



## CELLULAR AND MOLECULAR BIOLOGY

# Morphological characterization of blue-and-yellow macaw (*Ara ararauna*) seasonal testicular variations in captivity

MARIANA G. MOREIRA, EDSON J. BENETTI, ANA C.S. REBELO, GESSICA C. SOUSA, MAX V.M. MEDEIROS, LUCÉLIA G. VIEIRA, RENATA C. MAZARO & KARINA SIMÕES

**Abstract:** The reproduction of monogamous wild birds in captivity it's difficult and the apparent low fertility in males requires more investigations. The objective of the study was to test the hypothesis that wild bird species in captivity would present low reproductive potential, through the analysis of the morphological characteristics of *Ara ararauna* testicles, maintained in captivity, correlating them with the climate variations in the Cerrado Biome. For that, testicles were captured in April (dry) and October (rainy). The right and left testicles showed mean weight, gonadosomatic index, longer axis, and volume similar between the dry and rainy season. Only the shorter axis demonstrated higher values during the rainy season. The morphometric variables of the seminiferous tubules have also higher values during the rainy season. By these histological and morphometric characteristics of the seminiferous epithelium we can conclude that, during the rainy season, the testicles were in gonadal recrudescence, which precedes the reproduction phase. During the dry season, the testicles were in the rest phase of the seminiferous epithelium. Therefore, we concluded that the species in captivity, under Cerrado environmental conditions, have kept their reproductive potential, presenting a complete spermatogenic cycle during the rainy season, which can guarantee the species perpetuation.

**Key words:** Conservation, reproduction, spermatogenesis, wild birds.

## INTRODUCTION

Vertebrates reproduction in under endogenous regulation and is, generally, dependent of environmental factors. Thus, the reproductive models are developed with the alternance of active and quiescent gonadal periods. In birds, the seasonal reproductive periods are constant, influenced by these factors, where the reproductive organs grow and regress with light, temperature, rainfall index and food availability (Kemp 1973), is also subjected to constant variations due to geographical latitude and altitude (Breucker et al. 1989).

Birds reproduction, with relation to season, has been widely studied in regions with temperate climate, where the seasons are very well defined (Hau 2001, Hau et al. 2008). In tropical areas, the studies are concentrated in regions that are the closest to the Equator (Hau et al. 1998, Hau 2001), where the temperature does not vary much and the rainfall index is very high during the entire year, as opposed to the Cerrado biome, the main area where the *Ara ararauna* is usually found and that possess well defined periods of dry and rainy seasons.

Studies performed in the tropical climate with domestic ducks (Simões et al. 2005, 2017)

and with quails (Baraldi-Artoni et al. 1997, Orsi et al. 2005, Amoroso et al. 2008) demonstrated a variation on the testicular morphology according to the annual phases of reproduction (proliferation), regression, quiescence (rest) and recrudescence, which succeeded throughout the year (Moore et al. 2006).

The objective of this study was to evaluate the morphological, biometric and morphometric characteristics of *Ara ararauna* testis, kept in captivity, under natural environmental conditions, correlating them with the climate variations of air temperature (°C), rainfall (mm) and luminosity (min/day) during the dry and rainy seasons of the Cerrado biome.

## MATERIALS AND METHODS

For the testicular morphological evaluation, we utilized six male specimens of *Ara ararauna* (blue-and-yellow macaw's) species, with body weight between 680 and 1115 grams, provided from seizures of the Wild Animal Screening Center (CETAS/IBAMA/GO), all healthy with periodic monitoring by IBAMA. For that, we conducted three animal captures during the month of April (Cerrado dry season) and three during the month of October (Cerrado rainy season). The study was performed in the city of Goiânia/ Goiás/Brasil (16°40'43"S; 49°15'14"W), area of the Cerrado biome, with an average temperature of 23.1°C and average annual rainfall of 1414mm. The birds were maintained in appropriate cages at CETAS, with food and water *ad libitum*.

For the male separation from the entire squad, we conducted the sex screening by PCR (Polymerase Chain Reaction), where we randomly selected six individuals from the total male population. Three of them were euthanized during the dry season, in the month of April 2016, and the other three during the rainy season, in the month of October 2016.

The euthanasia procedure in both cases was initiated with the anesthetic Midazolam (0.4mL/ Kg) in a spray form, followed by the saturation with the anesthetic Sodium Pentobarbital (1mL/ Kg) with Potassium Chloride (2nmol/Kg) through the puncture of the superficial ulnar vein.

The study was approved by the Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO, n°50963) and by the Ethics Committee of Animal Use (CEUA n°093/15) from the Goiás Federal University.

### Macroscopic analysis

The body length of *Ara ararauna* was measured with measuring tape (in centimeters) from the top of the head to the point of the tail and all of them were weighted with a commercial weight scale soon after euthanasia. The testis were retrieved using an abdominal laparoscopy and gastrointestinal tract evisceration. In the abdominal cavity we measured the longer (longitudinal) and shorter (transversal) right and left testis axis length, utilizing a precise digital pachymeter (Mitutoyo, Japan). For the calculation of each test's total volume we applied the ellipsoid formula:  $V = 4/3 \cdot \pi \cdot a \cdot b^2$ , where  $a$  is the longer semi-axis and  $b$  the shorter semi-axis (Miraglia & Hayashi 1993). We also calculated the gonadosomatic index (GSI) by the sum of the testis weight ( $P_{test}$ ), divided by body weight ( $P_{corp}$ ) and multiplied by 100. according to the formula:  $IGS = (P_{test}/P_{corp}) \times 100$  (Villagra 2012).

### Microscopic analysis

After retrieval, the testis was weighted in a semi-analytical scale (Acculab, USA), transversally cut and fixed by a Metacarn solution for 3 hours and embedded in Paraplast (Leica). Histology sections with thickness ranging from 5 to 3µm were stained with Hematoxylin-Eosin and Toluidine Blue at 1% and by Schiff's periodic acid reaction (PAS). All these materials were analyzed and photo-documented by Photomicroscope (Leica, DM 3000. Berlin, Germany).

For the morphometric analysis of the seminiferous tubules and seminiferous epithelium height, we took six histological sections at random per animal and measured the longer and shorter axis ( $\mu\text{m}$ ) of 30 seminiferous tubules and obtained 150 measurements of the seminiferous epithelium height ( $\mu\text{m}$ ) per animal, using the Computer Program of Image Analysis LAS (Leica, Berlin, Germany).

### Climate data

Monthly climate data of air temperature ( $^{\circ}\text{C}$ ), rainfall (mm) and luminosity (min/day) of Cerrado biome, in the Goiânia area, GO, Brazil, during the year of 2016, were provided by the Evaporimetry Station / School of Agronomy, Goiás Federal University (From: <http://www.agro.ufg.br>).

### Statistical analysis

For the statistical analysis we used the BIO ESTAT 5.0 Program. The Shapiro-Wilk test was utilized to evaluate the normal distribution of data. The biometric and morphometric data showed normal distribution and were described as mean and standard deviation. We used the T-test for independent samples in comparing the variables between dry and rainy seasons. Besides, we also applied the paired student T-test for the comparison between the right and left testis. Chi-square tests were used to compare the values found for the right and left testis, during the dry and rainy seasons, between the evaluated parameters. In all analysis we adopted a significant level of 5% ( $p \leq 0.05$ ).

## RESULTS

### Climate data

Climate data of temperature and luminosity intensity during the testis collection, in the year

of 2016, little varied between the month of April (dry), with respective values of  $24.53^{\circ}\text{C}$  ( $\pm 0.34$ ) and of 513.6 min/day ( $\pm 20.90$ ) and October (rainy), with values of  $25.53^{\circ}\text{C}$  ( $\pm 0.34$ ) and 413.2 min/day ( $\pm 30.99$ ). On other hand, the rainfall values showed several variations throughout the year, where during the dry season it did not rain at all (0mm) and, during the Cerrado rainy season, the value for October was 217.8mm of rain (Figure 3).

### Macroscopic analysis

Macroscopically, *Ara ararauna* testis demonstrated an elongated format with round and tapered extremities. They were located in the interior of the celomatic cavity, ventrally, about the kidneys and dorsally about the gastrointestinal tract, being the right one situated more cranially in comparison to the left one (Figure 1).

The analysis of *Ara ararauna* biometric aspect, average body weight (g) during the rainy season was from 1095.0g ( $\pm 20.0$ ), and in the rainy seasons presented 843.3g ( $\pm 151.7$ ). The total body length, however, did not differ significantly between the dry and rainy seasons (Table I). The *Ara ararauna* of the dry season showed average biometric data for weight, longer axis and volumes of the right and left testicle, similar to the data found during the rainy season ( $p > 0.05$ ), but with a statistically significant difference for the variable longer axis of the left testis (Table I). Qui-square tests did not demonstrate significant differences ( $p > 0.05$ ) when comparing the values found for the right and left testis of *Ara ararauna* during the dry and rainy season, between the evaluated parameters, probably because the photoperiod is very similar in both seasons (513 minutes and 413 minutes).



**Figure 1.** Anatomical location of the right (RT) and left testicle (LT) in the celomic cavity, cranio-ventrally about the kidneys (K).

**Table I.** Body weight (g), body size (length) (cm), weights from the right and left testicles (g), longer axis (mm), shorter axis, volume (mL) and gonadosomatic index from the *Ara ararauna* right and left testicles between the dry and rainy seasons.

Variables	Dry (n=3)	Rainy (n=3)	p Value
Body Weight (g)	843.3±151.77	1095.00±20.00	0.04
Size (Length) (cm)	66.67±12.50	71.33±7.09	0.30
Right Testicle			
Weight (g)	0.58±0.51	0.56±0.22	0.47
Longer Axis (mm)	8.55±3.04	9.40±2.52	0.36
Shorter Axis (mm)	3.95±2.72	4.21±0.64	0.44
Volume ( $\frac{4}{3} \pi a b^2$ ) (mL)	0.83±1.12	0.73±0.33	0.44
Left Testicle			
Weight (g)	0.32±0.28	0.62±0.16	0.08
Longer Axis (mm)	10.22±6.49	9.16±0.96	0.40
Shorter Axis (mm)	Shorter Axis (mm)	Shorter Axis (mm)	Shorter Axis (mm)
Volume ( $\frac{4}{3} \pi a b^2$ ) (mL)	0.49±0.60	0.71±0.21	0.28
Gonadosomatic Index TD ( $10^4$ )	7.70±7.97	5.14±1.98	0.62
Gonadosomatic Index TE ( $10^4$ )	4.04±3.97	5.70±1.43	0.53

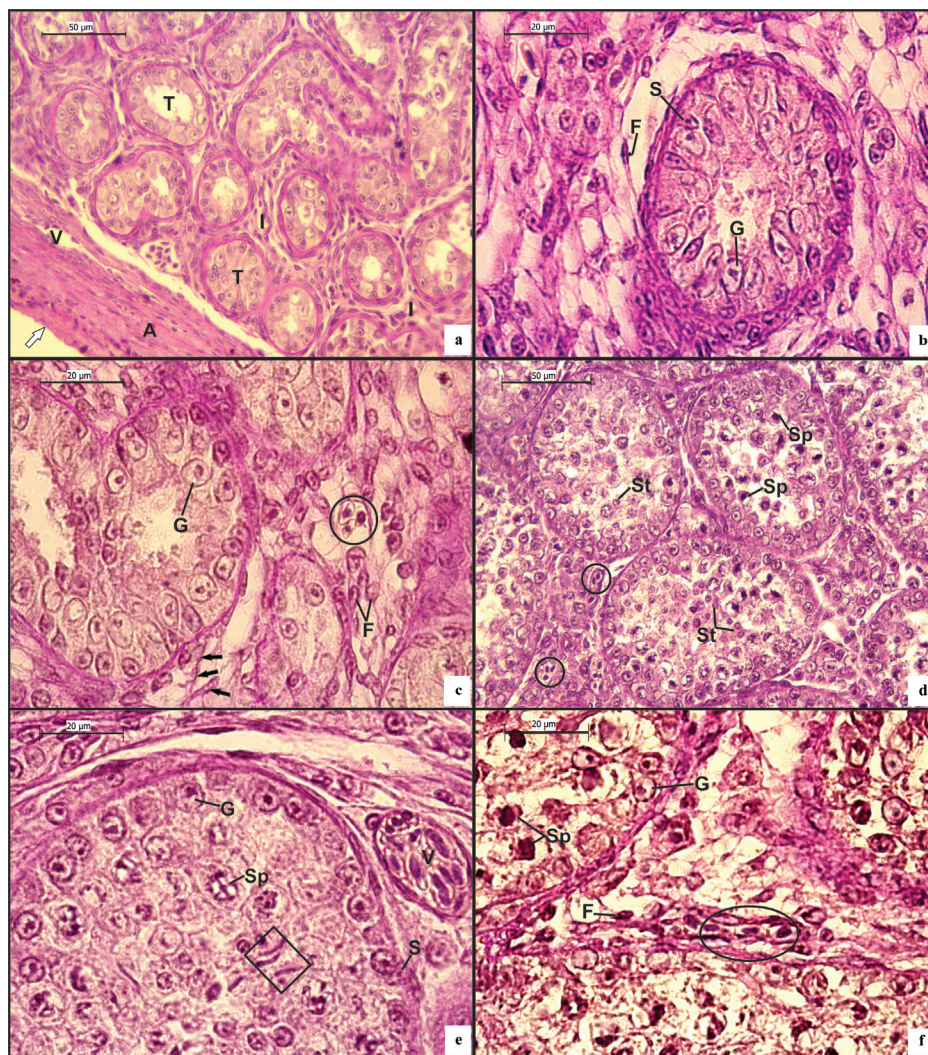
Comparison between the dry and rainy seasons, T-test for independent samples,  $p < 0.05$ . n= number of animals; values expressed as mean ± standard deviation. TD= Right Testicle; TE= Left Testicle.

### Microscopic analysis

Microscopically, the testis showed parenchyma constituted by irregularly entangled seminiferous tubules, involved by the testicular capsule, which covers the epididymal area and the testicle. Histologically, the testicular capsule was constituted by the most external layer to the

most inner layer, by the serous and albuginea tunics (Figure 2a). We did not observe connective tissue septa coming from the albuginea tunic between the seminiferous tubules.

Differences in the morphology of the seminiferous tubule walls during the dry and rainy seasons were found out. In the harvested



**Figure 2.** Histological section from *Ara ararauna* testicle during the dry season in a, b, c and during the rainy season in d, e and f. In a: testicular capsule with its serous tunic (arrow) and albuginea tunic (A), with collagen fibers and blood vessels (V), interstitium (I) and seminiferous tubules (T). In b: the seminiferous tubule presenting only spermatogonia (G) and Sertoli cells (S), fibroblast (F) in the interstitium. In c: interstitium constituted by Leydig cells (circle), fibroblasts (F) and collagen fibers (arrow); spermatogonia (G). In d: several cell types in the tubular wall: primary spermatocytes (Sp), round spermatids (St), Leydig cells (circle) in the interstitium. In e: Sertoli cell (S), spermatogonia (G), elongated spermatids (square), a blood vessel in the interstitium (V). In f: Leydig cells (circle) and fibroblasts (F) in the interstitium, spermatogonia (G) and primary spermatozoa (Sp) in the seminiferous tubules.

testis from the dry season (April), the epithelium of the seminiferous tubules demonstrated to be thin, showing only spermatogonia and Sertoli cells (Figures 2a and b). The peritubular tissue and the albuginea tunic were fully developed (Figure 2a), as well as the interstitium with the presence of Leydig cells, collagen fibers and fibroblasts (Figure 2c).

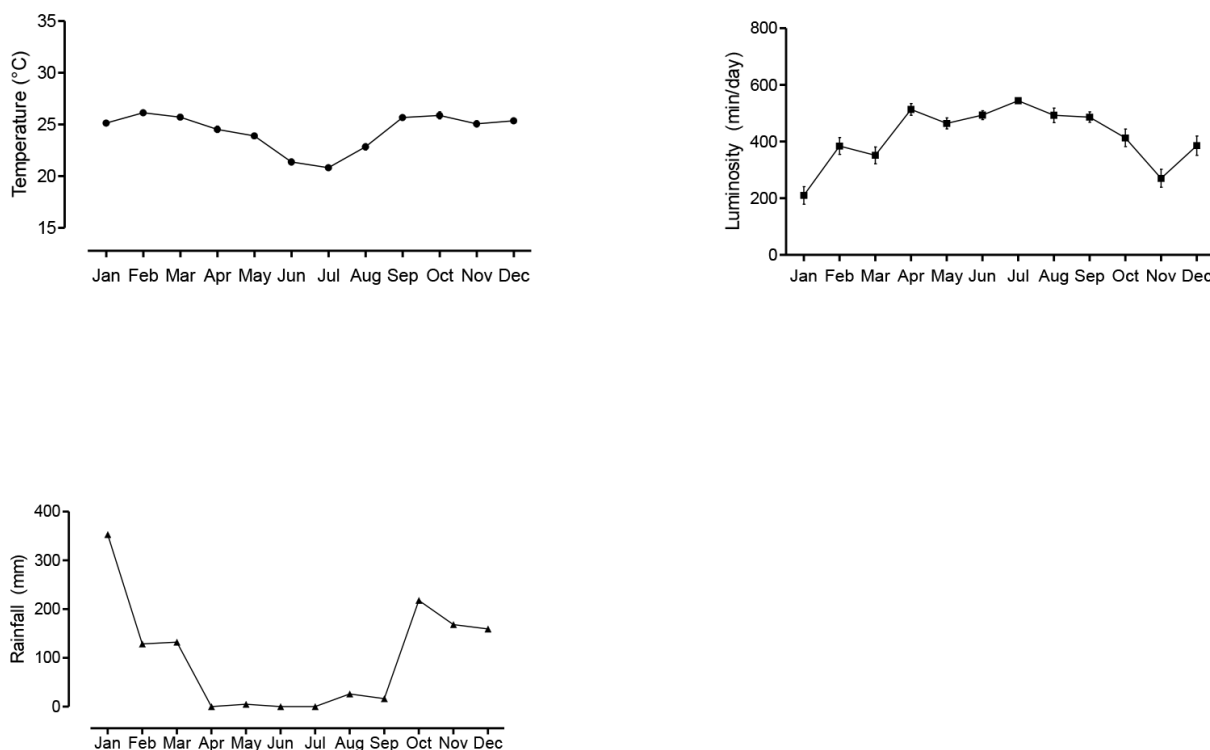
During the rainy season (October), the seminiferous epithelium showed stratification, with a reduced lumen and the presence of all cell types from the spermatogenesis lineage, with exception of spermatozoa; spermatogonia, primary spermatocytes in different phases of meiotic division, round and elongated spermatids, besides Sertoli cells. Around the seminiferous tubules, the interstitium demonstrated a size reduction when compared to the dry season, however, still showing

fibroblasts, Leydig cells and collagen fibers (Figures 2d, e and f).

In the morphometric analysis, we measured the longer and shorter axis from the seminiferous tubules and the seminiferous epithelium height (Figures 4 and 5). All analyzed variables showed significant differences in comparing the dry and rainy seasons of the Cerrado biome, being all the morphometric values, greater during the rainy season ( $p < 0.001$ ).

## DISCUSSION

The blue-and-yellow macaw testicular capsule paved the epididymal region as well as the testicle. Histologically, we could identify only two layers of this testicular capsule, or in other words, the serous and albuginea tunics, differently from Carvalho et al. (2015), who work



**Figure 3.** Monthly climate variations of air temperature (°C), luminosity (min/day) and rainfall (mm) from Cerrado biome, in 2016, in the region of Goiânia/GO.

with emus, that possess the inner vascular tunic belonging to the testicular capsule. The testis of the *Ara ararauna* were contained in a covering, the tunica albuginea. The capsule did not give off septa and therefore seminiferous tubules were not separated by true septa, similar to that found in *Numida meleagris* (Abdul-Rahman et al. 2017).

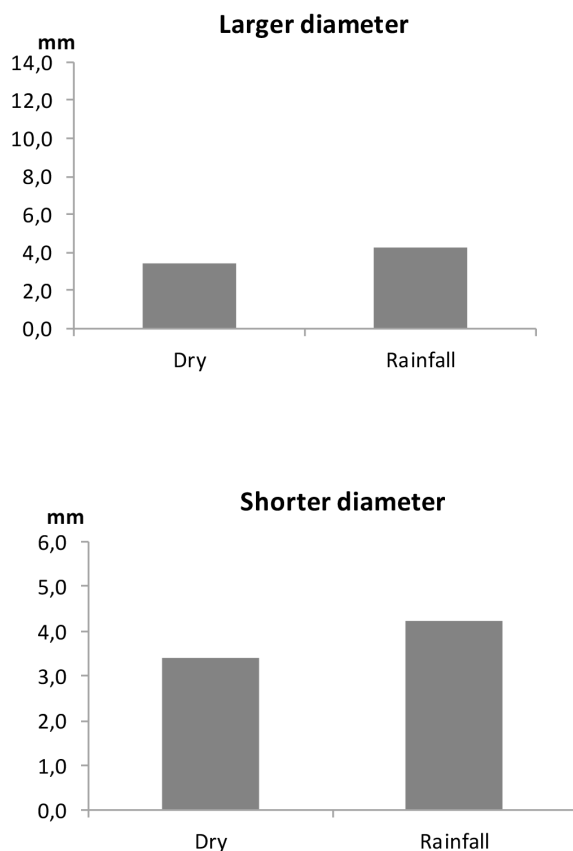
Differently from what Carvalho et al. (2015) reported in his study with emus, the external morphology of *Ara ararauna* testis did not demonstrate larger differences between the dry (April) and rainy (October) seasons, mainly about length and width, as well as the total weight and volume. However, the right testicle

presented itself, in both climate periods, bigger and thinner than the left testicle, similar to what Bull et al. (2007) observed in the domestic cock.

The blue-and-yellow macaw parenchyma, as well as the emus (Carvalho et al. 2015), during both Cerrado biome characteristic climates, was formed by the interstitium and various seminiferous tubules, without septa or lobes dividing the parenchyma. In the *Ara ararauna*, the interstitium in both, dry and rainy seasons, was replenished by fibroblasts, fibrocytes, collagen fibers and Leydig cells, however, it showed more development during the dry season and regressed during the rainy season, a similar pattern found in the domestic duck (Simões et al. 2005, 2017) and the guinea fowl (Dharani et al. 2018).

Baraldi-Artoni et al. (1997) defined four phases in the domestic quail's annual reproductive cycle, according to the months of the year and morphometric parameters such as: seminiferous tubules diameter, seminiferous epithelium height, quantification of mature spermatids and spermatozoa present in the seminiferous tubule's lumen, besides the characterization of the testicular weights. These phases were described as gonadal rest or quiescence (end of Spring), spermatogenesis recrudescence or retake (Autumn), reproduction (Winter and beginning of Spring) and testicular regression (Summer). This pattern found in domestic quail (Baraldi-Artoni et al. 1997) was similar to the one found in the domestic duck (Simões et al. 2005, 2017) and emus (Carvalho et al. 2015).

Breucker (1982) and Breucker et al. (1989) considered the increase and decrease of the seminiferous tubule diameter as a parameter to evaluate the spermatogenic activity in their studies about the annual reproductive cycle of the European swan and Andean duck. The European swan seminiferous tubules exhibited



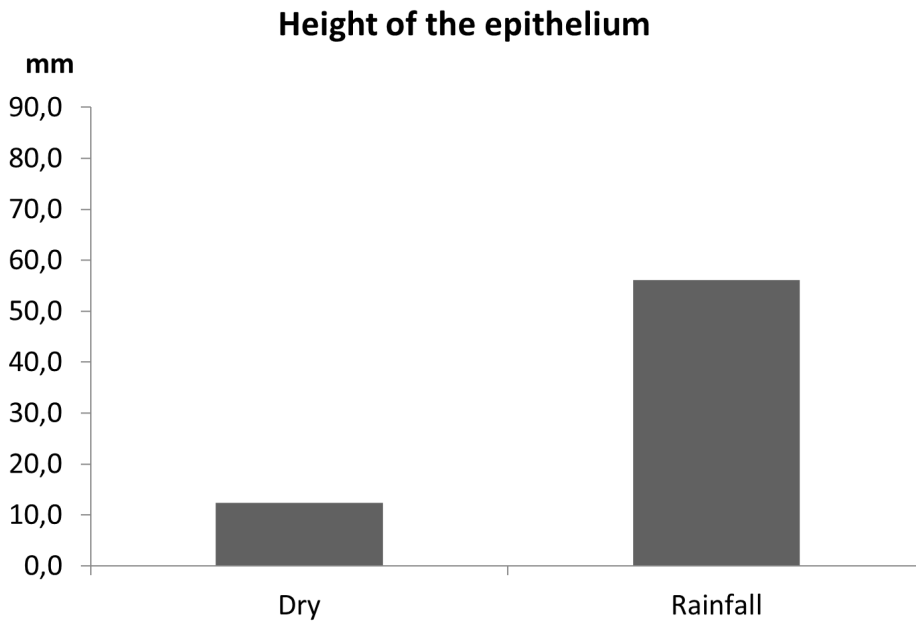
**Figure 4.** Mean and standard deviation of the longer (mm) and shorter (mm) axis of seminiferous tubules during the dry and rainy seasons. T-test for independent samples ( $p < 0.0001$ ).

a progressive increase in their diameter during the Winter, showing the greater value during the Spring their reproductive period, and from that on, a subsequent decrease during the Summer and Autumn, periods of testicular regression and quiescence. In the Andean duck, a small decrease in the seminiferous tubule's diameter was observed during the Summer (October to December) and in Winter (July to August). The differences in the morphometric parameters between these species were probably due to the photoperiod change in both hemispheres. In blue-and-yellow macaw's, the seminiferous tubule longer and shorter axis, besides the seminiferous epithelium height, were statistically bigger during the rainy season (October), when compared to the dry season (April), being these, very characteristic of the testicular cycles of recrudescence and rest in birds.

Comparing *Ara ararauna* testicular histology with the one from the domestic duck studied by Simões et al. (2005), the reproductive cycle on which the species was found during the dry season (April), a month belonging to the

Autumn season in the South Hemisphere, was similar to that found in the domestic duck in the rest phase, occurring during the months of January and February (Summer), where the seminiferous tubules possess only Sertoli cells and spermatogonia. During the rainy season (October), a Spring season, the blue-and-yellow macaw's phase of testicular cycle was in the period of recrudescence, because the seminiferous tubules were ample and demonstrated all the cell types of the seminiferous epithelium, except spermatozoa.

Breucker et al. (1989) reinforce that the seasonal reproductive cycles are subject to not only the climate variations such as rainfall, temperature, and food availability but also to factors such as geographic latitude and altitude, altering the phases of the reproductive cycle in birds. To the climate data obtained during the dry and rainy seasons of the Cerrado biome, only the rainfall did demonstrate significant differences, being possibly be correlated to the *Ara ararauna* testicular responses.



**Figure 5. Mean and standard deviation of the heights of seminiferous epithelium between the dry and rainy seasons. T-test for independent samples ( $p < 0.0001$ ).**



In the domestic duck (Simões et al. 2005, 2017), the recrudescence phase extended throughout the Autumn, from March to June, while reproduction began in July (Winter), peaking in October. Although the studies of testicular cycling with domestic duck (Simões et al. 2005, 2017) and Japanese quail (Baraldi-Artori et al. 1997) were conducted in the Cerrado biome and with captivity species, similarly to the blue-and-yellow macaw's, the geographic latitude and altitude, rainfall, photoperiod length and temperature, were too much difference between the regions of these studies, possibly leading to an alteration of the testicular response to the year's seasons, characterizing a distinct testicular cycle for each analyzed species.

Therefore, we concluded that, according to the analyzed parameters, the blue-and-yellow macaw's in captivity in the Cerrado biome, demonstrated averaged weight, testicular longer axis and volume, similar between the periods of dry (April) and rainy (October). However, the variable shorter axis exhibited a statistically significant increase during the rainy season concerning the dry ones. The seminiferous tubule longer and shorter axis and epithelium height showed higher values during the rainy season and, during this period, all types of spermatogenesis cells were also found, except spermatozoa, which characterized the recrudescence period, or, the retaking of spermatogenesis that preceded the reproductive phase. During the dry season, only spermatogonia were found together with Sertoli cells, characterizing the rest phase of the seminiferous epithelium.

With this, we concluded that the studied species, even in captivity, under the Cerrado natural environmental conditions, have kept their reproductive potential. Complementary future studies about the reproductive biology of wild birds, kept in captivity, will be fundamental

for a better understanding of the testicular kinetics and reproductive process, ensuring the elaboration of conservational plans for these species living in different biomes.

### Acknowledgments

We acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Goiás Federal University (Post-Graduation Course in Animal Biodiversity) for the project financing and the Center of Wild Animal's Screening (CETAS-GO) for their technical and scientific assistance.

### REFERENCES

- ABDUL-RAHMAN II, OBESE FY & JEFCOATE IA. 2017. Developmental changes in the histological structure of the testis, and testosterone profiles in male guinea fowls (*Numida meleagris*). *Theriogenol* 10: 14-122.
- AMOROSO L, BARALDI-ARTONI SM, MORAES VMB, PERECIN D, FRANZO VS & AMOROSO P. 2008. Influência da espermatogênese e dos níveis de testosterona no aspecto reprodutivo de codornas. *Rev Bras Zootec* 37: 61-66.
- BARALDI-ARTONI SM, ORSI AM, CARVALHO TL & LOPES RA. 1997. The Annual Testicular Cycle of the Domestic Quail (*Coturnix japonica*). *Anat Histol Embryol* 26: 337-339.
- BREUCKER H. 1982. Seasonal spermatogenesis in the mute swan (*Cygnus olor*). *Anat Embryol Cell Biol* 72: 1-91.
- BREUCKERH, SCHAFFERE & OBREGONEB. 1989. Spermatogenesis of *Lophonetta specularioides* (Anatidae, Aves) in the high plateau of the southern Andes. A light and electron microscopic study. *Micr Electr Biol Cel* 13: 167-182.
- BULL ML, MARTINS MRFB, CESÁRIO MD, PADOVANI CR & MENDES AA. 2007. Anatomical study on Domestic fowl (*Gallus domesticus*) reproductive system. *Int J Morphol* 24: 709-716.
- CARVALHO SFM, FRENEAU BN & FRERNEAU GE. 2015. Aspects of the Macroscopic Testicular and Epididymal Morphology in the Greater Rhea, Rhea Americana (Linnaeus – 1758) Birds. *Anat Histol Embryol* 44: 255-261.
- DHARANI P, USHAKUMARY S, SUNDARAM V, JOSEPH C & RAMESH G. 2018. Morphological Analysis of Testis of the Guinea Fowl (*Numida meleagris*) Under Tropical Savannah Climate of India. *Int J Morphol* 36: 909-914.

HAU M. 2001. Timing of breeding in variable environments: tropical birds as model systems. *Horm Behav* 1: 281-290.

HAU M, PERFITO N & MOORE IT. 2008. Timing of breeding in tropical birds: mechanisms and evolutionary implications. *Ornitol Neotrop* 19: 39-59.

HAU M, WIKELSKI M & WINGFIELD JC. 1998. A neotropical Forest bird can measure the slight changes in tropical photoperiod. *Proc Biol Sci* 265: 89-95.

KEMP AC. 1973. Environmental factors affecting the onset of breeding in some Southern African hornbills, *Tockus spp.* *J Reprod Fertil* 19: 319-331.

MIRAGLIA SM & HAYASHI H. 1993. Histomorphometry of immature rat after heating. *J Morphol* 217: 65-74.

MOORE IT, BENTLEY GE, WOTUS C & WINGFIELD JC. 2006. Photoperiod-independent changes in immunoreactive brain gonadotropin-releasing hormone (GnRH) in a free living, tropical bird. *Brain Behav Evol* 68: 37-44.

ORSI A, STEFANINI M, VIEGAS K, SIMÕES K & ARTONI SMB. 2005. Aspectos morfológicos do ciclo testicular anual de codorna doméstica (*Coturnix coturnix*) da variedade italiana. *Braz J Vet Res An Sci* 42: 163-170.

SIMÕES K, ORSI AM, SCHIMMING B & BENETTI EJ. 2017. Seasonal reproductive cycle and variability in plasma testosterone levels in the domestic duck (*Anas platyrhynchos*). *Biosci J* 33: 53-164.

SIMÕES K, ORSI AM, URBINATI EC, GONÇALVES FD, CRUZ C & NATALI MRM. 2005. Estudo morfológico do ciclo reprodutivo e variabilidade nos níveis de glicose, de glicogênio e de lipídeo ao longo do ano em pato doméstico (*Anas platyrhynchos*). *Acta Scient* 27: 319-325.

VILLAGRA ALI, CISINT SB, CRESPO CA, MEDINA MF, RAMOS I & FERNÁNDEZ SN. 2012. Spermatogenesis in *Leptodactylus chaquensis*. *Histological study*. *Zygote* 22: 291-299.

#### How to cite

MOREIRA MG, BENETTI EJ, REBELO ACS, SOUSA GC, MEDEIROS MVM, VIEIRA LG, MAZARO RC & SIMÕES K. 2021. Morphological characterization of blue-and-yellow macaw (*Ara ararauna*) seasonal testicular variations in captivity. *An Acad Bras Cienc* 93: e20190557. DOI 10.1590/0001-3765202120190557.

*Manuscript received on June 25, 2019;  
accepted for publication on April 13, 2020*

#### MARIANA G. MOREIRA<sup>1</sup>

<https://orcid.org/0000-0002-8361-6454>

#### EDSON J. BENETTI<sup>1</sup>

<https://orcid.org/0000-0002-2529-2682>

#### ANA C.S. REBELO<sup>1</sup>

<https://orcid.org/0000-0002-9214-5025>

#### GESSICA C. SOUSA<sup>1</sup>

<https://orcid.org/0000-0002-2927-9661>

#### MAX V.M. MEDEIROS<sup>1</sup>

<https://orcid.org/0000-0003-4727-9822>

#### LUCÉLIA G. VIEIRA<sup>1</sup>

<https://orcid.org/0000-0003-3116-3374>

#### RENATA C. MAZARO<sup>2</sup>

<https://orcid.org/0000-0002-0198-2910>

#### KARINA SIMÕES<sup>1</sup>

<https://orcid.org/0000-0001-5018-2760>

<sup>1</sup>Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Morfologia, Avenida Esperança, s/n, 74690-900 Goiânia, GO, Brazil

<sup>2</sup>Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Farmacologia, Avenida Esperança, s/n, 74690-900 Goiânia, GO, Brazil

Correspondence to: **Karina Simões**

E-mail: [simoesk@ufg.br](mailto:simoesk@ufg.br)

#### Author contributions

Mariana G. Moreira, Renata M. Costa and Karina Simões contributed to data acquisition and writing of the manuscript, data interpretation and critical revision of the manuscript. Mariana G. Moreira, Renata M. Costa, Karina Simões, Edson J. Benetti, Ana C.S. Rebelo, Gessica C. Sousa, Max V.M. Medeiros and Lucélia G. Vieira designed the study and contributed to data acquisition. All authors discussed the results and approved the final version of the manuscript.

