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RESEARCH ARTICLE

Effects of Seasonal Variation in Different Reproductive Phases on the Cellular Response of Bursa and Testes in Japanese Quail (*Coturnix japonica*)

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ABSTRACT

In this study effects of seasonal variation on the cellular response of bursa and testes in Japanese quail (*Coturnix japonica*) have been studied. Thirty mature male Japanese quail, 10 birds in each reproductive phase, namely reproductive active phase, regressive phase and non-active phase were sacrificed. Total leukocyte and lymphocyte counts were performed and serum testosterone levels were measured. The macroscopic and microscopic dimensions of testes and bursa of Fabricius were measured. Automated computer software Image J® was used for histometric analysis. The results revealed that morphometrical parameters such as the length, circumference, thickness, and width of the testis showed a variable pattern in different seasonal phases e.g. in the active phase they were significantly ($P<0.01$) larger and decreased at regular pattern in the regressive and the non active phase. In contrast the immune parameters were significantly ($P<0.01$) at higher levels during the non active phase and the regressive phase and decreased at regular pattern in the active phase. The serum testosterone levels were also high during the active phase and decreased at regular pattern in the regressive and the non active phase. The histometric analysis revealed similar results such as diameters of seminiferous tubules showing a significant ($P<0.01$) rise during the active phase as compared with the regressive and the non active phase, and a significant declined was record during the regressive and the non active phase. In conclusion, the increased reproductive activity in Japanese quail coincided with enhanced steroid hormone production during summer which suppressed the immune response. Moreover, during the winter decreased gonadal activity resulted in elevated immune function that helped to combat the harsh environment.

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INTRODUCTION

In quail farming, the reproductive and immune status of birds is of prime importance to obtain good production. Both are interrelated to each other and modulated by seasonal variations in Japanese quail (*Coturnix japonica*) because of its seasonal breeding. It is evident from literature that there exist a strong inter-relationship between the neuroendocrine and immune systems, and a functional correlation between the lymphoid organs and gonads. The birds alter their physiology and behavior according to photoperiods (day's lengths) which are helpful for adjustments for climatic effects in different seasons (Ono *et al*., 2009; Jalees *et al*., 2011).

Environmental factors such as changes in light durations rise and fall in temperatures, changes in relative humidity, i.e. during an annual cycle, modulate the immune system. The photoperiod and temperature play a significant role in the modulation of lymphoid organs such as the spleen and thymus and immune parameters, such as total leukocyte and lymphocyte counts in animals (Bilbo *et al*., 2003). The brighter light intensity could improve health status and provide opportunities for more normal behavioral rhythms (Blatchford *et al*., 2009).

The birds respond to change in day-length for adaptation to seasonal variation in their climate. The day-length induce thyrotropin in the pars tuberalis of the pituitary gland act as a major factor modulating reproductive activity by GnRH secretion from the hypothalamus in seasonal breeders (Ikegami K and T Yoshimura, 2012). Gonadotrophin-releasing hormone-1 and Kisspeptin neurons in birds and mammals respectively, show major modulation in expression that is positively associated with breeding state (Stevenson and Ball, 2011).

The seasonal fluctuation in temperature and light duration lead to changes in hormone levels, which are needed in metabolic activities that lead to proper management for a particular season. This alteration may be due to hormones such as melatonin and steroidal hormones of the gonads. The long photoperiod and high concentration of steroids from gonads in circulation cause modulation in reproductional activities in birds and lower the immunological parameters (Schuurs and Verheul, 1990; Singh and Haldar, 2005).

The birds have different aspects of immunity in respect of the organs, the cells, or the molecular and the gene levels of immunity although they are found in the same regions as occupied by the other animals (Kaiser *et al*., 2009). In birds there are special responses for the development of gonads and the reproductive activities occur during the longer photoperiod or long days in summer. In contrast, the reproductive activities decrease during the shorter photoperiod or days in winter. This could be due to decreased secretion of gonadotropins releasing hormone (GnRH) and a mechanism of timing in brain of the birds control the reproduction by starting gonadal developments in the influence of photoperiod (Nicholls *et al.*, 1988; Halawani *et al*., 2009).

The bursa of Fabricius is a primary lymphoid organ which plays an important role in the in the maturation of the B cells (Glick, 1956). It has a distinct anatomical structure and regulates the total leukocyte and lymphocyte count through B-cell differentiation and proliferation (Glick, 1995).

The lymphocytes produced in the bursa of Fabricius consist of antibody producing B-cells. The mature B-cells are transported via the circulating blood to the secondary lymphoid organs, where they encounter and respond to foreign antigen, thus regulating the humoral immune response (Singh, 2000).

MATERIALS AND METHODS

Experimental design: A total of 30 clinically healthy male Japanese quail (*Coturnix japonica*) 14 weeks of age having an average body weight of 140 g were obtained from the Avian Research and Training Center of University of Veterinary and Animal Science, Lahore. The birds were kept for two weeks in a shed, so the birds were subjected to natural environmental conditions such as natural sunlight, temperature, humidity and rainfall in summer (reproductive active phase), autumn (regressive phase) and winter (non active phase). The birds had full access to feed and water all time of keeping.

Parameters studied: Meteorological data of each season was collected from the Climatology Laboratory, University of Agriculture, Faisalabad, Pakistan. Ten birds in each season were sacrificed with a sharp metallic knife. Blood samples were taken from each bird at slaughter in two different test tubes. Blood with anticoagulant (EDTA) was used to perform a total leukocyte count (TLC) and lymphocyte count (LC) by Medonic M series (MERCK, Stockholm, Sweden). Blood with without anticoagulant was subjected to extract serum which was used for serum testosterone measurement by RIA (Radioimmunoassay) using a commercially available test kit (IMMUNOTECH, Marseille, France).

After slaughtering, the testes and bursa of Fabricius were weighed. Testis volume was determined by the water displacement technique. The length, circumference, thickness and width of both testis and bursa of Fabricius were measured in centimeters using Vernier's caliper.

The specimens of testes and bursa of Fabricius were washed with normal saline solution. Tissue samples of 3-5 mm thickness of testes and bursa of Fabricius were fixed in Bouin's solution and 10% buffered formaldehyde. Then specimens were washed in running tap water for a period of 6 hours to remove the fixative. The specimens of testes and bursal tissues were processed by the paraffin tissue preparation technique. After slide preparation, the diameter of seminiferous tubules of each testis was measured (µm) with the help of automated image analysis system Image J® version 1.43n (Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA).

Statistical analysis: The results obtained were subjected to one way analysis of variance (ANOVA). Group means were compared with help of least significance difference (LSD) test and differences were considered significant at $P \leq 0.05$.

RESULTS

Gross anatomical parameters: The mean length, circumference, thickness, volume, weight and width of the left and right testis during each season (reproductive phase) are given in table 1. The comparison of left and right testicular length, circumference, thickness, volume, weight and width with reproductive phases showed that they were statistically significantly ($P<0.01$) higher in the summer (reproductive active phase) and gradually declined in autumn (regressive phase) and winter (non active phase), and were statistically not significantly different in the left and right testis (Fig. 1A).

The mean length, circumference, thickness, weight and width of the bursa of Fabricius during each season are given in the Table 1. As compared to the testis, the comparison of bursa of Fabricius length circumference, thickness, weight and width during the reproductive phases showed that they were statistically significantly ($P<0.01$) higher in the winter (reproductive non active phase) and gradually declined in the autumn (regressive phase) and summer (active phase) (Fig. 1B).

Microscopic parameters: The histological findings revealed that the bursa of Fabricius had maximum count of regressing follicles during the summer (reproductive active phase), relatively a lower number of regressing follicles were recorded during the autumn (regressive

Table 1: Means ± SEM of gross anatomical parameters of testis and bursa of Fabricius of Japanese quail (*Coturnix japonica*) during different reproductive seasons of the year

Parameters	Seasons					
	Summer		Autumn		Winter	
	Testis	Bursa of Fabricius	Testis	Bursa of Fabricius	Testis	Bursa of Fabricius
Length (cm)	2.75±0.092	0.975±0.047	1.95±0.154	1.050±0.052	0.11±0.011	1.335±0.059
Width (cm)	1.81±0.058	0.174±0.019	1.21±0.094	0.229±0.029	0.53±0.026	0.345±0.015
Thickness (cm)	1.73±0.049	0.180±0.018	1.17±0.090	0.226±0.029	0.51±0.027	0.581±0.269
Circumference (cm)	5.50±0.200	0.894±0.037	3.76±0.307	0.910±0.050	1.65±0.095	1.250±0.078
Weight (g)	4.53±0.370	0.033±0.003	1.56±0.304	0.041±0.006	0.78±0.048	0.101±0.012
Volume (cm^3)	4.41±0.323		1.74±0.266		0.18±0.015	

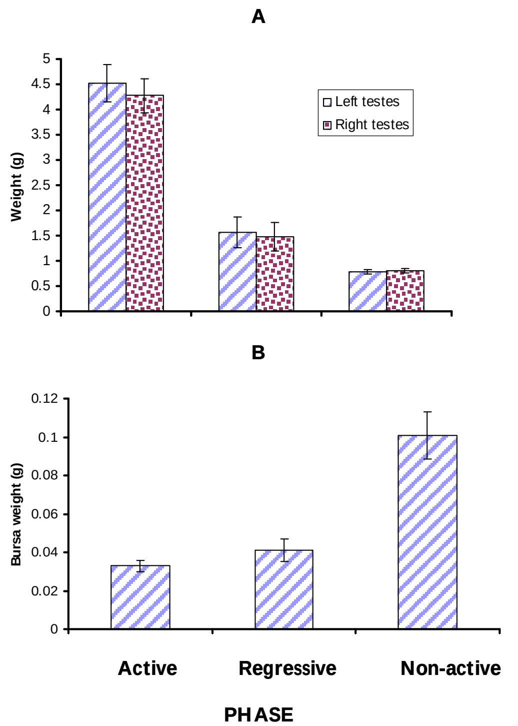

Fig. 1: Effect of seasonal variation on (A) the weight of testes and (B) weight of bursa of Fabricius during the reproductive active, regressive and non active phases of Japanese quail (*Coturnix japonica*). Each bar represents means ± SEM.

Table 2: Immune parameters and hormonal levels (mean±SEM) of Japanese quail (*Coturnix japonica*) during different reproductive seasons of the year

Seasons	Total Leukocyte Count TLC (k/μL)	Lymphocyte Count LC (k/μL)	Serum Testosterone (ng/mL)
Active Phase	107.60±1.840	89.80±2.255	2.31±0.028
Regressive Phase	153.35±2.960	113.95±3.036	0.58±0.019
Non Active Phase	184.29±6.306	132.79±4.864	0.23±0.008

phase), and the highest number of bursal follicles with high density of lymphocytes in the medulla and cortex of bursa of Fabricius were observed in the winter (non-active phase) as shown in (Fig. 3: A, B and C).

The histometric analysis of testis showed a statistically significant ($P<0.01$) rise in the seminiferous tubule diameters during the active phase as compared with the regressive and the non active phases, the comparison of left and right testicular diameters of seminiferous tubules; however, remained unaltered (Fig. 2: A, B and C).

The immune parameters including TLC and LC were significantly higher ($P<0.01$) during the winter (non active phase) as compared to those in autumn and summer (regressing and active phases) as described in Table 2.

Hormonal analysis: The serum testosterone concentrations were significantly ($P<0.01$) higher during the summer (active phase), decreased during the autumn (regressive phase) and declined to the baseline during the winter (reproductive non active phase) (Table 2). In addition, the climatic parameters, such as increase in temperature and rainfall were positively correlated with the increase in serum testosterone concentrations, but there was a negative correlation between the relative humidity and serum testosterone concentrations.

DISCUSSION

The Japanese quail has not been used as a source of interrelationship of neuroendocrine, immune and reproductive systems studies. As a result researchers using this species have to rely upon similar studies performed in other avian species. The high temperature accelerates the testicular maturation in the Japanese quail, as birds show a better reproductive performance in summer as described in willow tits (*Parus montanus*) (Silverin and Viebke 1994).

The present study revealed that the morphological parameters namely weight, length, circumference, thickness, volume, and width of the left and right testis as well as the histometrical diameters of the seminiferous tubule were significantly ($P<0.01$) higher during the summer (reproductive active phase) as compared to the autumn (regressive) and the winter (non-active phase). These results are in line with some previous findings (Haldar and Ghosh, 1990; Maitra and Dey, 1993; Cockrem, 1995; Haldar and Rai, 1997; Wikelski *et al.,* 2000; Haldar and Singh, 2001).

Contrary to these findings, weight, length, circumference, thickness, and width of bursa of Fabricius were significantly ($P<0.01$) higher during winter (non active reproductive phase) which gradually declined in the autumn (regressive phase) and reached to the bottom line during the summer (active reproductive phase). The histological findings revealed that the bursa of Fabricius possess maximum count of regressing follicles during the reproductive active phase, relatively lower number of regressing follicles were recorded during the regressive phase, and the highest number of bursal follicles with high density of lymphocytes were observed in the medulla and cortex of busa of Fabricius as described by Haldar and Singh (2001), in Indian jungle bush quail (*Perdicula asiatica*) and Bently *et al*. (1998) in European starlings (*Sturnus vulgaris*).

The results of current study agreed with previous reports suggesting that the testosterone was modulated by climatic or seasonal variations that activated the gonadal

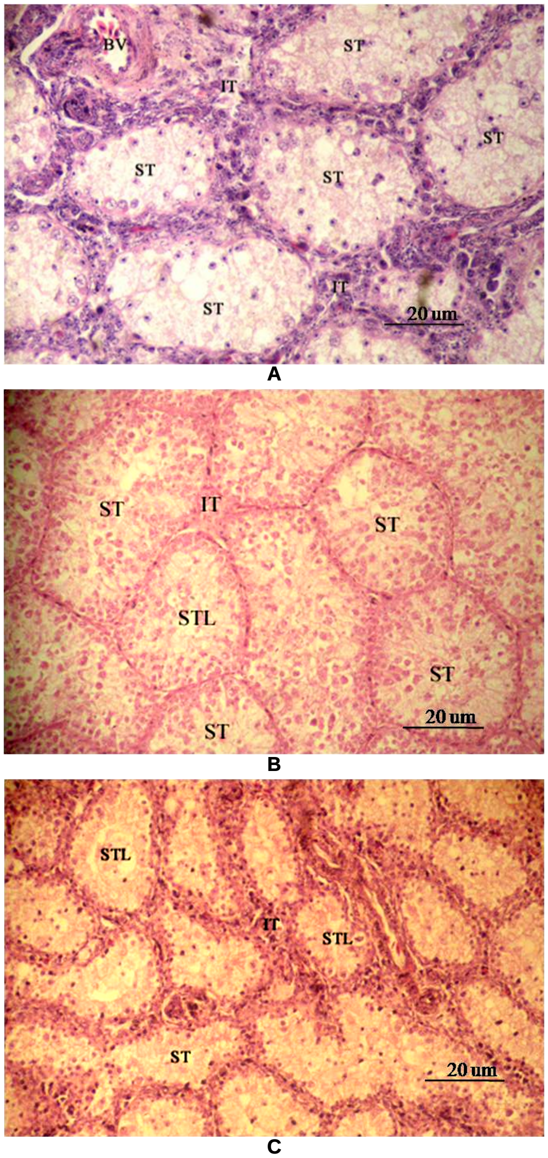

Fig. 2: Photomicrographs of testis of the Japanese quail (*Coturnix japonica*) (A) during the active reproductive phase showing larger diameters of seminiferous tubules (ST) and intertubular tissue (IT) along with some blood vessels (BV) (B) during the regressive reproductive phase showing medium diameters of (ST) along (IT) and seminiferous tubules lumen (STL) (C) during the non active reproductive phase showing lower diameters of (ST) and (IT). H&E; X200.

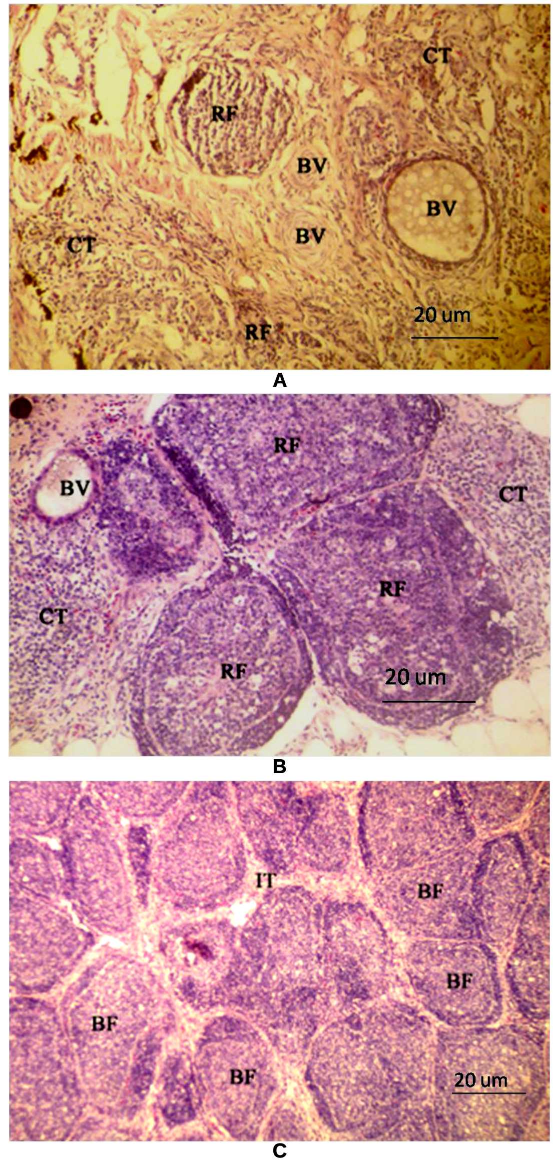

Fig. 3: Photomicrographs of bursa of Fabricius of the Japanese quail (*Coturnix japonica*) (A) during the active reproductive phase showing regressing follicles (RF) filling with connective tissue (CT) along some blood vessels (BV) (B) during the regressing reproductive phase showing high density of (RF) along (CT) (C) during the non active reproductive phase showing high density of bursal follicles (BF) having lymphocytes. H&E; X200.

activities. The testosterone concentrations were significantly higher (P<0.01) during the summer (active phase) which decreased during the autumn (regressive phase) and declined to the baseline during the winter (reproductive non active phase) as described by Haldar and Singh (2001) and also noted in mammals by Lochmiller and Ditchkoff (1999), Haldar and Singh (2000) and Haldar *et al*. (2002).

The immune parameters, i.e. TLC and LC were significantly higher (P<0.01) during the winter (reproductive non active phase) as compared with the autumn (reproductive regressing) and summer (reproductive active phase) of Japanese quail (*Coturnix japonica*) as described by Haldar and Singh (2001), in Indian jungle bush quail (*Perdicula asiatica*) and Giannessi *et al*. (1992) in chicken (*Gallus gallus*).

It can be conceived from the present findings that seasonal changes influenced the testis and the bursa of Fabricius; and changed the total leukocytes and lymphocyte counts along with serum testosterone level. Moreover, enhanced gonadal activity resulting in increased production of steroid hormone and suppressed immune function during the active reproductive phase; and suppressed gonadal activity but elevated immune function enabling survival during unfavorable climatic conditions during the inactive phase were also noticed.

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