. 2-a. It is clear also that the size and number of the crypts of Lieberkühn were greater in the ileum section of hens in the mating group of young males with old females (Fig. 2-b) compared with the old x old group (Fig. 2-a). It is difficult to attribute these histological changes to male's age, rather than the productive performance of females.

In young females (Fig. 2-c and 2-d), the number and size of the crypts were increased along with a hypertrophy of the epithelial lining of the villi. The height and diameter of the villi were detected with an obvious high columnar epithelial cells indicative of hyperactivity. This may responsible for an improvement in nutrients absorption and utilization in these young female groups, regardless male age.

Concerning the effect of AgNps administration on ileum histology, the present sections showed different magnitude depending on hens age. In old females (Fig. 2-e and 2-f) the histological examination showed considerable changes in the size of the muscular layer, number and size of the crypts, the presence of more or less lymphocytes along with same degenerative areas in the brush border of the epithelial surface. This effect was also observed, but with mild or little changes in young females' sections (Fig. 2-g and 2-h) which may reflect age-related response to AgNps administration. It appears from these observations that the combined effect of age and AgNps administration, showed a significant change in the ileum histology of laying quails. These changes include the number and size of the crypts of Lieberkühn accompanied by increased height of villi and their epithelial lining.

Fig. (2-a) T. S. in the ileum of hens from the mating group 2, without AgNps administration (H&E x 200).

Fig. (2-b) T. S. in the ileum of hens from the mating group 4, without AgNps administration (H&E x 200).

Fig. (2-c) T. S. in the ileum of hens from the mating group 1, without AgNps administration (H&E x 200).

Fig. (2-d) T. S. in the ileum of hens from the mating group 3, without AgNps administration (H&E x 200).

Fig. (2-e) T.S. in the ileum of hens from the mating group 2, with AgNps administration (H&E x 200).

Fig. (2-f) T.S. in the ileum of hens from the mating group 4, with AgNps administration (H&E x 200).

Fig. (2-g) T.S. in the ileum of hens from the mating group 1, with AgNps administration (H&E x 200).

Fig. (2-h) T.S. in the ileum of hens from the mating group 3, with AgNps administration (H&E x 200).

B: blood vessels, cr: crypts of Lieberkühn, g: goblet cells, ep: epithelial cells, il: intestinal lumen, La: Lamina propria, Ly=Lymphocytes, m: muscularis layer and v: villi.

It is well known that the crypts of Lieberkühn had the ability to secrete fluids containing different vital substances essential for the internal micro-environment of the small intestine segments (Hodges, 1974). These fluids are rapidly absorbed from the villi lumina, making a circulation from crypts to villi which results in a watery vehicle supply for improving nutrients absorption, elaboration and production of antibodies and lymphocytes along with an increase in the number and size of goblet cells which secrete substances responsible for reducing the pH of the intestinal lumen, allowing for better environment to lactic acid bacteria. This may explain the changes in the productive performance of different groups. The negative effect of AgNps on ileum histology was also reported by Grodzik and Sawoza (2006), Sawosz *et al.* (2007) and Ahmadi *et al.* (2009).

Testis histology:

Histological examination of the testis sections as influenced by male age and AgNps administration are illustrated in Figures 3-a to 3-h. It is clear from Fig. 3-a and 3-b that the seminiferous tubules of old males were large with the lumen became wider and filled with numerous spermatozoa (Fig. 3-a). On the other hand, when old males were mated to young females, the seminiferous tubules (Fig. 3-b) appears to contains few spermatozoa in the lumen, however, the number of round or elongated spermatids was greatly increased. Microscopic sections showed also that the seminiferous epithelium lies on a connective tissue membrane, within the basement membrane is multilayered epithelial cells representing the successive stages of spermatogenesis. Sertoli cells are also presented in all sections with their pale-staining nuclei.

In young males, histological sections Fig. 3-c and 3-d showed a progressive proliferation of germ cells as indicated by many spermatocytes, spermatids and spermatozoa of different developmental stages. The dark-stained matured germ cells may reflect hyperactivity and progressive spermatocytogenesis, especially in young males that mated with old females (Fig- 3-d). It appears that these histological observations are related to age change, and may be regulated by the level of plasma testosterone. It may be also that mating frequency and behaviors is different between old and young males.

Fig. (3-a) T.S. in the testis from the mating group 2, without AgNps administration (H&E x 200).

Fig. (3-b) T.S. in the testis from the mating group 1, without AgNps administration (H&E x 200).

Fig. (3-c) T.S. in the testis from the mating group 3, without AgNps administration (H&E x 200).

Fig. (3-d) T.S. in the testis from the mating group 4, without AgNps administration (H&E x 200).

Fig. (3-e) T.S. in the testis from the mating group 2, with AgNps administration (H&E x 200).

Fig. (3-f) T.S. in the testis from the mating group 1, with AgNps administration (H&E x 200).

Fig. (3-g) T.S. in the testis from the mating group 3, with AgNps administration (H&E x 200).

Fig. (3-h) T.S. in the testis from the mating group 4, with AgNps administration (H&E x 200).

Bm: Basement membrane, L: Lumen, S1: primary spermatocytes, S2: Secondary spermatocytes St: Spermatids, Sp: Spermatozoa, Sg: Spermatogonia and Sr: Sertoli cells.

The role of females age was not consistent in the present study, and needs further research. However, some earlier investigations demonstrate that sexual receptivity of females varies and plays a part in inducing successful mating and then stimulating some endocrine and behavioral responses in male chickens and

quails (Wood-Gush, 1955; Sharp *et al.*, 1964; Lake, 1984; Ottinger and Balthazart, 1986). Furthermore, the age-related changes in the histological structure of testis may be due to loss of gonadal steroids as there was a significant decrease in sexual behavior of males with age. This confirms the recent findings by Chen *et al.*, (2004); Ottinger *et al.* (2002 a,b) and Ottinger *et al.* (2004).

Concerning the effect of AgNps administration combined with age at mating on the histological feature of testes, the present sections showed variable changes as illustrated in Figures, 3-e to 3-h. The seminiferous tubules of old males showed irregular distribution of the spermatogenic cells (Fig. 3-e) which may be due the combined effects of age and AgNps administration. The lumen of the tubules was larger with some morphologic and apoptotic aspects of testis (Fig. 3-f). This structure was more obvious in the testis section of old males that mated with old females, (Fig. 3-e), but it is mild in old males mated with young females (Fig. 3-f). Similar observations were detected, but with little detrimental effects on the spermatogenesis process. The germinal epithelium undergoes several proliferation and maturation processes, regardless AgNps administration (Fig. 3-g and 3-h). Since, there are numerous sperms, spermatids of different maturation stages, and Sertoli cells in the gross sections of young males, regardless of females age.

In the present study, there were no clear effect of AgNps on the histology of testis in quail males. It is probable that AgNps can induce some histological changes in tests histology, and this was more relevant in old males. These observations are consistent with the findings of Braydich-Stolle *et al.* (2005) and Sawosz *et al.* (2007) who found dramatic changes in spermatogonial cells induced by AgNps administration. In addition to necrotic areas, indicating that apoptosis had occurred.

Sperm storage tubules (SST):

Storage of sperms occurs in the so-called sperm-host glands or sperm storage glands, which are tubular glands lying within the corium of the mucosal folds of the utero-vaginal junction (Fig. 4-a1). There are about 25000 SST in the hen oviduct especially in the utero-vaginal junction (UVJ) each which may contain between a few or several hundred spermatozoa (Tingari and Lake, 1973; Etches, 1996).

Histological examination of SST from the UVJ of quail breeder hens as influenced by age at mating and AgNps administration are shown in Figures 4-a to 4-h. Fig. 4-a1 and 4-a2 showed that the UVJ folds in old females containing varying numbers of SST with some spermatozoa in the tubular lumen. The epithelial lining of the SST is columnar epithelium being non-ciliated in appearance. A similar observation was detected for old female in Fig. 4-b but with numerous spermatozoa being filled the SST lumen. These hens were mated with young males, which may explain the higher number of spermatozoa. There are also many lymphocytes in the lamina propria of the UVJ folds. In young females, the results show that the epithelial cells of the vaginal fold are very active, being highly columnar with a dark-stained nuclei (Fig. 4-c), however, the number of SST in Fig. 4-d (young ♂ x young ♀) was abundant and contained large number of spermatozoa either in groups or mass.

They may be due to the effect of male age on the number of spermatozoa per ejaculate, which undoubtedly increased in young males. Also, histological sections revealed that AgNps administration had no effect on the SST structure along with the UVJ folds, however, many pale straining areas could be seen in Fig. 4-e (old ♀ x old ♂) and also in Fig. 4-f (old ♀ x young ♂). Both of which containing few spermatozoa and more lymphocytes. This effect may be related to hens age and AgNps treatment.

Fig. (4-a1) T.S. showing the SST in the oviduct from mating group 2, without AgNps administration (H&E x 200).

Fig. (4-a2) T.S. showing the SST in the oviduct from mating group 2, without AgNps administration (H&E x 200).

Fig. (4-b) T.S. showing the SST in the oviduct from mating group 4, without AgNps administration (H&E x 200).

Fig. (4-c) T.S. showing the SST in the oviduct from mating group 1, without AgNps administration (H&E x 200).

Fig. (4-d) T.S. showing the SST in the oviduct from mating group 3, without AgNps administration (H&E x 200).

Fig. (4-e) T.S. showing the SST in the oviduct from mating group 2, with AgNps administration (H&E x 200).

Fig. (4-f) T.S. showing the SST in the oviduct from mating group 4, with AgNps administration (H&E x 200).

Fig. (4-g) T.S. showing the SST in the oviduct from mating group 1, with AgNps administration (H&E x 200).

Fig. (4-h) T.S. showing the SST in the oviduct from mating group 3, with AgNps administration (H&E x 200).

ep: Epithelial surface, Sp: Spermatozoa and Ly: Lymphocytes.

In young females, either mated with old males (Fig. 4-g) or with young male (Fig. 4-h), the epithelial surface area is very active, being high columnar in the sections with numerous well-developed SST lying within the base of the vaginal folds. Many sperm masses could be seen, especially in Fig. 4-h which reflect the effect of male age in this respect.

From the previous results it appears that quail SST are well developed, functional and contains numerous spermatozoa in their lumen. These results confirm the recent findings by Holm and Ridderstrale (2002); Bakst (1992, 1998, and 2011) and Bakst *et al.* (2010). They studied the role of SST in fertility, their ultrastructure development and function in different avian species including Japanese quail. Furthermore, AgNps did not show any damaging properties on SST especially in young females, however, when hens age was considered, some histological changes could be seen.

CONCLUSION

From the results of the present study, it could be concluded that it is possible to improve the productive performance and healthy status of aged Japanese quail breeder stocks if the old age breeder is mated with younger ones. The magnitude of this effect could be achieved through the administration of AgNps in their drinking water.

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الأداء الإنتاجي والاستجابات الهيستولوجية للعمر عند التزاوج وإضافة جزيئات النانو فضة للسمان الياباني

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تم استخدام عدد 64 من ذكور و192 من إناث السمان الياباني البالغ وتم تقسيمهم إلى مجموعتين من أعمار مختلفة (الطيور الكبيرة بعمر 52 أسبوعا والطيور الصغيرة بعمر 10 أسابيع). وقد استخدمت هذه المجموعات الفرعية في سلسلة من أربع مجموعات تزاوج طبيعية بطريقة منتظمة ومتبادلة. في مجموعتي التزاوج الأولى والثانية؛ تم تزاوج الذكور الكبيرة بالعمر مع الإناث الصغيرة والكبيرة بالعمر على التوالي. بينما في المجموعتان الثالثة والرابعة؛ تم تزاوج الذكور الصغيرة بالعمر مع الإناث الصغيرة والكبيرة بالعمر على التوالي. تم إخضاع المجموعتين الأولى والثانية من كلا العمرين إلى المعاملة بإضافة 20 جزء في المليون من جزيئات النانو فضة في مياه الشرب، أما المجموعتين الثالثة والرابعة فقد استخدمتا كمجموعات مقارنة من خلال استخدام مياه شرب بدون أي إضافات. قياسات إنتاج البيض، استهلاك العلف ونسبة التحويل الغذائي تم تسجيلها كل أسبوعين. في الأسبوع الأخير من الفترة التجريبية (الأسبوع الثامن) تم وزن الطيور وذبحها وتشريحها لتقدير الوزن النسبي لبعض الأعضاء الداخلية، كما تم عمل الفحوصات الهيستولوجية لكل من الكبد، اللفانفي، الخصية وأنابيب تخزين الحيوانات المنوية.

أوضحت النتائج أن أوزان البيض تأثرت معنويا بزيادة العمر في قطعان السمان الياباني بالمقارنة بالقطعان الصغيرة في العمر. كما أن إضافة جزيئات النانو فضة إلى مياه الشرب أدت إلى زيادة معنوية في وزن الجسم الحي وأيضا وزن البيضة لأمهات السمان الياباني. كما أظهرت النتائج حدوث تداخل غير معنوي بين العمر عند التزاوج وإضافة جزيئات النانو فضة إلى مياه الشرب وذلك على مستوى قياسات استهلاك الغذاء، معامل التحويل الغذائي ووزن الجسم الحي. كما أوضحت الفحوصات الهيستولوجية حدوث بعض الآثار السلبية الطفيفة على أنسجة الكبد واللفانفي لأمهات السمان الياباني.

ويمكن أن نستخلص من الدراسة الحالية أنه من الممكن تحسين الأداء الإنتاجي والحالة الصحية لقطعان السمان الياباني إذا تم تزاوج الإناث الكبيرة بالعمر مع الذكور الأصغر سنا، ويمكن تحقيق هذا التأثير أيضا من خلال إضافة جزيئات النانو فضة إلى مياه الشرب.