

How Does Oocyte Uptake Occur? A Macroscopic Study of the Ovarian and Oviductal Modifications for Egg Capture in the Coral-Snake *Micrurus corallinus*

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ABSTRACT

The processes of follicular development, ovulation, egg capture, and egg transport vary among vertebrates. Therefore, analysis of the reproductive tract of several lineages is needed for understanding the evolutionary changes of the reproductive system. In turtles, the ovulated eggs are released into the coelomic cavity and taken up by one of the two oviducts, a phenomenon called *extrauterine migration of eggs*. However, the process of egg uptake in lizards is different. The egg is ovulated directly into the infundibulum, and oocyte uptake by the contralateral oviduct rarely occurs. The same pattern has been hypothesized to occur in snakes. To test this hypothesis, we analyzed the reproductive tract of female *Micrurus corallinus* preserved in zoological collections. We examined the anatomical characteristics of the infundibulum and ovary and compared the reproductive output between the ovaries to verify the mechanism of egg capture and the anatomical viability of extrauterine migration of eggs. The reproductive output of the right ovary was higher than that of the left ovary, and the higher number of eggs in the right oviduct is due exclusively to the production of the ipsilateral ovary. Several anatomical features prevent extrauterine migration of eggs, including the asymmetry of the reproductive system, the arrangement of the ovarian follicles in a single row, and the formation of a wrapping around the ovary and infundibulum by the visceral pleuroperitoneum membrane (preventing against ectopic eggs). Therefore, the hypothesis of egg capture by the contralateral oviduct is anatomically infeasible in *M. corallinus* and possibly in other snakes. Anat Rec, 301:1936–1943, 2018. © 2018 Wiley Periodicals, Inc.

Key words: Elapidae; reproduction; ovulation; egg uptake

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Research on reproductive biology of female snakes usually investigates the timing of reproductive events (e.g., mating, vitellogenesis, oviposition, and hatching) and the morphophysiological changes in the reproductive tract associated with reproduction (Mathies, 2011; Almeida-Santos et al., 2014). Examination of oviducts in different lineages is important for understanding the evolutionary changes that occurred in the squamate reproductive system and drawing comparisons with other vertebrate groups (Blackburn, 1998b).

The reptilian oviduct performs several functions throughout the reproductive cycle including egg uptake, sperm storage, fertilization, provision of albumen, and egg-shell formation (Blackburn, 1998b; Girling, 2002). This variety of functions results in a regionalization of the tasks across each portion of the oviduct (Perkins and Palmer, 1996; Blackburn, 1998b). The oviduct of snakes can be divided into anterior infundibulum, posterior infundibulum, glandular uterus, nonglandular uterus, and vagina (Siegel and Sever, 2008; Siegel et al., 2011). The anterior infundibulum (the most cranial region of the oviduct) is a thin, translucent funnel-shaped membrane (Perkins and Palmer, 1996). It opens to the pleuroperitoneal cavity through the infundibular ostium, whose function is to capture the mature oocyte released from the ovary (Perkins and Palmer, 1996; Girling, 2002; Rojas et al., 2015). The mechanism of egg uptake by the oviduct of squamates has rarely been discussed in the literature (Cuellar, 1970; Blackburn, 1998b).

The release of oocyte into the coelomic cavity may entail problems as increases in the number of ectopic embryos and unfertilized eggs (Shine, 1977). Although there are reports of reabsorption of ectopic eggs, evidences of this mechanism are weak (Blackburn, 1998a). The issue of eggs shed in coelomic cavity is highlighted in snakes that lost the left oviduct, but both ovaries apparently remain functional (e.g., *Tantilla* and *Typhlops*; Robb, 1960; Clark Jr., 1970; Fox, 1977).

The ovarian follicles of snakes can be classified into two stages of yolk deposition. Primary vitellogenic follicles are small and exhibit a slow rate of yolk deposition, whereas secondary vitellogenic follicles exhibit a higher rate of yolk accumulation (Dessauer and Fox, 1959; Aldridge, 1979). Primary and secondary vitellogenic follicles are easily distinguished macroscopically (Almeida-Santos et al., 2014), allowing comparisons of the productivity of each ovary between follicular stages. Several studies have investigated differences in ovarian productivity (or ovarian reproductive output) in reptiles by comparing the number of corpora lutea in each ovary with the number of eggs in the ipsilateral oviduct. This comparison does not always match. In some cases, the numerical difference is offset by the number of eggs in the opposite oviduct, as previously reported in turtles (Legler, 1958; Duda and Gupta, 1982; Miller et al., 2003), lizards (Parker, 1971; Goldberg, 1972), and snakes (Betz, 1963; Shine, 1977; Shiroma, 1993). The hypothesis proposed to explain this phenomenon is that the egg is released into the coelomic cavity and taken up by the contralateral oviduct, a process called *extrauterine migration of eggs* (Betz, 1963; Cuellar, 1970; Shine, 1977; Miller et al., 2003).

The process of ovulation in lizards differs from that of turtles. In lizards, the egg is transferred directly into the infundibulum, instead of being released into the coelomic cavity. However, due to follicle allocation and

oviductal displacement, the egg can be taken up by the contralateral oviduct during ovulation (Cuellar, 1970). A similar pattern is hypothesized to occur in snakes (Cuellar, 1970). However, the mechanism of egg uptake and movements of the oviduct during follicular growth may differ among species. Therefore, this issue needs to be investigated in different species to make generalizations across taxa (Blackburn, 1998b). Specimens preserved in zoological collections provide an excellent opportunity to verify the seasonal anatomical changes related to the reproductive cycle (Almeida-Santos et al., 2014).

Here, we examined the reproductive tract of several specimens preserved in museums to investigate how the snake oviduct captures the ovulated eggs. As a model system, we used the coral snake *Micrurus corallinus*, a species in which female reproductive biology is well known (Marques, 1996; Almeida-Santos et al., 2006; Marques et al., 2013). Specifically, we aim to test the hypothesis of extrauterine migration of eggs and verify the anatomical viability of this process by analyzing the anatomical characteristics of the infundibulum and the ovaries during follicular growth until ovulation.

MATERIALS AND METHODS

Micrurus corallinus is a semi-fossorial elapid snake that inhabits Atlantic forest areas in southeastern South America (Marques, 1996; Marques et al., 2013). This species is oviparous, and females lay a clutch of 2–12 eggs between late spring and early summer (Marques, 1996). We investigated the reproductive system of 46 adult females (snout-vent length > 400 mm) of *M. corallinus* preserved in Brazilian herpetological collections (Appendix).

Specimens were measured using a tape measure and then dissected through midventral incision in the distal third of the body to expose the reproductive system. We measured the diameter of ovarian follicles using a digital caliper. Follicular stages were classified as primary vitellogenesis (follicles <12 mm) and secondary vitellogenesis (follicles >12 mm). Nomenclature for the female reproductive tract followed Siegel et al. (2011). However, we adopted the terms “anterior uterus” and “posterior uterus” instead of “glandular uterus,” and “nonglandular uterus” (*cf.*, Aldridge, 1992), because distinguishing between glandular regions would require histological analysis. Specimens were photographed using a Nikon L180 digital camera. Additional photographs were taken using a digital camera (Leica DFC295; Leica Microsystems, Wetzlar, Germany) mounted on a stereomicroscope (Leica MZ16). We measured the area of preovulatory follicles and infundibular ostium directly on digital images using Image-Pro Plus 6.0 software (Media Cybernetics, Silver Spring, MD). After data collection, we sutured all specimens to preserve their internal structures (Hoyos et al., 2015).

We examined reproductive output of the ovaries in *M. corallinus* at three reproductive stages: primary vitellogenesis, secondary vitellogenesis, and gravidity. All data were tested for normality and homogeneity of variances before statistical analysis. We applied Box-Cox data transformations to satisfy assumptions of normality, in the data of the primary and secondary vitellogenic follicles. We used paired *t* tests to compare the number of

follicles between the left and right ovaries and the number of eggs between the left and right oviducts. The significance level was set at $P < 0.05$. Statistical analyses were performed using Minitab, version 17.

RESULTS

The female reproductive system of *M. corallinus* consists of paired ovaries, a pair of oviducts, and a vagina. The oviducts are macroscopically divided into three regions: a cranial infundibulum (subdivided into anterior and posterior portion) followed by the uterus (subdivided into anterior and posterior portion and vagina). The architecture of the oviduct is asymmetric. The right oviduct is longer than the left, and the right ovary is situated more cranial to the left ovary (Fig. 1A,B).

Two serous membranes line the body cavity. Externally, the parietal pleuroperitoneum attaches to the abdominal wall. Internally, the visceral pleuroperitoneal membrane overlays the organs and supports both the digestive tract and the reproductive system (Fig. 1C). From the dorsal midline of the body, the oviducts are suspended by the mesotubarium and the ovaries by the mesovarium membrane. The visceral and parietal pleuroperitoneal membranes interconnect at the medial axis of the body. The region of this interconnection attaches to the digestive tract, partially separating the right side of the body from the left side. Abdominal fat bodies (in each side of the abdominal cavity) are observed between the visceral and parietal pleuroperitoneal membranes. After removing the abdominal fat bodies, we observed that the visceral pleuroperitoneal membrane envelops the infundibulum and the ovary together, forming a wrapping that

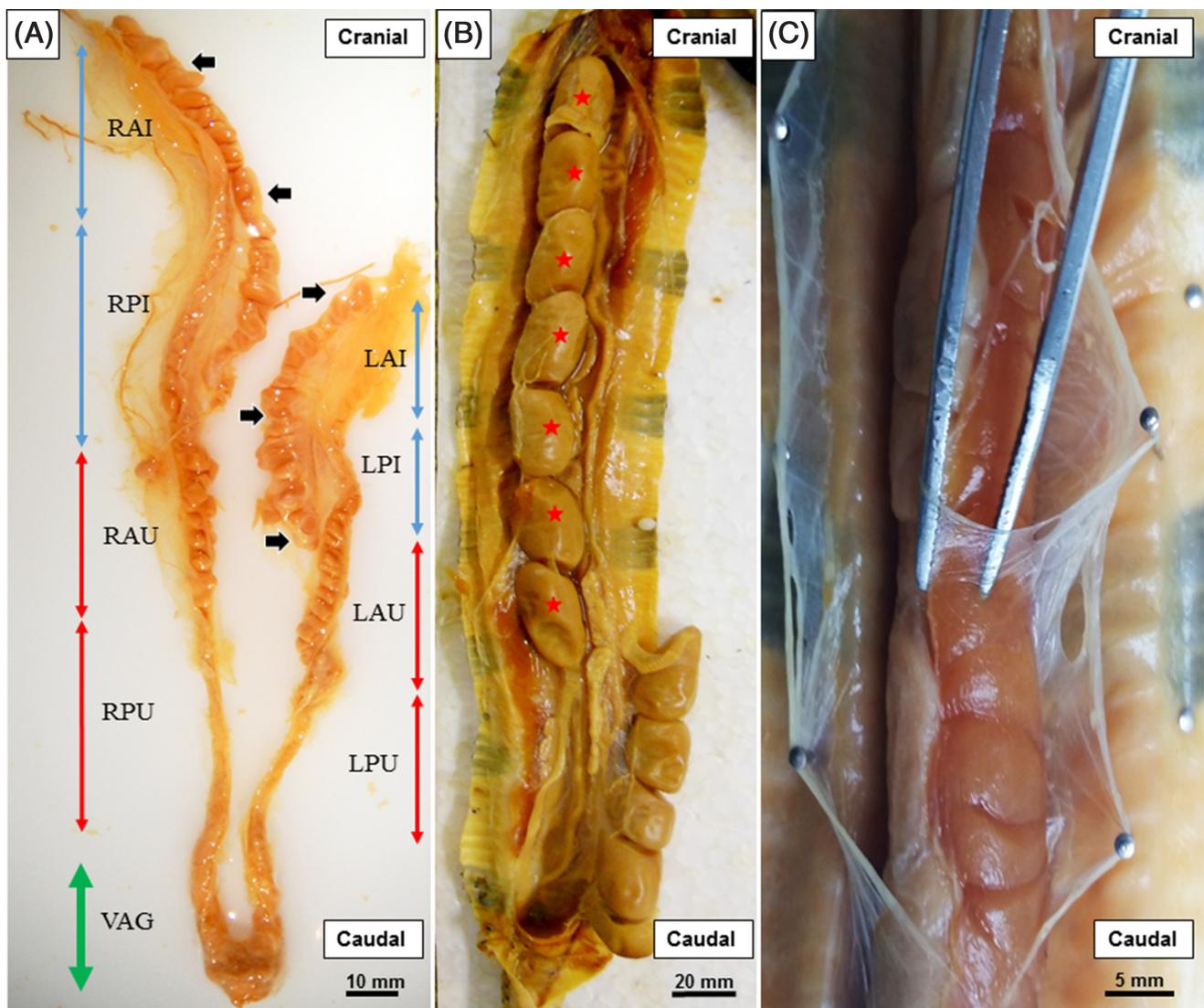


Fig. 1. Female reproductive system of *Micrurus corallinus*. (A) Ovaries (black arrows) and subdivisions of the oviduct. (B) Gravid oviduct. The red stars indicate the eggs in the right oviduct. (C) The parietal pleuroperitoneal membrane (pinned to the body wall) and the visceral pleuroperitoneal membrane stretched by the tweezers. RAI, right anterior infundibulum; LAI, left anterior infundibulum; RPI, right posterior infundibulum; LPI, left posterior infundibulum; RAU, right anterior uterus; LAU, left anterior uterus; RPU, right posterior uterus; LPU, left posterior uterus; VAG, vagina.

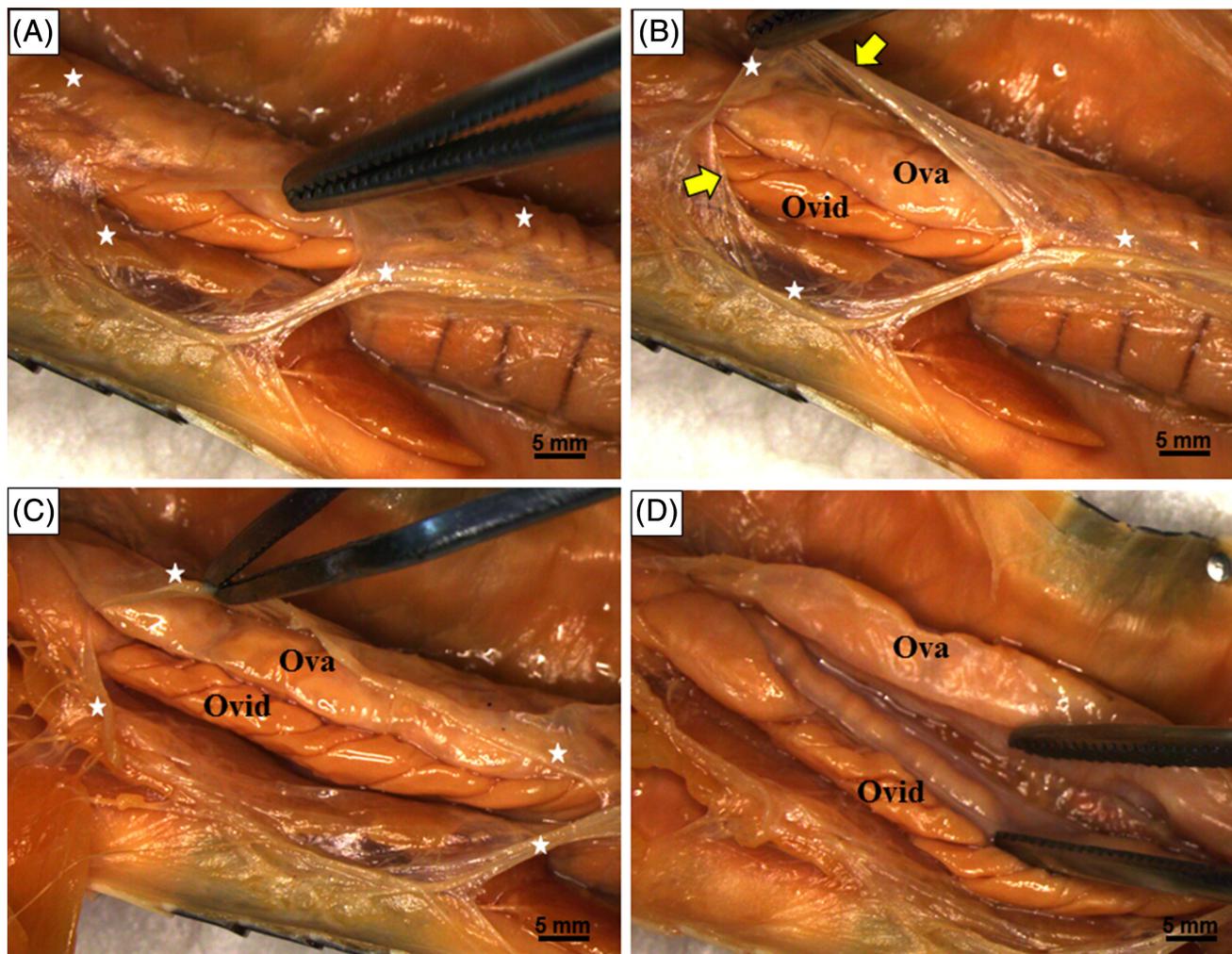


Fig. 2. Dissection of the wrapping formed by the visceral pleuroperitoneal membrane of the right side of the body of a female *Micrurus corallinus*. (A–C) The presence of the wrapping involving the ovary and the infundibulum allows an approximation and a virtual isolation of the coelomic space. (D) The wrapping is now fully opened exposing the ovary and the folded anterior infundibulum. Yellow arrow, the opening of the wrapping; white star, delimitation of the wrapping. Ova, ovary; Ovid, oviduct.

compacts and shortens the distance between these two structures (Fig. 2). This wrapping is often difficult to observe because the visceral pleuroperitoneal membrane is extremely thin and easily broken, becoming unnoticed if the incision to remove the fat bodies is not performed carefully.

The ovaries are positioned at the median axis of the body, and the follicles are arranged in a single row. The ovaries of nonreproductive females have only primary vitellogenic follicles (Fig. 3A), whereas the ovaries of reproductive females exhibit primary and secondary vitellogenic follicles simultaneously. These follicles are generally intercalated, and small overlaps are observed as vitellogenesis proceeds (Fig. 3B,C).

Asymmetry of the ovaries becomes more evident as vitellogenesis advances. There are only small overlaps between the most caudal follicle of the right ovary and the most cranial follicle of the left ovary. Regardless of the reproductive status of the adult specimens, the posterior infundibulum and the anterior uterus are pleated

and have a concertina-like appearance, which results in a compact arrangement of the entire oviduct. The anterior infundibulum of nonreproductive females is also folded in the wrapping. In reproductive females, these folds dilate thus increasing the diameter of the oviduct. In preserved specimens, primary vitellogenic follicles are light cream and have a thin theca layer. Internally, these follicles are light yellow, exhibit a granular appearance and some fissures inside. Secondary follicles are cylindrical, and the theca appears as a thick and coriaceous structure. Internally, the yolk is opaque, dark yellow and has a compact, nongranular appearance. Shortly before ovulation, secondary vitellogenic follicles shift to the first positions in the row of ovarian follicles, probably because this location is strategically close to the infundibular ostium. Follicular growth during secondary vitellogenesis eventually causes the ovaries to expand craniocaudally (Fig. 4).

A vitellogenic female (specimen ZUEC-566) had three secondary vitellogenic follicles in the left ovary positioned sequentially in a row. The ostium of the ipsilateral

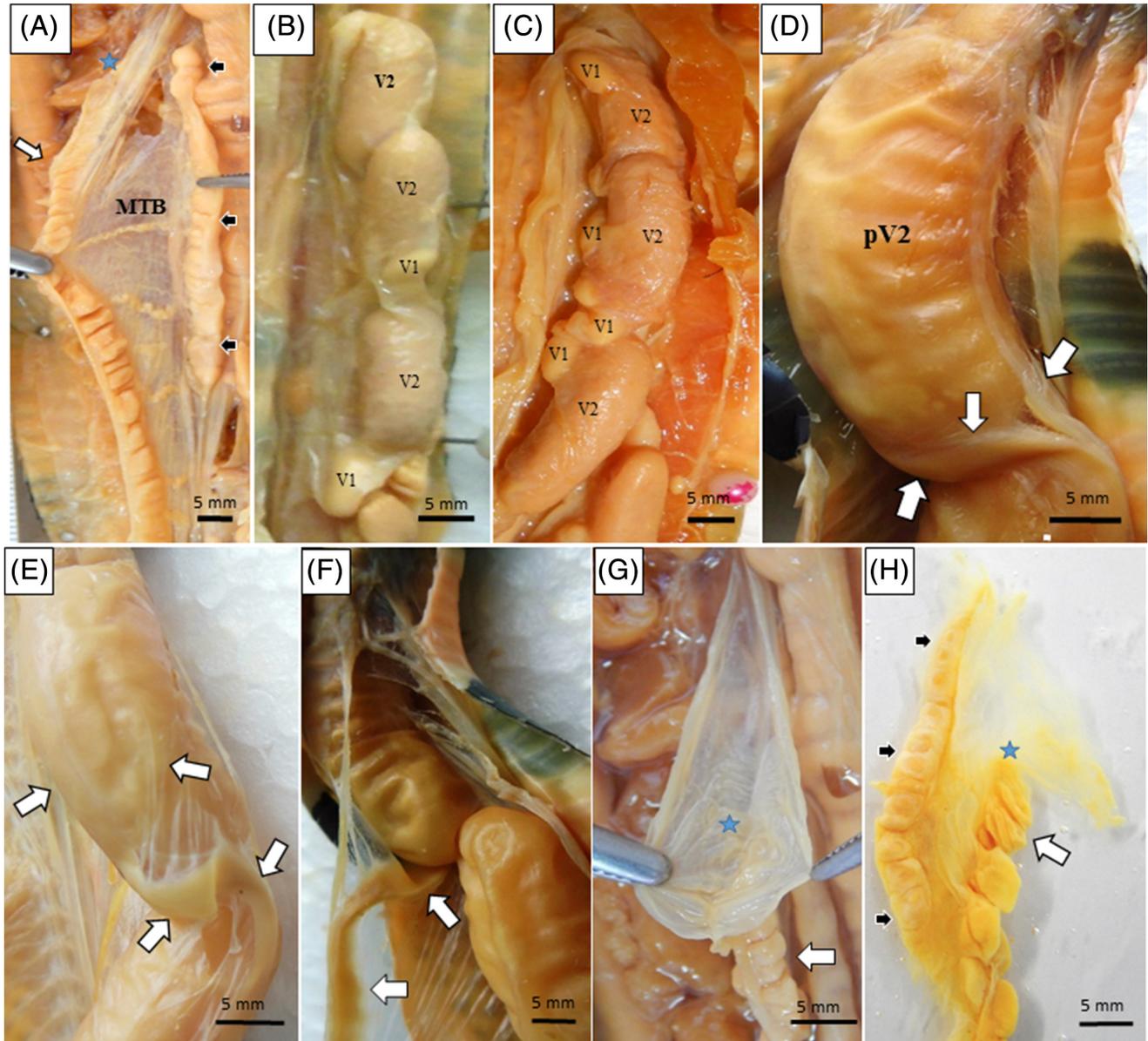


Fig. 3. Distribution of the follicles in the ovary and enveloping process performed by the anterior infundibulum in *Micrurus corallinus*. (A) Nonreproductive specimen presenting only primary vitellogenic follicles in the right ovary. (B) Left and (C) right ovary containing primary vitellogenic follicles intercalated with secondary vitellogenic follicles. (D) Ventral view of a follicle in the left ovary being enveloped dorsally by the anterior infundibulum. (E) Dorsal and (F) lateral views of the follicle being dorsally enveloped by the ipsilateral anterior infundibulum. (G) Front view of the anterior infundibulum detailing the ostium opening. (H) Ovary and anterior infundibulum (left side) in lateral view. The white arrows indicate the anterior infundibulum, and black arrows indicate the ovary. The blue stars indicate the infundibular ostium. MTB, mesotubarium; V1, primary vitellogenic follicles; V2, secondary vitellogenic follicle; pV2, preovulatory follicle.

anterior infundibulum expanded and embraced the entire dorsal region of the first follicle in the row, located cranial to the ovary (Fig. 3D–F). The ostium opening had 195.4 mm² area and 30.6 mm of length, and the preovulatory follicle had 351.6 mm² area and 30.2 mm of length (Fig. 3G,E). This specimen had no oviductal eggs.

In both preovulatory stages, the right ovary of *M. corallinus* had a higher number of follicles than the left ovary (paired *t* test, primary vitellogenesis: $t = 2.14$, $df = 35$, $P = 0.039$ and secondary vitellogenesis: $t = 2.29$, $df = 25$, $P = 0.033$; Fig. 5). The number of eggs in gravid

females ranged from 6 to 12 (mean = 8 ± 4 s.d.; $N = 8$ females). The right oviduct had a higher number of eggs than the left oviduct (paired *t* test, $t = 3.05$, $df = 7$, $P = 0.018$; Fig. 5). In all gravid specimens, eggs of the left oviduct were always closer to the cloaca than eggs of the right oviduct (Fig. 1B).

DISCUSSION

The processes of ovulation and egg uptake by the oviduct underwent specific adaptations in reptiles (Cuellar,

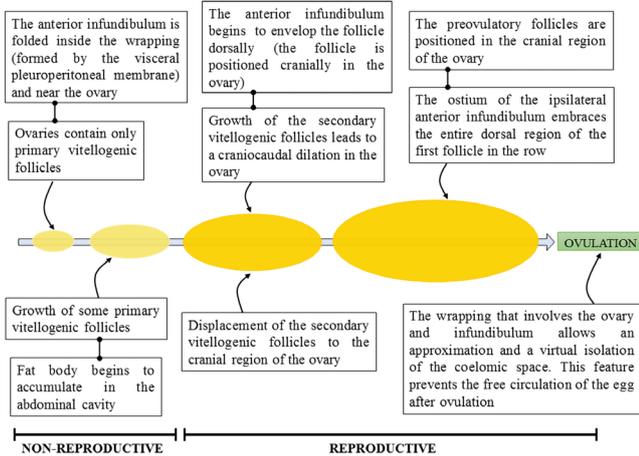


Fig. 4. Diagram illustrating the main modifications of the reproductive tract of *Micrurus corallinus* for egg capture from follicular development to ovulation.

1970), possibly due to changes in female body shape (Rivas and Burghardt, 2001) and reproductive strategies adopted by the species. Turtles usually have a compact and semicircular body shape, and many species have large clutch sizes (Legler, 1958; Miller et al., 2003; Uller and Olsson, 2008). Eggs are released into the coelomic cavity before entering the oviduct (Legler, 1958), and oviductal motility and displacement of the infundibular ostium around the ovary assist in the uptake of eggs (Wyneken, 2004). One of the ovaries usually has higher productivity compared with its antimere. Consequently, extrauterine migration of eggs would be a mechanism to improve egg distribution in oviducts thus avoiding

injuries to the oviduct wall or egg deformation (Legler, 1958). Lizards have an ellipsoid body shape, and although clutch size varies greatly among species, it is generally smaller than in turtles (Vitt and Caldwell, 2009). The strategic positioning of the infundibulum near the ovary assists in egg encapsulation; consequently, oocytes are ovulated directly into the infundibulum, rather than being released into the coelomic cavity (Cuellar, 1970). However, even if the egg is released directly into the infundibulum, it can sometimes be captured by the contralateral oviduct (Cuellar, 1970; Blackburn, 1998b).

Snakes exhibit an elongated cylindrical body shape, with the paired organs arranged asymmetrically (Fox, 1977). In *M. corallinus*, we found that egg uptake begins before ovulation, as evidenced by the differentiated positioning of the anterior infundibulum to envelop the preovulatory follicle. Therefore, the egg is released directly into the infundibular ostium, similar to that described in lizards (Cuellar, 1970). However, unlike lizards, there is no ventral communication between the sides of the body *M. corallinus*. A single follicle is likely to be ovulated and captured at a time because the dimensions of the infundibular ostium and the encapsulated preovulatory follicle were very similar. Due to the shape of the ovary, the size of the secondary vitellogenic follicles, and their arrangement in a single row, at ovulation, the preovulatory follicles likely displace to the cranial region of the ovary rather than the anterior infundibulum move longitudinally to envelop the entire ovary.

During vitellogenesis, the enlarged ovarian follicles are arranged in clusters resembling a “bunch of grapes” in lizards (*cf.*, Siegel et al., 2014), turtles (*cf.*, Dobie, 1971), and Crocodylia (*cf.*, Lance, 1989). Lizards exhibit a ventral communication between the right and left coelomic cavities (Schildger et al., 1999). As follicular growth occurs, secondary vitellogenic follicles may

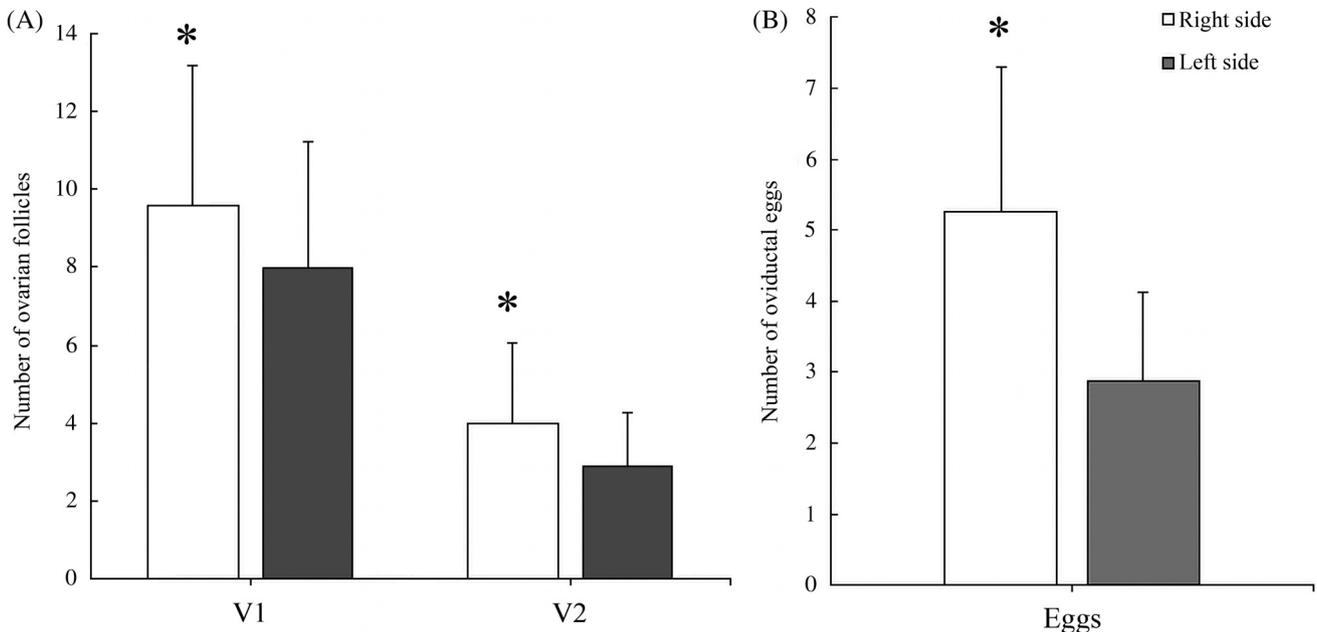


Fig. 5. Reproductive output in *Micrurus corallinus*. (A) Comparative analysis of the production of primary vitellogenic follicles (V1) and secondary vitellogenic follicles (V2). (B) Distribution of eggs in the oviducts. Asterisks indicate significant differences ($P < 0.05$) and error bars denote standard deviation.

occupy the coelomic cavity of the opposite side and be captured by the contralateral oviduct (Cuellar, 1970). A cylindrical body favors the linear arrangement of the follicles in the ovary, as observed in amphisbaenids (Navega-Gonçalves, 2009) and *M. corallinus* (this study). Although the asymmetry of the reproductive tract is accentuated in snakes (Blackburn, 1998b; Di Girolamo and Mans, 2016), we observed that a slight overlap of opposing ovarian follicles may still occur, as previously observed in *M. tenere* (Quinn, 1979). However, the cranio-caudal dilation of the ovary during vitellogenesis reduces this overlap. In addition, a parallel alignment of the follicles at late stages of vitellogenesis might cause a blockage in the digestive tract.

In most squamates, the right ovary is larger and more productive than the left, but some lizards (*cf.*, Cuellar, 1970) and amphisbaenians (*cf.*, Navega-Gonçalves, 2009) do not follow this pattern. Therefore, verification of this sort of data in different squamate lineages is extremely relevant. In many snakes, the right ovary produces more eggs than the left (Aldridge, 1979; Shiroma, 1993; Blackburn, 1998b), including many species of Elapidae (Shine, 1977; Quinn, 1979). However, some authors suggest that the higher number of eggs in the right oviduct may also be due to extraoviductal migration of eggs (Betz, 1963; Shine, 1977; Shiroma, 1993). Our observations do not corroborate this idea. In *M. corallinus*, primary and secondary vitellogenesis can be distinguished macroscopically. The differences we observed in follicle color is likely due to the differential deposition of yolk components between primary and secondary vitellogenesis (Dessauer and Fox, 1959; Aldridge, 1979). The higher number of eggs in the right oviduct matches the higher number of follicles in the right ovary. Because the right oviduct is longer than the left, it provides more space for egg allocation. Therefore, the higher number of eggs in the right oviduct is a direct result of the production of the ipsilateral ovary. The clutch size we observed (between 6 and 12 eggs) is within the range previously reported for the species (*i.e.*, 2–12 eggs; Marques, 1996). By taking into account the number of corpora lutea, comparisons of the reproductive output between the ovaries may be inadequate if they are not accompanied by anatomical examination of the reproductive system of the species and an investigation of the viability of egg uptake by the contralateral oviduct. In parallel, degeneration of a corpus luteum or even the overlap of these structures in the ovary may mislead inferences on reproductive output.

In snakes, the infundibulum is positioned anterior to the ipsilateral ovary (Blackburn, 1998b). The pleuroperitoneal wrapping that involves the ovary and infundibulum allows an approximation and a virtual isolation of the coelomic space. This feature prevents the free circulation of the egg after ovulation in case of failure in egg uptake. Ectopic eggs can block the passage of other eggs and, consequently, compromise reproduction, as evidence of reabsorption is uncertain (Blackburn, 1998a). Because of the marked asymmetry, a successful egg capture by the contralateral oviduct would be possible only if the oviduct is highly mobile (so that the infundibulum reaches the contralateral ovary) and the mesotubarium is highly distensible. Such movement would be possible with the aid of an attached longitudinal muscle band, but this has not been demonstrated in any species (Blackburn, 1998b).

We conclude that in *M. corallinus*, the reproductive output of the right ovary is higher than that of the left ovary, and the higher number of eggs in the right oviduct is due exclusively to the production of the ipsilateral ovary, rather than extrauterine migration of eggs or egg uptake by the contralateral oviduct. The anatomical characteristics observed in *M. corallinus* (as the asymmetry of the reproductive system) hinder follicles to overlap. Consequently, the oviduct is unlikely to move to the cranial position of the contralateral ovary without the presence of an attached musculature. Furthermore, the oocyte is unlikely to move within the coelomic cavity because of the presence of barriers as the wrapping formed by the visceral pleuroperitoneum, the mesocolon in the dorsal region, and the digestive tract in the ventral region. Finally, we conclude that snakes and lizards share the same pattern of egg uptake by the infundibulum. However, egg uptake by the contralateral oviduct is infeasible in *M. corallinus* and possibly in other snakes whose both oviducts and ovaries are functional.

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APPENDIX

Voucher specimens of *Micrurus corallinus* analyzed in this study (N = 46): **Museu de Zoologia da Universidade Estadual de Campinas (ZUEC; N = 7)**: 1338, 1,076, 1,077, 1,078, 2,642, 566, 75. **Museu de História Natural Capão da Imbuia (MHNCI; N = 4)**: 14072, 11,533, 10,513, 5,990. **Museu Nacional/UFRJ (MNRJ; N = 18)**: 4845, 7,052, 4,295, 6,329, 7,357, 6,450, 4,293, 8,244, 8,246, 2,840, 23,832, 19,319, 18,113, 20,714, 22,034, 15,948, 15,947, 4,292. **Coleção Herpetológica da Universidade Federal de Minas Gerais (CHUFMG; N = 3)**: 804, 1,604, 1,697. **Coleção Herpetológica da Universidade Federal de Santa Catarina (CHUFSC; N = 4)**: 362, 491, 976, 1,530. **Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCT-PUCRS; N = 10)**: 4896, 16,305, 16,308, 7,374, 16,304, 2,617, 16,303, 16,307, 3,145, 16,334.