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Morphometric Dynamics of Selected Male Reproductive Organs in Rose-Ringed Parakeets (*Psittacula Krameri*) in Faisalabad, Pakistan

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ABSTRACT

This project aimed to study the adult male reproductive organs in *Psittacula krameri* (rose-ringed parakeets) during the breeding and non-breeding seasons (n= 6). Once the reproductive organs were collected, they were fixed and processed by paraffin tissue preparation technique. The testes and vas deferens were carefully collected from each bird to record the weight and dimensions (length, width, diameter and volume), determined with a digital weighing balance and Vernier's calipers. Data were analyzed using ANOVA to determine significant differences. The results showed maximum mean weights of right (290.63±5.69mg) and left (177.20±6.63mg) testis during the breeding season and decreased weights (56.73±2.48mg) and (51.37±1.40mg) in the non-breeding season, respectively. The lengths of the left and right testes during the breeding season were (9.00±0.57mm) and (6.55±0.28mm) and for the non-breeding season were (6.92±0.12mm) and (6.55±0.28mm), respectively. The width of left and right testes during the breeding season was recorded as (6.50±0.56mm) and (5.49±0.49mm) and during the non-breeding season was (3.94±0.08mm) and (3.41±0.0mm). The mean volume during the breeding season was recorded as (0.32±0.03cm^3) and (0.25±0.02cm^3) and during non-breeding season as (0.24±0.0 cm^3) and (0.06±0.005cm^3) for the left and right testes, respectively. The mean length of left and right ductus deferens was (1.41±0.1mm) and (1.21±0.12mm) for the breeding season and (1.40±0.06mm) and (1.00±0.01mm) during the non-breeding season. The microscopic study showed the maximum diameter of seminiferous tubules and the germinal layer thickness (200.09±7.1µm) and (34.31±2.4µm) during the breeding season and minimum values (149.2±10.4µm) and (23.45±0.99µm) during non-breeding season respectively**.** These stark differences in the values of macroscopic and microscopic parameters suggest the physiological adaptations of rose-ringed parakeets for optimum reproductive efficiency.

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INTRODUCTION

The rose-ringed parakeet (*Psittacula krameri*), known as a ringed-neck parakeet (or Gani wala tota), in South Asian countries (Pakistan, Bangladesh, and India). This species has invasive potential and adaptability to urban environments. Understanding the morphometric dynamics of its male reproductive organs provides valuable insights into its reproductive success and ecological adaptability. Many variables affect spermatogenesis, testicular growth and development, and the morphology of the testes, including age, season, temperature, photoperiod, diet, and disease in this species (Uchechukwu *et al.*, 2015). Among other things, photoperiod has an important role in synchronizing reproductive activity (testicular growth, spermatogenesis, and plasma sex steroid synthesis) with the optimal environment in seasonal breeders via the neuroendocrine system (gonadotropin production) (Qureshi *et al.*, 2016b).

Breeding seasonality in avian species is crucial in optimizing reproductive success, ensuring that offspring are born during periods of peak resource availability. This seasonality is an evolutionary adaptation that aligns reproductive cycles with favorable environmental conditions, such as food abundance and optimal weather. This synchronization enhances the survival rates of both offspring and adult birds by reducing the risks associated with raising young during periods of scarcity or harsh climates (Mingozzi et al., 2022; Soanes et al., 2021).

The breeding and non-breeding seasons reveal noticeably varied testicular values, in many avian (*Coturnix japonica*, *Numida meleagris*) and mammalian species. The highest values were discovered during the full breeding season, and then markedly fell during the low breeding and non-breeding periods. In comparison to low breeding and non-breeding seasons, the hypsometrical examination of the seminiferous tubules in the right and left testis showed a considerably fast increase in dimensions during the full breeding season (Ali *et al.*, 2015; Qureshi *et al.*, 2019; Hayat *et al.*, 2022; Masood *et al.*, 2022; Ahmed *et al.*, 2023).

The seasonal variations in the anatomy and histology of the reproductive organs in rose-ringed parakeet has not been studied yet in Pakistan; therefore, the current study aimed to investigate the physical and morphometric characteristics of the testes and ductus deferens of the rose-ringed parakeet about the breeding seasonality.

MATERIALS AND METHODS

The Institutional Biosafety and Bioethics Committee (IBBC), University of Agriculture, Faisalabad, approved the animal trials vide letter no. 3763/ ORIC, dated 28/07/2022.

Experimental design

Six healthy, adult male rose-ringed parakeets (three birds during the breeding season (December to February) and three birds during the non-breeding season (April to June) were bought from a local bird market in Faisalabad. The birds were brought to the animal unit of the Department of Anatomy, Faculty of Veterinary Sciences, the University of Agriculture Faisalabad in a typical ventilated cage.

Collection of samples

Immediately before euthanasia, the live body weight of each bird was determined to the nearest gram with a digital weighing balance. The parakeets were euthanized according to the rules and regulations of the Bio-Ethics Committee of the University of Agriculture Faisalabad, and testes and vas deferens were collected. The dimensions of the testis and vas deferens (length, circumference, and width) and weight were measured to the nearest millimeter (mm) and milligram (mg) using a Vernier caliper and an electronic weighing balance. The volume of testes was estimated by the water displacement method with a graduated measuring cylinder.

Microscopic analysis: After collection, tissues were immersed in Bouin's solution for 72 hours. Subsequent to fixation, the tissues underwent dehydration, infiltration, embedding in paraffin blocks, sectioning, and mounting. For histological analysis, 5µm slices were prepared and stained with hematoxylin and eosin (H&E) (Bancroft and Layton, 2019). The diameter of the seminiferous tubules, germinal epithelium thickness, and luminal diameter of the seminiferous tubules of each testes of each bird were determined using image analysis software Image J®. All the recorded data was computed using Microsoft Excel® and analyzed by analysis of variance (ANOVA) to find the statistical differences ($P<0.05$), with statistical software Minitab®. The Tukey's honestly significant difference (HSD) test was used to compare the group means.

RESULTS

Gross Morphology

The results showed that both the testes were ovoid in shape and yellowish in color (Plate 1). Statistical analysis revealed significantly ($P<0.05$) higher values of the gross anatomical parameters (absolute and relative weights, volume, and various dimensions including length, width, and circumference) for both right and left testis during the breeding season and significantly decreased values during the non-breeding season (Table 1, Plate 1). The width of the vas deferens showed significantly ($P<0.05$) lower values in the non-breeding season as compared to the breeding season. There was no significant difference ($P<0.05$) in the length of vas deferens during breeding and non-breeding seasons (Table 1).

Microscopic Analysis

The histomorphometric evaluation of the seminiferous tubules diameters and germinal epithelium thicknesses, during the breeding and non-breeding seasons showed that these were significantly ($P<0.05$) higher during the breeding season than non-breeding season. This contrast of values with regards to breeding seasonality ($P<0.05$) is presented in Table 1 and Plate 2.

DISCUSSION

The present study revealed significant seasonal variations in the macroscopic and microscopic structure of testes. The gross anatomical parameters including absolute weight, relative weight, length, circumference, thickness, volume, and width of left and right testes were significantly ($P<0.05$) higher during the breeding season and decreased during the non-breeding season. Previously, the values of paired testes of rose-ringed parakeets were recorded as (414.56±21.62 mg) (Maitra and Mitra, 2008), higher than our breeding season values. Our results showed that testes were about 3-6 times heavier in breeding season than non-breeding season. For the Japanese quails, *Coturnix japonica*, the weights of the left and right testes were recorded as (4.27±0.33 g) and (0.78±0.048 g), respectively (Qureshi *et al.*, 2017). In the guinea fowl, the weight of testes was recorded in breeding and non-breeding seasons as (1.39±0.157 g) and (0.18±0.023 g) (Qureshi *et al.*, 2016a). Like many previous reports on birds and mammals (Calhim and

Montgomerie, 2015; Umar *et al.*, 2017; Hayat *et al.*, 2022; Masood *et al.*, 2022). The left testis was significantly heavier than the right, suggesting functional asymmetry in testicular activity (Rani et al., 2023). Different testes weights correspond to different body sizes and weights in these different species. The species differences could also depict different reproductive potentials in these species. Furthermore, these findings align with hormonal studies showing elevated testosterone levels during breeding months (Patel et al., 2022).

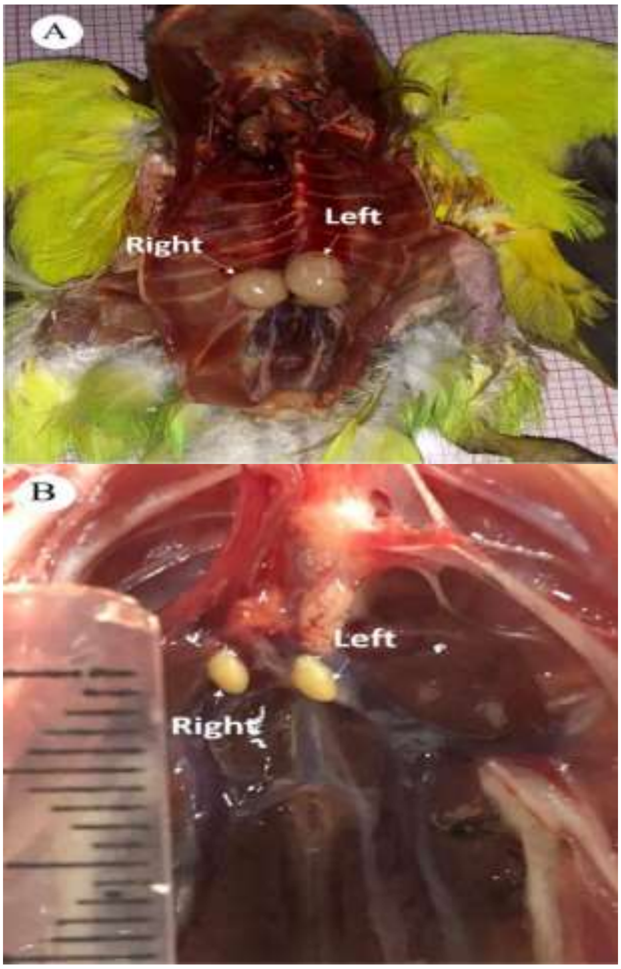


Fig. : Tests of rose ringed after removel of different abdominal Organs. Notice the size variation in A (Breeding season) and B (Non-Breeding season).

The relative weights of testis were also significantly higher during breeding season than non-breeding season. However, our reported values in *Pscittacula krameri* are quite smaller as compared to other birds (Calhim and Montgomerie, 2015; Hayat *et al.*, 2022). This could be attributed due to this species seasonally monogamous reproductive behavior (Baehaqi *et al.*, 2018; Baker *et al.*, 2020).

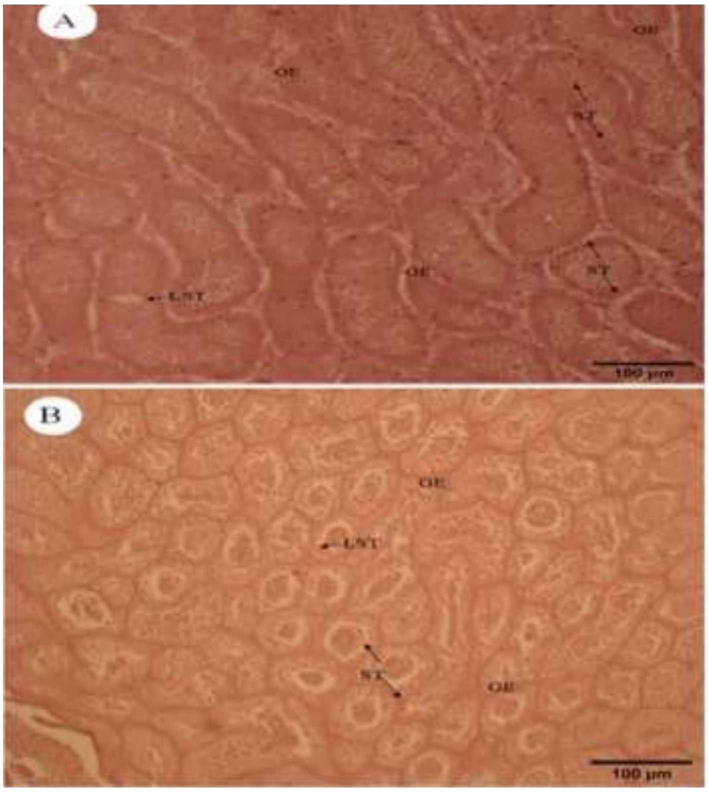
Significantly ($P<0.05$) higher values of length for right and left testes during the breeding season and significantly decreased during the non-breeding season were observed in the present study. Previously, the lengths of left and right testes in budgerigar *Melopsittacus undulatus* was recorded as (6.28 mm) and (5.21 mm) (Herrera-Barragán *et al.*, 2020) without mention of breeding/ non-breeding season. In guinea fowl, the length of testes was recorded during the breeding season (1.69±0.077 cm) and during the non-breeding season as (1.08±0.062 cm) (Qureshi *et al.*, 2016). In guinea cock *Numida meleagris*, it was recorded during the breeding season (18.2 ± 0.5 mm) and during the non-breeding season as (13.2 ± 0.4 mm) (Abdul-Rahman *et al.*, 2016). These values correspond to testis weight in that particular species and may depict functional capacity of these organs during breeding/ non-breeding seasons.

Statistical analysis revealed significantly ($P<0.05$) greater values of the width for both right and left testis during the breeding season and significantly decreased during the non-breeding season (table 1). The values of testes length recorded in Budgerigar were lower than the rose-ringed parakeet due to less body size and weight. In Saker Falcon *Falco cherrug*, the width of the left and right testis during the breeding season (9.6 ± 0.2 mm) and (9.4 ± 0.3 mm) and during the non-breeding season (8.6 ± 0.2 mm) and (5.8 ± 0.2 mm) (Dixon *et al.*, 2021). In Japanese quail, the width of the left and right testis during the breeding season was (1.81±0.058 cm) and (1.72±0.045 cm) and during the non-breeding season (0.53±0.026 cm) and (0.51±0.025 cm) (Qureshi *et al.*, 2017).

The volume of testes showed significantly ($P<0.05$) greater values during the breeding season and significantly decreased during the non-breeding season (table 1). The volume of left testis in Swift Parrots *Lathamus discolor* was recorded in December (25.56±1.82 mm^3) and June (2.56±0.83 mm^3) (Gartrell, 2002). The volume of testes of the rose-ringed parakeet was higher than the swift parrot because of their greater body weight. In Japanese quail, the volume of the left and right testes was recorded during the breeding season (4.41±0.323 cm^3) and (4.28±0.263 cm^3) and non-breeding season (0.18±0.015 cm^3) and (0.18±0.013 cm^3) In guinea cocks, the volume of the testis was recorded during the breeding season (1.0±0.8 cm^3) and non-breeding season (0.4±0.03 cm^3) (Abdul-Rahman *et al.*, 2016).

The values of testicular circumference were significantly ($P<0.05$) greater for both the right and left testes during the breeding season and significantly decreased during the non-breeding season (table 1). In Japanese quail, the mean circumference for the left and right testes during the breeding season was recorded as (5.50±0.20 cm) and (5.20±0.18 cm) and during non-breeding was as (1.65±0.095 cm) and (1.66±0.069 cm) (Qureshi *et al.*, 2017). The circumference of the testes in Japanese quail was greater than the rose-ringed parakeet due to higher body weight. Furthermore, the invasive populations in temperate regions (e.g., Europe) exhibit pronounced photoperiod-induced testicular growth, suggesting phenotypic plasticity in reproductive timing in *Pscittacula krameri* (Patel et al., 2022).

The length of the ductus deferens didn't significantly ($P>0.05$) differ concerning breeding and non-breeding seasons (table 1). In the Khaki Campbell duck *Anas platyrhynchos domesticus*, the mean length of the ductus deferens was recorded as (16.65 ± 0.10 cm) (Khatun and Das, 2019), irrespective of the season.



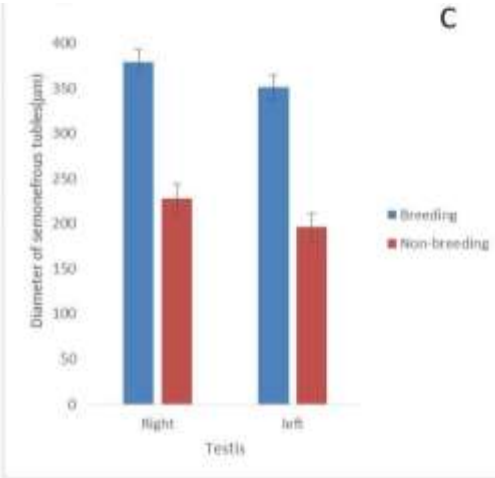


Plate 2. (A) Photomicrograph of testis during the breeding phase of cycle showing maximum spermatic activity and seminiferous tubules having larger diameter. **(B)** Photomicrograph of testis during the resting phase showing that (ST) diameter of tubules decreases significantly.
(LST) lumen seminiferous tubule.
GE germinal epithelium. Hematoxylin and Eosin stains (H&E) 100X.
C: Graphic presentation of seminiferous tubule diameters during breeding and non-breeding seasons.

Table 1: Gross anatomical and histomorphometric parameters of testes and vas deferens of rose ringed parakeet (*Psittacula krameri)* during breeding and non-breeding seasons (means ± SEM)

Parameters/ Organs	Breeding Season				Non-Breeding Season			
	Left Testis	Right Testis	Left Ductus deferens	Right Ductus deferens	Left Testis	Right Testis	Left Ductus deferens	Right Ductus deferens
Weight (mg)	290.63±5.69^a	177.20±6.63^b			56.73±2.84^c	51.37±1.40^c		
Relative weight (%)	0.22±0.002	0.13±0.07			0.05±0.02	0.04±0.02		
Length (mm)	9.00±0.57 ^{a}	6.55±0.28 ^{b}	35.01±0.07	35.00±0.05	6.92±0.12^{bc}	5.15±0.36^c	34.96±0.08	34.86±0.08
Width (mm)	6.50±0.56 ^{a}	5.49±0.49^{ab}	1.41±0.12^a	1.21±0.12^b	3.94±0.08^{bc}	3.41±0.07^c	1.40±0.006^a	1.00±0.01^b
Diameter (mm)	20.38±0.58 ^{a}	17.52±1.27 ^{a}			10.99±0.20^b	9.88±0.22^b		
Circumference (mm)	63.99±1.83 ^{a}	55.01±3.98^a			34.51±0.65^b	23.54±3.27^b		
Area (mm^2)	45.25±2.24^a	38.9±2.8^a			24.4±0.46^b	21.94±0.48^b		
Volume (cm^3)	0.32±0.03 ^{a}	0.25±0.02 ^{a}			0.24±0.01^b	0.16±0.05^b		
Diameter of seminiferous tubules	200.09±7.1^a				149.2±10.4^b			
Thickness of Germinal Epithelium (µm)	34.31±2.4a				23.45±0.99b			

Significantly greater values ($P<0.05$) of the width for ductus deferens during the breeding season and significantly decreased during the non-breeding season (table 1). In the Khaki Campbell duck, the mean length of the ductus deferens was recorded as (0.45 ± 0.01 cm) (Khatun and Das, 2019). The width of the ductus deferens of the rose-ringed parakeet was lower than the Khaki Campbell duck because of the lower body weight and size of the rose-ringed parakeet. This adaptation facilitates efficient sperm storage and transport during peak reproductive periods.

Morphometric evaluation of the diameter of seminiferous tubules revealed variation in the diameter of tubules and thickness of the germinal epithelium (table 1) varied in breeding and non-breeding seasons. The widest tubular diameters were observed during breeding season when the germinal epithelium was thicker and had a larger number of spermatozoa. During the non-breeding season, the seminiferous tubules showed reduced diameter, it is associated with a germinal epithelium of reduced thickness. However, the value of seminiferous tubules in rose ringed parakeets was recorded as (208.99±10.32 µm) (Maitra and Mitra, 2008), in agreement with our breeding season values. We have previously reported seminiferous tubules diameter in guinea fowl, Japanese quail (Ali et al., 2015; Qureshi et al., 2017; Hayat et al., 2022), higher than present values, suggestive of body weight and species physiology and habitat differences.

Conclusions

The present findings provide valuable insights into the morphometric dynamics of male reproductive organs in *Psittacula krameri*. It indicates that seasonality and photoperiod influence the histomorphology of the male reproductive organs in the rose-ringed parakeet. The absolute and relative weights, along with the dimensions (length, width, circumference, area, and volume) of both the right and left testes, increased during the breeding season and decreased during the non-breeding season. These macroscopic structural changes were accompanied by a thickened germinal epithelium and wider luminal diameter of the vas deferens. These adaptations enhance reproductive success, allowing this avian species to breed during favorable climatic conditions in spring when temperatures are optimal, and food availability is abundant. Future research should explore the molecular mechanisms governing these morphological changes to enhance reproductive management strategies for both wild and captive populations.

DECLARATIONS

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Conflicts of Interest: All authors of the manuscript declare that they have no financial or personal interests.

Data Availability: All the data is available in this paper

Ethics Statement: This research was executed following the guidelines regarding the use and welfare of laboratory animals.

Authors Contribution: SR designed the project, supervised lab work and finalized the manuscript. AR, ASQ, UA, MU, RK performed laboratory sampling, ZU and MU, MUS, SS, HYA conducted statistical analysis and prepared preliminary write up.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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