

The effect of age on growth and development of the gonads pre- to post sexual maturity of Japanese quail (*Coturnix japonica*)

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Abstract

A total number of 300 unsexed Japanese quail chicks one day old were randomly distributed in the metal cages to determine the optimal age of sexual activity for quail in Iraq by studying the growth and development of the gonads pre-to post sexual maturity. Data were collected during 4, 6, 8 and 10 weeks. Steroids hormone and gonads and oviduct development were studied. Highest testosterone and estrogen hormone levels were recorded on 8 and 10 weeks of age. Left and right testis weight, height, width and volume were recorded highest values on 8 and 10 weeks compared with 4 and 6 weeks . Histological parameter of testes sections indicated a complete development for a seminiferous tubules beginning 8 to 10 weeks . Ovary weight with follicles and oviduct weight were given highest values at 10 weeks old. It has been concluded that the optimal age of sexual activity for quail in Iraq could be situated around 8 weeks.

Keywords : (Age , Quail , Qonads , Sexual maturity).

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Introduction

Japanese quail birds are a laboratory animals (2). In most birds, secondary sexual traits (comb, wattle, pin and feather traits) have been used as external indicators of sex and determine of sexual maturity (4).Growth of combs and wattles is stimulated by testosterone hormone (30 and 17). While japanese quail does not have it. Sexual signs of puberty in males of quail appear to produce foam from the cloacal gland, and egg first egg produce for females (32).Birds fertility depends on gonadal development and activity of spermatozoa (13). Age at sexual maturity was also depended with the weight of testes and levels of male hormones (15). in addition There was a positive correlation between weight and age of the bird puberty (29). Moreover, there was correlation between age of puberty and weight of the testes in males(36) . Gonads growth and development depends on the level of sex hormones in blood, as well

as the testes growth and development depends on the concentration of testosterone in the blood, as there was a positive correlation between the concentration of testosterone and the size of the testicles coefficient (3), as well as the estrogen hormone play a key role in stimulating the development of the female reproductive system (5). Jacob and Pescatore (19) showed that female birds had two ovaries and oviducts but the right decay during fetal development and weren't functional in birds adult, however , the left one has developed to replace it . The physiological and anatomical were important signs in the clinical estimate since hatched birds and when sexual maturity, moreover being featured indicators to evaluation growth rates and its relation to the functions physiological birds with natural growth and abnormal (11). As the changes in the physiological signs of the important indicators in the showed of bird growth rates (35).

Noirault *et al* ; (25) explained that the development of the testes an important signs for estimate the growth rate in birds, because birds testes are located within the body cavity. It makes difficulty to follow testes development only by taking them out after removing the viscera as intestine (1) So present study aimed to determine the optimal age of sexual activity for quail in Iraq by studying the growth and development of the gonads pre-to post sexual maturity.

Materials and methods

This study was carried out at the quail farm, Faculty of Agriculture ,University of Basrah. A total of 300 unsexed Japanese quail one day old were randomly distributed on the 20 cages per one 15 birds . The metal cages (71 × 71 × 51 cm) were used in brooding chicks locally manufactured consists of three floors. Feed and water were allowed *ad libitum*. Feed containing approximately 20.62% crude protein and 2904 metabolizable energy (kcal/kg).

Ingredients and chemical composition of diet were shown in Table (1). The temperature degree and humidity percentages were daily measured and recorded approximately 35 ± 2.0 C° and $65 \pm 3.0\%$ as averages at the first week, then the temperature degree was gradually decreased with age until quail chicks were acclimatized to the environmental condition. At 21 day of age,, sexes were determined by the breast feathers and segregated. Onset of puberty was assessed in females as the first day of egg production. In males, onset of puberty was measured as the first day of foam production from the proctodeal gland. (28) , The weight of males and females were calculated at puberty age . At the 4 , 6 , 8 , 10th weeks of age , 40 birds (20 male and 20 female) were randomly selected for slaughtering. After slaughtering, the testes , ovaries and oviducts were weighed . The length and diameter of both testis were measured in millimeter (mm) using Vernier's caliper.. The size

of both testis were collocated by : $(4/3 \times 3.5414 \times a^2)$ where a = half the longitudinal axis of the testicle and b = half transverse axis (8 and 24). According Gridley, (16) After weighing, the testes They were fixed in 4% neutral formaldehyde, embedded in paraffin, and the sections were stained with haematoxylin and eosin at room temperature. The stained cells were analyzed using a light microscope with a camera attached. Measurements of different histological structures of the testes tissues were performed by the calibrated stage micrometer in micrometer(μm). The blood samples were collected after birds slaughtering in dry clean centrifuge tubes . Blood samples were separated by centrifugation at 3000 rpm for 15 minutes. Serum was separated and stored at -20 C° until it was assayed for testosterone (ng/ml) and estrogen (pg/ml) hormone that measurement by Radioimmunoassay (RIA) using a commercially available test kit (Immunotech, Marseille, France)

(26 and 27). Collected data were subjected to one way analysis of variance (ANOVA) and differences were considered to be significant L.S.D if P was < 0.05 . SPSS Statistics 18 .0 (33) was used for statistical analysis.

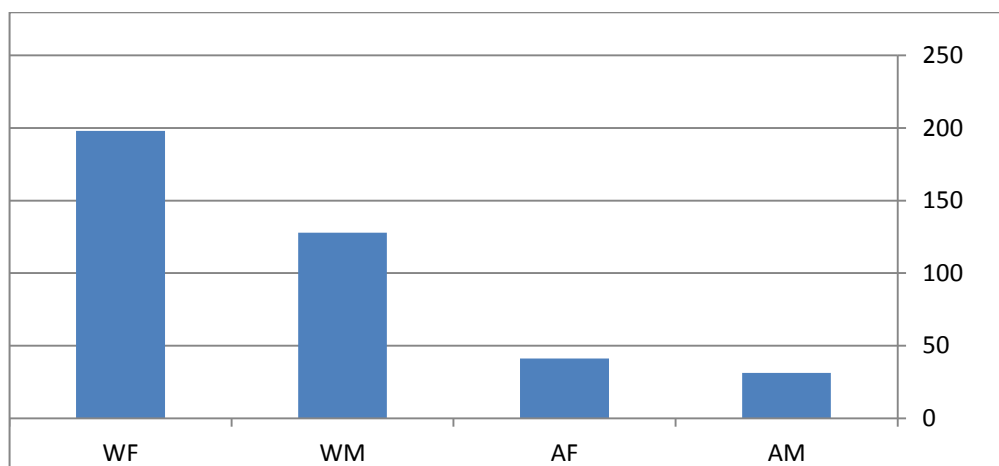
Results and Discussion

Age and weight of males and females at sexual maturity was summarized in Figure 1. The mean values of age and weight was higher in females compared with males.

The mean weight, length, width and volume of the right and left testis during 4 , 6 , 8 , 10 week of age are given in Table 2 . The mean right and left testicular weight , length, width and volume, showed that they were a significantly ($P<0.05$) as age increased the birds with higher mean values at eighth and tenth week as age to left testes. The dimensions and shape of the testes changed as the birds approached sexual maturity, appearing greatly enlarged , the combined weight of testes was highly correlated with

age several reports are available in the literature stating that left testes are larger than right testes in most avian species such as Japanese Quail, (36), chickens (24 and 37), Turkeys (7). This study showed that the left testes were larger than the right testes in all parameters and under all investigated ages. Deviche *et al.* (10) reported this asymmetry in growth seems to be

likely due to low sensitivity of the least developed testis to gonadostimulating factors. The rapid testicular growth up to 10 weeks is due to the high proliferation of Sertoli cells, their increase in size results in the development of the seminiferous tubules leading to a gradual increase in testicular weight and volume (22, 9 and 30).



WF (gram)	WM (gram)	AF (day)	AM (day)
197.92	127.77	41.25	31.37

Fig. 1. Age and weight of males and females at sexual maturity (averages = average for 35 value).

males n =35 , females n= 35 , * where to (WM and WF: weight of males and females at maturity respectively. *AM and AF: ages of males and females at maturity respectively.

Table 1: The ingredients and composition of experimental diets:

Ingredient (%)	%	Chemical analysis (%)	
Yellow Corn	56	¹ ME (Kcal /Kg) diet	2904
Wheat	4.0	Crude protein %	20.03
Soybean meal (44%)	28	Ether extract %	3.93
*Protein Concentration	5.0	Crude fiber %	3.49
Oil plant	1.0	Calorie : protein ratio	144.98
Limestone	4.4	Calcium %	2.31
Dicalcium Phosphate	1.0	Phosphorus available %	0.46
Vitamin / mineral premix	0.3	Methionine %	0.38
Common Salt	0.3	Lysine %	1.06
total	100	Methionine + Cystine %	0.83

*Provides per kg of diet: Tryptophan 10 mg , Tyrosine 30 mg Phenylalanine 10 mg , Arginine 40 mg , valine 40 mg , threonine 20 mg , methionine 40 mg , leucine 40 mg , isoleucine 40 mg , lysine 60 mg , and histidine 10 mg , trans-retinol: 3125 µg , cholecalciferol: 75 µg , α-tocopherol acetate : 50 mg, Vit K3: 5 mg, Vit B1: 3 mg, Vit B2: 6 mg, Vit B6: 5 mg, Vit B12: 0.003 mg, Pantothenic acid: 10 mg, Niacin: 50 mg, Folic acid: 1 mg, Biotin: 0.1 mg, Cu: 5 mg, I: 2 mg, Co: 0.5 mg, Se: 0.15 mg, Mn: 90 mg, Fe: 50 mg, Zn: 70 mg.

¹ME (Kcal . Kg⁻¹) diet = Metabolized energy.

Table (2) Effect of age on right and left testis development (mean± st. error).

Traits Ages	Weight of testes (g)		Length of testes (mm)		Width of testes (mm)		Volume of testes (mm ³)	
	right	Left	right	Left	right	Left	right	left
Fourth Week	0.44 ± 0.04d	0.46± 0.04c	10.02± 0.18c	10.03± 0.26b	6.17± 0.19 b	6.20 ± 0.21c	234.6± 15.63c	244.3± 17.86b
Sixth Week	2.52± 0.14c	2.81± 0.10b	20.11± 0.60b	22.37± 0.50a	10.71 ± 0.26a	10.80 ± 0.19b	1416.9± 87.97b	1916.8± 116.65a
Eighth Week	2.99 ± 0.07a	3.08± 0.09ab	23.10± 0.35a	23.17± 0.26a	10.96 ± 0.22a	11.65 ± 0.17a	1691.9± 3.53a	2020.1± 98.41a
Tenth Week	3.08 ± 0.13a	3.38± 0.15a	23.17± 0.41a	23.18± 0.26a	11.10 ± 0.19a	11.82 ± 0.23a	1719.8± 69.12a	2042.2± 85.50a
significant	*	*	*	*	*	*	*	*

* a,b,c means in the same Colum with no superscript are different significantly (p<0.05).

The increase in these measurements may be due to the increased in height and diameter of seminiferous tubules, as well as in Leydig and interstitial cells number (6 and 30). In addition, through the pre-maturity period, testicular development is highly correlated with the number and size of Sertoli cells, while during puberty, it best correlated with germ cells(21).

Testosterone and estrogen hormone concentrations during 4 , 6 , 8 and 10 week of age are given in table 3 . Testosterone concentrations were significantly increased ($P < 0.05$) with age , the results revealed that higher concentration obtained between 8 and 10 weeks while less testosterone concentration were recorded at four and six weeks of age Table 3. Testosterone concentrations at week 4 and 6 were also significantly ($P < 0.05$) lower than value obtained at week 8 and 10 . In birds, testosterone is responsible for the growth and development of the male testes, secondary sexual traits , in addition

spermatogenesis. (22). The results showed that testosterone level was low in the 4th week. However, it increased significantly from the 10th week. This increase might be that quail could have reached maturity around 6 weeks. This results according to (17, 27 , 30 and 20). In addition the male Japanese quail begin secreting LH hormones at 3-4 weeks of age which is crucial for initiating the secretion of testosterone which, in turn, stimulates the rapid growth of testes (14 and 31). Estrogen concentrations were non significant between age of 8 ($169.244 \text{ pg.ml}^{-1}$) and 10 ($170.851 \text{ pg.ml}^{-1}$) weeks . Estrogen concentrations at week 4 and 6 were also significantly ($P < 0.05$) lower than at week 8 and 10. The increase in estrogen levels during this period stimulated thyroid function in birds (34). So that the increased number of thyrotropic cells during this period might be related to an increased estrogen level produced by the maturing ovarian follicles in quail (26) . Morphometric evaluation of

seminiferous tubules revealed that different parameters increased significantly ($P < 0.05$) growing old (Fig.2 and Table 3). However, values recorded at 4th and 6th week old were significantly ($P < 0.05$) lower than those recorded lately. Seminiferous tubule diameter and the germinal cell layer thickness were equally similar ($P < 0.05$) at 8 and 10 weeks old. Seminiferous tubules diameter and the germinal cell layer thickness at 8 and 10 weeks of age were significantly ($P < 0.05$) higher than the value obtained at 4 and 6 weeks. Seminiferous tubules showed a diversity of form but the majority has circular shape. The diameter of seminiferous tubules significantly increased and reached 322.96 micrometer at 10 weeks old. Same observation was obtained for the germinal cell layer thickness (100.51 micrometer) at the same period. According to Mather and Wilson (23) that the stage of spermatogenesis may be promoted on the basis of testes weight and showed 10 week of age

testes in full spermatogenesis in their results. Growth rate of testes is in relationship with an increase of volume, diameter and length of seminiferous tubules (18).

Weight of ovary with follicles and weight of oviduct increased significantly ($P < 0.05$) with growing old (table 4). However, values recorded at 6 and 8 week old were similar in weight of ovary and oviduct but significantly ($P < 0.05$) lower than those recorded at 10 weeks of age, on other hand , there were a significant differences between them at this age, while, the lowest values recorded at four weeks of age. The significantly increase ($P < 0.05$) in weight with growing old might be that quail could have reached sexual maturity may be causes to gonadal hormones regulate the rapid development of the oviduct, which occurs before and during sexual maturation. Treatment of immature Japanese quail and young female chickens with estradiol enhances growth of the oviduct and formation of tubular secretory

Table (3) Effect of age on testosterone and estrogen concentration and seminiferous tubules development (mean ± st. error)

Traits	Testosterone Concentration	Estrogen Concentration	Diameter of seminiferous tubular (µm)	Thickness of spermatogenesis (µm)
Ages	In males (ng.ml ⁻¹)	In females (pg.ml ⁻¹)		
Fourth week	0.497 ± 0.061 d	137.286 ± 6.789 c	137.52 ± 10.359 c	40.28 ± 2.169 c
Sixth week	1.944 ± 0.235 c	153.539 ± 4.561 b	265.95 ± 14.835 b	84.89 ± 6.827 b
Eighth week	2.993 ± 0.131 a	169.244 ±6.063 a	303.22 ± 9.210 a	93.43 ± 1.836 ab
Tenth week	3.023 ± 0.154 a	170.851 ± 4.772 a	322.96 ± 9.925 a	100.51 ± 4.353 a
Significant	*	*	*	*

* a,b,c means in the same Colum with no superscript are different significantly (p<0.05).

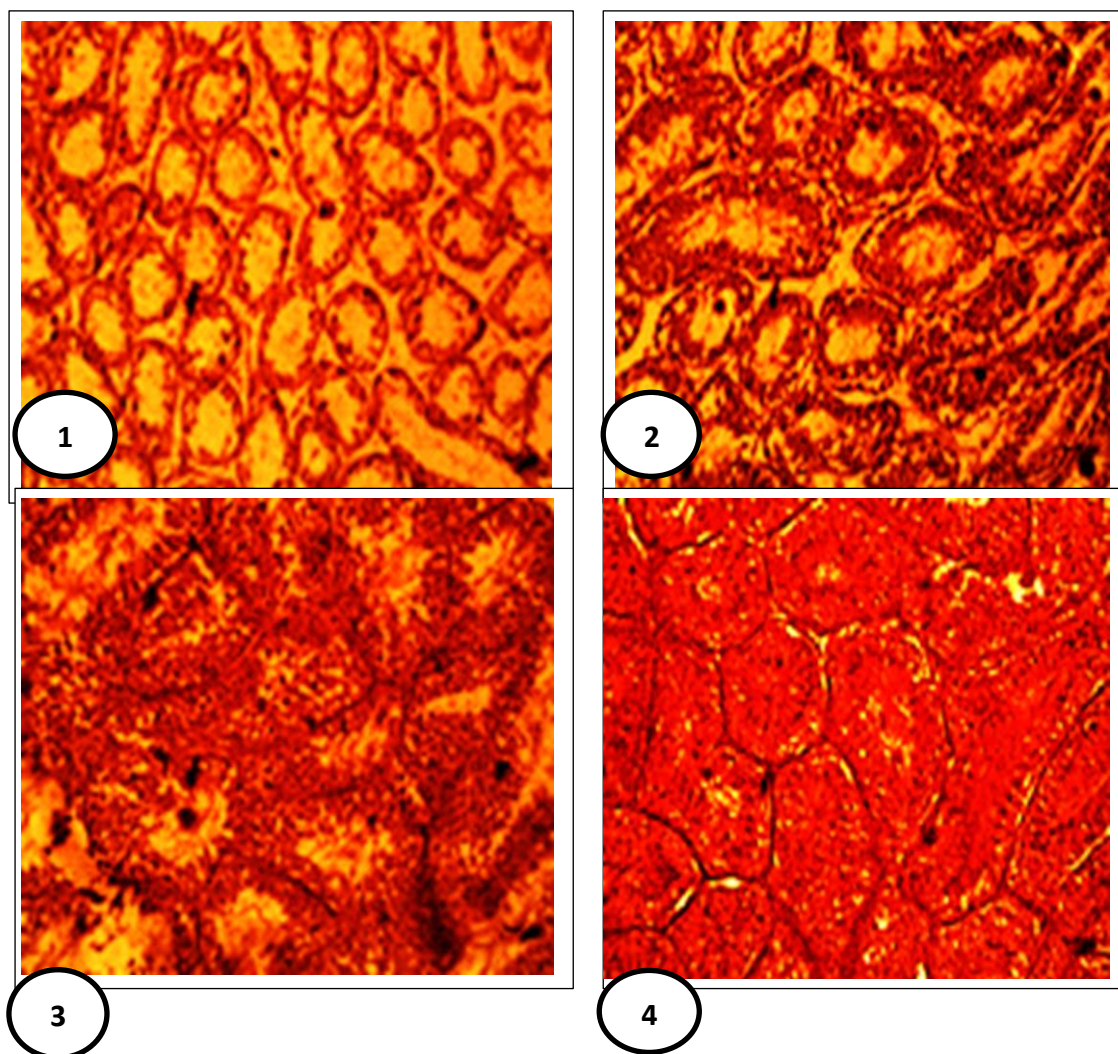


Fig. 2. Histological evolution of seminiferous tubules of quail testes sections from 4 to 10 weeks old. 1. Growing and nonfunctional seminiferous tubules (Week 4, 100×), 2. Seminiferous tubules initiating spermatogenesis (Week 6, 100×), 3. Active seminiferous tubules (Week 8, 100×), 4. Spermatozoa in the seminiferous tubules lumen (Week 10, 100×).

glands and epithelial differentiation (12). Also there is high significant correlation between body weight and ovary weight and size of

follicles (2) . The increase in estrogen secretion during this period promotes ovary and reproductive tract in females (5).

Table(4)Effect of age on ovary and oviduct weight(mean ± st. error)

Ages Traits	Fourth week	Sixth week	Eighth week	Tenth week	Significant
Weight of ovary with follicles (g)	0.2714 ± 0.0088 c	6.6123 ± 0.2299 b	7.0774 ± 0.1973 b	8.2074 ± 0.3186 a	*
Weight of oviduct (g)	0.2663 ± 0.0197 c	6.0314 ± 0.1674 b	6.2900 ± 0.2157 b	7.8217 ± 0.2036 a	*

* horizontally different letters mean significant differences at a significance level (0.05).

Conclusion:

We conclude that the best age of obtaining high fertility of Japanese quail in Iraq is 8-10 weeks due to completely development of testes and ovaries.

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تأثير العمر في نمو وتطور الغدد التناسلية قبل واثناء وبعد النضج الجنسي لطيور السمان

الياباني (*Coturnix japonica*)

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المستخلص:

استعمل في الدراسة الحالية 300 طير من طيور السمان الياباني غير المجنسة بعمر يوم واحد ، وزعت عشوائياً في اقفاص معدنية محلية الصنع لغرض تحديد العمر الامثل للنضج الجنسي للطيور تحت الظروف المحلية وذلك من خلال دراسة نمو وتطور الغدد التناسلية قبل واثناء وبعد النضج الجنسي ، جمعت بيانات الدراسة عند 4 و6 و8 و10 اسبوع من العمر اذ تم دراسة كل من الهرمونات الجنسية وتطور الغدد التناسلية وقناة البيض ، اظهرت نتائج الدراسة اعلى مستويات لهرموني التستستيرون والاستروجين عند عمر 8 و 10 اسبوع فضلاً عن ارتفاع اوزان واطول و عرض وحجم الخصيتين عند هذين العمرين مقارنة بالعمرين 4 و 6 اسبوع ، و اظهرت الدراسة النسيجية للخصى اكتمال تطور النبيبات المنوية بدءاً من 8 ولغاية 10 اسبوع ، وسجلت اوزان المبايض مع الحويصلات المبيضية وقناة البيض اعلى الاوزان عند عمر 10 اسبوع ، نستنتج مما تقدم ان العمر الامثل للفعالية الجنسية لطيور السمان في العراق تقع عند عمر 8 اسبوع. الكلمات المفتاحية : (العمر ، السمان ، النضج الجنسي ، الغدد التناسلية).

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