

SEASONAL VARIATIONS IN SPERMATOGENIC ACTIVITY AND SERUM TESTOSTERONE CONCENTRATION IN JAPANESE QUAILS

Anas Sarwar Qureshi^{1,*}, Muhammad Usman Shafi², Zeeshan Akbar³ and Malik Zohaib Ali¹

¹Department of Anatomy, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan;

²Department of Biosciences, Faculty of Veterinary Sciences, Bahauddin Zakriya University, Multan, Pakistan;

³Department of Veterinary Biomedical Sciences, PMAS Arid Agriculture University, Rawalpindi, Pakistan.

*Corresponding author's e-mail: anas-sarwar@uaf.edu.pk

The present study investigates the influence of seasonal variations on testicular histomorphometric parameters of 15 adult male Japanese quails (*Coturnix japonica*) and testosterone concentrations over a year. The gross anatomical and histomorphometric study of both left and right testes was conducted. Serum testosterone concentration was measured by RIA technique. Statistical analysis revealed that the testicular weight and morphological parameters of testes including volume, length, width, thickness, and circumference were significantly ($P < 0.01$) higher during peak breeding season (June–July). In contrast, all morphologic and histomorphometric parameters were significantly ($P < 0.01$) lower during low breeding (Sept.–Oct.) and non-breeding season (Jan.–Feb). A significantly ($P < 0.01$) higher serum testosterone concentration was recorded during peak breeding season which showed a gradual decline during regressive breeding season and finally reached to minimum during non-breeding season. Serum testosterone concentration showed a strongly significant positive correlation ($r = 0.855$) with all studied testicular parameters, however a strongly negative correlation ($r = -0.819$) was ascertained between the relative humidity and serum testosterone concentration throughout the study period. It is concluded that Japanese quails demonstrate an annular reproductive cycle. The testosterone secretion clearly elevates and stimulates gonadal function to its peak in Japanese quails during summer season which gradually declines to bottom during harsh season of winter. It is conceivable that the pineal gland (melatonin hormone) responds to environmental influences, particularly changes in length of the daily photoperiod, and in turn exerts regulatory effects on the activity of the testis.

Keywords: Japanese quail, testis, breeding season, histology, testosterone

INTRODUCTION

Environmental factors are known to be important in controlling the reproduction, especially in animals and birds of the tropical zone, in which there exists a wide variation in light, rainfall, humidity and temperature. The climatic changes ultimately lead to physiological and behavioral alterations which are helpful for adjustments in different seasons for good survival and reproduction (González-Morán *et al.*, 2008; Ono *et al.*, 2009; Jalees *et al.*, 2011).

In the seasonal environments, variation of ambient temperature can be used as a secondary cue to predict future breeding of birds. Variations in testicular size and weight influenced by photoperiodic changes are species-specific, associated with differences in breeding phenology (Pitcher *et al.*, 2005; Garamszegi *et al.*, 2005).

In climatic conditions where the availability of food is relatively predictable and flexible, the supplementation of food can enhance testicular growth. So the food availability may also serve as a secondary cue for reproduction as well (Hau *et al.*, 2000; Hahn *et al.*, 2008). There is a typical response for gonadal growth in birds and breeding occur under long photoperiods and for the short days of winter

there is a decreased gonadotrophin secretion (Busso *et al.*, 2013).

In most of avian species, testes attain maximum development during the peak breeding season, followed by reproductive quiescence period during which testes do not produce sperms (Dawson and Sharp, 2007; Leska and Dusza, 2007). Like some other avian species, various aspects of quail behavior are influenced by photoperiod. One of the most important physiological effects of long day length is to stimulate gonadal growth and increase plasma sex steroids by stimulating gonadotrophin production and release. Therefore,

MATERIALS AND METHODS

A total of forty five pairs of testes from clinically healthy fully matured male Japanese quails of 16 weeks were obtained from the Avian Research and Training Station (ARTS), University of Veterinary and Animal Sciences (UVAS), Lahore, during three different phases of annular testicular cycle viz., peak breeding (June–July), low breeding (Sept.–Oct.) and non-breeding seasons (Jan.–Feb). Each seasonal group was comprised of 15 birds. The birds

were subjected to natural environmental conditions like natural sunlight, temperature, relative humidity and rainfall in each breeding season. The birds had full access to feed and clean drinking water all the time.

Data on ecological factors including temperature, relative humidity and rainfall for each season collected by the metrological center of Department of Agronomy University of Agriculture Faisalabad was used. Immediately before slaughtering, live body weight of birds was determined to the nearest milligram with electrical weighing balance. Following slaughter, blood samples were collected in test tubes from each bird without any anticoagulant agent to extract serum by centrifugation and stored at -20°C for measuring serum testosterone concentration till analysis. The testes from each bird were removed by laparotomy.

Different dimensions of testis (length, circumference, thickness and width) and weight were recorded in nearest centimeters (cm) and grams (g) with the help of Vernier's caliper and electric weighing balance. The volume of testes was estimated by water displacement method with the help of graduated measuring cylinder.

Following collection, tissues were put into Bouin's solution for 72 hours. After fixation, the tissues were dehydrated, infiltrated, embedded in paraffin blocks, sectioned and mounted. For histological studies sections of 5µm thick were obtained and stained with haematoxylin and eosin (H&E) stain. Percentage area of interstitial cells and diameter of the seminiferous tubules, germinative epithelium thickness and diameter of lumen of the seminiferous tubules of testis of each bird was determined at 200X using Image J® Analysis System following Akbar *et al.*, (2012).

The serums were extracted from each blood samples for serum testosterone concentration analysis. The serum was collected with help of centrifugation 1500 rpm for 10 minutes and was stored at -20°C till analysis. The serum concentrations of testosterone hormone in all birds were

estimated by using (radioimmunoassay RIA kit obtained from the IMMUNOTECH® at NIAB Faisalabad. The sensitivity of the testosterone assay was 0.1/ml (defined as the lowest). The inter- and intra-assay coefficient of variances (CVs) of 10 and 15% for reference sera.

Statistical analysis: Mean ± standard error means (SEM) and ranges were calculated for each parameter under study with the help of computer software Microsoft Excel. The means of parameters were compared with one way analysis of variance (ANOVA). Group means were compared with help of Least Significance Difference (LSD) test. The concentration of significance were 1 and 5% concentrations.

RESULTS

The mean (±SEM) of all these gross anatomical parameters of right and left testis were recorded for peak breeding (June –July), low breeding (Sept.-Oct.) and non-breeding (Jan.-Feb.) seasons. Statistical analysis revealed that highly significant (P<0.0.1) greater values of the gross parameters including (absolute and relative weights, volume and various dimensions including length, width, thickness and circumference) for both right and left testis during peak breeding season (June –July) as compared to low and non-breeding seasons. All gross parameters of the testis showed significantly (P<0.01) lower values during non-breeding season (Sept.-Oct.) as compared to low breeding season (Jan.-Feb) as depicted in Table 1.

There was no significant difference of all gross anatomical parameters observed between right and left testis in each breeding season of the year. The mean (±SEM) of all these gross anatomical parameters and dimensions of right and left testis are presented in Table 1.

The histomorphometric evaluation of the diameter of seminiferous tubules, germinative epithelium, lumen diameter of seminiferous tubules and percent area of

Table 1. Gross anatomical and hitomorphometric parameters of testes of Japanese quail (*Coturnix japonica*) during different reproductive breeding seasons of the year (means ± SEM).

Testicular Parameters	Breeding Seasons					
	Peak Breeding		Low breeding		Non-breeding	
	Right Testis	Left Testis	Right Testis	Left Testis	Right Testis	Left Testis
Absolute Weight (g)	4.27±0.330A	4.53±0.370A	1.47.±0.28B	1.560±0.30B	0.810±0.42C	0.780±0.42C
Relative weight (g/100g)	3.50±0.255A	3.27±0.290A	1.065±0.32B	1.114±0.28B	0.586±.253C	0.565±0.24C
Length (cm)	2.85±0.090A	2.67±0.100A	1.950±0.15B	1.78±0.120B	0.11±0.010C	0.11±0.010C
Thickness (cm)	1.68±0.049A	1.73±0.049A	1.05±0.078B	1.17±0.090B	0.50±0.030C	0.51±0.027C
Width (cm)	1.72±0.045A	1.81±0.058A	1.08±0.077B	1.21±0.094B	0.51±0.025C	0.53±0.026C
Volume (cm3)	4.28±0.263A	4.41±0.323A	1.60±0.227B	1.74±0.266B	0.18±0.013C	0.18±0.015C
Circumference (cm)	5.20±0.183A	5.50±0.200A	3.41±0.289B	3.76±0.307B	1.66±0.069C	1.65±0.095C
Diameter of seminiferous tubules (µm)	205.94A	199.07±70.4A	142.8±4.70B	139.3±16.1B	101.2±13.3C	99.5±11.63C
Area of Interstitial tissue (%)	8.95C	8.47±1.19C	19.25±2.02B	19.16±2B	36.32±4.28A	36.37±4.30A
Germinative Epithelium Thickness (µm)	67.58A	64.78A	48.786B	47.897B	38.345C	37.865C
Lumen of seminiferous tubules (µm)	3.85A	3.67A	1.95B	1.89B	0.98C	0.86C
Serum testosterone conc. (ng/ml)	2.31±0.028A		0.58±0.019B		0.23±0.08C	

interstitial tissue revealed a cyclic annual variation during each breeding season of the year. The diameter of seminiferous tubules, germinative epithelium and lumen diameter of seminiferous tubules were significantly ($P<0.01$) higher during peak breeding, as compared to all other breeding seasons of the year. Their values then decreased progressively and highly significant ($P<0.01$) lower values were observed during low breeding season and lowest values recorded during non-breeding season of the year. The percent area of interstitial tissue was lower significantly ($P<0.01$) during the peak breeding season than increased during regressive breeding season and ultimately reached its significantly ($P<0.01$) higher values at non-breeding season, showing a reverse relation with diameter of seminiferous tubules in the testicular parenchyma as indicated in (Table 1, Fig. 1-3).

The mean serum testosterone hormones concentration during peak breeding season was as 2.31 ± 0.028 ng/mL, during the low breeding season was as 0.58 ± 0.019 ng/mL and during the non-breeding season was as 0.23 ± 0.008 ng/mL. The comparison of the serum testosterone hormone concentrations among different breeding seasons showed that testosterone hormone concentration was statistically significantly ($P<0.01$) higher in the peak breeding season as compared to other seasons of the year. The significantly lowest values ($P<0.01$) were observed in the non-breeding season as compared to low breeding season in Japanese quail (*Coturnix japonica*).

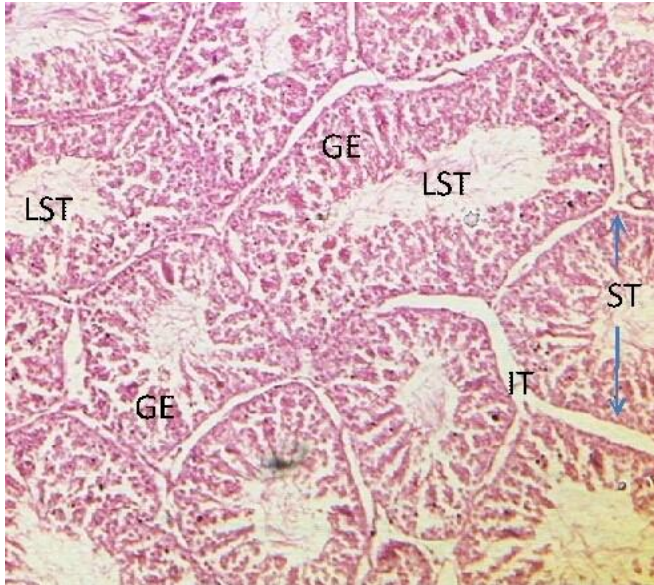


Figure 1. Photomicrograph of testis during peak breeding season showing maximum spermatic activity and seminiferous tubules having larger diameter (ST), germinative epithelium (GE) and lumen of seminiferous tubules (LST), and

small extent of interstitial tissue (IT). (H&E; 200X).

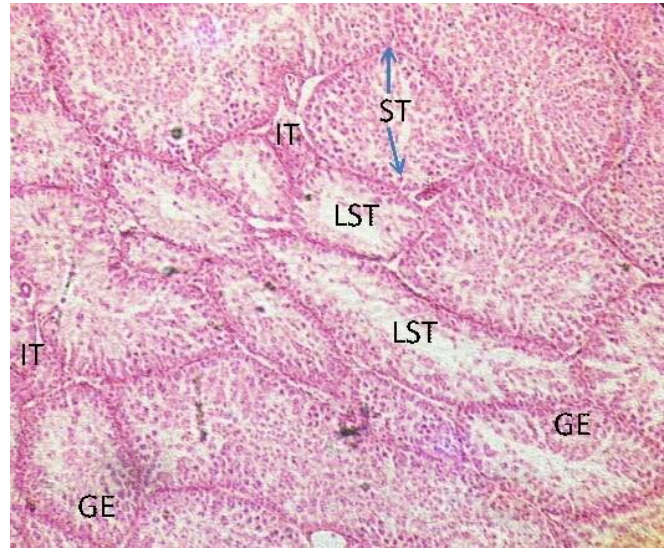


Figure 2. Photomicrograph of testis during low breeding season showing less spermatic activity and decreased diameter of tubules (ST), germinative epithelium (GE) and lumen of seminiferous tubules (LST), along with interstitial tissue (IT). (H&E; 200X).

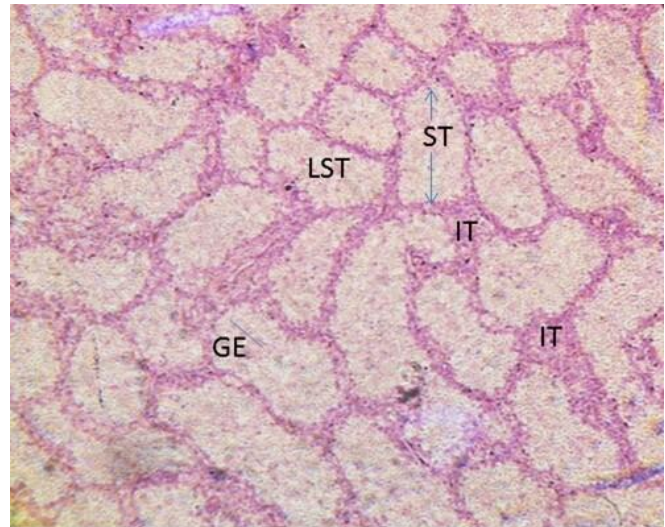


Figure 3. Photomicrograph of testis during non-breeding season showing diameter of tubules (ST), germinative epithelium (GE) and decreased lumen of seminiferous tubules (LST) and increased interstitial tissue (IT). (H&E; 200X).

DISCUSSION

Present study revealed that gross anatomical parameters like weight, absolute weight, length, circumference, thickness, volume and width of left and right testes were significantly ($P<0.01$) higher during the peak breeding season which followed a progressive decline in the low/regressive breeding season and reached to bottom line in the non-breeding season. A similar trend was recorded in Indian jungle bush quail (Haldar and Singh, 2001), rose ringed parakeets (*Psittacula krameri*) (Maitra and Day, 1993) and New Zealand birds (Cockrem, 1995). The gland's weights were observed higher in the reproductive active phase as described by Sudhakumari *et al.* (2001). The weights were decreased during the gonads regression during the reproductive regressive phases as reported by Sudhakumari and Haldar (2001). The breeding period influences by multiple climatic factors such as day length, temperature, food availability, mates in appropriate physiological condition, and a suitable breeding habitat (Haldar and Singh, 2001). Of these factors, environmental changes have been shown in many cases to be of more importance (Wingfield, 2007). Statistical analysis revealed a significant ($P<0.01$) rise in the seminiferous tubule diameters, germinative epithelium and lumen diameter of seminiferous tubules during the peak breeding season as compared with the low and the non-breeding season, while there exist a reverse pattern of percentage area of interstitial tissue adjacent to seminiferous tubules in testicular parenchyma. These results are comparable with Baraldi-Artoni *et al.* (1997, 1999) who studied these parameters in domestic quail. Serum testosterone concentrations were found significantly ($P<0.01$) higher during the peak breeding season which decreased during the low breeding season and declined to the baseline during the non-breeding season. In addition, the interrelationship between the values of hormonal profile and gonadal activity remained strongly positive, however a strongly negative correlation ($r=-0.819$) was determined between hormonal gonadal profiles and metrological parameters. These results are in line with previous reports in Indian jungle bush quail (Haldar and Singh, 2001) and mammals (Lochmiller and Ditchkoff, 1999) as they observed increased weight of testes and plasma testosterone concentration during summer i.e. long photoperiod followed by minimum concentrations during short photoperiod. In fact, the pineal gland responds to environmental influences, particularly changes in length of the daily photoperiod, and in turn exerts regulatory effects on the activity of the testis. This relation is much more sensitive where lack of adequate illumination stimulates pineal antigonadal activity to produce inhibition of both testicular gametogenesis and androgenesis. The evidence indicates that pineal function in the quail is also primarily related to the regulation of testicular endocrine function. Consequently, its physiological role may be associated with seasonal changes in libido in relation to environmental influences, by virtue of

the action of pineal factors on androgen status. Pineal factors influence testicular function by interaction with the neuroendocrine system to affect pituitary gonadotropin secretion.

It is conceivable from the above findings that during summer season testosterone secretion clearly elevates and stimulates gonadal function to its peak in Japanese quails whereas it gradually reverses during harsh season of winter.

REFERENCES

- Akbar, Z., A.S. Qureshi and S.U. Rahman. 2012. Effects of seasonal variation in different reproductive phases on the cellular response of bursa and testes in Japanese quail (*Coturnix japonica*), Pakistan. Pak. Vet. J. 32:525-529.
- Baraldi-Artoni, S.M.B., A.M. Orsi, T.L.L. Carvalho and R.A. Lopes. 1997. The annual testicular cycle of the domestic quail (*Coturnix coturnix japonica*). *Anatomia Histologia. Embryolog.* 26:337-339.
- Baraldi-Artoni, S.M.B., A.M. Orsi, T.L.L. Carvalho, C.A. Vicentini and M.A. Stefanini. 1999. Seasonal morphology of the domestic quail (*Coturnix coturnix japonica*) testis. *Anat. Histolog. Embryolog.* 28:217-220.
- Boon, P., H. Visser and S. Daan. 2000. Effect of photoperiod on body weight gain, and daily energy intake and energy expenditure in Japanese quail (*Coturnix c. japonica*). *J. Physiol. Behavior.* 70:249-260.
- Busso, J.M., M.F. Dominchin, R.H. Marin and R. Palme. 2013. Cloacal gland, endocrine testicular, and adrenocortical photoresponsiveness in male Japanese quail exposed to short days. *Domestic Animal Endocrinol.* 44:151-156.
- Cockrem, J.F. 1995. Timing of seasonal breeding in birds, with particular reference to New Zealand birds. *Reprod. Fertil. Dev.* 7:1-19.
- Dawson, A. and P.J. Sharp. 2007. Photorefractoriness in birds-photoperiodic and non-photoperiodic control. *Gen. Comp. Endocrinol.* 153:378-384.
- Garamszegi, L.Z., M. Eens, S. Hurtrez-Bousses and A.P. Moller. 2005. Testosterone, testis size, and mating success in birds: a comparative study. *Horm. Behav.* 47:389-409.
- González-Morán, M.G., C. Guerra-Araiza, M.G. Campos and I. Camacho-Arroyo. 2008. Histological and sex steroid hormone receptor changes in testes of immature, mature, and aged chickens. *Domestic Animal Endocrinol.* 35:371-379.
- Haldar, C. and S.S. Singh. 2001. Melatonin and immunological functions/ expression by the bursa of Fabricius in Indian jungle bush quail *Perdica asiatica*. *Avian Endocrinol.* 1:427-435.
- Hahn, T.P., J.M. Cornelius, K.B. Sewall, T.R. Kelsey, M. Hau and N. Perfito. 2008. Environmental regulation of

- annual schedules in opportunistically breeding songbirds: adaptive specializations or variations on a theme of white-crowned sparrow? *Gen. Comp. Endocrinol.* 157:217-226.
- Hau, M., M. Wikelski and J.C. Wingfield. 2000. Visual and nutritional food cues fine-tune timing of reproduction in a neotropical rainforest bird. *J. Exp. Zool.* 286:494-504.
- Jalees, M.M., M.Z. Khan, M.K. Saleemi and A. Khan. 2011. Effects of cottonseed meal on hematological, biochemical and behavioral alterations in male Japanese quail (*Coturnix japonica*). *Pak. Vet. J.* 31:211-214.
- Leska, A. and L. Dusza. 2007. Seasonal changes in the hypothalamo-pituitary-gonadal axis in birds. *Reprod. Biol.* 7:99-126.
- Lochmiller, R.L. and S.S. Ditchkoff. 1999. Environmental influence on mass dynamics of the cotton rat (*Sigmodon hispidus*) thymus gland. *Biol. Rhythm Res.* 9:206-212.
- Maitra, S.K. and M. Dey. 1993. Cytological studies of the pineal in relation to the annual cycle in the testis and climatological variables of free living rose ringed parakeets (*Psittacula krameri*). *J. Int. Cyc. Res.* 24:43-55.
- Ono, H., N. Nakao and T. Yoshimura. 2009. Identification of the photoperiodic signaling pathway regulating seasonal reproduction using the functional genomics approach. *Gen. Compar. Endocrinol.* 163:2-6.
- Pitcher, T.E., P.O. Dunn and L.A. Whittingham. 2005. Sperm competition and the evolution of testis size in birds. *J. Evol. Biol.* 18:557-567.
- Sudhakumari, C.C. and C. Haldar. 2001. Effects of photoperiod alterations on adrenocortical, pineal and gonadal activity in nocturnal bird, *Athene brama* and diurnal bird, *Perdicula asiatica*. *Zool. Sci.* 18:71-79.
- Sudhakumari, C.C., C. Haldar and B. Senthilkumaran. 2001. Seasonal changes in adrenal and gonadal activity in the quail, *Perdicula asiatica*: involvement of the pineal gland. *Compar. Biochem. Physiol.* 128:793-804.
- Wingfield, J.C. 2007. Organization of vertebrate annual cycles: implications for control mechanisms. *Phil. Trans. R. Soc. B.* 10:21-49.
- Zann, R.A., S.R. Morton, K.R. Jones and N.T. Burley. 1995. The timing of breeding by zebra finches in relation to rainfall in central Australia. *Emu.* 95:208-222.