http://dx.doi.org/10.1590/1678-4162-12657

Arq. Bras. Med. Vet. Zootec., v.74, n.5, p.853-861, 2022

Testicle histology of the *Epicrates cenchria*: a morphological and reproductive biology analysis

[Histologia dos testículos de Epicrates cenchria: uma análise morfológica e da biologia reprodutiva]

H.J. *Bento*[1,3], A. *Ferreira*[2], G.A. *Iglesias*[1,3], F.F. *Curcio*[2], H. A.S. *Lima*[3], T.G. *Araújo*[4], A.H. *Kuczmarski*[1,3], R.C.R. *Paz*[1,3]

[1]Graduate, Universidade Federal do Mato Grosso, Faculdade de Medicina Veterinária, UFMT, Cuiabá, MT, Brasil
[2]Universidade Federal do Mato Grosso, UF,T. Instituto de Biociências, Cuiabá, MT, Brasil
[3]Universidade Federal do Mato Grosso, Faculdade de Medicina Veterinária, Favet-UFMT, Cuiabá, MT, Brasil
[4]Graduate, Universidade Federal de Alfenas, Unifal, Alfenas, MG, Brasil

ABSTRACT

The lack of information about anatomy, physiology and reproductive biology in many snake species makes the understanding of these free-living animals' reproduction and reproductive biotechnics application in captivity difficult. The present study aims to evaluate the *Epicrates cenchria's* testicle morphology and correlate these findings with environmental aspects and reproductive biology. The testicles of five specimens of *E. cenchria* were histologically evaluated, and it was possible to observe seasonality in sperm production, with the presence of mature spermatozoa in the wettest and warmest periods of the year, as well as the highest testicular volume in these periods. Correlating these findings with that reported in the literature on copulation period presupposes a prenuptial (or associated) pattern in *E. cenchria*.

Keywords: snakes, spermatogenesis, reproduction, reproductive pattern

RESUMO

A falta de informações sobre anatomia, fisiologia e biologia reprodutiva em muitas espécies de serpentes impossibilita compreender melhor a reprodução desses animais em vida livre e aprimorar as biotécnicas reprodutivas nessas espécies em cativeiro. O presente trabalho tem como objetivo avaliar a morfologia dos testículos de Epicrates cenchria *e correlacionar esses achados com aspectos ambientais e da biologia reprodutiva. Os testículos de cinco espécimes de* E. cenchria *foram avaliados histologicamente, sendo possível observar sazonalidade na produção espermática, com presença de espermatozoides maduros nos períodos mais chuvosos e quentes do ano, bem como o maior volume testicular nesses períodos. Correlacionando-se esses achados com o relatado em literatura sobre período de cópulas, pressupõe-se um padrão pré-nupcial (ou associado) em* E. cenchria.

Palavras-chave: serpentes, espermatogênese, reprodução, padrão reprodutivo

INTRODUCTION

Boidae snakes have a worldwide distribution, and are found in the Americas, South Pacific islands, India, Central Africa, and South Asia. They are divided into two subfamilies: Boinae (genera *Boa*, *Candoia*, *Corallus*, *Epicrates*, *Eunectes* and *Sanzinia*) and Erycinae (genera *Charina* and *Eryx*), presenting a robust body, with well-developed musculature, necessary to kill their prey by constriction (Zug *et al.*, 2001; Garcia *et al.*, 2015).

The Boidae family has several species, including *Epicrates cenchria*, known as Rainbow Boa. These snakes are found from Costa Rica to Argentina, with wide distribution in Brazil. It presents terrestrial and arboreal, day and night habits, feeds on small mammals, birds, and eggs

Corresponding author: heitorjbento@gmail.com
Submitted: January 29, 2021. Accepted: July 15, 2022

of birds and small lizards. It is not a venomous species and presents a peculiar coloring aspect, becoming an exotic pet and consequently demanding captive reproduction increase (Zug *et al.*, 2001; Grego *et al.*, 2014; Costa and Bérnils, 2018).

Snakes, as well as reptiles, present seasonal aspects in reproduction, showing continuous or discontinuous gametogenesis depending on the species. (Pizzatto *et al.*, 2006). It should be emphasized that in snakes, several environmental factors such as temperature, rainfall, humidity, photoperiod, and diet directly influence the reproductive aspects in individuals of different species, subspecies and even specimens inserted in different habitats, which justifies a better understanding of reproductive aspects. (Girons, 1982; Pizzatto *et al.*, 2006).

The snakes' reproduction in captivity (naturally or using biotechnologies) is difficult, due to the lack of basic information about the anatomy, physiology, and reproductive biology of individuals in this group (Zacariotti and Guimarães, 2010).

Histological and ultrasound evaluation, for example, are used to understand morphological variations that occur in testicle sizes in seasonal individuals, to identify the different reproductive periods (Bertona and Chiaraviglio, 2003; Ibargüengoytía *et al.*, 2006; Garcia *et al.*, 2015). Mature animals' determination, in addition to gonadosomatic indices and macroscopic observations data in these species can be better understood through the histological characterization of gametogenesis (Hernández-Gallegos *et al.*, 2002).

The present study aims to evaluate morphological aspects of the testicles of *E. cenchria*, correlating these findings with environmental factors and aspects of reproductive biology of this species.

MATERIALS AND METHODS

This study was conducted with authorization from the Ethics Committee on The Use of Animals (CEUA/UFMT) no. 23108.050100/2019-01.

Five testicles' *E. cenchria* specimens at Laboratory of Zoological Collections – Herpetology Sector of the Institute of Biosciences of UFMT, from different regions of the state of Mato Grosso, Brazil, and named 2384 (captured at Vale de São Domingos, on May 19, 2002), 3897 (captured at Claudia, on February 5, 2004) , 3920 (captured in at Claudia, on March 20, 2003), 4187 (captured at Aripuanã, on August 15, 2006) and 5489 (captured at Alta Floresta, on January 10, 2007) were used. It was not possible to collect both testicles in all specimens, because, in some of them, one of the testicles had already been removed for previous projects or didactic purposes.

The testicles collected were measured with a caliper, and the testicular volume was calculated based on the ellipsoid volume, according to Méndez and Villagrán (1998). Rostro-cloacal length was measured with a measuring tape.

Climatic data from the capture sites for the study period were obtained from the Meteorological Database for Teaching and Research (BDMEP) of the National Institute of Meteorology (Banco de Dados Meteorológicos para Ensino e Pesquisa, 2020). Data from available weather stations were searched based on proximity or climatic similarity, according to Souza *et al.* (2013), between the collection site and the location of the weather station, in a period of approximately six months before and six months after the capture date.

To perform histological processing, the tissues were initially fragmented and packed in Eppendorf's. Subsequently, the tissue samples were processed for dehydration in two stages: 1. Samples were kept in 70º ethyl alcohol and refrigerated for a minimum period of 24 hours; 2. 70º ethyl alcohol was removed and then 97º ethyl alcohol was added for a period of 4 hours kept refrigerated.

After the dehydration procedure, the samples were incorporated in plastic resin type methacrylate glycol according to the manufacturer's recommendations.

The incorporated samples were cut into sections of 3µm thickness in microtome, fixed in histological lamina and stained with 1% Toluidine Blue, aqueous solution, pH ≅ 6.0. To

stain, the dye was dripped over the histological sections, with the Pasteur pipette aid and waited three minutes. The excess dye was removed, and the histological lamina dried on a heating plate. After that, the slides were prepared for photo documentation.

RESULTS

The rostro-cloacal length of 5489 specimens was 101 cm, the testicle volume of the left testicle was 2.41cm³ and right testicle was 2.09cm³. This animal was captured in January, a period characterized by high rainfall and temperature rates (Fig. 1). In the histological analysis (Fig. 2A) the testicles presented seminiferous tubules with evident lumen, without spermatozoa presence. The germinative epithelium presents Sertoli cells, type A and type B spermatogonia, and primary spermatocytes in reduced amounts when compared to other individuals. Interstitial tissue is little evident, with dense and organized collagen fibers, and evident and grouped Leydig cells. In the morphometric evaluation (Fig. 3) seminiferous tubules presented diameter of 159.5µm ± 25.29µm, germinative epithelium with height of 41.97µm ± 11.23µm, and interstitial tissue with height 10.68µm ± 5.83µm.

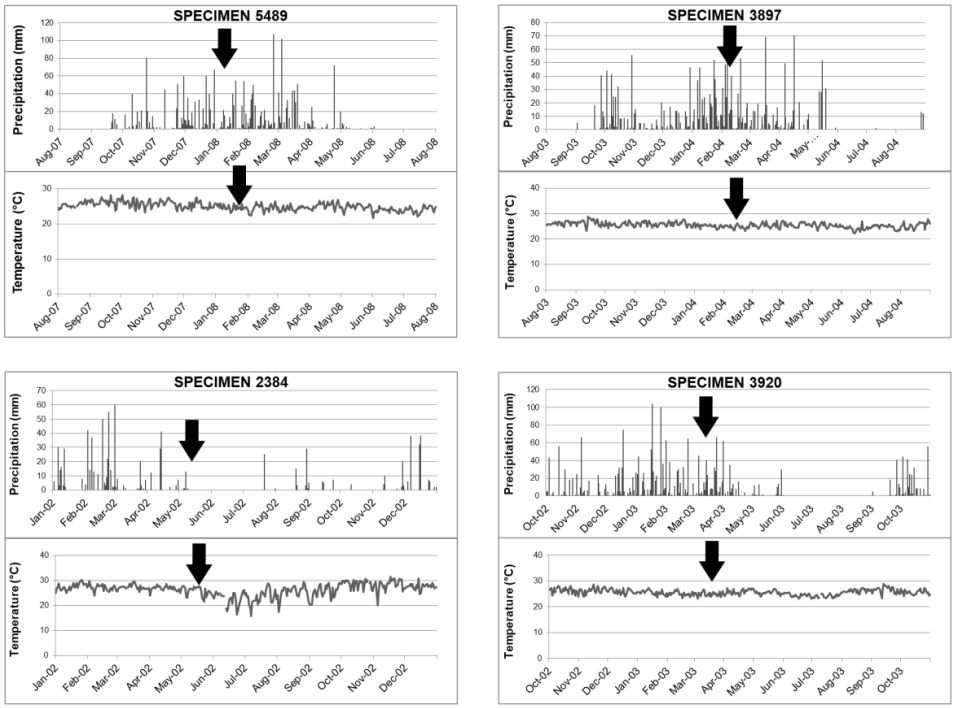

Figure 1**.** Rainfall (above) and temperature (below) average of the capture period of specimens 5489 (weather station 83214), 3897 (weather station 83214), 2384 (weather station 83405) and 3920 (weather station 83214). The arrows indicate the capture periods. (Banco de Dados Meteorológicos para Ensino e Pesquisa, 2020).

The rostro-cloacal length of 3897 specimens was 106 cm, the testicle volume of the right testicle was 2.41cm³ and the left testicle was absent. This animal was captured in February, a period characterized by high temperatures and high rainfall (Fig. 1). In histological analysis (Fig. 2B) the testicles present seminiferous tubules with narrow lumen, high density of mature spermatozoa, and absence of cellular remains. The germinative epithelium presented cells in different gametogenesis phases. In the morphometric evaluation (Fig. 3) this animal presented seminiferous tubules with a diameter of 232.15µm ± 40.54µm, germinative epithelium with height of 111.61µm ± 15.69µm, and interstitial tissue with height of 11.89µm ± 2.94µm.

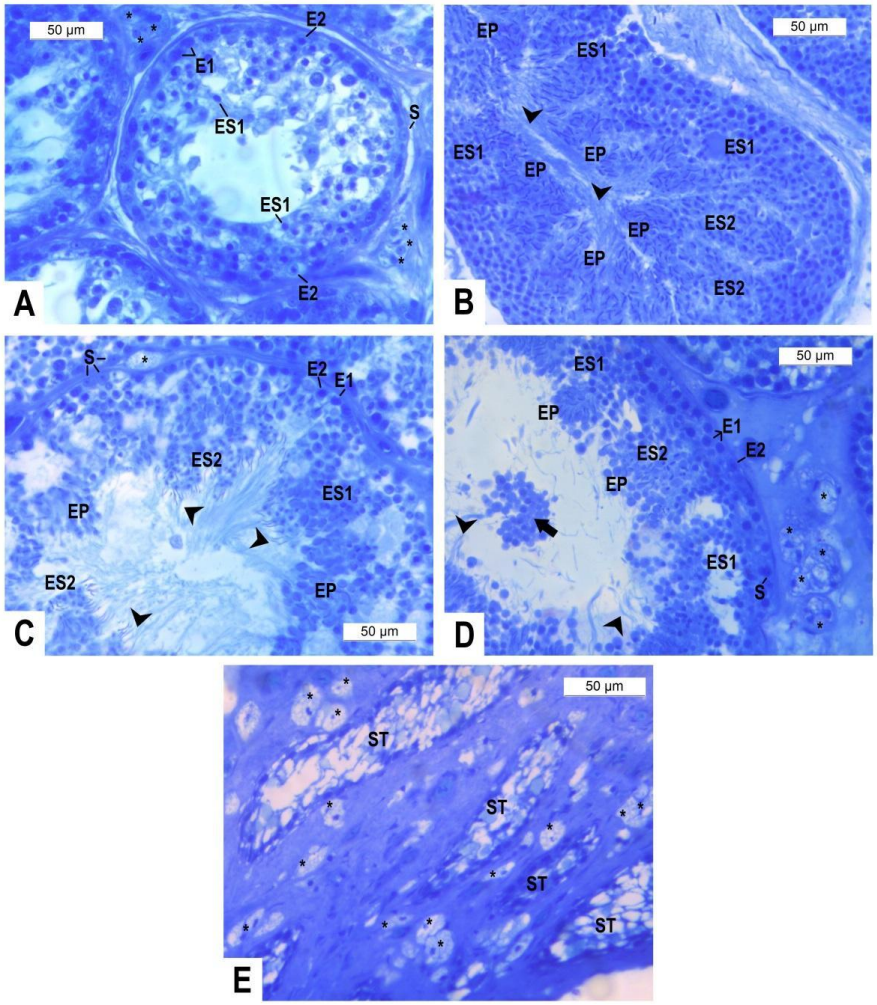

Figure 2. *Epicrates cenchria* testicles histology. **[A]** Exemplar 5489 (stage 2), captured in a high rainfall and temperature rates period. Seminiferous tubules with evident lumen, without the presence of sperm. The germ epithelium presents Sertoli cells, type A and type B spermatogonia, and primary spermatocytes. Interstitial tissue is little evident, with dense and organized collagen fibers, and evident and grouped Leydig cells. Toluidine blue 1%, obj. 40x. **[B]** Exemplar 3897 (stage 6), captured in a high temperatures and rainfall period. Seminiferous tubules with little lumen evident and high density of spermatozoa (arrowhead), and cellular debris absence. The germinative epithelium presents cells in different gametogenesis phases. Toluidine blue 1%, obj. 40x. **[C]** Exemplar 2384 (stage 6), captured in higher temperatures and rainfall period. Seminiferous tubules with evident lumen and spermatozoa presence (arrowhead). Germinative epithelium with cells in different gametogenesis phases, and not evident interstitial tissue, with Leydig cells presence. Toluidine blue 1%, obj. 40x. **[D]** Exemplar 3920 (stage 6), captured in a high rainfall and temperatures period. Seminiferous tubules with evident lumen, spermatozoa presence (arrowhead) and cellular remains (arrow). The germinative epithelium presents cells in different gametogenesis phases, and evident interstitial tissue, with Leydig cells presence. Toluidine blue 1%, obj. 40x. **[E]** Exemplar 4187 (stage 8), captured in lower rainfall and temperature rates period. Testicles with seminiferous tubules without evidence, lumen absence, no spermatozoa and cellular debris. In the seminiferous tubules we observe germinative cells in apparent pos-reproductive period apoptosis process, characterized by homogeneous aspect, with chromatin condensed and cytoplasm fragmented and collapsed, without Sertoli cells evident. The interstitial tissue is evident, with Leydig cells grouped. Toluidine blue 1%, obj. 40x. Legend: (S) Sertoli cells, (E1) spermatogonia type A, (E2) spermatogonia type B, (ES1) primary spermatocyte, (ES2) secondary spermatocyte, (EP) spermatid, (ST) seminiferous tubule, (*) Leydig cell.

The rostro-cloacal length of 2384 specimens was 117 cm, the testicle volume of the left testicle was 29.68cm³ and the right testicle was absent. This animal was captured in May, with higher temperatures compared to other periods of the year and high rainfall levels, close to the transition period with the driest time of the year (Fig. 1). In the histological analysis (Fig. 2C) the testes presented seminiferous tubules with evident lumen and spermatozoa presence, germinative epithelium with cells in different gametogenesis phases, and interstitial tissue not evident with the presence of Leydig cells. In the morphometric evaluation (Fig. 3) seminiferous tubules presented diameter of 247.3µm ± 38.38µm, germinative epithelium with height of 98.89µm ±19.07µm, and interstitial tissue with height of 6.61µm ±2.51µm.

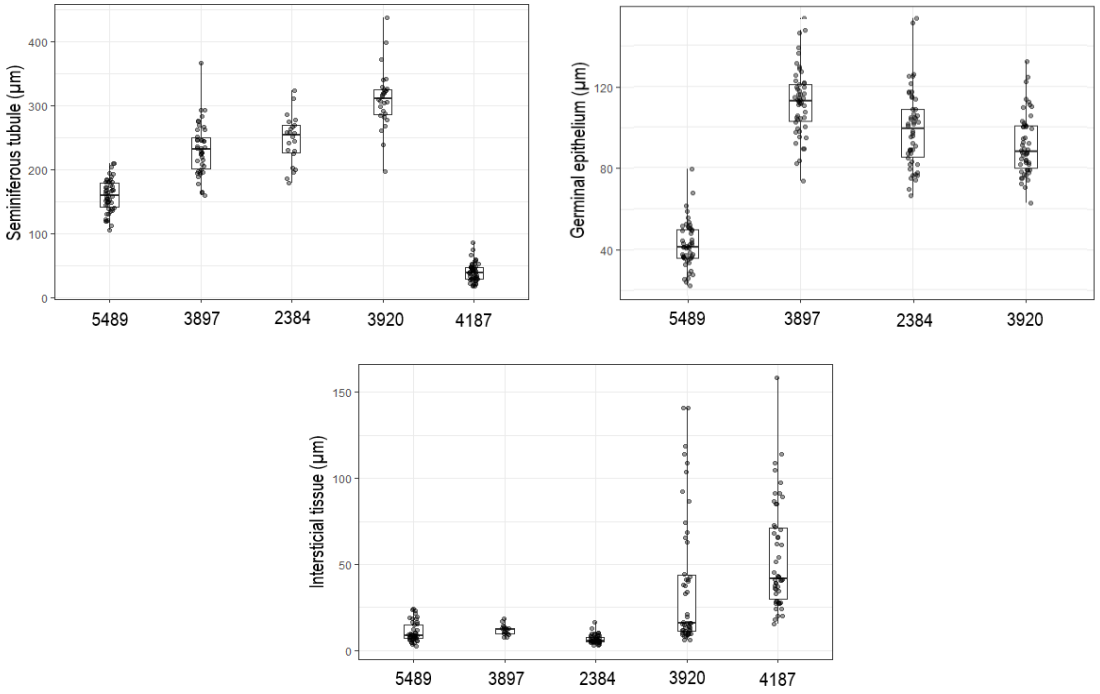

Figure 3. *Epicrates cenchria* testicles morphometric analysis graphic representation in different reproductive periods: seminiferous tubules diameter, germinative epithelium height and interstitial tissue height. The boxes represent the mean values (middle line) and the standard deviation, and the lines above and below represent the upper and lower bounds, respectively.

The rostro-cloacal length of 3920 specimens was 130cm, the testicle volume of the right testicle was 17.25cm³ and the left testicle was absent. This animal was captured in March, a period characterized by high rainfall rates and high temperatures

(Fig. 1). In histological analysis (Fig. 2D) the testes presented seminiferous tubules with evident lumen, spermatozoa presence and cellular remains, and germinative epithelium with cells in different gametogenesis phases. The interstitial tissue was evident with Leydig cells. In the morphometric evaluation (Fig. 3) seminiferous tubules presented diameter of 311µm±45.83µm, germinative epithelium with height of 91.28µm ± 15.04µm, and interstitial tissue with height of 37.71µm ±38.19µm.

The rostro-cloacal length of 4187 specimens was 113 cm, the testicle volume of the left testicle was 1.42cm³ and right testicle was 1.51cm³. The site of animal was capture did not present accurate meteorological data, but according to Souza *et al*. (2013), the capture site presents climatic parameters similar to the capture sites of specimens 3897 and 3920; thus, the capture period of this individual (August) is characterized by lower rainfall and temperature rates. Histological analysis (Fig. 2E) of the testicles presented seminiferous tubules and lumen without evidence, absent spermatozoa and

cellular remains. In the seminiferous tubules we observe cells in apoptosis process post-reproductive period, which are characterized by homogeneous aspect, with condensed chromatin and cytoplasm in fragmentation and collapse, Sertoli cells were difficult to observe. The interstitial tissue is very apparent, with Leydig cells evident and grouped. In the morphometric evaluation (Fig. 3) seminiferous tubules presented a diameter of 40.15μm±14.64μm, and interstitial tissue with height of 54.07μm±30.59μm. Due to the apoptosis process, it is not possible to measure the height of germination epithelium.

DISCUSSION

The present study highlights *E. cenchria* reproductive seasonality in relation to the gametes male's production evaluated through the histological analysis of the testicle. The maximum sperm production occurring in the wettest periods of the year, with high temperatures, according to as observed in other snakes of the boidae family, such as *Boa c. occidentalis* (Bertona and Chiaraviglio, 2003; Ibargüengoytía *et al.*, 2006) and *Boa c. constrictor* (Bento *et al.*, 2019).

The spermatogenic cycle, according to Ballinger and Nietfeldt (1989), is classified into eight gametogenesis stages expressed as follows: (juvenile – stage 0) non-apparent germ cells; (increasing - stage 1) division of germ cells without lumen development; (early spermatogenesis - stage 2) primary spermatocytes in the lumen; (spermatogenesis - stage 3) secondary spermatocytes in the lumen; (spermiogenesis - stage 4) undifferentiated spermatids in the lumen; (mature - stage 5) spermatozoa metamorphosis in the lumen; (reproductive - stage 6) mature spermatozoa; (post-reproductive - stage 7) early regression and presence of cellular remains in the lumen; (inactive - stage 8) complete regression, without cell division and without lumen.

The specimen 5489 (Fig. 2A) presents type A and type B spermatogonia and primary spermatocytes, indicating a recrudescent phase, period of early spermatogenesis – stage 2. In specimen 3897 (Fig. 2B) we observe cells in different gametogenesis phases, with sperm metamorphosis and mature spermatozoa presences in the lumen, being this individual in an initial phase of the reproductive period – stage 6. In specimen 2384 (Fig. 2C) we observed the presence of mature sperm in the tubule lumen, being in the reproductive period - stage 6. In specimen 3920 (Fig. 2D) we observe the presence of mature sperm in the tubules lumen, but with abundant presence of cellular debris, indicating that this individual is in the reproductive stage – stage 6, but may already be in an advanced phase of the reproductive stage or close to the regression period, more specifically at the post-reproductive stage. Finally, absence of dividing cells and apparent apoptosis process characterizes specimen 4187 (Fig. 2E) in an inactive period - stage 8. The spermatogenesis stages and the morphological and environmental aspects are expressed in Table 1.

Table 1. *Epicrates cenchria* spermatogenesis: reproductive stages, morphological aspects, and environmental data

Stage[1]	Specimen	SVL[2] (cm)	Testes volume (cm³)		Precipitation	Temperature
			Left	Right		
2	5489	101	2,41	2,09	elevated	elevated
6	3897	106	absent	22,21	elevated	elevated
6	2384	117	29,68	absent	moderate*	elevated
6	3920	130	absent	17,25	elevated	elevated
8	4187	113	1,42	1,51	low	moderate

*Rainy season transition to the driest period of the year, with moderate rainfall indexes.
[1]Spermatogenic cycle according to Ballinger and Nietfeldt (1989).
[2]Snout–vent length.

In the present study we observed that *E. cechria* presented recrudescent (initiating gametogenesis) and reproductive stage (mature spermatozoa in the lumen of the seminiferous tubules) at different times of year with greater rainfall. On basis on rainfall and temperature graphs, and spermatogenic cycle chronology, we observed that the reproductive period (mature spermatozoa) in *E. cenchria* occurs in the higher rainfall and temperature rates period, as observed in other Boidae (Bertona and Chiaraviglio, 2003; Ibargüengoytía *et al.*, 2006; Bento *et al.*, 2019).

We should consider that different environmental factors (temperature, rainfall, humidity, photoperiod, and diet) influence the metabolism of these animals and consequently their reproduction (Girons, 1982; Pizzatto *et al.*, 2006). Considering that food availability and dietary frequency directly influence spermatogenesis in snakes due to the importance of energy accumulation (fat) in the period preceding reproduction (Pizzatto *et al.*, 2006), annual changes may occur, affecting food availability and justifying the spermatogenesis period postponement or anticipation observed in this study.

In addition, we observed an inactive individual (specimen 4187), without cell division in the germinative epithelium and with lower testicular volume, in the months characterized by lower rainfall indexes and mild temperatures as shown in the graphs. In addition, the gametogenesis and environmental relationship, we observed germ cells in an apoptosis process. Although in some species the apoptosis occurrence is related to problems in normal spermatogenesis, affecting spermatozoa quality (Liu *et al.*, 2017), in different species of birds, reptiles, fish and mammals, especially with a seasonal or cyclic reproduction, the occurrence of germ cell apoptosis is reported as a normal part of the spermatogenesis process, showing that this event is essential to maintain the ratio between germ cells and Sertoli cells, influencing directly quality seminal as well as the sperm produced (Young and Nelson, 2001; Gribbins *et al.*, 2005; Zhang *et al.*, 2008; Mahfouz *et al.*, 2009; Shaha *et al.*, 2010; Liu *et al.*, 2017).

The testicular volume variation observed in the present study is similar to the ultrasound evaluation data in *E. cechria* (Garcia *et al.*, 2015). The testicular volume variation is also reported in other snake species, as *Boa c. constrictor* (Garcia *et al.*, 2015; Bento *et. al.*, 2019), *Boa c. occidentalis* (Bertona and Chiaraviglio, 2003; Ibargüengoytía *et al.*, 2006), *Eunectes murinus* (Garcia *et al.*, 2015) and *Gloydius halys caucasicus* (Salehi *et al.*, 2018).

Although we observed the testicular volume correlation with gametic production period in several snakes, as well as in the species studied here, this shouldn't be considered for all snakes. There are some species, such as *Crotalus scutulatus*, that present the maximum testicles volume in the regression period due to endocrine activity (Schuett *et al.*, 2002).

In the morphometric analysis we observed that the seminiferous tubules diameter is increasing from stage 2 to stage 6, presenting a larger diameter in the individuals in reproductive stage, and smaller diameter in inactive stages. In the germinative epithelium we observed a dynamic where it presents lower in stage 2 and at a higher height in stage 6, with a decline as the reproductive stage progresses. Although in the inactive individual it was not possible to determine accurately the germinative epithelium height, the seminiferous tubule diameter considerably smaller in relation to the germinative epithelium height in the other phases makes clear the decrease in this period. This dynamic that occurs in seminiferous tubules diameter and germinative epithelium height was observed in *E. cenchria*, with an increase in reproductive periods and decrease in no reproductive phases, as a result of the mitosis and meiosis that occur in gametogenesis, reported in different species of snakes (Goldeberg and Parker, 1975; Gribbins *et al.*, 2008).

Seasonal gametic production snakes are classified as prenuptial or associated (gamete production occurs simultaneously or preceding copulation period) and postnuptial or dissociated (gamete production occurs after the copulation period, with sperm stocking in the vas deferens until the next copulation) (Girons, 1982; Pizzatto *et al.*, 2006).

According to Bertona and Chiaravaglio (2003), in *Boa c. occidentalis* males the gametogenesis occurs in the rainy periods of the year, while

synchronization and copulation occur in the dry periods, suggesting a prenuptial pattern. In *Boa c. constrictor*, Bento *et al.* (2019) reports the same reproductive pattern.

In the present study, histological evaluations of the *E. cechria* testicles showed gametogenesis in the rainy periods of the year, while the copulation period is reported in autumn-winter (Garcia, 2012), a period of the year characterized by lower rainfall indices and mild temperatures. Thus, when observing gametogenesis period associated with copulations period, we assume that *E. cenchria* presents a prenuptial reproductive pattern. In studies conducted with Brazilian snakes with postnuptial pattern, the maximum testicular mass occurs in autumn (dry season) and copulation in spring (rainy season) (Pizzatto *et al.*, 2006), a condition not observed in this study for *E. cenchria*, reinforcing the prenuptial pattern for this species as observed in other Boidae.

CONCLUSION

Based on histological findings, we concluded that *Epicrates cenchria* spermatogenesis occurs in the increased rainfall and temperatures periods, with higher testicular volume in the reproductive period or near stages. In the reproductive stage it is possible to observe an increase in the diameter of the seminiferous tubules and the height of the germinative epithelium. The histological spermatogenesis evaluation in conjunction with copulations period reported in the literature presupposes a prenuptial pattern for this species.

ACKNOWLEDGEMENTS

The authors thank the Laboratório de Coleções Zoológicas – Setor de Herpetologia do Instituto de Biociências da UFMT, for making available the specimens used in this study; and the Laboratório de Análises Morfológicas e Morfométricas do Instituto de Biociências da UFMT, for providing the structure and intellectual support for the development of this research.

REFERENCES

BALLINGER, R.E.; NIETFELDT, J.W. Ontogenetic stages of reproductive maturity in the viviparous tizard, *Sceloporus jarrovi* (Iguanidae). *J. Herpetol.*, v.23, p.282-292, 1989.

BANCO de Dados Meteorológicos para Ensino e Pesquisa. Brasilia: INMET, 2020. Available in: http://www.inmet.gov.br/portal/index.php?r=bdmep/bdmep. Accessed in: 25 Dec. 2021.

BENTO, H.J.; FERREIRA, A.; CURCIO, F.F. *et al*. Aspectos da biologia reprodutiva de *Boa constrictor constrictor*: um estudo histológico dos testículos nos períodos reprodutivos de quiescência e máxima atividade. *Arq. Bras. Med. Vet. Zootec.*, v.71, p.1551-1557, 2019.

BERTONA, M.; CHIARAVIGLIO, M. Reproductive biology, mating aggregations, and sexual dimorphism of the Argentine boa constrictor (*Boa constrictor occidentalis*). *J. Herpetol.*, v.37, p.510-516, 2003.

COSTA, H.C; BÉRNILS, R.S. Répteis do Brasil e suas Unidades Federativas: Lista de espécies. *Herpetologia Brasileira*, v.7, n.1, p.11-57, 2018. Available in: http://www.sbherpetologia.org.br/. Accessed in: 4 Jan. 2021.

GARCIA, V.C. *Avaliações ultrassonográficas dos ciclos reprodutivos das serpentes Boidae Neotropicais.* 2012. 101f. Dissertação (Mestrado em Anatomia dos Animais Domésticos e Silvestres) - Universidade de São Paulo, São Paulo, SP.

GARCIA, V.C.; VAC, M.H.; BADIGLIAN, L.; ALMEIDA-SANTOS, S.M. Avaliação ultrassonográfica do aparelho reprodutor em serpentes vivíparas da família Boidae. *Pesqui. Vet. Bras.*, v.35, p.311-318, 2015.

GIRONS, H.S. Reproductive cycles of male snakes and their relationships with climate and female reproductive cycles. *Herpetologica*, v.38, p.5-16, 1982.

GOLDEBERG, S.R.; PARKER, W.S. Seasonal testicular histology of the colubrid snakes, *Masticophis taeniatus* and *Pituophis melanoleucus*. *Herpetologica*, v.31, p.317-322, 1975.

GREGO, K.F.; ALBUQUERQUE, L.R.; KOLESNIKOVAS, C.K.M. Squamata (Serpentes). In: CUBAS, Z.S.; SILVA, J.C.R.; CATÃO-DIAS, J.L. (Eds.). *Tratado de animais selvagens*: medicina veterinária. 2.ed. São Paulo: Roca, 2014. p.186-218.

GRIBBINS, K.M.; HAPP, C.S.; SEVER, D.M. Ultrastructure of the reproductive system of the black swamp snake (*Seminatrix pygaea*). V. The temporal germ cell development strategy of the testis. *Acta Zool.*, v.86, p.223-230, 2005.

GRIBBINS, K.M.; RHEUBERT, J.L.; COLLIER, M.H.; SIEGEL, D.S.; SEVER, D.M. Histological analysis of spermatogenesis and the germ cell development strategy within the testis of the male Western Cottonmouth Snake, *Agkistrodon piscivorus leucostoma*. *Ann. Anat.*, v.190, p.461-476, 2008.

HERNÁNDEZ-GALLEGOS, O.; CRUZ, F.R.M.; CRUZ, M.V.; ANDREWS R.M. Continuous spermatogenesis in the lizard *Sceloporus bicanthalis* (sauria: phrynosomatidae) from high elevation habitat of central Mexico. *Herpetologica*, v.58, p.415-421, 2002.

IBARGÜENGOYTÍA, N.R.; BERTONA, M.; CHIARAVIGLIO, M. Seasonal changes in testicular activity of the protected cites i *Boa constrictor occidentalis* (Serpentes: Boidae): a histological study. *S. Am. J. Herpetol.*, v.1, p.143-148, 2006.

LIU, T.; WANG, L.; CHEN, H. *et al*. Molecular and cellular mechanisms of apoptosis during dissociated spermatogenesis. *Front. Physiol.*, v.8, p.1-17, 2017.

MAHFOUZ, R.Z.; SHARMA, R.K.; SAID, T.M.; ERENPREISS, J.E.; AGARWAL, A. Association of sperm apoptosis and DNA ploidy with sperm chromatin quality in human spermatozoa. *Fertil. Steril.*, v.91, p.1110-1118, 2009.

MÉNDEZ, F.R.; VILLAGRÁN, M. Reproducción asincrónica de *Sceloporus palaciosi* (Sauria: Phrynosomatidae) en México, con comentarios sobre sus ventajas y regulación. *Rev. Biol. Trop.*, v.46, p.1159-1161, 1998.

PIZZATTO, L.; ALMEIDA-SANTOS, S.M.; MARQUES, O.A.V. Biologia reprodutiva de serpentes brasileiras. In: OLIVEIRA, M.E.; BARRETO, L. (Eds.). Herpetologia no Brasil. Piraquara: Sociedade Brasileira de Herpetologia, 2006. v.2, p.201-221.

SALEHI, A.; TODEHDEHGHAN, F.; ESLIMIESFAHANI, D. Testosterone level, testicular histology and reproductive events of caucasian pit viper, *Gloydius halys caucasicus* (Serpentes: Viperidae). *J. Adv. Biol.*, v.11, p.2155-2162, 2018.

SCHUETT, G.W.; CARLISLE, S.L.; HOLYCROSS, A.T. *et al*. Mating system of male mojave rattlesnakes (Crotalus scutulatus): seasonal timing of mating, agonistic behavior, spermatogenesis, sexual segment of the kidney, and plasma sex steroids. In: SCHUETT, G.W.; HOGGREN, M.; DOUGLAS, M.; GREENE, H. (Eds.). *Biology of vipers*. Eagle Mountain: Eagle Mountain Publishing, 2002. p.515-532.

SHAHA, C.; TRIPATHI, R.; MISHRA, D.P. Male germ cell apoptosis: regulation and biology. *Philos. Trans. R. Soc.*, v.365, p.1501-1515, 2010.

SOUZA, A.P.; MOTA, L.L.; ZAMADEI, T. *et al*. Classificação climática e balanço hídrico climatológico no estado de Mato Grosso. *Nativa*, v.1, p.34-43, 2013.

YOUNG, K.A.; NELSON, R.J. Mediation of seasonal testicular regression by apoptosis. *Reproduction*, v.122, p.677-685, 2001.

ZACARIOTTI, R.L.; GUIMARÃES, M.A.B.V. Aplicações da biotecnologia na reprodução de serpentes. *Rev. Bras. Reprod. Anim.*, v.34, p.98-104, 2010.

ZHANG, L.; HAN, X.K.; QI, Y.Y.; LIU, Y.; CHEN, Q.S. Seasonal effects on apoptosis and proliferation of germ cells in the testes of the Chinese soft-shelled turtle, *Pelodiscus sinensis*. *Theriogenology*, v.69, p.1148-1158, 2008.

ZUG, G.R.; VITT, L.J.; CALDWELL, J.P. Snakes, In: _____. (Eds). *Herpetology*. 2.ed. San Diego: Academic Press, 2001. p.503-595.