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Morphological Analysis of Testis of the Guinea Fowl (*Numida meleagris*) Under Tropical Savannah Climate of India

Análisis Morfológico de Testículos de las Aves de Guinea (*Numida meleagris*) Bajo el Clima de la Sabana Tropical de la India

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SUMMARY: The present study aimed to document the seasonal variations in the testicular morphology of the adult guinea fowl (*Numida meleagris*) under tropical savannah climate of India. The study was conducted in 24 adult healthy guinea cocks. The testes were ovoid in shape throughout the year. The consistency of the testes was moderately firm with great resilience and creamy white in colour during summer and monsoon-I whereas soft in consistency and dull white to yellowish tinge in colour during monsoon-II and winter seasons. The testicular weight, length, width, thickness, volume and circumference were higher during summer and monsoon I than the winter and monsoon II seasons. The mean body weight of the birds did not show significant difference among the seasons. The testicular weight, length, width, thickness, volume and circumference were higher during the summer and monsoon I than the winter and least during monsoon II in the current study. The gonadosomatic index (GSI) and paired testicular weight were high and showed no significant difference during the summer and monsoon-I whereas during winter and monsoon-II, the values were low and showed significant difference between them. The diameter of the seminiferous tubules and its lumen, the diameter of the sustentacular cells (Sertoli cells) were much higher during summer and Monsoon I than winter and monsoon II seasons. The percentage area of interstitial cells and the thickness of the testicular capsule appeared less during the summer and monsoon I seasons. The decreased size of seminiferous tubules, increased amount of cellular debris in the tubular lumen, degenerating germ cells and collapse of seminiferous tubules observed during monsoon II and winter in the present study.

KEY WORDS: Guinea fowl; Testis; Season; Morphology; Morphometry.

INTRODUCTION

Reproduction in birds is a cyclic phenomenon and mainly depends on the photoperiod, rainfall, humidity, temperature and food availability. The timing of the reproductive periodicity depends on the seasonal day length in temperate climates (Lake, 1957) The majority of the species living outside tropics depend on a food source available to feed their young ones become available on a predictable period of each year. The length of the breeding season was highly variable among the species, but most of them were asymmetrical with the changes in the photoperiod.

The guinea fowl is a long day seasonal breeder and exhibits peak breeding activity during summer while no breeding activity takes place during winter (Ali *et al*., 2015; Shil *et al*., 2015). The breeding season of male guinea fowl is limited to the spring months of April to July in Europe whereas in Africa, there are two seasons in areas with bimodal rainfall patterns and only one in those with monomial rainfall patterns (Awotwi, 1987). The breeding season is not determined primarily by changes in day length, but by external, unpredictable factors (non-photic cues), such as food availability or rain as demonstrated in guinea fowls by Awotwi. Similarly, in small ground finches, only rainfall, but not photoperiod, light intensity during the day, or ambient temperature, correlated with the pattern in gonad development (Hau *et al*., 2000). The most important factors in the seasonality of the environment are the amplitude of the seasonal fluctuations and precision with which these fluctuations occur each year (Wingfield *et al*., 1992). Several studies were done on seasonality of reproduction in birds in temperate zones (Wingfield *et al*.; Hahn *et al*., 1997) and very few studies done on the tropics.

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India is a big tropical country and is famous for its diverse climatic features. India experiences variety of climates ranging from tropical in the south to temperate and alpine in the Himalayan north. The elevated areas receive sustained snowfall during winters. The Himalayas and the Thar Desert strongly influence the climate of the country. The Himalayas work as a barrier to the frigid katabatic winds, which blow down from Central Asia. The Tropic of Cancer passes through the middle of the country and this makes its climate more tropical. Tropical wet and dry climate or the savannah climate is most common in the country and prevails mainly in the inland peninsular region of the country except for some portion of the Western Ghats. The summers are extremely hot and the rainy season extends from the month of June to September. There is little research work on the seasonality of reproduction of guinea fowls in this climate of India. Therefore, the study was conducted to evaluate the effect of season on the morphology of the testis in tropical savannah climate in India.

MATERIAL AND METHOD

Experimental site. The study was conducted from the birds collected from Poultry Research Station, Madhavaram Milk Colony, Tamilnadu Veterinary and Animal Sciences University, Chennai (India) lies on altitude 13.0827° N, 80.2707° E. The temperatures are generally high with minimum and maximum values of 21 to 36.8 throughout the year respectively. The rainfall was binomial with mean annual rainfall of 945 mm which is mainly due to southwest monsoon and Northeast monsoon of which 48 % is through the North-East monsoon, and 32 % through the South-West monsoon from July to December (Shanmugasundaram, 1984). The long dry season from late December to June and rainy season is from July to early December. Advance rainfall from the tropical cyclones was also common. The area lies on the south-eastern coast of India in the north-eastern part of Tamil Nadu state on a flat Eastern Coastal Plain with tropical savanna climate, has nearly equal amounts of light and darkness (12L:12D) throughout the year. The meteorological data during the experimental period (Tamilnadu Agricultural weather report) is tabulated on Table I.

Collection of birds. A total of 24 healthy breeding cocks (*Numida meleagris*) with 1.3 to 1.4 Kg average body mass were collected from the Poultry Research Station, Madhavaram Milk Colony, Tamilnadu Veterinary and Animal Sciences University, Chennai (India) during different seasons of the year 2016. Six birds in each season were weighed and euthanized by cervical dislocation for the collection of the testes. The testes were examined for shape, consistency, colour and size.

Gross Morphometry. The length, width and thickness were measured by using a Vernier's calliper. The volume of each testis was measured by volume displacement technique (Ali *et al*.). The weight of each testis was recorded by using electronic weighing balance. The gonado somatic index (GSI), calculation of the testicular mass as a proportion of total body mass was calculated by the formula: GSI= Paired testicular weight / Total Body Weight x 100 (Kouatcho *et al*., 2015).

Tissue processing. The tissue samples washed with normal saline and fixed in different fixatives namely, neutral buffered formalin, Bouin's and Davidson's fixative for the micrometrical study. The tissue samples were processed as per the standard procedures (Suvarna *et al*., 2013). The tissue sections of 5-8 µm thickness were cut and used for routine Haematoxylin and Eosin (H&E) technique.

Histology and Histometry. The photographs were taken by using the Olympus microscope (ProgRes). The 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 interstitial cell (Leydig cell) diameter at 1000X using Olympus microscope (ProgRes) microscope. Only round tubules of perfectly clear transverse section were measured for diameter of seminiferous tubules. The diameter of the seminiferous tubules (n=20 tubules per testis) was measured across the minor and major axes and mean diameter was obtained.

Statistical analysis. Student's t-tests were used to compare between the paired groups. The one-way analysis of variance (ANOVA) with SPSS Statistics 17.0 software was used to compare the differences in weight, volume, circumference and length of testes, thickness of testicular capsule and seminiferous epithelium, diameter of seminiferous tubules and its lumen, diameter and percentage area of interstitial cells. The results were presented as arithmetic mean ± standard deviation. The differences were considered to be significant if P was ≤0.01.

RESULTS AND DISCUSSION

Gross appearance: The testes of the guinea fowls were ovoid in shape in all the seasons in the current study whereas Islam *et al*. (2010) reported that significant shape difference existed between the testes of jungle crows. The testes in the present study were moderately firm in consistency with great resilience during summer and monsoon-I whereas soft during Monsoon-II and winter seasons. The firm consistency and great resilience are the indicators of good semen quality and active testicular function (Etim, 2015).

The testes were creamy white in colour in summer and Monsoon-I seasons and relatively dull white to yellowish tinge in monsoon II and winter seasons. The colour variability is reported among the sexually active testes viz., yellow in Denizli cock (Keskin & Ili, 2011) and white in ducks (Elbajory *et al*., 2013). Age related colour changes were also recorded as bright yellow in juveniles and grey white in adults in ostrich (Zhang *et al*., 2011) and in quails (Kannan *et al*., 2008). The bright yellow colour of the juveniles was due to the accumulation of lipids in interstitial cells and grey-white colour due to the dispersal of the interstitial cells by the enlarging seminiferous tubules after sexual maturity (Dharani *et al*., 2017a). The creamy white colour in summer and Monsoon-I seasons in the present study indicated that the active spermatogenesis occurs during the summer and

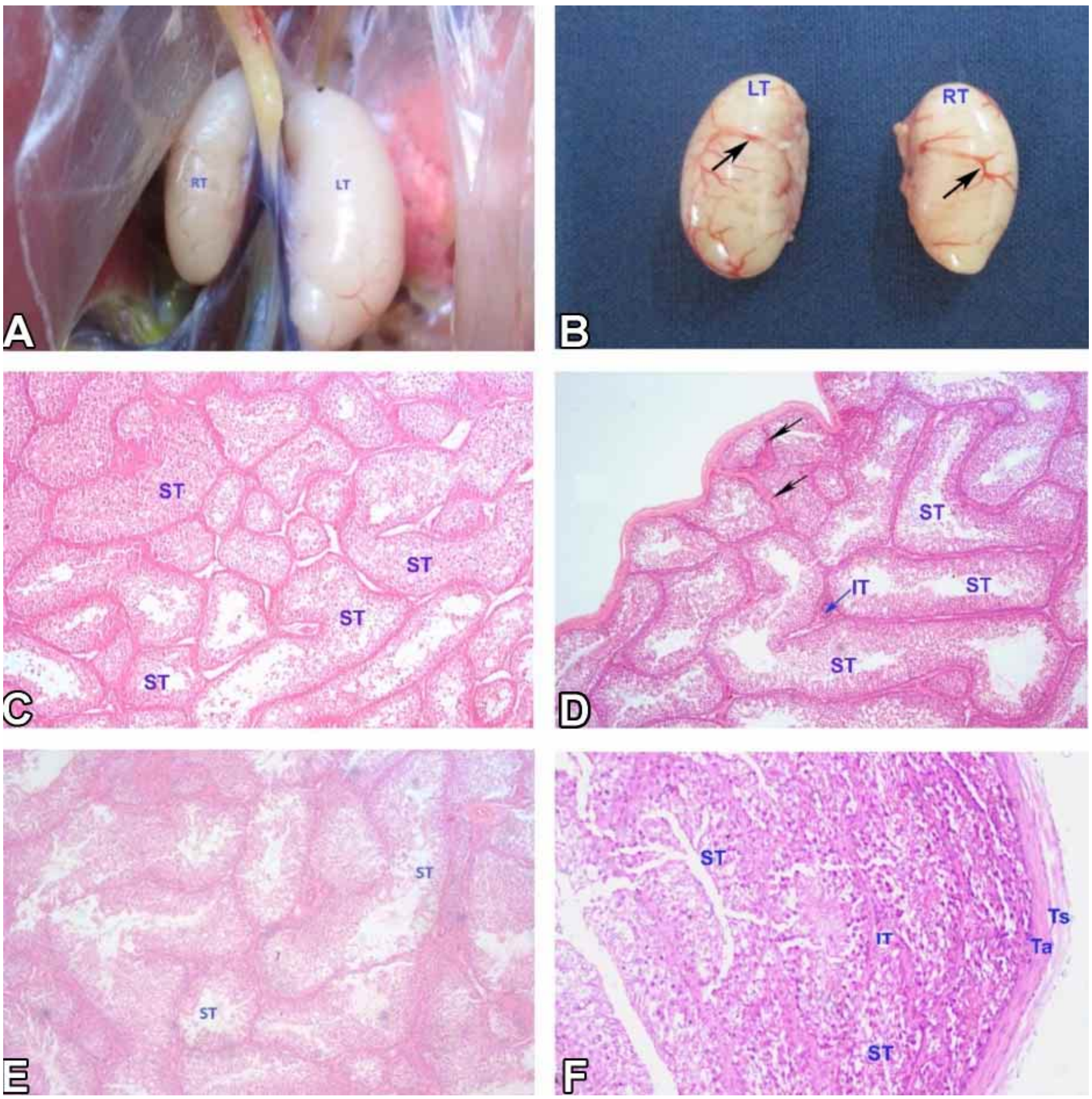

Fig.1. (A) The testes of the adult guinea fowl showing its topographical relationship in the coelomic cavity. Testis; L- Lungs; V-Vas deferens; P-Proventriculus; G-Gizzard; K-Kidney. (B).The testes showing asymmetry in the size. RT-Right Testis; LT- Left testis; L-Lungs; K-Kidney. (C, D, E & F) showing variations in the luminal diameter of seminiferous tubules in winter, summer, monsoon I and II respectively. Ts-Tunica serosa; Ta-Tunica albuginea; ST-Seminiferous epithelium and IT-Interstitial cells.

Monsoon-I, i.e from March to September in guinea fowls which is further confirmed by rich vascularisation (Fig. 1B) on the surface of the testes during this period as reported by Carvalho *et al*. (2015) in greater rhea.

Testicular size is the main factor determining the number of sperm and volume of ejaculate in males. The testicular measurements indicate that reproduction potential in males (Lanna *et al*., 2013). In the current study, the left testis was larger in size and weighed heavier than the right (Fig. 1a) but did not show significant difference within the seasons (P<0.01) (Table II). The basis for testicular asymmetry is said to remain unknown but may be due to an unequal number of primordial germ cells incorporated into the embryonic gonads as opined by Tyler & Gous (2008). Daviche *et al*. (2011), stated that the asymmetrical growth of left and right testis was attributed to the low sensitivity of smaller testis to the gonad stimulating factors. Lanna *et al*. stated that the capacity of sperm production is similar between testes of Japanese quails despite the difference in morphology, since sperm production is directly related to testicular weight which is contrary to the reports of Obidi *et al*. (2008), who verified that greater sperm reserve in the left testis in roosters due to their large size in broiler cocks.

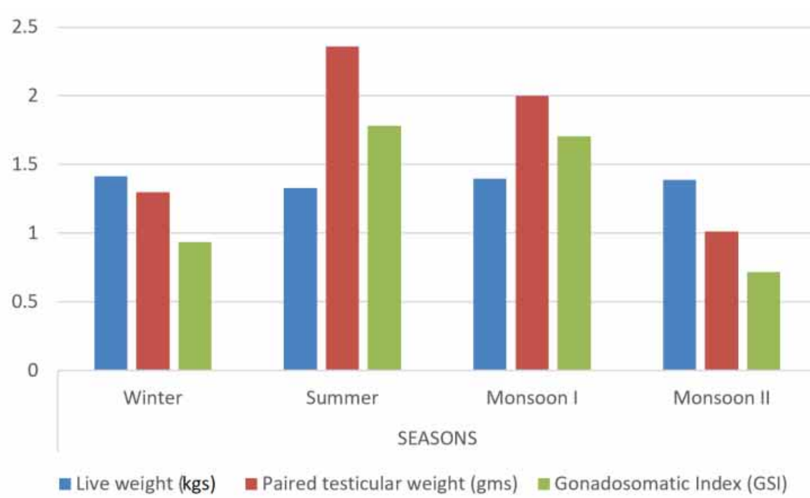

Fig. 2. Gonadosomatic index (GSI) of guinea fowl (*Numida meleagris*) in different seasons in tropical savannah climate

Morphometry of testis by season and daylight: A high positive correlation between testicular weight, length, width, height, volume and circumference was observed within the seasons in the present study. The high correlation and relative simplicity of obtaining such measurements by using ultrasonography, the width and thickness of the left testicle can be the best parameter for reproductive selection. The testicular weight, length, width, thickness, volume and circumference were higher during the months of March to September (Summer and Monsoon I) and lower in December to February (Winter) and least during October to February (Monsoon II) in the current study (Table I). The high values during summer are similar to the findings of Ali *et al*. and Abdul-Rahman *et al*. (2016) in guinea fowl due to the active reproductive phase. Shil *et al*. recorded that these parameters higher during summer and in rainy seasons in adult quails. The low rainfall season (Monsoon-I) did not affect the testicular size of guinea fowls in the present study. During heavy rainfall season (Monsoon-II) the testicular size much lower and during winter (December to February) the size started increasing. The higher anatomical biometric traits in breeding males may be due to higher testosterone synthesis in the breeding than non-breeding males as high gonadotrophin and/or testosterone concentrations have been reported in birds with bigger testes during the breeding season (Malecki *et al*., 1998).

Gonadosomatic index (GSI): The mean body weight of the birds was recorded as 1.41 ± 0.09 kg in winter, 1.33 ± 0.04 gm in summer, 1.39 ± 0.06 kg in monsoon-I and 1.38 ± 0.05 kg in monsoon II and there was no significant difference in weight between the seasons. The mean paired testicular weight were recorded as 1.30 ± 0.14 gm in winter, 2.36 ± 0.19 gm in summer, 2.07 ± 0.36 gm in monsoon-I and in 1.01 ± 0.14 in Monson-II. The GSI was recorded as 0.93 ±0.03, 1.78 ±0.17, 1.70 ± 0.28 and 0.71 ± 0.08 during winter, summer, monsoon-I and monsoon-II respectively. Both GSI and paired

Table I. Meteorological data during experimental period (Tamilnadu Agricultural weather report).

Seasons	Month	Average range of temperature (in centigrade)	Average relative humidity (in percentage)	Rainfall (mm)	Average Monthly sunshine hours
Winter	January -February	22 - 31	70-73	439	268.2
Summer	March -May	28 - 36.8	62-69	56	291.3
Monsoon I (South	June -September	26 - 33	66-72	147	203.6
Monsoon II (North	October to	21 - 29	77-78	664	204.5

Table II. Morphometric parameters of the testes guinea fowl (*Numida meleagris*) in different seasons in tropical savannah climate.

Morphometric Variables of the testes	Winter		Summer		Monsoon I South west monsoon		Monsoon II North East Monsoon	
	Lt	Rt	Lt	Rt	Lt	Rt	Lt	Rt
Weight (gm)	0.73^{b}±0.01	0.68^{b}±0.05	1.34^{a}±0.08	1.13^{a}±0.16	1.29^{a}±0.19	1.18^{a}±0.18	0.61^{b}±0.08	0.50^{b}±0.06
Length (cm)	1.47^{a}±0.11	1.30^{ab}±0.07	1.94^{b}±0.09	1.75^{c}±0.04	1.80^{a}±0.03	1.69^{ab}±0.04	1.28^{a}±0.07	1.18^{b}±0.07
Width (cm)	0.91^{b}±0.01	0.83^{a}±0.02	1.84^{c}±0.05	1.68^{b}±0.07	1..68^{ab}±0.01	1.53^{a}±0.01	0.64^{ab}±0.02	0.54^{a}±0.05
Height (cm)	0.61^{b}±0.01	0.55^{a}±0.01	0.74^{c}±0.01	0.71^{b}±0.04	0.61^{b}±0.02	0.57^{a}±0.03	0.47^{a}±0.08	0.44^{a}±0.04
Volume (cm^3)	0.81±0.23	0.75±0.11	1.47^{a}±0.01	1.42^{a}±0.12	1.33^{a}±0.34	1.21^{a}±0.11	0.62^{a}±0.12	0.59^{a}±0.05
Circumference (cm)	2.65^{b}±0.06	2.45^{b}±0.21	3.65^{b}±0.11	3.36^{c}±0.25	3.20^{b}±0.10	3.06^{b}±0.20	2.25^{a}±0.13	2.05^{a}±0.18

Mean (±SEM) bearing same superscript did not differ significantly ($p \leq 0.01$).

Table III. Histometric parameters of guinea fowl (*Numida meleagris*) in different seasons in tropical savannah climate

Histometric variables of testis	Winter	Summer	Monsoon I South West monsoon	Monsoon II North East Monsoon
Diameter of ST (µm)	950.34^{a}± 12.56	1300.67^{b} ± 29.37	1280.59^{b} ± 8.47	750.28^{c} ± 10.87
Diameter of the lumen of ST (µm)	350.67^{a} ± 4.89	808.49 ^{b} ± 13.34	757.60 ^{b} ± 6.39	250.29^{c} ± 8.42
Thickness of GE (µm)	587.93^{a} ± 9.42	512.85^{b} ± 7.34	468.59^{b} ± 9.76	379.56^{c} ± 6.21
Diameter of interstitial cells (µm)	16.76^{a}±13.34	27.21^{b} ± 2.97	25.12^{b} ±1.37	12.26^{c} ± 2.23
Percent area of interstitial cells (µm)	29.34^{a}±2.37	20.58^{b} ± 1.23	22.34^{b} ± 1.12	46.78^{c} ± 4.22
Capsule thickness (µm)	70.35^{a} ± 0.49	63.45^{b} ± 0.76	58.24^{b} ± 0.35	86.24^{c} ± 4.12

ST- Seminiferous Tubules; GE-Germinal Epithelium. Mean (±SEM) bearing same superscript did not differ significantly ($p \leq 0.01$).

testicular weight were high and showed no significance difference during March to September i.e summer and Monsoon-I whereas during winter and monsoon-II the values were low and showed significant difference between them. The GSI is said to indicate the sperm production efficiency as reported by Okpe *et al*. (2010), in Nigerian local breed of chicken, the results of the GSI in the current study indicated that active reproductive phase in guinea fowl was during March to September (summer and monsoon I) in the present study (Fig. 2).

Histology and Histometric analysis the testis: The diameter of the seminiferous tubules and its lumen were much higher during summer and Monsoon I than winter and monsoon II (Table II). The thickness of the germinal epithelium was higher than the luminal diameter of the seminiferous tubules in monsoon II and winter seasons than the other seasons (Figs. 1C-F). Based on their value, it can be interpreted that the winter season is progressive phase and monsoon II is resting phase. However, Spermatogenic activity was present throughout the year in the current study. The diameter of the sustentacular cells (Sertoli cells) were higher in summer and Monsoon I and very low during monsoon II with the presence of degenerating sustentacular cells with darker cytoplasm, nuclear compaction and more cytoplasmic inclusions. But they regained their normal appearance during the winter. The percentage area of interstitial cells and the thickness of the testicular capsule appeared less during the summer and monsoon I seasons. This may be due to the enlarged seminiferous tubules and active spermatogenesis whereas their area is increased during the monsoon II and stated increasing during the winter. The decreased size of seminiferous tubules, increased amount of cellular debris in the tubular lumen, degenerating germ cells and collapse of seminiferous tubules observed during monsoon II and winter in the present study have also been reported in other non-breeding birds (Dharani *et al*., 2017b).

CONCLUSION

The results of the present study clearly showed that development of the gonad is greatly influenced by the availability of the photoperiod. Though, some spermatogenic activity was observed throughout the year in the tropical savannah climate in the present study, the gonadal activity was very high during the month of March to September i.e during summer and monsoon I. So, it can be concluded that the artificial illumination during the month from September to February i.e winter and monsoon II may keep the birds sexually active throughout the year in the tropical savannah climate.

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RESUMEN: El presente estudio tuvo como objetivo documentar las variaciones estacionales en la morfología testicular de la gallina de Guinea (*Numida meleagris*) en el clima de la sabana tropical de la India. El estudio se realizó en 24 gallos adultos sanos de Guinea. Los testículos fueron ovoidedurante todo el año. La consistencia de los testículos fue moderadamente firme con gran elasticidad y color blanco cremoso durante el verano y durante el Monzón I, mientras que de consistencia blanda y color blanco pálido a amarillento durante el Monzón II y las temporadas de invierno. El peso testicular, la longitud, el ancho, el grosor, el volumen y la circunferencia fueron más altos durante el verano y el Monzón I en comparación con las temporadas de invierno y Monzón II. El peso corporal medio de las aves no mostró una diferencia significativa entre las estaciones. El peso testicular, la longitud, el ancho, el grosor, el volumen y la circunferencia fueron más altos durante el verano y el Monzón I en comparación con el invierno y menos durante el Monzón II. El índice gonadosomático (GSI) y el peso testicular apareado fue alto y no mostraron diferencias significativas durante el verano y el Monzón I, mientras que durante el invierno y el Monzón II, los valores fueron bajos y mostraron diferencias significativas entre ellos. El diámetro de los túbulos seminíferos y su luz, el diámetro de las células sustentaculares (células de Sertoli) fue mucho mayor durante el verano y el Monzón I que en las estaciones de invierno y el Monzón II. El área de porcentaje de células intersticiales y el grosor de la cápsula testicular aparecieron menos durante las temporadas de verano y Monzón I. Se identificaron en el presente estudio disminución del tamaño de los túbulos seminíferos, el aumento de la cantidad de restos celulares en la luz tubular, la degeneración de las células germinales y el colapso de los túbulos seminíferos observados durante el Monzón II y el invierno.

PALABRAS CLAVE: Gallina de Guinea; Testículo; Temporada; Morfología; Morfometría.

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