

## CHANGES IN TESTICULAR HISTOMORPHOLOGY AND SERUM TESTOSTERONE CONCENTRATION OF HELMETED GUINEA FOWL (*NUMIDA MELEAGRIS*) DURING DIFFERENT REPRODUCTIVE PHASES IN PAKISTAN

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### ABSTRACT

The objective of this study was to peruse the annular variations in testicular histomorphology and serum testosterone of guinea fowl (*Numida meleagris*) during different reproductive phases *viz.*, resting, progression and peak breeding in Pakistan. Thirty healthy male birds were slaughtered: samples of testes were stained with H&E and histometric analysis was made with Image J<sup>®</sup>. Serum testosterone was measured by radioimmunoassay. Results revealed significantly ( $P<0.01$ ) greater values of weight, volume, length, width, thickness and circumference of testes during peak breeding as compared to other phases. Histometric parameters like diameter of seminiferous tubules and its lumen, thickness of germinal epithelium and diameter of leydig cells showed significantly ( $P<0.01$ ) higher values during peak breeding than other phases. Conversely, thickness of testicular capsule and percentage area of interstitial cells were significantly ( $P<0.01$ ) higher during resting in contrast to progression and peak phases. Serum testosterone showed significantly ( $P<0.01$ ) higher value ( $4.60\pm 0.44$ ) during peak breeding which declined significantly ( $P<0.01$ ) in progression ( $1.73\pm 0.19$ ) and resting ( $0.62\pm 0.07$ ) phases. Moreover, all histomorphometric changes were positively correlated while percentage area of interstitial cells and thickness of testicular capsule were negative with hormonal profile during each phase. In conclusion, different reproductive phases influence annular testicular histomorphology and hormonal profile. Peak breeding activity of this bird is coincided with the increased steroid hormone synthesis under suitable circumstances.

**Key words:** Guinea fowl, histomorphology, seminiferous tubule's epithelium, serum testosterone, testicular cycle.

### INTRODUCTION

Considering the high nutritive value of guinea fowl meat and eggs in comparison to other domestic fowls (Moreki and Seabo, 2012), its commercialization has expanded in many countries like United States, France and Belgium (Nahashon *et al.*, 2006). In Pakistan, guinea fowl is raised for being a decorative pet and meat and egg producing purposes in villages and towns (Khan, 2004).

Seasonal changes are caused by ecofactors like light (photoperiod), temperature, rainfall, humidity etc. These eco-factors are known significantly important in controlling the reproduction in animals and birds especially of the tropical zone where there exists a wide variation in them. Birds have a highly sophisticated mechanism to predict seasonal changes which ultimately leads to the physiological and behavioral alterations helpful for their adjustment in different seasons for good survival and reproduction (Jalees *et al.*, 2011). Among different factors, photoperiod plays a vital role in synchronization of the reproductive activity (testicular growth, spermatogenesis and plasma sex steroid synthesis) through neuroendocrine system (gonadotropin

production) with suitable environment in seasonal breeders (Boon *et al.*, 2000).

In view of germ cell transfer technique and production of transgenic progeny, the use of spermatogenesis is of great importance (Dobranski, 2005). Many studies have been conducted to see the influence of photoperiod on the reproductive functions of male birds in view of their economic interest. Earlier work described the germ cell morphology, spermeogenesis, seminiferous epithelium and sertoli cell differentiation in chicken and duck (Gunawardana, 1977, Aire *et al.*, 1980). Recent work describes that seminiferous epithelium and duration of meiosis are same in turkey, chicken and guinea fowl (Noirault *et al.*, 2006). However, limited information is available about the testicular cycle of this bird along with the histological changes occurring inside the gonads in response to ecofactors.

The purpose of this study was to elucidate histomorphological changes in testes and serum testosterone of male helmeted guinea fowl (*Numida meleagris*) during different phases of its annular testicular cycle. In addition, interrelationship between histomorphometrical indices and serum testosterone values was ascertained.

## MATERIALS AND METHODS

**Experimental design:** A total of thirty healthy male helmeted guinea fowls (*Numida meleagris*) at age of 24-28 weeks having 1Kg average body mass were obtained from the backyard poultry houses in Faisalabad during annual reproductive cycle. The reproductive cycle consists of resting phase (December-January), progression phase (March-April) and peak breeding phase (June-July). The selected birds were reared under natural environmental conditions of sunlight, temperature, humidity and rainfall at open poultry house of Faculty of Veterinary Science, University of Agriculture Faisalabad. The birds had full access to feed and clean drinking water *ad-libitum*. Meteorological data during the experimental period was obtained from Climatology laboratory, University of Agriculture Faisalabad.

**Collection of Samples:** Ten birds in each reproductive phase were slaughtered to obtain samples of testes. Five ml blood was collected from each bird without anticoagulant agent to extract serum for estimation of testosterone by Radioimmunoassay (RIA) using a commercially available test kit (IMMUNOTECH®, Beckman Coulter Company, USA). Analytical sensitivity of the test was up to 0.025ng/ml. The antibody used in the immunoassay was highly specific for testosterone and measurement range was 0.025-20ng/ml. The inter- and intra assay CVs were 9 and 13.5% for reference.

**Histomorphometric Analysis:** Morphological characteristics including length, width, thickness and circumference of testes were recorded using a Vernier's caliper. The weights were determined by using an electrical weighing balance. The volume was measured by water displacement method. Testicular tissues were cut, washed and fixed in Bouin's solution. Slides were prepared by paraffin tissue preparation technique (Bancroft *et al.*, 2008). Histological measurements like thickness of testicular capsule and seminiferous epithelium, diameter of seminiferous tubules and its lumen, diameter and percentage area of interstitial cells in each testis were measured with the help of automated image analysis system Image J®, version 1.46 (Research Services Branch, NIMH, Bethesda, Maryland, USA). Percentage area of interstitial cells in relation to seminiferous tubules of each testis was measured at 200X

while leydig cell diameter at 1000X using Nikon Optiphot 2 microscope, Japan. Only round tubules of perfectly clear transverse section were measured for diameter of seminiferous tubules. Histomorphometrical changes were correlated with hormonal profile during each phase.

**Statistical analysis:** The means of parameters were compared with one way analysis of variance (ANOVA). Group means ( $\pm$ SEM) were compared with least significance difference (LSD) with level of significance at  $\leq 0.05$ . The correlation between histomorphometric parameters and serum testosterone was measured by Pearson's Correlation Sig. (2-tailed) method.

## RESULTS

**Morphological variations:** Statistical analysis revealed that reproductive phases significantly ( $P < 0.01$ ) affected the testicular morphology. The highest values of testicular weight, volume, length, width, thickness and circumference were found during peak breeding phase but these values declined significantly ( $P < 0.01$ ) during progression and resting phases (Table 1; Fig. 1). Right and left testis showed a non-significant difference in all morphological variations.

**Histological variations:** Histological variations including diameter of seminiferous tubules and its lumen, thickness of germinal epithelium and diameter of leydig cells showed significantly ( $P < 0.01$ ) higher values during peak breeding phase as compared to progression and resting phases. However, thickness of testicular capsule and percentage area of interstitial cells showed a reverse trend with significantly ( $P < 0.01$ ) highest values found during resting phase (Table 2; Fig. 2).

**Hormonal profile:** Serum testosterone analysis depicted significantly ( $P < 0.01$ ) higher value during peak breeding phase and this value showed significantly ( $P < 0.01$ ) declining trend from progression to resting phase (Table 2).

Furthermore, all histomorphological variations depicted a positive correlation with reproductive phases and hormonal profile except thickness of testicular capsule and percentage area of interstitial cells which were negatively correlated.

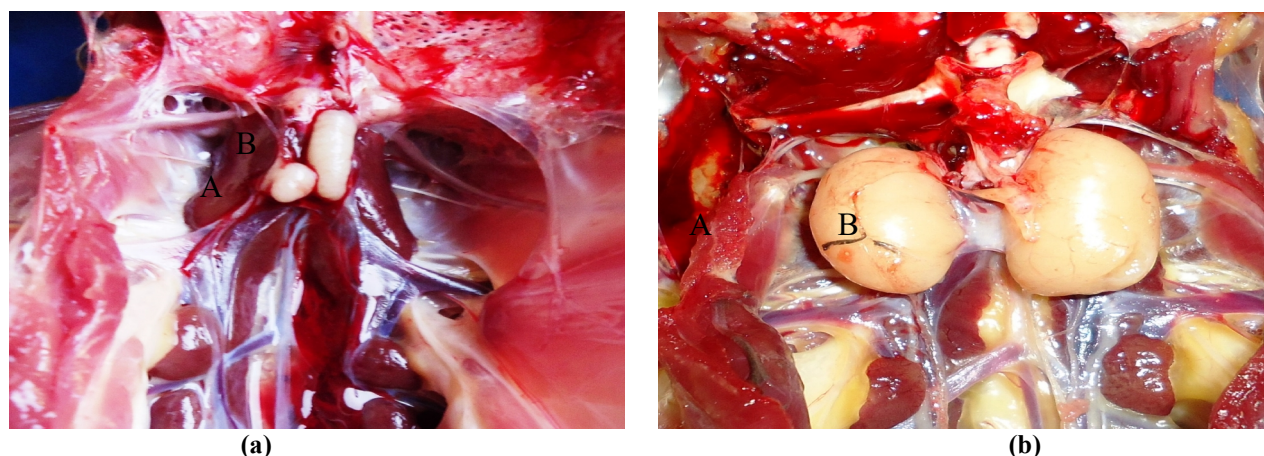
**Table 1. Morphometric parameters of guinea fowl (*Numida meleagris*) in different reproductive phases of annular testicular cycle in Pakistan.**

Parameter	Peak breeding phase	Progression phase	Resting phase
Weight (g)	1.39±0.157 <sup>a</sup>	0.58±0.064 <sup>b</sup>	0.18±0.023 <sup>c</sup>
Volume (cm <sup>3</sup> )	1.47±0.175 <sup>a</sup>	0.67±0.075 <sup>b</sup>	0.24±0.019 <sup>c</sup>
Length (cm)	1.69±0.077 <sup>a</sup>	1.53±0.084 <sup>b</sup>	1.08±0.062 <sup>c</sup>
Width (cm)	1.34±0.085 <sup>a</sup>	0.86±0.043 <sup>b</sup>	0.62±0.036 <sup>c</sup>
Thickness (cm)	0.97±0.058 <sup>a</sup>	0.72±0.053 <sup>b</sup>	0.49±0.030 <sup>c</sup>
Circumference (cm)	3.42±0.162 <sup>a</sup>	2.71±0.184 <sup>b</sup>	1.76±0.122 <sup>c</sup>

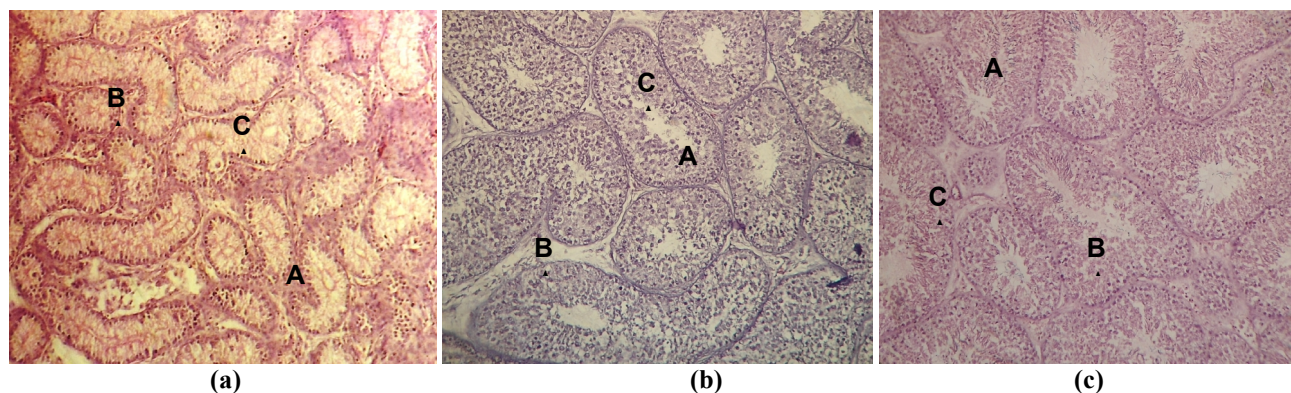
**Table 2. Histometric parameters of guinea fowl (*Numida meleagris*) in different reproductive phases of annular testicular cycle in Pakistan.**

Parameter	Peak breeding phase	Progression phase	Resting phase
Diameter of seminiferous tubules (µm)	1245.9±59.19 <sup>a</sup>	598.7±61.16 <sup>b</sup>	358.5±36.19 <sup>c</sup>
Lumen diameter of seminiferous tubules (µm)	741.8±41.10 <sup>a</sup>	258.5±20.00 <sup>b</sup>	99.6±9.30 <sup>c</sup>
Percent area of interstitial cells	16.90±0.80 <sup>c</sup>	34.63±1.80 <sup>b</sup>	56.26±1.45 <sup>a</sup>
Thickness of germinal epithelium (µm)	686.7±40.51 <sup>a</sup>	331.6±30.60 <sup>b</sup>	152.7±16.49 <sup>c</sup>
Diameter of leydig cells (µm)	30.7±01.67 <sup>a</sup>	14.9±0.51 <sup>b</sup>	9.5±0.48 <sup>c</sup>
Thickness of testicular capsule (µm)	6.03±0.48 <sup>b</sup>	6.23±0.48 <sup>b</sup>	8.21±0.57 <sup>a</sup>
Serum testosterone level (ng/ ml)	4.60±0.44 <sup>a</sup>	1.73±0.19 <sup>b</sup>	0.62±0.07 <sup>c</sup>

Mean (±SEM) values bearing the same superscripts in a row do not differ significantly (P<0.01)



**Figure 1. Photograph of testis during (a) resting phase showing less volume (b) peak breeding phase showing more volume of both right – A and left testes – B**



**Figure 2. Photomicrograph of testis during (a) resting phase (b) progression phase (c) peak breeding phase: showing A-Lumen of Seminiferous tubule B-Interstitial tissue C-Germinal epithelium. HE; X200.**

## DISCUSSION

In present study some aspects of reproductive behavior of adult male helmeted guinea fowl were studied under the effect of seasonal photoperiodicity. Long days stimulated testicular growth and increased plasma testosterone by stimulating gonadotropin production as described by Boon *et al.*, 2000. Different results have been reported in the literature about the testicular kinetics of young/ adult birds submitted to natural/ artificial lighting conditions or investigated for testicular growth, sperm production and other histomorphometric parameters of testes. In this project, investigation of quantitative parameters of helmeted guinea fowl permitted us to define an annual testicular cycle with three distinct successive phases similar to those reported by Akbar *et al.*, 2012; Shil *et al.*, 2015 in Japanese quail (*Coturnix japonica*) and Islam *et al.*, 2010 in Jungle crow (*Corvus macrorhynchos*).

Morphometric studies including weight, volume, length, width, thickness and circumference of testes showed sharp variations over the year. Results revealed significantly highest values ( $P<0.01$ ) in peak breeding which declined significantly ( $P<0.01$ ) in progression and resting phases as described by findings of Ali *et al.*, 2015; Hien *et al.*, 2011 in Guinea fowl; Shil *et al.*, 2015 in Japanese quail; Madhu and Manna, 2009 in domestic pigeon. The increased diameter of seminiferous tubules contributed to increase values of morphological parameters. Moreover, left testis was seen non-significantly heavier than the right testis in 80% of the birds. The basis for testicular asymmetry remains unknown but may be due to an unequal number of primordial germ cells incorporated into the embryonic gonads (Tyler and Gous, 2008).

Histological variations in seminiferous tubule parameters (total diameter, lumen diameter and thickness of seminiferous epithelium) and diameter of leydig cells showed significantly ( $P<0.01$ ) higher values in peak breeding phase than other phases. However, thickness of testicular capsule and percentage area of interstitial cells showed a reverse trend with significantly ( $P<0.01$ ) highest values in resting phase. The development of seminiferous tubules and regression of interstitial cells are under the control of increased secretion of gonadotropins releasing hormone (GnRH) and a mechanism of timing in brain of the birds control the reproduction by starting gonadal developments under the influence of photoperiod. These results are in accordance with the studies of Akbar *et al.*, 2012; Shil *et al.*, 2015 in Japanese quail; Islam *et al.*, 2010 in Jungle crow. Right and left testis showed a non-significant difference in all histological variations.

Results of serum testosterone analysis indicated a positive correlation with the reproductive phases. The value was found significantly ( $P<0.01$ ) higher in peak

breeding phase ( $4.60\pm 0.44$  ng/ ml) with the declining trend ( $P<0.01$ ) in progression phase ( $1.73\pm 0.19$  ng/ ml) and resting phase ( $0.62\pm 0.072$  ng/ ml). These results were strongly supported by Ali *et al.*, 2015 in *Numida meleagris*; Yadav *et al.*, 2011, Yadav and Halder 2013 in *Perdica asiatica*; Bharucha and Padate, 2009 in house sparrow *Passer domesticus*. Photoperiod (long days) influences the rate of biosynthesis of gonadotropin hormones, which in turn act on the testes to promote more spermatogenic activity and elevated serum testosterone level (McGuire *et al.*, 2011).

**Conclusion:** Present data revealed a substantial variation in testicular histomorphology and serum testosterone level in guinea fowl over the year under the seasonal effect. It is conceivable from these findings that during peak breeding phase elevated level of testosterone helps seasonal reproducing birds to adapt their physiology for maximum sexual activity.

**Authors' contribution:** ASQ designed the project, supervised lab work and finalized manuscript. MHS, MZA and RK performed laboratory sampling, statistical analysis and prepared preliminary write up.

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