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Sperm Storage Potential and Daily Sperm Production of Brown Male Japanese Quails for Three Different Physiological Age Groups

***Ewuola, E. O and Esan O. J**

Animal Physiology and Bioclimatology Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria

*Corresponding author Email: eoewuola@gmail.com; GSM: +2348060862361

Target audience: Quail Farmers, Avian breeders, Poultry nutritionists and Researchers

Abstract

Thirty brown male Japanese quail birds consisting of 10 each of pubertal mature and adult quails were used to determine their sperm storage potential and daily sperm production at the three different physiological age groups. The birds were randomly allotted to treatment groups (pubertal mature and adult) in a completely randomised design. All animals were sacrificed and their reproductive organs excised and weighed. Testes and epididymis were homogenised separately in 0.154M NaCl. Sperm reserves in the homogenates were determined. Sperm production efficiency and daily sperm production were also determined from testicular homogenates and epididymal sperm reserves from epididymal homogenate. The results showed that pubertal quail had significantly (P<0.05) higher paired epididymal weight (0.25±0.05g) than the mature (0.17±0.07g) and adult (0.23±0.05g). The pubertal quails had highest (P<0.05) daily sperm production per testis (259.29 x10^6) and had a significantly (P<0.05) higher sperm production efficiency (97.57x10^6) than matured (44.84x10^6) and adult groups (10.95x10^6). The mature group had a significantly (P<0.05) higher right epididymal sperm reserve (88.46 x10^6) than the adult (28.62 x10^6) while the pubertal group had highest (P<0.05) paired epididymal sperm reserves (237.54 x10^6) and the age groups. The live weight of the birds was significantly (P<0.05) and positively correlated with the weight of the paired epididymis (r = 0.52) and with the paired testicular weight (r = 0.60; P<0.01). Daily sperm production per testis and right testicular sperm reserve per testis were observed to be significant and positively correlated (r=0.91 P<0.01). This study revealed that the brown male Japanese quail has its peak reproduction potential at puberty.

Keywords: Quail sperm storage, sperm production

Description of Problems

The efficiency of spermatogenesis is key to reproductive success in the male. Male fertility is characterized by many important contributing factors. One of the most important factors is the number of spermatozoa available for fertilizing oocytes. Morphometric analysis of the

testis of any species or breed is necessary in assessing and estimating quantitative changes in testicular components and spermatogenic function arising from factors such as age, season, temperature and diseases (1). Nutrition also exerts some influence on testicular morphometric parameters and on gonadal sperm reserves in Corriedale rams (2). For this reason, Gage and Freckleton (3) described the mammalian testes as infallible predictors of spermatozoa production. The authors further asserted that knowledge of the basic morphometric characteristics of the reproductive organs is mandatory for assessment and prediction not only of sperm production but also of the storage potential and fertilizing ability of the breeder male. Direct counts of maturing spermatids in the testes have been carried out in various domestic animals and the results expressed as gonadal sperm reserves, while extra gonadal sperm reserves represents sperm stored in the caput, corpus and cauda epididymis. Almquist and Amman (4) developed a method for the estimation of gonadal sperm reserves and extra gonadal sperm reserve in bulls. This method with suitable modifications has been used to determine gonadal and extra gonadal sperm reserves for various animal species such as goats (5), rats (6), wild boar (7), rabbits (8), partridge (9), sheep (10), donkey (11), domestic fowl (12), Japanese quail (13), bulls (14), rams (15), pigeons (16) and elephants (17).

Species extinction rates seem to have accelerated in recent decades (18). The Japanese quail is a near threatened species (19). To prevent the permanent loss of this individual's potential contribution to the genetic variability of a rare or endangered species, it is feasible to collect sperm before or even shortly after death by retrieval from the ejaculate, epididymis or testes, and cryopreserve the sperm for future use in assisted reproduction (20, 21).

Due to the paucity of information on the reproductive capacity of the Japanese quails, conscious effort is required for its genetic improvement programs. The information on the gonadal sperm reserve, daily sperm production, testicular and epididymal weights, body weight and sperm production capacity in male Japanese quail has not been adequately documented. Therefore, sperm storage potential and daily sperm production of the brown Japanese quail at three different physiological age groups were investigated.

Materials and Methods

Experimental materials and operation

Thirty birds were purchased at the poultry section of the Teaching and Research Farm, University of Ibadan. The pubertal group of birds at 7-10 weeks of age, the mature at 15-20 weeks of age and the adults above 24 weeks of age were used for the study (22).

The experimental design was completely randomized design and the layout of the experiment was as follow;

Treatment 1 (T_1) - Pubertal birds (7-10 weeks old)

Treatment 2 (T_2) - Mature birds (15-20 weeks old)

Treatment 3 (T_3) - Adult birds (above 24 weeks old)

Determination of testicular and epididymal sperm reserve

All birds were weighed, sacrificed, dissected and their right and left testes and epididymis were carefully removed. The weight and volume of each testis and weight of the epididymis were obtained. Each testis and epididymis was homogenized with 1ml of normal saline (0.154M NaCl), and filtered with gauze. The homogenate was diluted with normal saline at ratio 1:30 the diluted homogenate was charged on the haemocytometer and then viewed under microscope at a magnification of x400, to obtain the sperm count.

Determination of daily sperm production (DSP)

The daily sperm production was estimated from the testicular sperm reserves. The estimation of DSP from testicular homogenate is based on the fact that the nuclei of elongating spermatid are resistant to physical destruction at some point during spermatogenesis. The DSP of the Japanese quails was therefore calculated with the formula proposed by Clulow and Jones (23) as follows:

$$\text{Daily Sperm Production} = \frac{\text{Testicular sperm reserve}}{\text{Time divisor (1day)}}$$

Sperm production efficiency

The sperm production efficiency was estimated from the daily sperm production. It was calculated using the formula as follows:

$$\text{Sperm Production Efficiency} = \frac{\text{Daily Sperm Production}}{\text{Weight of the testis}}$$

Data analysis

All data obtained were analysed using Analysis of Variance of the Statistical Analysis System software (24) and mean separation was done using Duncan Multiple Range Test option of the same software.

Results

The result of the morphometric characteristics of the brown male Japanese quail at three different physiological age groups is presented in Table 1. It was observed that there was no significant difference in the live weight of the birds. The pubertal brown male had significantly ($P<0.05$) higher right epididymis weight (0.15±0.06g) than other groups, however there was no significant difference observed in the testes weight. The pubertal group also had significantly ($P<0.05$) higher paired epididymal weight (0.25±0.05g) than the mature group (0.17±0.07g) and adult group (0.23±0.05g).

Table 1. Weight characteristics of the testes and epididymis of the brown Japanese male quail at three physiological age groups

Parameters	Treatments		
	Pubertal (7-10wks old)	Mature (15-20wks old)	Adult (above 24 wks)
Weight of bird (g)	.....	135.65±19.28	144.01±11.52
Weight of Right Testis (g)	2.62± 0.38	2.46±0.81	2.44±0.51
Weight of Left Testis (g)	2.70± 0.48	2.46±0.79	2.40± 0.47
Paired Testis (g)	5.32±0.72	4.92±1.59	4.84±0.95
Weight of Right Epididymis (g)	$0.15±0.06^{a}$	$0.09±0.04^{b}$	$0.11±0.05^{b}$
Weight of Left Epididymis (g)	$0.13±0.04^{a}$	$0.09±0.04^{b}$	$0.13±0.04^{a}$
Paired Epididymis (g)	$0.29±0.05^{a}$	$0.17±0.07^{c}$	$0.23±0.05^{b}$
Right testis volume (cm^3)	2.49 ±0.36	2.60±0.81	2.17±0.53
Left testis volume (cm^3)	2.57±0.45	2.75±0.92	2.23±0.52

abc: Means in the same row with different superscript are significantly ($P<0.05$) different.

The result of the testicular sperm reserve of the brown male quails at three different physiological ages is as presented in Table 2. The pubertal brown quail had significantly (P<0.05) higher left and right testicular sperm reserve per testis (133.38x10^6) and (125.92x10^6), respectively than other age groups. The left and right testicular sperm reserve/g testis of the pubertal brown quails (49.22±35.92/g testis) and (48.35±13.11/g testis), respectively were significantly higher than other age groups. It was also observed that the pubertal brown quails had highest (P<0.05) daily sperm production per testis (259.29 x10^6). The pubertal group also had a significantly (P<0.05) higher sperm production efficiency (97.57 x10^6) than matured and adult groups

Table 2. Testicular sperm reserve of brown Japanese male quails at three physiol ogical age groups

Parameters (x10^6/ml)	Treatments		
	Pubertal (7-10wks old)	Mature (15-20wks old)	Adult (above 24 wks old)
Right testicular sperm reserve per testis	125.92±38.94^a	43.97±11.64^b	16.59±3.98^c
Left testicular sperm reserve per testis	133.37±102.79^a	41.18±17.13^b	13.30±2.85^b
Paired testicular sperm reserve per testis	259.30±129.21^a	85.15±23.38^b	26.16±10.13^b
Right testicular sperm reserve /g testis	48.35±13.12^a	23.48±21.79^b	7.04±2.02^c
Left testicular sperm reserve /g testis	49.22±35.92^a	21.35±18.92^b	15.47±1.00^b
Paired testicular sperm reserve / g testis	97.57±40.36^a	44.84±39.98^b	10.95±4.07^c
Daily sperm production / testis	259.29±129.21^a	85.15±23.38^b	26.16±10.13^b
Sperm production efficiency	97.57±40.36^a	44.84±39.98^b	10.95±4.07^c

abc: Means in the same row with different superscript are significantly (P<0.05) different.

The epididymal sperm reserve of the brown male quails at three different physiological ages is presented in Table 3. The mature group had a significantly (P<0.05) higher right epididymal sperm reserve (88.46 x10^6), than the adult group (28.62 x10^6). There was however no significant difference between the right epididymal sperm reserve of the mature and the pubertal groups of quails or that of the adult group and the pubertal group. The mature group had a significantly (P<0.05) higher right epididymal sperm reserve/g epididymis (1001.54 x10^6) than the adult group (287.0 x10^6). There was however, no significant difference in the right epididymal sperm reserve/g epididymis of the mature and the pubertal quails neither was there any significant difference in the right epididymal sperm reserve/g epididymis of the adult and pubertal groups. However, the pubertal group had a significantly (P<0.05) higher paired epididymal sperm reserves (237.54 x10^6). The pubertal quails had a significantly (P<0.05) higher left epididymal sperm reserve/g epididymis (15.14 x10^6) than other age groups.

The correlation analysis of some selected morphometric parameters of the male brown quails is shown in Table 4. It was observed that the live weight of the birds was significantly and positively correlated with the weight of the paired epididymis of the birds (r = 0.52;

P<0.05). The live weight of the birds was also observed to have a very significant, a fairly strong and positive correlation with the paired testicular weight (r = 0.60; P<0.01). However, a non significant but positive correlation existed between paired testes weight and the weight of the paired epididymis (r=0.26; P>0.05). There was a highly significant and positive correlation between the volume of the right testis and the volume of the left testis (r = 0.01; P<0.001).

Table 4. The correlation coefficient of morphometrics characteristics of testis and epididymis of the brown male Japanese quails

Parameters	Live Weigh i	Right Testicul ar Wt	Left Testicul ar Wt	Paired Testicula r Wt	Right Epidid ymal Wt	Left Epidid ymal Wt	Paired Epidid ymal Wt	Right Testicula rVolume	Left Testicula r Volume
Live weight	1	0.55*	0.59**	0.60**	0.46*	0.29^{NS}	0.52*	0.52*	0.37*
Right Testicular Weight		1	0.84***	0.96***	0.05^{NS}	0.20^{NS}	0.16^{NS}	0.89***	0.82***
Left Testicular Weight			1	0.96***	0.27^{NS}	0.22^{NS}	0.33^{NS}	0.80***	0.88***
Paired Testicular Weight				1	0.17^{NS}	0.22^{NS}	0.26^{NS}	0.88***	0.89***
Right Epididymal Weight					1	0.08^{NS}	0.80***	0.05^{NS}	0.04^{NS}
Left Epididymal Weight						1	0.66***	0.12^{NS}	0.04^{NS}
Paired Epididymal Weight							1	0.11^{NS}	0.01^{NS}
Right Testicular Volume								1	0.83***
Left Testicular Volume									1

NS = Not Significant; * Significant (P<0.05); **Very Significant (P<0.01); ***Highly Significant (P<0,0001)

The correlation coefficients of sperm production potential of the quails are presented in Table 5. The left testicular sperm reserve per testis showed a very significant, strong and positive correlation with the right testicular sperm reserve per testis (r=0.76, P<0.01). Also, a very significant, strong and positive correlation was observed between paired testicular sperm reserves per testis and right testicular sperm reserves per testis (r=0.91, P<0.01). Right testicular sperm reserve per g testis had a very significant, strong and positive correlation with right testicular sperm reserve per testis (r=0.83, P<0.01). The left testicular sperm reserves per g testis was significantly and positively correlated with the right testicular sperm reserve per testis (r=0.72, P<0.01). A significant, very strong positive correlation existed between paired testicular sperm reserve per g testis and right testicular sperm reserve per testis (r=0.84, P<0.01), while daily sperm production per testis and right testicular sperm reserve per testis were observed to be significant and positively correlated (r=0.91, P<0.01).

Table 5. The correlation coefficients of sperm production potential of brown Japanese quails

Parameters	RTSRPT	LTSRPT	PTSR	RTSRPGT	LTSRPGT	PTSRPGT	RESR	PESR	DSP/T	SPE
RTSRPT	1	0.76**	0.91**	0.83**	0.72**	0.84**	0.24	0.29	0.91**	0.84**
LTSRPT		1	0.96**	0.50**	0.92**	0.81**	-0.01	0.12	0.96**	0.81**
PTSR			1	0.68**	0.89**	0.88**	0.11	0.23	1.00**	0.88**
RTSRPGT				1	0.68**	0.89**	0.11	0.19	0.68**	0.89**
LTSRPGT					1	0.94**	-0.10	0.01	0.89**	0.94**
PTSRPGT						1	0.01	0.13	0.88**	1.00**
RESR							1	0.87**	0.11	0.012
PESR								1	0.23	0.13
DSP/T									1	0.88**
SPE										1

NS = Not Significant; * Significant (P<0.05); **Very Significant (P<0.01); ***Highly Significant (P<0.0001)
RTSRPT =Right Testicular Sperm Reserve Per Testis, LTSRPT= Left Testicular Sperm Reserve Pe r Testis, PTSR=Paired Testicular Sperm Reserve, RTSRPGT=Right Testicular Sperm Reserve/g Testis, LTSRPGT=Left Testicular Sperm Reserve/g Testis, PTSRPGT=Paired Test icular Sperm Reserve/g Testis, RESR=Right Epididymal Sperm Reserve, LESR=Left Epididymal Spe rm Reserve, PESR=Paired Epididymal Sperm Reserve, DSP/T=Daily Sperm Production Per Testis, SPE=Sperm Production Efficiency

Discussion

The Japanese quail is primarily ground living specie which can be housed in rooms similar to garages (25). The live weight of the quails was not influenced by the physiological age examined. This could be attributable to the growth rate being almost similar in all the age groups of quails due to the fact that animals have reached matured life weight at puberty. The live weight recoded in this study for the groups of quails is consistent with the live body weight recorded by Arora (26), who observed that the live weight of laying quails established at 140-150g. The consistent live weight could also be attributed to the balanced diet and optimum utilization of the nutrient by the birds. This is corroborated by the finding of Akinola and Sese (27), who reported a consistently high weight of the female quails. Yildiz *et al*. (28) however, reported that growth and sexual maturity became delayed when animals were fed restricted diet.

Physiological age did not significantly influence the weight of the testes among the age groups. The finding in the current study is consistent with the findings of Li *et al*. (29) and Akinola and Sese (27). Age at sexual maturity has been suggested to be associated with high sperm production. This may be due to the fact that quails at the pubertal age have not been engaged in active mating as compared to mature and adult age. The significant influence of physiological age group on testicular sperm reserve is attributed to early sexual maturity (30). High testicular weight with a consequential increase in the blood concentration of testosterone and luteinizing hormone has been reported (31). Gonzalez-Moran and Soria-Castro (31) reported a positive correlation between high testosterone and luteinizing hormone production and increase in the ley dig cell volume, which in conjunction with sertoli cells are responsible for sperm production. Testicular weights have also been reported to have a high correlation with sperm reserves in the testes or epididymis and this is a direct reflection of testicular integrity for sperm production (32, 33).

The highly significant and very strong positive correlation observed between testicular and epididymal weight supports the high sperm reserve in the testis and epididymis. These organs play crucial function in spermatogenesis (34), maintenance of sperm maturation and development, and stimulation of optimal sperm movement such as rapid forward progression (33). Highly significant correlation existing between right and left testis volume and paired testes weight suggest that male quails have a high propensity for sperm production. The strong correlation observed for paired testicular sperm reserve per gram testis and paired testicular sperm reserve corroborates the findings of Etches (35), and Thurston and Kam (36). The weight of the testis has been established to have a significant relationship with the efficiency of sperm production and sperm reserves (32). This could be attributed to highly develop seminiferous tubules.

Highly significant correlation between daily sperm production efficiency and testicular sperm reserves suggested that the male quail's reproductive organs are actively functioning and thus are able to

perform spermatogenetic activities at an optimum level.

Conclusion and Application

1. In this study, the sperm storage potential and daily sperm production was influenced by the age of the quail bird.
2. Testicular and epididymal sperm reserve, daily sperm production and sperm production efficiency was better and enhanced in pubertal brown male Japanese quails among the age groups examined.
3. It was also discovered in this study that the storage potential and sperm production are age dependent.

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