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Research Article

Physiological Responses of Japanese Quail Breeders to Age at Mating and Silver Nanoparticles Administration

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ABSTRACT

A total of 64 males and 192 females from Japanese quail breeders (Coturnix coturnix japonica) were divided into two groups of different ages (old birds; 52 weeks of age (WOA) and young birds; (10 WOA). 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 old and young females, respectively. While the 3rd and the 4th groups; young males were paired with old and young females, respectively. The first and the second subgroups of both ages (old and young) 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 (8^{th} wk), birds were weighed and slaughtered, autopsied and some organs were weighed. Some blood constituents were determined for quail breeder.

The results showed that, young age quail showed significantly higher plasma level of TP, globulin, Cho, LDL and P values compared to those produced from the elder ones, especially for young females mated with old males. Similar trend was observed for the concentration of gonadal hormones E_2, P_4 and T. While, the older females had the highest plasma TL, HDL and Ca values regardless of males age. The administration of AgNps improved significantly plasma Alb, TG, Cho, LDL and A/G ratio compared with control without AgNps supplementation. The differences between the treatments in plasma TP, TL, Ca and E_2 were not significant for silver nanoparticles administration. The interaction between age at mating and AgNps administration was not significant for plasma TL, Ca, LDL, P_4 and E_2. It could be concluded from the present study that, it is possible to improve reproductive 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.

Key words: Age at mating, Physiological responses, Silver nanoparticles, 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 reproduction are age, body weight, hormones and sex ratio (El-Wardany *et al*., 2016). The quail breeder age and age at mating are important factors affecting egg weight and egg production (El-Wardany *et al*., 2016).

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.

Supplementation of silver nanoparticles (AgNPs) improved the stomach internal environment for aged birds. 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. But, only metallic particles can resist high acidity stomach of birds and remain effective in the blood stream.

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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 microbial flora and morphology of enterocytes of duodenal villi in quail (Farzinpour and Karashi, 2012 and El-Wardany *et al*., 2016).

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 physiological and histological responses of Japanese quail breeder.

MATERIALS AND METHODS

This study was carried out at Faculty of Agriculture, Ain Shams University. A total of 64 males and 192 females from Japanese quail breeders flock (*Coturnix coturnix japonica*) represent two equal groups of different ages (old birds; 52 weeks of age (WOA) and young birds; 10 WOA) 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. 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 nutrient requirements 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 8h dark throughout the experimental period.

Blood sampling

A total of 48 blood samples were collected at the end of the experimental period, into heparinized tubes and then centrifuged at the speed of 4000 r.p.m. for 15 min. Plasma samples were decanted into Ependorf tubes (1.5 ml) stoppered tightly and stored in a deep freezer at -20ºC until further assays.

Blood constituents

Plasma total proteins (g/dl) were determined according to the method described by Henry (1974). the determination of plasma albumin (g/dl) based on a colorimetric method described by Doumas *et al.* (1971). Globulin was calculated by subtraction of plasma albumin from plasma total protein, and then A/G ratio was calculated. Total Plasma lipids (mg/dl) were determined according to the method of Knight *et al.* (1972). Triglycerides (mg/dl) were determined by the method of Stein and Myers (1995).

Cholesterol (mg/dl) and high density lipoprotein (mg/dl) were determined according to the method of Watson (1960). Low density lipoprotein was calculated by the following equation:

LDL (mg/dl) = Total Cholesterol – Cholesterol in the supernatant

Plasma calcium and phosphorus

The determination of plasma calcium (mg/dL) was measured, as described by Tietz (1990). Phosphorus (mg/dl) was determined according to the method of Henry (1974).

Plasma hormonal assay

Plasma estradiol-17β (E) (pg/ml) was determined by radioimmunoassay technique according to the method described by Senior (1974). Progesterone (P) (ng/ml) was determined by radioimmunoassay technique according to the method of Williams and Sharp (1977). Testosterone (T) (ng/ml) was determined by radioimmunoassay technique according to the method of Sharp *et al.* (1977). Rabbit antiserum for E, P, and T was prepared and used as described by Bluhm *et al.* (1983).

Statistical analysis

Data were subjected to a two-way analysis of variance with age at mating and drinking water sliver nanoparticles treatment (N) as the main effects using the General Linear Models (GLM) procedure of SAS User's guide (1998).

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

Some Plasma Hormones (Estrogen, E2 and Progesterone, P4)

Data in Table 1 revealed that Japanese quail breeder hens of the young age groups (M3 and M4) had significantly ($P<0.01$) higher plasma levels of both E2 and P4 compared with the old hens of groups (M2 and M4).

Whereas, the administration of AgNps in drinking water had a significant effect on plasma levels of E2 but not P4 compared to those without administration.

The interaction between age at mating and the administration of AgNps treatments was significant for the plasma levels of both E2 and P4. The significant decrease in E_2 and P_4 concentration in aged females may be due to increased loss of ovarian function and reduced hypothalamic responsiveness to ovarian steroids.

Table 1: Effect of age at mating and silver nanoparticles administration on plasma level of gonadal hormones of Japanese quail breeder hens.

Nano-silver (N)	Mating system (M) 1	2	3	4	Overall mean	Significance M	N	M*N
			Estrogen(pg/ml)					
N_0	179.50±1.50	173.00±3.00	293.00±3.00	181.50±3.50	206.75^b			
N_1	275.00±2.21	194.00±2.00	282.66±9.06	183.0± 3.35	233.50^a			
Overall mean	227.25^b	183.50^c	286.80^a	182.40^c	-	**	**	**
			Progesterone(ng/ml)					
N_0	2.45± 0.350	2.40± 0.200	3.90± 0.300	3.45± 0.150	3.05			
N_1	3.80± 0.100	2.80± 0.230	4.20± 0.230	2.40± 0.200	3.34			
Overall mean	3.12^b	2.64^b	4.08^a	2.92^b	-	**	NS	**

1, (old ♂x young ♀); 2, (old ♂x old ♀); 3, (young ♂ x young ♀); 4, (young ♂x old ♀). N0, (Zero nano-silver); N1, (20ppm nano-sliver). *P≤ 0.05, ** P≤ 0.01, NS= non-significant**;** Mean within a column (having capital letters) or row (having small letters) are significantly different.

This was supported by the findings by Johnson (1990) who reported that both the prehierarchial follicles along with the preovulatory follicles have a steriodogensis activity. Also, Ottinger *et al.* (2004) indicated that during aging of chicken and Japanese hens, the hypothalamic response to gonadal steroids diminished, resulting in a reduced preovulatory LH surge. Since, aging females have irregular egg laying and low ovarian weight, which in close agreement with the present results, where old females had the lowest ovarian weights (%) as shown previously in Table 2.

The previous results may suggest a synergetic effects between the ovarian steroid hormones and the productivity of females and the biochemical constituents of blood.

It is well known that progesterone is secreted mainly by the granulosa cells of F1 and F2 hierarchical follicles in laying hens; its secretion is induced by LH secreted by the anterior pituitary (Gue mene and Williams, 1999).

This suggests that the large number of follicles equal to or greater than the size of the F1 follicle in polycystic ovarian follicle hens is the source of the increased P4 and results in a blockage of surge secretion of LH in polycystic ovarian follicle hens.

Moreover, the plasma progesterone concentration significantly increased from 6 to 10 weeks of age, and plasma estradiol levels also tended to increase between 6 and 13 weeks of age, but the alteration was not signifcant (Tsutsui *et al.,* 1998).

The onset of egg-laying in quail, which is synonymous to the onset of sexual maturity, depends on several biological factors, such as body weight (Zelenka *et al*. 1984) and age (Yannakopoulos *et al*., 1995), which support our results.

Plasma total protein (TP), albumin (Alb), globulin (G) and A/ G ratio

Results in Table 2, showed that plasma concentration of total proteins were significantly higher for young females compared to old females regardless of males age. The increase of plasma TP in young females may be due to estrogen secretion at the onset of egg production in laying hens.Although, the administration of AgNps in drinking water has no effect on plasma concentration of total proteins of female Japanese quail breeder hens, the interaction between mating treatments and AgNps was significant. Plasma albumin (Alb) and A/G ration were not significantly different between all mating groups. However, the AgNps increased significantly plasma albumin and A/G ratio compared to those without AgNps administration. Also, in the present study, there were significant differences for the overall mean of globulin concentration in mating groups especially young females with either young or old males, which may reflect greater improvement in the immunity of young females than old ones.But the interaction between both age at mating and AgNps administration had highly significant effect on plasma total proteins, albumin and globulin of female Japanese quail breeder hens. This result is in close agreement with the findings of Bahie El-Deen *et al*. (2009) who reported that TP values of Japanese quails decreased with age. However, the increase of plasma TP and Alb may be due to estrogen secretion at the onset of egg production and the consequent increases in plasma levels of protein bound calcium. In this respect, multiple serum proteins, including very low density lipoprotein (VLDL), vitellogenin and some egg white were reported to be present in large quantities in the bloodstream of chickens during the laying period (Ito *et al*., 2003 and Liou *et al*., 2007). precursors (such as ovotransferrin, lysozyme and ovoinhibitor).

Plasma Total Lipids (TL) and its fraction

Plasma total lipids and triglycerides of quails differed significantly (P≤0.0001) for different mating groups (Table 3). The mating between different ages showed significant (P≤0.001) increases in plasma total lipids and triglycerides concentrations compared to those of the same age. Similar trend was nearly observed for triglycerides concentration.

The administration of AgNps in drinking water had no effects on plasma concentration of total lipids of female Japanese quail breeders hens. While, there were significant differences in triglycerides concentration by the administration of AgNps in drinking water.

Interaction between age at mating and AgNps administration had a significant effect on mean values of plasma triglycerides but not for plasma total lipids.

These increases in plasma lipids can be attributed to the fact that estradiol activate of total lipids metabolism during vitellogensis as reported by Walzem (1996). Moreover, Tufa *et al*. (2001) found that in laying Leghorn hens the triacylglycerol concentration during the laying period was about 12-fold higher than in the growing period. The phospholipids, cholesterol, glycerol and nonesterified fatty acid in the laying period were also higher than those in the growing period.

Results in Table 3 clearly show that old females group had significantly decreased plasma cholesterol and LDL concentrations regardless of their males age. While, HDL concentration was significantly increased for this group.

In this respect, Mady (1990) stated that the decrease in serum cholesterol as egg production level increased might be due to a cholesterol-shift from the blood to the ovarian tissue for egg yolk formation. The magnitude of such trend appeared to be a metabolic phenomenon.

Converse trend was reported in broiler breeder chickens by Dikmen and Sahan (2007). They found that the mean blood cholesterol levels were 165.1, 166.5 and 175mg/dl at 28, 45 and 65 wks of age, respectively.

Moreover, in the present study quails received 20 ppm AgNps in drinking water had significantly higher plasma cholesterol, HDL and LDL concentrations than those without AgNps administration. Also, a highly significant interaction between age at mating and nanoparticles silver administration on plasma cholesterol and HDL concentrations was recorded (Table 3).

Blaszczyk *et al.* (2006) suggested that no marked changes in mean plasma cholesterol content of Pharaoh quail from 6-10 wks of age. However, Blaszczyk *et al.* (2002) claimed that there was circadian rhythm in the blood cholesterol concentration.Moreover, cholesterol as well as other blood lipid components may depend on climatic conditions, feeding, animal condition and sexual activity (Itoh *et al.*, 1998).

Table 2: Effect of age at mating and silver nanoparticles administration on plasma total protein, albumin, globulin and A/G ratio of Japanese quail breeder hens.

Nano-silver (N)	Mating system (M)				Overall mean	Significance		
	1	2	3	4		M	N	M*N
			Total proteins (g/dl)					
N_0	3.32±0.190	3.65± 0.110	4.56±0.147	3.01±0.422	3.66			
N_1	4.45±0.40	3.01± 0.091	3.42±0.130	3.57±0.150	3.52			
Overall mean	4.00^a	3.25^b	3.99^a	3.23^b	-	*	NS	**
			Albumin (g/dl)					
N_0	1.19±0.065	1.46± 0.041	1.51±0.240	0.84±0.230	1.25^b			
N_1	1.72±0.196	1.25± 0.065	1.44±0.109	1.84±0.105	1.49^a			
Overall mean	1.51	1.33	1.48	1.24	-	NS	*	**
			Globulin (g/dl)					
N_0	2.12±0.120	2.19±0.147	3.05±0.332	2.17±0.222	2.40^a			
N_1	2.73±0.247	1.75±0.072	1.97±0.078	1.72±0.045	2.02^b			
Overall mean	2.48^a	1.91^b	2.51^a	1.99^b	-	*	*	**
			A/G ratio					
N_0	0.56±0.005	0.67±0.065	0.52±0.145	0.38±0.080	0.53^b			
N_1	0.63±0.051	0.72±0.056	0.73±0.062	1.06±0.031	0.75^a			
Overall mean	0.60	0.70	0.63	0.65	-	NS	**	**

1, (old ♂x young ♀); 2, (old ♂x old ♀); 3, (young ♂ x young ♀); 4, (young ♂x old ♀). N0, (Zero nano-silver); N1, (20ppm nano-sliver); * P≤ 0.05, ** P≤ 0.01, NS= non-significant; Mean within a column (having capital letters) or row (having small letters) are significantly different.

Table 3: Effect of age at mating and silver nanoparticles administration on plasma total lipids and its fraction of Japanese quail breeder hens.

Nano-silver (N)	Mating system (M)				Overall mean	Significance		
	1	2	3	4		M	N	M*N
			Total lipids (mg/dl)					
N_0	1141.8±20.99	589.3±14.46	900.0± 18.43	1069.9± 14.19	954.81			
N_1	1255.3± 33.47	538.67± 12.22	537.14±18.23	918.5± 16.33	844.08			
Overall mean	1209.95^a	564.00^c	744.54^c	1002.66^b	-	**	NS	NS
			Triglycerides (mg/dl)					
N_0	185.1± 4.64	136.8±2.22	153.6±9.0 0	158.2± 12.97	158.90^b			
N_1	145.6±5.0 0	293.3±6.00	89.33± 8.00	222.4±7.22	191.55^a			
Overall mean	169.33^a	199.47^a	121.50^b	190.33^a	-	**	*	**
			Cholesterols (mg/dl)					
N_0	284.61±15.38	250.00±19.23	273.07± 19.23	255.55±15.45	264.67^b			
N_1	410.71± 13.09	173.07± 3.84	323.07±23.07	248.80±13.09	313.92^a			
Overall mean	397.67^a	211.54^d	298.08^b	252.86^c	-	**	**	**
			HDL (mg/dl)					
N_0	29.79± 2.94	18.98± 4.58	15.05± 0.65	35.79± 7.15	26.11^b			
N_1	27.16± 4.25	24.88±7.85	54.34± 7.85	56.96± 0.031	40.84^a			
Overall mean	28.48^b	21.93^b	34.70^{ab}	44.26^a	-	*	**	*
			LDL (mg/dl)					
N_0	140.56±2.28	92.85± 6.96	89.74± 9.23	99.14±3.63	104.86^b			
N_1	226.09± 5.40	81.41± 3.13	189.74± 2.69	133.42± 2.78	207.67^a			
Overall mean	233.33^a	87.13^b	189.74^{ab}	112.86^b	-	*	*	NS

1, (old ♂x young ♀); 2, (old ♂x old ♀); 3, (young ♂ x young ♀); 4, (young ♂x old ♀). N0, (Zero nano-silver); N1, (20ppm nano-sliver); * P≤ 0.05, ** P≤ 0.01, NS= non-significant**.** Mean within a column (having capital letters) or row (having small letters) are significantly different.

Table 4: Effect of age at mating and silver nanoparticles administration on plasma calcium (Ca) and phosphorus (P) levels of Japanese quail breeder hens.

Nano-silver (N)	Mating system (M)				Overall mean	Significance		
	1	2	3	4		M	N	M*N
Calcium (mg/ dl)								
N_0	13.85± 0.46	16.45±2.09	16.22±1.90	19.22±1.90	16.44			
N_1	13.91± 0.28	21.01± 1.73	15.64±1.70	15.18±1.30	16.23			
Overall mean	13.88^{b}	18.27^{a}	15.87^{ab}	16.80^{ab}	-	*	NS	NS
Phosphorus (mg/ dl)								
N_0	13.22± 0.06	10.96± 0.01	12.90±0.79	6.01±0.41	10.51^{a}			
N_1	13.63± 0.49	7.51± 0.17	12.82±1.23	4.51±0.51	9.05^{b}			
Overall mean	6.43^{a}	4.23^{c}	5.87^{a}	5.26^{b}	-	**	*	**

1, (old ♂x young ♀); 2, (old ♂x old ♀); 3, (young ♂ x young ♀); 4, (young ♂x old ♀). N0, (Zero nano-silver); N1, (20ppm nano-sliver); * P≤ 0.05, ** P≤0.01, NS= non-significant**.** Mean within a column (having capital letters) or row (having small letters) are significantly different.

Plasma calcium (Ca) and phosphorus (P)

A highly significant increase in plasma Ca concentration was found with old age females comparable to the young ones (Table 4). Conversely, there were significant decreases in plasma phosphorus levels. Concerning the AgNps effect, the present results showed that there were insignificant differences in levels of Ca for quails received 20 ppm AgNps in drinking water compared with those without AgNps administration. While, there were significant decrease in plasma levels of phosphorus for quails received 20 ppm AgNps in drinking water. Moreover, the interaction between age at mating and AgNps administration in this respect was significant.

Increasing the level of calcium with increasing egg production may be due to steroid hormones, which are implicated in regulation of Ca metabolism in laying hens. This regulation of the steroid hormones is throughout several modes of action such as deposition of Ca within the medullary portion of long bones and increases serum levels of protein bound calcium. A considerable increase in plasma Ca levels at the beginning of laying period of hens and subsequent gradual increase was observed by Gyenis *et al*. (2006) and Pavlik *et al*. (2009). While, Eren *et al*. (2004) reported decreasing plasma calcium levels in laying hens from 22 to 28 WOA.

However, Pavlik *et al*. (2009) found that plasma P concentration in laying hens was decreased from the 22-75 wks of age. The same trend for P concentration was found in Fayoumi hens by Abdel Magid (2006) who indicated that P concentration increased at the peak of egg production and decreased thereafter.

As hens aged, there are apparent breakdown in their ability to maintain optimum calcium levels which appears as thin eggshells; fragile, easily broken bones etc. Data from Hansen (2002) showed a profound effect of age on the ability of duodenal tissue to uptake calcium.

Conclusions

It could be concluded from the present study that, it is possible to improve reproductive 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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