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Sperm storage in *Crotalus durissus* (Serpentes: Crotalinae): histological insights about the female reproductive tract of pit vipers

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Abstract

The reproductive cycle of *Crotalus durissus* is markedly seasonal and synchronous between individuals. The start of vitellogenesis occurs at the end of the summer and coincides with copulation. However, given that the copulation is dissociated from ovulation, sperm storage is obligatory in females. In viperids, sperm storage in the female reproductive tract is reported to occur in two regions: (1) the posterior infundibulum, which presents sperm storage glands; and (2) the nonglandular uterus where sperm is stored in crypts by means of the uterine muscular twisting (UMT). The mechanisms that allow the survival of sperm in the female reproductive tract of snakes are still unknown. In this study, we investigated five regions of the reproductive tract of *C. durissus*, searching for the presence of spermatozoa and sperm storage structures in different oviductal portions. Additionally, we used histological techniques to verify the occurrence of hypertrophy of the infundibular and uterine glands during the processes of vitellogenesis, as well as histochemical techniques to investigate the nature of the secretion produced in the nonglandular uterus and posterior infundibulum. Storage sperm were observed in the nonglandular uterus and although the posterior infundibulum had storage receptacles, sperm were not observed in that region. Both sperm storage regions presented granules testing positive for acidic and neutral polysaccharides, in vitellogenic and previtellogenic females. This presence of guaranteeing conditions for sperm storage. Histochemical analysis revealed the possible storage capacity of sperm in the nonglandular uterus. In addition, the UMT was observed in all the females with storage sperm, which assures the maintenance of sperm in the nonglandular uterus until ovulation.

Keywords Rattlesnake · Reproduction · Vitellogenesis · Uterus · Spermatozoa

Introduction

The female reproductive tract (*Agkistrodon*, *Bothrops*, *Crotalus*) of pit vipers presents two ovaries and two oviducts arranged asymmetrically (Almeida-Santos and Orsi 2002; Siegel et al. 2011; Barros et al. 2014). The anatomy of the oviduct has been divided into anterior infundibulum, posterior infundibulum, nonglandular uterus, glandular uterus, and pouch (Siegel et al. 2011). Regarding the relationship among morphology and reproductive cycles, the posterior infundibulum and the nonglandular uterus can be considered one of the most intriguing and less studied regions of the oviduct (Siegel et al. 2011). It has been suggested that these two oviduct regions represent

the locations that allow the impressive long period sperm storage in snakes (Girling 2002; Holt and Fazeli 2016; Levine et al. 2021). Therefore, several studies have described the morphological and histochemical characteristics of the oviduct throughout the reproductive cycle (Hoffman and Wimsatt 1972; Rojas et al. 2015; Silva et al. 2019). So far, it is well known that the oviduct of vitellogenic females increases the production of secretory granules (e.g., polysaccharides, proteins) in the presence of sperm (Siegel and Sever 2008; Rojas et al. 2015; Silva et al. 2019). However, specificities and roles of these secretions in storing and keeping viable sperm cells are still poorly known.

In general, pit vipers present sperm storage in the nonglandular uterus occurring in crypts, which can be facilitated through uterine contractions, known as uterine muscular twisting (UMT) (Ludwig and Rahn 1943; Almeida-Santos and Salomão 1997; Barros et al. 2012; Silva et al. 2019). On the other hand, sperm storage in the posterior infundibulum occurs in specialized tubular

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receptacles (Siegel and Sever 2008; Silva et al. 2019). Some authors have presented evidence that the oviductal secretion provides a chemical refuge and a place of nutrition for the stored sperm (Siegel and Sever 2008; Marinho et al. 2009). The vitellogenesis phase of the South American rattlesnake, *Crotalus durissus* (Linnaeus 1758), is markedly seasonal and synchronic between individuals (Almeida-Santos and Orsi 2002; Barros et al. 2012). The onset of vitellogenesis occurs at the end of summer and autumn and coincides with the mating period (Almeida-Santos and Orsi 2002; Barros et al. 2012). However, because of the dissociation between copulation and ovulation periods, sperm storage is mandatory for at least four months (Almeida-Santos and Orsi 2002; Barros et al. 2012).

Several histochemical and ultrastructural aspects of the oviduct of pit viper snakes are still little explored (Siegel and Sever 2008; Silva et al. 2019; Souza and Almeida-Santos 2022), and the posterior infundibulum of *C. durissus* has never been properly studied. The morphological description of the oviduct of *C. durissus* and its sperm receptacles is a key improvement for the understanding of the reproductive strategies of *Crotalus*. The sperm storage in this genus is the longest known among vertebrates, reaching up to six years (Booth and Schuett 2011; Levine et al. 2021). Moreover, the stored spermatozoa can allow consecutive parturitions in this genus (Levine et al. 2021). Specifically in *C. durissus*, spermatozoa have been found in the nonglandular uterus (Almeida-Santos and Salomão 1997; Barros et al. 2012), and present a uterine contortion until now exclusive to viper snakes (Nilson and Andrén 1982; Yamanoye et al. 2004; Muniz-da-Silva et al. 2018; Silva et al. 2019).

In this study, we provide new evidence about the anatomy and reproductive cycle of *C. durissus*. We investigate five regions of the female reproductive tract (pouch, nonglandular uterus, glandular uterus, posterior infundibulum, and anterior infundibulum), inspecting the presence of spermatozoa and sperm storage structures in these different oviductal portions. Furthermore, we also use histological techniques to verify the occurrence of hypertrophy of the infundibular and uterine glands during the processes of vitellogenesis. Finally, we use histochemical techniques to investigate the histochemical nature of the secretion produced in the nonglandular uterus and in the posterior infundibulum.

Materials and methods

We examined 55 females of *C. durissus* collected throughout the southeastern region of Brazil and preserved in scientific collections (Appendix I). We measured snout–vent length (SVL) of each specimen before performing dissections.

We recorded the diameter of the largest ovarian follicle using a digital caliper (± 0.1 mm) and the presence of UMT. We conducted histological analyses of the right side of the oviduct (pouch, nonglandular uterus, glandular uterus, posterior infundibulum, anterior infundibulum). Tissue samples were dehydrated through a series of increasing ethanol concentrations, cleared in xylene, embedded in paraffin, and stained with hematoxylin and eosin (Junqueira and Carneiro 2008). In addition, we collected the tissues only from adult females according to the morphological characteristics of the ovaries (presence of vitellogenic follicles or corpus luteum) and/or characteristics of the oviducts (presence of embryos, UMT, or folded oviducts indicating parturition; Silva et al. 2023).

To assess the nature of the secretion produced in the nonglandular uterus and posterior infundibulum, we performed several histochemical techniques. The periodic acid-Schiff (PAS) reaction was performed to identify neutral carbohydrates, and Alcian blue (AB) 8GX (pH 2.5) was used to identify carboxyl and sulfate–ester groups of acid mucosubstances (Kiernan 2008). To detect proteins, we used Coomassie brilliant blue (CBB) R250 (Kiernan 2008) diluted at 0.04 mg/mL (Braz et al. 2018).

We calculated the mean diameter of the uterine and infundibular glands by taking 5–10 measurements from females in vitellogenesis (follicles > 10 mm) and previtellogenesis (follicles ≤ 10 mm; see Almeida-Santos and Orsi 2002; Vieira et al. 2010) using the software ImageJ, version 1.51d (Abràmoff et al. 2004). Values for all measured variables were then averaged to obtain a mean value per individual. Before the analysis, all variables were tested for normality of distribution using the Kolmogorov–Smirnov test. Linear regression was performed to analyze the relationship between the independent variable (follicle diameter) and the dependent variable (secretory infundibular gland diameter and uterine gland diameter).

Results

To facilitate the description, we divided the infundibulum of *C. durissus* into anterior and posterior portions. The anterior infundibulum histologically presented thin muscular tissue, irregular folds in the epithelium, and the absence of glands in the connective (Fig. 1a). The epithelial tissue is presented as a simple cuboidal aggregated, consisting mainly of ciliated cells interspersed with secretory cells. On the other hand, the posterior infundibulum differed from the anterior infundibulum by presenting many ciliated and secretory glands in the connective tissue (Fig. 1b). The epithelium of the posterior infundibulum presented ciliated and secretory cells with oval and rounded nuclei. The epithelium of posterior infundibulum of *C. durissus* showed histochemical
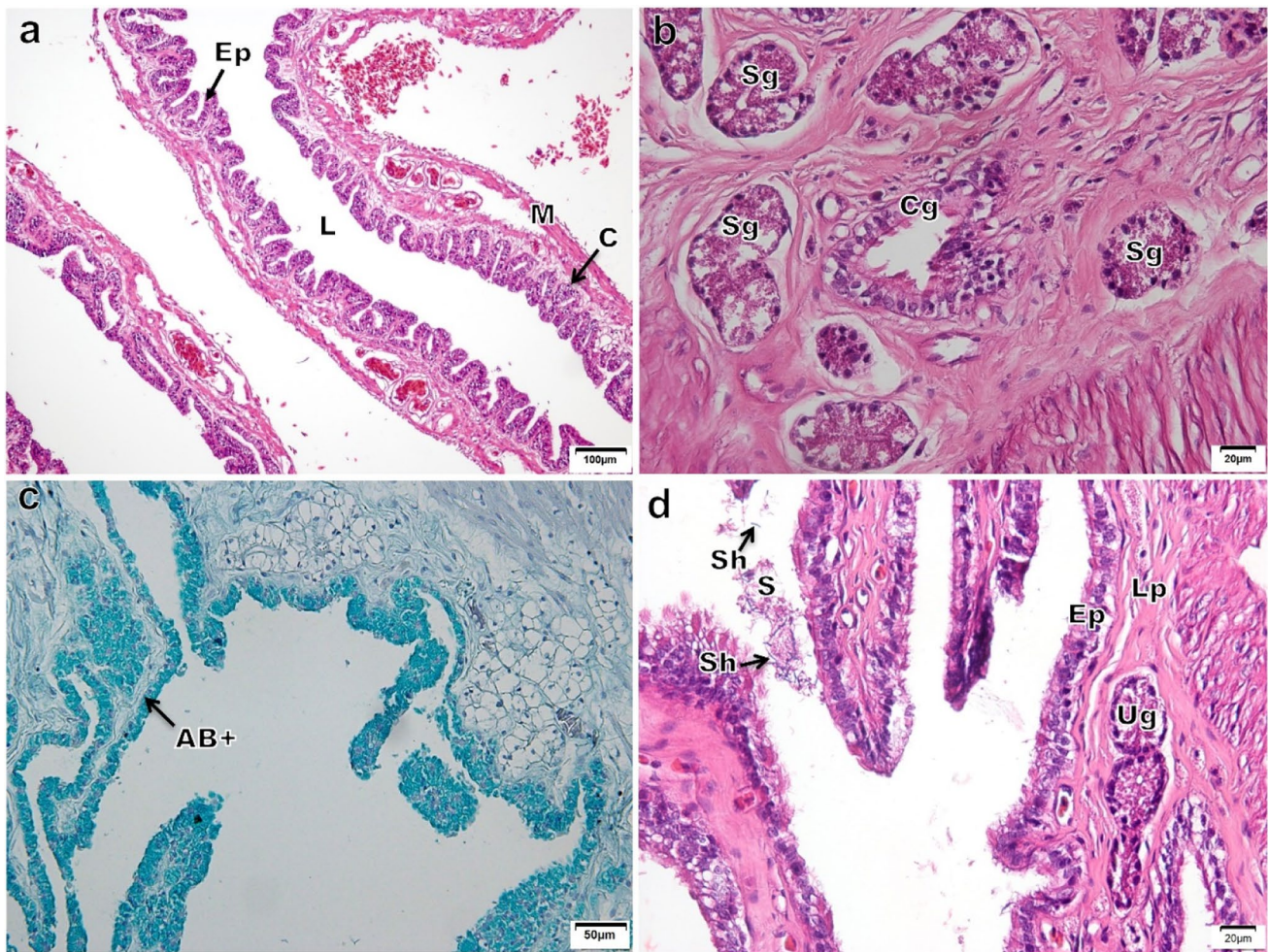
Fig. 1 Photomicrograph of the infundibulum and uterus of *Crotalus durissus*. The anterior infundibulum (**a**). The posterior infundibulum highlights the presence of glands in the connective tissue (**b**). The posterior infundibulum showing epithelial cells stained for AB (**c**). The glandular uterus with spermatozoa in the lumen (**d**). *AB+* positive reaction to alcian blue, *C* connective tissue, *Ep* luminal epithelium, *Cg* ciliated tubular glands, *L* lumen, *Lp* lamina propria, *M* muscular tissue, *S* spermatozoa, *Sg* secretory tubular glands, *Sh* sperm head, *Ug* uterine glands

reaction to AB and PAS in vitellogenic and previtellogenic females (Fig. 1c).

In addition, the posterior infundibulum presented thicker lamina propria with deeper tubular-like folds forming glandular grooves. The secretory tubular glands in posterior infundibulum (Fig. 1b) indicated seasonal variation in relation to their diameters, showing hypertrophy and increased secretions in vitellogenic females (Table 1) only in the months of January, March, May, June, July, September, and November. These secretory glands presented a significant correlation with the diameter of the ovarian follicle ($r = 0.5$, $P = 0.03$).

We did not find spermatozoa stored in the lumen or within the infundibular glands (receptacles of sperm). However, we observed spermatozoa in the glandular uterus in the months of July and September, in two females

Table 1 Mean ovarian follicular diameters of *Crotalus durissus* compared with mean diameters of tubular ciliated glands (DTCG) and tubular secretory glands (DTSG) of the posterior infundibulum

Ovarian follicle stage	Follicle diameter (mm)	DTCG (μm)	DTSG (μm)
Previtellogeneses $n = 10$	7 ± 2 (5–10)	15 ± 4 (11–18)	24 ± 6 (13–36)
Vitellogeneses $n = 9$	22 ± 7 (12–34)	14 ± 4 (11–21)	31 ± 5 (23–38)

with ovarian follicles measuring 27 mm and 35 mm, respectively, indicating that spermatozoa ascend to the infundibular region before ovulation (Fig. 1d).

The glandular uterus of *C. durissus* presented a lamina propria and a well-developed muscular layer (Fig. 2a, b). The inner layer of the glandular uterus was constituted by ciliated and secretory cells of the simple cuboidal type, which is the most abundant in this portion of the oviduct. The connective tissue of the glandular uterus showed many uterine glands (or eggshell glands). These uterine glands were bigger in vitellogenic females (mean 33 ± 4 mm of diameter, range $= 27$–36, $n = 4$) than in previtellogenic females (mean 17 ± 5 mm of diameter, range $= 11$–26, $n = 5$). Additionally, the uterine glands presented a significant correlation with the diameter of the ovarian follicle ($r = 0.8$, $P = 0.01$).

The nonglandular uterus of *C. durissus* presented folds in the mucosa and deep crypts, which are lined with simple columnar to pseudostratified epithelium constituted by ciliated cells with spherical or elongated nuclei, and several mucus-secreting goblet cells (Fig. 3a, b). The epithelium of nonglandular uterus of *C. durissus* indicated histochemical reaction to AB and PAS in vitellogenic and previtellogenic females (Fig. 3c, d). Muscle tissue was well developed, composed of smooth muscle with fusiform cells and central nucleus. The innermost layer was circular, and the outermost layer was longitudinal.

The nonglandular uterus presented a UMT in vitellogenic and previtellogenic females (Figs. 3a, 4a). Spermatozoa in this region were observed in three previtellogenic females (January and August) and nine vitellogenic females (January, May, July, August, and September) (Fig. 3b). Frequently, spermatozoa were associated with the epithelium with their heads oriented toward the cilia (Fig. 3b). All females with stored sperm presented UMT (Figs. 3a, 4a). Remarkably, the female of July presented spermatozoa in three regions of the oviduct, in the pouch (Fig. 4b), in the nonglandular uterus (Fig. 3b), and in the lumen of the glandular uterus (Fig. 1d). The pouch (Fig. 4a, c) was characterized by an epithelium composed of columnar secretory cells with basal nuclei (Fig. 4b), a thick lamina propria, and external musculature.

Discussion

The sperm storage has been commonly observed in the nonglandular uterus in vitellogenic and previtellogenic females of *C. durissus* (Almeida-Santos and Salomão 1997; Barros et al. 2012). Our study extends the knowledge about sperm storage in pit vipers through the identification of spermatozoa in three regions (pouch, nonglandular uterus, and glandular uterus) of the reproductive tract of *C. durissus*. These histological results—found only in July—may suggest that some females could have mated recently, because they still present spermatozoa in the pouch (Fig. 5). On the other hand, the presence of spermatozoa in the glandular uterus in some individuals may indicate that sperm can ascend to the infundibular region before ovulation (Fig. 5). Therefore, for *C. durissus*, sperm storage can occur in different portions of the oviduct, a strategy that can increase the reproductive success in this species. Sperm storage in both regions, nonglandular uterus and posterior infundibulum, has been described for several species (Braz and Almeida-Santos 2022), and these morphological adaptations in the oviduct have been described as essential to the strategy of storage of sperm derived from different males (Hoggren and Tegelstrom 1995; Friesen et al. 2020).
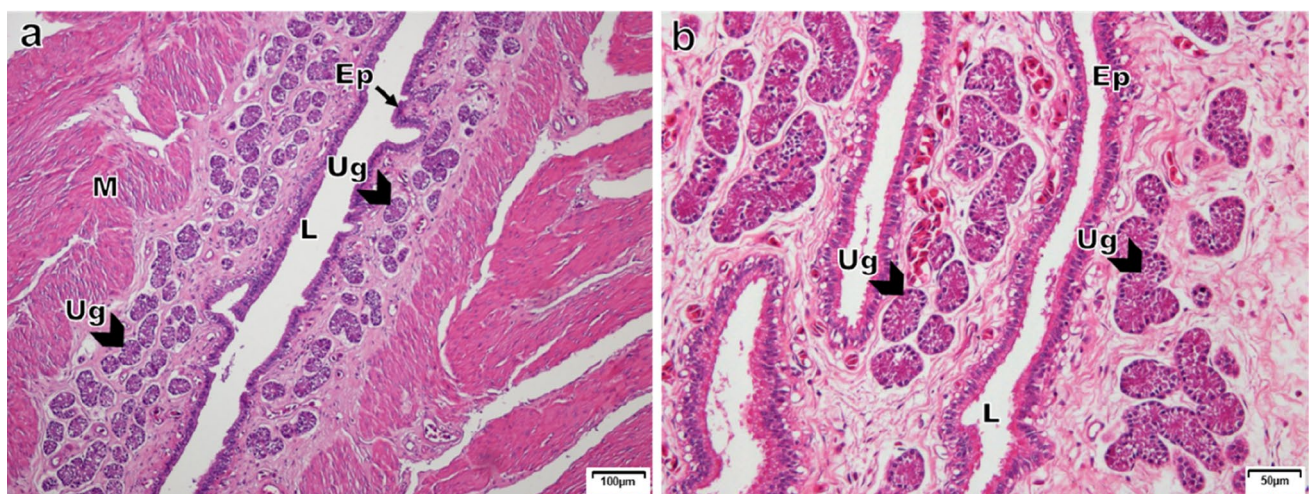

Fig. 2 Photomicrograph of the glandular uterus of *Crotalus durissus*. The glandular uterus showing a well-developed muscular layer and many glands in the lamina propria (**a**). Higher magnification of the lamina propria (**b**). *Ep* luminal epithelium, *L* lumen, *M* muscular tissue, *Ug* uterine glands
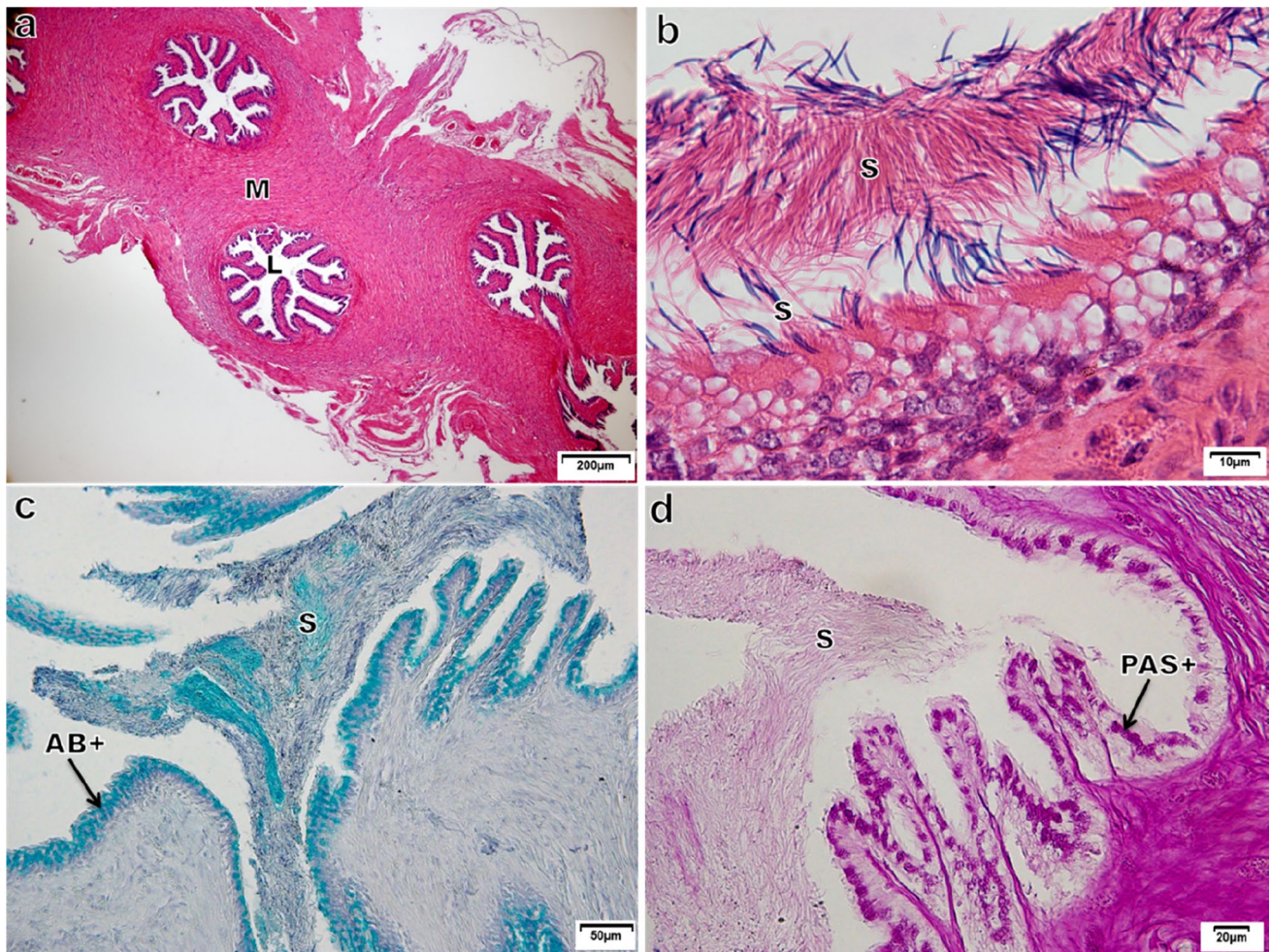
Fig. 3 Photomicrograph of the nonglandular uterus of *Crotalus durissus*. Midsagittal section showing uterine muscular twisting of the nonglandular uterus. Note that the lumen is not presented in linear arrangement, instead it is presented as a curved structure. The curves are showed sectioned as three independent lumina (**a**). Higher magnification of the nonglandular uterus with storage sperm (**b**). The nonglandular uterus showing epithelial cells stained for AB (**c**). The nonglandular uterus showing epithelial cells stained for PAS (**d**). *AB+* positive reaction to alcian blue, *L* lumen, *Lp* lamina propria, *M* muscular tissue, *S* spermatozoa, *PAS+* positive reaction to periodic acid-Schiff

The UMT is another remarkable morphological adaptation of the female reproductive tract of pit vipers, which is usually macroscopically noticeable, especially in adult females in the vitellogenesis stage (Yamanouye et al. 2004; Muniz-da-Silva et al. 2018). Our findings about the presence of UMT in vitellogenic and previtellogenic females in *C. durissus* indicate that its presence is not directly related with ovulation. However, the UMT is under hormonal influence—a balance between estradiol and progesterone—suggesting its dependence of particular stages of the reproductive cycle that can be associated with the maintenance and survival of spermatozoa in the reproductive tract (Yamanouye et al. 2004).

The contact between the spermatozoa acrosome and the oviduct epithelium has been described as a necessary phenomenon to activate the synthesis of substances related to the maintenance and survival of spermatozoa in the oviduct (Holt and Fazeli 2016). Usually, when we identified sperm storage in the nonglandular uterus of *C. durissus*, the acrosomes were observed orientated toward the oviduct epithelium—and the presence of UMT was also observed. Studies have shown that acidic glycoproteins can reduce the phagocytosis of spermatozoa by polymorphonuclear leukocytes (Holt and Fazeli 2016). The neutral and acidic glycoproteins can stain to PAS and AB, and in *C. durissus*, we identified that the epithelial cells of the nonglandular uterus synthesize AB and PAS-positive secretory granules (Almeida-Santos and Salomão 1997). Although we are not able to confirm the specific mechanisms that provide the environment for sperm survival in the oviduct, through morphological and histochemical analyzes of the nonglandular uterus of *C.*
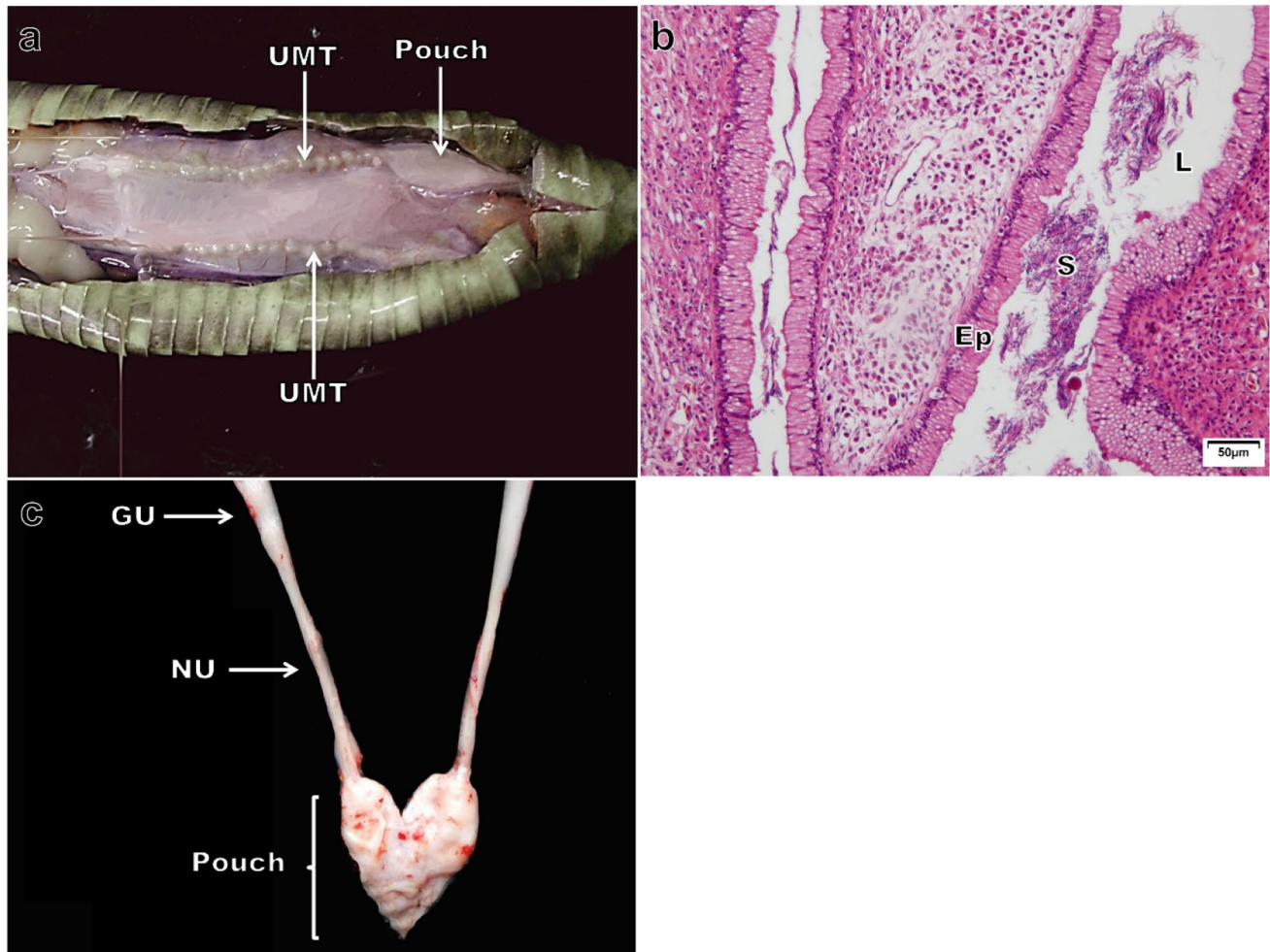
Fig. 4 Photographs of the tract reproductive of *Crotalus durissus*. Nonglandular uterus presenting the uterine muscular twisting (**a**). Pouch lumen with spermatozoa (**b**). Macroscopic view of the pouch with a part of the uterus (**c**). *Ep* luminal epithelium, *GU* glandular uterus, *L* lumen, *NU* nonglandular uterus, *S* spermatozoa, *UMT* uterine muscular twisting

durissus, we can conclude that this region presents the necessary glycoproteic conditions to store spermatozoa for a long period.

The posterior infundibulum of *C. durissus* has several infundibular glands (receptacles of sperm), which represent small invaginations into the lamina propria, forming simple tubular glands (Siegel et al. 2009, 2011). Other species of vipers of the genera *Agkistrodon*, *Crotalus*, *Bothrops*, *Cerastes,* and *Vipera* also present sperm storage in tubular glands (Saint-Girons 1957, 1962; Siegel and Sever 2008; Silva et al. 2020). These glands and the epithelium of the posterior infundibulum of several groups of snakes have positive secretory granules composed by polysaccharides and proteins (Siegel and Sever 2008; Rojas et al. 2015; Silva et al. 2019). In *Agkistrodon piscivorus* (Lacépède 1789), the infundibular glands have a lipoid material produced in the secretory cells that are either diffuse and unorganized or tightly packed into denser lipid droplets (Siegel et al.

2009). The presence of these granules is often associated with sperm storage, as a possible sperm survival mechanism (Siegel and Sever 2008; Rojas et al. 2015, 2017; Silva et al. 2019). For example, in *Bothrops* and *Agkistrodon*, infundibular glands are positive for PAS in the presence of sperm (Siegel and Sever 2008; Silva et al. 2019). Although we did not find spermatozoa in the infundibular glands of *C. durissus*, we found several sperm receptacles in the infundibulum. Based on the presence of these glands in the posterior infundibulum, together with the histochemical results, we suppose that *C. durissus* can store spermatozoa in this region, in a similar way presented by its congeners (Siegel and Sever 2008; Silva et al. 2019, 2020).

Crotalus durissus shows the presence of glands in the posterior infundibulum and glandular uterus that undergo hypertrophy during vitellogenesis. It is noteworthy that the infundibular secretory glands and the uterine glands increase substantially in diameter under the influence of the
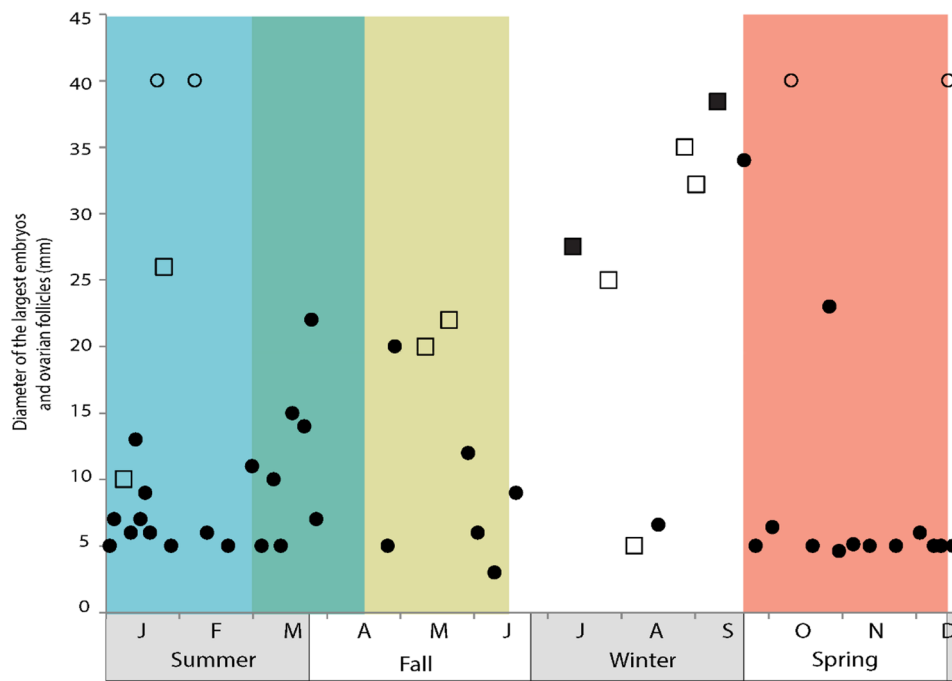
Fig. 5 Overview of female reproductive aspects of *Crotalus durissus*. Solid circles: diameter of the largest ovarian follicle of females without spermatozoa in the oviduct. Open circles: diameter of the largest embryo. Open squares: diameter of the largest ovarian follicles of females presenting sperm storage in the nonglandular uterus. Solid squares: diameter of the largest ovarian follicles of females presenting spermatozoa in the nonglandular uterus, and in the lumen of the glandular uterus (some females also presented spermatozoa in the pouch in July). Shaded rectangles indicate the period of reproductive events as described by Barros et al. (2012). Blue rectangle indicates period related to the birth of neonates; yellow rectangle indicates the mating period; Green rectangle indicates the overlap period between birth and mating; and red rectangle indicates the period of ovulation

vitellogenesis processes. Therefore, it is probable that these glands are hypertrophy under the influence of increased levels of plasmatic estradiol. The glandular uterus and the sperm storage tubules are the only two regions of the oviduct that exhibit dramatic seasonal changes in diameter in pit vipers (e.g., *Agkistrodon piscivorus*; Siegel and Sever 2008).

The relationship between the vitellogenesis processes and the hypertrophy of the infundibular and uterine glands is supported by several studies (Rojas et al. 2017; Braz et al. 2018). In *C. durissus*, the estradiol levels are significantly higher in vitellogenic females (Almeida-Santos et al. 2004) similar to the results presented for other species of Viperidae (Bonnet et al. 1994, 2001; Schuett et al. 2004; Taylor et al. 2004). The vitellogenesis process ends with a decrease in estradiol and a subsequent increase in progesterone (Taylor et al. 2004). These hormonal changes are associated with anatomic and secretory alterations in the oviduct, supporting the conclusion that the estradiol influences the vitellogenesis process, oviductal hypertrophy, increase in the number of mucous glands, oviductal secretory activity, and vascularization (Girling 2002).

In conclusion, our study indicates that the morphology of the female reproductive tract of *C. durissus* follows similar patterns already described for viperids (Siegel and Sever 2008; Barros et al. 2014; Silva et al. 2019). Sperm storage in *C. durissus* occurs in the nonglandular uterus and probably in the posterior infundibulum. Although spermatozoa were not observed in the latter, the region has tubular glands specialized in sperm storage. Furthermore, the observation of spermatozoa in the lumen of the glandular uterus before the period of ovulation indicates that sperm may also remain in the infundibular region. Because of the fragile nature of the spermatozoa, we suggest that more advanced microscopic techniques (e.g., electron microscopy, scanning microscopy) should be considered to advance the investigation of the infundibular region of animals fixed in scientific collections. Regarding some hypotheses about divergent sperm storage time among different regions, the pouch region likely has the shortest sperm storage time, while the nonglandular uterus region likely has the longest sperm storage duration. The infundibulum region likely presents a more variable sperm storage duration (maybe shorter than the nonglandular region), which can extend until the sperm migration before ovulation.

Table 2 Voucher specimens of *Crotalus durissus terrificus* analyzed in this study

Voucher	State	Locality	SVL (mm)	Reproductive status	Season
93,348	São Paulo	Botucatu	856	Previtellogeneses	Summer
93,347	São Paulo	Maria da Fé	820	Pregnant	Summer
93,346	São Paulo	Itapira	822	Postpartum	Summer
93,351	São Paulo	Itapira	950	Postpartum	Summer
93,350	São Paulo	Araraquara	860	Vitellogeneses	Summer
93,349	Minas Gerais	Varginha	1004	Postpartum	Summer
83,445	São Paulo	Sorocaba	955	Previtellogeneses	Summer
83,983	São Paulo	Sorocaba	930	Vitellogeneses	Fall
83,266	São Paulo	Atibaia	920	Previtellogeneses	Summer
82,433	São Paulo	Atibaia	710	Vitellogeneses	Fall
81,833	São Paulo	Atibaia	760	Previtellogeneses	Fall
81,829	São Paulo	Atibaia	830	Pregnant	Summer
86,501	São Paulo	Santa Isabel	800	Previtellogeneses	Summer
88,324	São Paulo	Itapira	810	Vitellogeneses	Fall
83,869	São Paulo	Itapira	785	Previtellogeneses	Fall
82,670	São Paulo	Itapira	850	Vitellogeneses	Winter
82,630	São Paulo	Itapira	850	Previtellogeneses	Winter
88,008	São Paulo	Botucatu	890	Previtellogeneses	Fall
88,006	São Paulo	Botucatu	900	Vitellogeneses	Fall
88,005	São Paulo	Botucatu	970	Vitellogeneses	Fall
85,508	São Paulo	São Luiz do Paraitinga	880	Previtellogeneses	Fall
79,308	São Paulo	Espírito Santo do Pinhal	730	Previtellogeneses	Summer
79,128	São Paulo	Espírito Santo do Pinhal	720	Previtellogeneses	Summer
79,126	São Paulo	Espírito Santo do Pinhal	850	Previtellogeneses	Summer
82,528	São Paulo	Valinhos	1000	Vitellogeneses	Summer
80,330	São Paulo	Valinhos	860	Vitellogeneses	Fall
86,613	São Paulo	Nazaré Paulista	780	Previtellogeneses	Summer
83,585	São Paulo	Nazaré Paulista	885	Previtellogeneses	Summer
81,375	São Paulo	Nazaré Paulista	1002	Previtellogeneses	Summer
85,091	São Paulo	Itu	930	Previtellogeneses	Summer
85,086	São Paulo	Itu	960	Previtellogeneses	Summer
85,085	São Paulo	Itu	950	Vitellogeneses	Summer
89,236	São Paulo	Jacareí	840	Previtellogeneses	Summer
89,237	São Paulo	Jacareí	940	Vitellogeneses	Summer
88,262	São Paulo	Jacareí	920	Previtellogeneses	Summer
80,922	São Paulo	Jacareí	870	Previtellogeneses	Winter
89,130	São Paulo	São José dos Campos	790	Pregnant	Summer
89,129	São Paulo	São José dos Campos	1000	Previtellogeneses	Summer
89,127	São Paulo	São José dos Campos	935	Previtellogeneses	Summer
78,239	São Paulo	Jundiaí	905	Previtellogeneses	Fall
84,144	São Paulo	Jundiaí	950	Previtellogeneses	Fall
82,325	São Paulo	Valinhos	850	Vitellogeneses	Fall
84,423	São Paulo	Itapira	1000	Vitellogeneses	Spring
87,500	São Paulo	Itu	710	Previtellogeneses	Spring
82,626	São Paulo	Atibaia	800	Previtellogeneses	Winter
84,423	São Paulo	Itapira	1000	Vitellogeneses	Spring
82,726	São Paulo	Pilar do Sul	920	Vitellogeneses	Spring
81,037	São Paulo	Socorro	1020	Previtellogeneses	Spring
89,535	São Paulo	Itatiba	1030	Pregnant	Spring
86,258	São Paulo	São João da boa vista	870	Previtellogeneses	Spring
84,600	São Paulo	Piracaia	710	Previtellogeneses	Spring

Table 2 (continued)

Voucher	State	Locality	SVL (mm)	Reproductive status	Season
86,245	São Paulo	Santa Isabel	700	Previtellogeneses	Spring
84,247	São Paulo	Jundiaí	790	Vitellogeneses	Winter
80,902	São Paulo	Itu	930	Vitellogeneses	Spring
81,017	São Paulo	Piedade	890	Previtellogeneses	Spring

Appendix 1

See Table 2.

Authors contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by RJ and KS. The first draft of the manuscript was written by RJ, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare that they have no conflict of interest.

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