

Caudal oviduct coiling in a viperid snake, *Crotalus durissus*

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Abstract

Sperm storage is common in the oviducts of female snakes and results in the decoupling of mating from ovulation and fertilization. In the majority of female snakes examined, sperm storage occurs in receptacles of the infundibular regions of the oviducts. In pit vipers (Viperida, Crotalinae), the storage of sperm was described in the caudal regions of the oviducts (utero-vaginal junction) through a mechanism termed uterine muscular twisting (UMT). Uterine muscular twisting was described as a twisting of the oviducts after copulation because of uterine contractions. The twisting remains until ovulation at which time the oviducts straighten and sperm migrate cranially to fertilize ovulated ova. Here, we demonstrate that the UMT is not formed by twisting (rotation around axis) of the oviducts of *Crotalus durissus* but rather coils formed by the inner layers of the oviducts at the utero-vaginal junction. Contrary to previous findings, coiling of the oviducts is present in females throughout the year, not only in the postcopulatory period; however, the degree of coiling is variable and may be linked to the seasonal reproductive cycle of *C. durissus*. We categorize the degree of coiling as *pronounced coil*, *discreet coil* or *absent coil*.

KEYWORDS

female, oviduct, reproduction, Serpentes, uterine muscular twisting

1 | INTRODUCTION

The reproductive tract of snakes consists of oviducts (derived from the paramesonephric ducts) and cloaca (Fox, 1997; Siegel, Miralles, Chabbarria, & Aldridge, 2011). The oviducts are paired, the right being longer than the left (Perkins & Palmer, 1996), with the left oviduct absent in some smaller species of snakes (Aldridge, 1992; Blackburn, 1998). The oviducts are divided into several regions according to structure and function, from cranial to caudal: infundibulum, tube, isthmus, uterus and vagina (Blackburn, 1998; Girling, 2002; Siegel, Miralles, Trauth, & Aldridge, 2011; Uribe, Gonzales-Porter, Palmer, & Guillette, 1998). However, terminology of the oviduct is variable (Girling, 2002); for example, the tube has been termed the posterior infundibulum by numerous authors (Almeida-Santos & Orsi, 2002; Blackburn, 1998; Fox, 1956, 1997; Halpert, Garstka, & Crews, 1982; Rojas, 2009;

Saint-Girons, 1975; Sever & Ryan, 1999; Siegel, Miralles, Chabbarria, et al., 2011). In an effort to standardize nomenclature of the oviduct, Blackburn (1998) simplified the terminology to three basic regions in snakes, from cranial to caudal: infundibulum, uterus and vagina (Blackburn, 1998). In a recent review, Siegel, Miralles, Chabbarria, et al. (2011) termed the uterus and vagina of (Blackburn, 1998) the glandular uterus and nonglandular uterus, respectively, owing to the fact that both regions appear to be derived from the paramesonephric ducts, with the cranial region (glandular uterus) possessing endometrial glands, which are absent in the more caudal region (nonglandular uterus). Thus, nonglandular uterus and vagina are often synonymous. Distended portions of the cranial region of the cloaca have also been termed the vagina, vaginal pouch or pouch in snakes, but the embryonic origin of these structure has not been assessed (for review see Siegel, Miralles, & Aldridge, 2011; Siegel, Miralles,

Chabarría, & Aldridge, 2011; Siegel, Miralles, Trauth, & Aldridge, 2011).

Sperm storage in the oviducts of snakes has been reported in glands located in the more cranially positioned posterior infundibulum (uterine tube; Fox, 1956; Saint-Girons, 1975; Fox, 1997; Halpert et al., 1982; Perkins & Palmer, 1996; Blackburn, 1998; Sever & Ryan, 1999; Siegel & Sever, 2008a, 2008b) or the more caudally positioned nonglandular uterus (vagina; Ludwig & Rahn, 1943; Halpert et al., 1982; Almeida-Santos & Salomão, 1997). Within the same species, sperm has been reported as present in both regions at different times; for example, short-term sperm storage in the nonglandular uterus after copulation and long-term sperm storage in the posterior infundibulum days after copulation and until ovulation (Halpert et al., 1982).

Sperm storage was previously noted by multiple authors in conjunction with coiling/twisting of the caudal region of the oviducts in pit vipers. Ludwig and Rahn (1943) identified a twisted region in the cranial portion of the vagina in *Crotalus viridis viridis* where a high concentration of sperm was found in comparison with other regions of the oviduct. Because of the coiled appearance of this oviduct region, the authors called it a “coiled tube.” Nilson and Andrén (1982) identified a similar morphology in the caudal oviduct of *Vipera berus*, which was probably the same structure observed by Ludwig and Rahn (1943), but these authors assumed copulatory plug function. Almeida-Santos and Salomão (1997) found a similar morphology in the caudal oviduct in *Crotalus durissus* and observed that this region of the oviduct of some females presented twisting between the time of mating and ovulation. Almeida-Santos and Salomão (1997) concluded that the twisting was most likely a mechanism to sequester sperm and termed the mechanism “uterine muscular twisting” (UMT).

Uterine muscular twisting has since been reported as a sperm storage mechanism in several species of pit vipers (e.g., Almeida-Santos & Salomão, 1997, 2002; Almeida-Santos, 2005; Barros, Sueiro, & Almeida-Santos, 2012; Barros, Rojas, & Almeida-Santos, 2014a, 2014b); however, Siegel and Sever (2006) found no evidence of oviduct twisting as a sperm storage mechanism in *Agkistrodon piscivorus*, *Sistrurus miliarius* and *Crotalus durissus*, as all three of these pit vipers possessed distinct sperm storage glands in the posterior infundibulum, a more cranial portion of the oviduct. Siegel and Sever (2006) questioned the existence of UMT as a sperm storage mechanism and suggested that twisting of the oviduct would be mechanically impossible, considering that the oviduct is connected to the dorsal body wall by a mesentery through which blood vessels and nervous tissues travel to reach the mucosa of the oviduct.

Although Siegel and Sever (2006) questioned the mechanics of UMT as presented, it is clear that twisting/coiling of the caudal oviduct is present, as numerous authors have depicted this structure through schematic drawings (e.g., Ludwig &

Rahn, 1943; Almeida-Santos & Salomão, 1997) or photographs of visceral organs from animals preserved in scientific collections (e.g., Barros et al., 2012, 2014a). Unfortunately, studies on the gross morphology of UMT were all conducted on specimens after fixation, which can dramatically alter morphological details due to variation in fixation techniques. The current study was undertaken to gain more knowledge about the caudal oviduct in snakes and its morphological features using biopsy of living tissue and multiple levels of microscopy. *Crotalus durissus* (Viperidae, Crotalinae), a viperid that has been documented multiple times as possessing UMT, was chosen as a model to further examine the morphology of the caudal oviduct in hopes of gaining a better understanding of the structure of UMT; that is, does the oviduct twist at any time of the reproductive cycle as depicted by Almeida-Santos and Salomão (1997), and if so, how is this morphologically possible?

2 | MATERIALS AND METHODS

Ten adult female and 10 juvenile female *Crotalus durissus* were obtained from captive collection of the Instituto Butantan and anesthetized with isoflurane (see Acknowledgements for protocol approval). After snakes were deemed unresponsive, an approximately 10 cm incision was made between the first two rows of lateral scales cranial to the cloaca to expose the caudal portion of the genital tracts. The morphology of the caudal portions of the oviducts was assessed through gross examination with a stereomicroscope without removal of

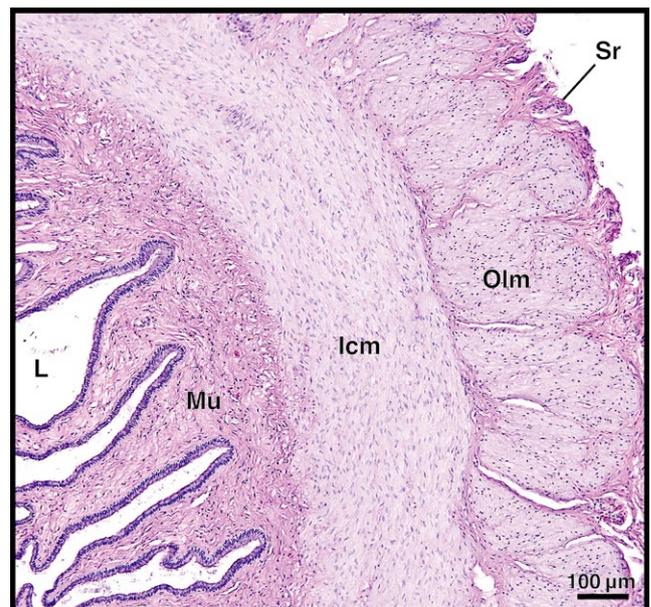


FIGURE 1 Basic histology of the utero-vaginal junction of *Crotalus durissus* (haematoxylin and eosin). Icm: inner circular muscle; L: lumen; Mu: mucosa; Olm: outer longitudinal muscle; Sr: serosa

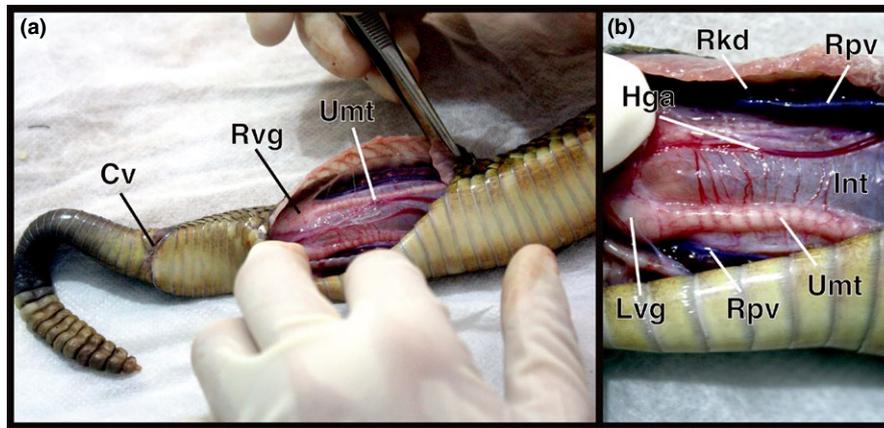


FIGURE 2 Gross examination of the utero-vaginal junction of *Crotalus durissus*. (a) Caudal third of the body of *Crotalus durissus* demonstrating ripples of the oviduct that were originally termed uterine muscular twisting at the utero-vaginal junction. (b) Higher magnification of (a) demonstrating greater details of the utero-vaginal junction and vasculature of the oviduct. Cv: cloacal vent; Hga: hypogastric artery; Int: intestine; Lvg: left vaginal pouch; Rkd: right kidney; Rpv: renal portal vein; Rvg: right vaginal pouch; Umt: folding at the utero-vaginal junction originally termed uterine muscular twisting

serosal and mesentery tissues, after removal of those tissues and after removal of the oviducts from the body cavity.

Of the 10 adult females, four were vitellogenic, three were pregnant, and three were not reproductive. After gross examination, tissues were fixed in 10% neutral-buffered formalin to preserve the morphology of the structures. After fixation, oviduct segments that were removed from the body cavity were sliced in half midsagittally to provide a gross view of the internal mucosa and muscle layers. Oviducts were then sliced into 1-cm segments and were prepared for sagittal examination with a bright-field microscope using the basic protocols outlined for histological preparation and haematoxylin and eosin staining by Junqueira, Bignolas, and Brentani (1979)

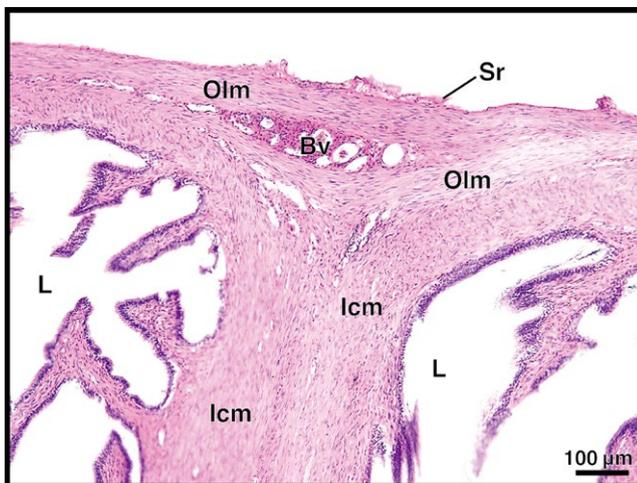


FIGURE 3 Sagittal section of oviduct folds showing the outermost layers in longitudinal arrangement (haematoxylin and eosin). Bv: blood vessels; Icm: inner circular muscle layer; L: lumen; Olm: outer longitudinal muscle layer; Sr: serosa

and Junqueira and Carneiro (2008) or sagittal examination with scanning electron microscopy following the protocols of Dykstra (1993). All 10 juveniles were prepared only for gross examination and light microscopy.

Tissues for bright-field microscopy were rinsed and then dehydrated through a graded series of EtOH. Samples were then cleared in xylene and embedded in paraffin wax. Paraffin blocks were cut with a rotary microtome at a thickness of 5 μm and affixed to albumenized slides. Slides were then stained with haematoxylin and eosin for general histological examination.

To perform scanning electron microscopy, samples were rinsed and dehydrated through a graded series of EtOH. The tissues were then dried through the critical point method using carbon dioxide as the transition fluid. Material was then mounted on stubs using double-sided adhesive tape and silver glue and sputter coated with gold. Images were analysed and captured on a FEI Quanta 250 scanning electron microscope.

3 | RESULTS

3.1 | Utero-vaginal junction

The utero-vaginal junction is part of the nonglandular uterus, found in the caudal portion of the oviducts and is located in the caudal third of the body cavity of snakes. What we term the vagina at the “utero-vaginal junction” is equivalent to the vaginal pouch, or pouch, described by Siegel, Miralles, Trauth, et al. (2011). What we term uterus is equivalent to the vagina described by Blackburn (1998) and nonglandular uterus by Siegel, Miralles, Chabarría, et al. (2011). Histologically, the utero-vaginal junction is formed by three layers, from the inner to the outermost: mucosa (endometrium)—subdivided into an inner epithelial lining and outer

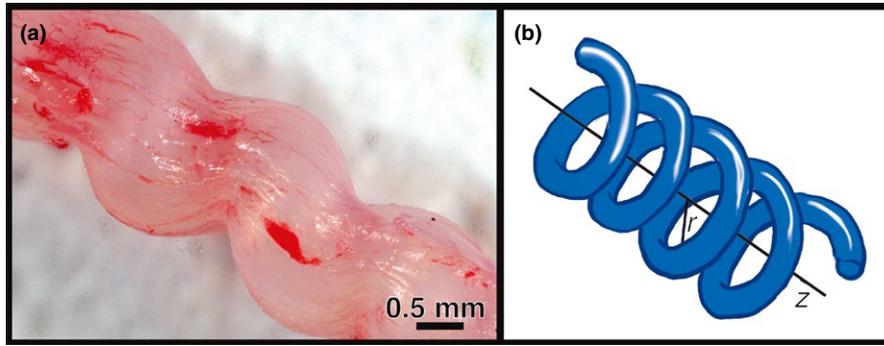


FIGURE 4 (a) Utero-vaginal junction after removal of some fibres of the outer longitudinal muscle layer. Note the waveform of the fibres of the remaining outer longitudinal muscular layer that is close to the circular muscular layer due to the coil formation of the innermost layers. (b) Schematic representation of the internal coil from (a) Note that rotation occurs far from the central axis because of the size of the radius of the coil. *r*: radius of the coil; *z*: central axis of the coil

lamina propria), muscularis externa (myometrium—subdivided into inner circular muscle and outer longitudinal muscle) and visceral serosa (perimetrium; Figure 1). Blood is

supplied to the oviducts by branches of the hypogastric artery and drained by branches of the renal portal vein (Figure 2a,b). When present, convolutions/folding of the oviducts similar to what was termed UMT are found only at the utero-vaginal junction (Figure 2a,b).

3.2 | Uterine muscular “twisting”

The utero-vaginal junction presents a false macroscopic aspect of twisting when what was termed UMT is present; that is, the oviducts appear twisted on their own axes (Figure 2b). In reality, the lumen of the oviduct and the inner layers (mucosa and inner circular musculature) are arranged in a counterclockwise coiling pattern, while the outermost layers (outer longitudinal musculature and visceral serous)

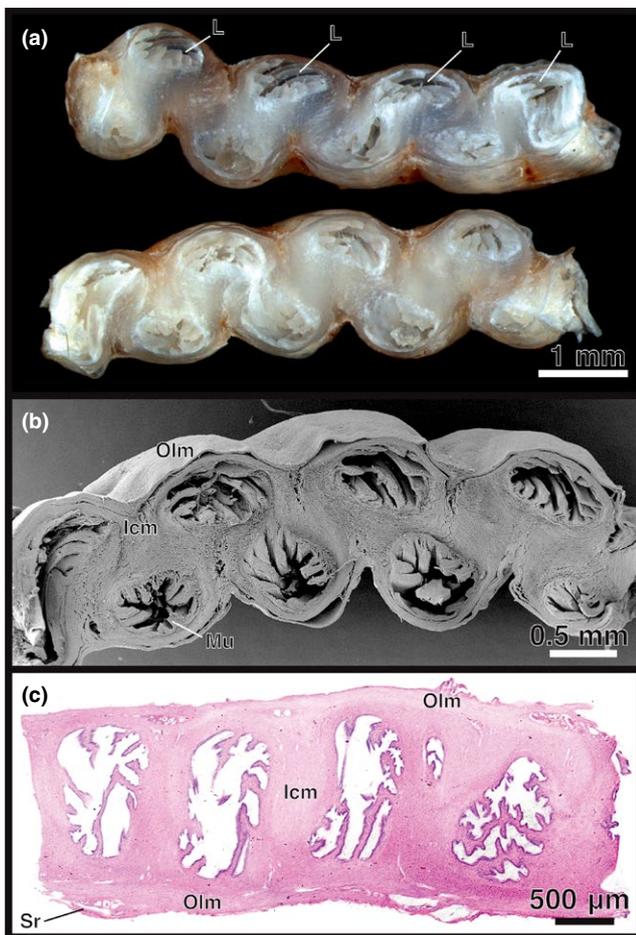


FIGURE 5 Midsagittal sections demonstrating coiling of the utero-vaginal junction by use of (a) gross examination and (b) scanning electron microscopy. Note that the lumen of the oviduct rotates around the central axis of the oviduct. (c) Paramedian sagittal section of coiling (haematoxylin and eosin) demonstrating the coalescence of coils at the periphery of the utero-vaginal junction. Icm: inner circular muscle layer; L: lumen; Mu: mucosa; Olm: outer longitudinal muscle layer; Sr: serosa

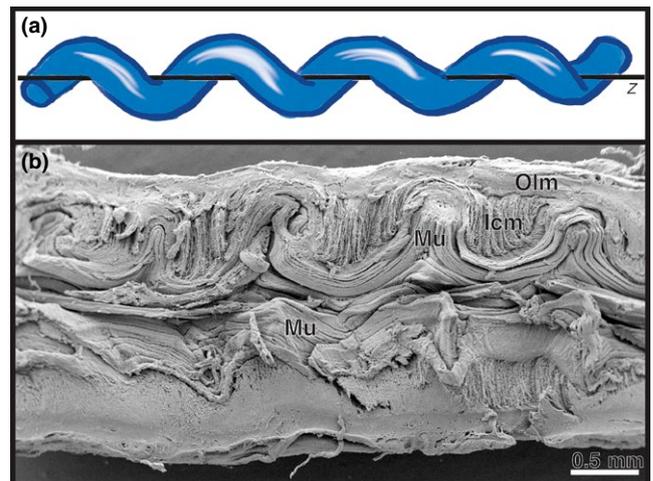


FIGURE 6 (a) Schematic representation of a coil where rotation occurs in contact with the central axis because the coil radius is equal to or close to zero. (b) Sagittal section of the utero-vaginal junction described in (a) with scanning electron microscopy. Note that the lumen of the oviduct exhibits a coil with a short radius. Icm: inner circular muscle layer; Mu: mucosa; Olm: outer longitudinal muscle layer; *z*: central axis of the coil

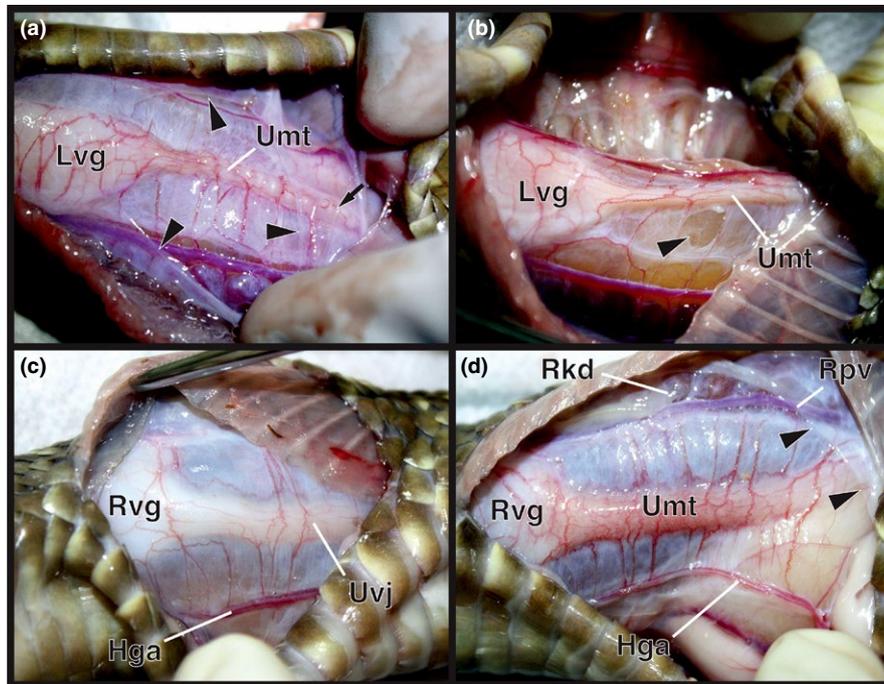


FIGURE 7 (a) Female with a visible oviduct coil through the serosa. Arrowheads point to the edge of the ruptured serosa. Note that the coil is visible even in the portion where the serosa covers the oviduct (arrow). (b) Female with an oviduct coil that is not visible because of the intact serosa. Arrowhead points to where the serosa is ruptured. (c) Female with no coiling at the utero-vaginal junction visualized with the serosa intact. (d) Same female as observed in Figure (c) with the serosa (arrowheads) excised. Hga: hypogastric artery; Lvg: left vaginal pouch; Rkd: right kidney; Rpv: renal portal vein; Rpv: right vaginal pouch; Umt: folding at the utero-vaginal junction originally termed uterine muscular twisting; Uvj: utero-vaginal junction

are arranged longitudinally (Figure 3). No twisting in any of the utero-vaginal junction tissue layers is present. When the visceral serosa and outer longitudinal muscular layers of the utero-vaginal junction are removed, the coiled formation of the internal structures becomes more visible (Figure 4a).

There is variation in the coiling that forms what was originally termed UMT. In some vitellogenic females, coils are present with large radii (Figure 4b), making the lumina of the organs rotate away from the central axes, and therefore, in a sagittal section, the lumina of the oviducts are not aligned parallel to the body (Figure 5a). This degree of coiling is observed in midsagittal sections of the oviducts as two parallel columns with several transverse sections formed by the mucosa and lumen arranged diagonally with transverse cuts of the contralateral column (Figure 5b). In paramedial sagittal sections, the mucosa and lumen are observed as a single row of transverse sections (Figure 5c). There are cases in which what was originally termed UMT is formed by coils with shorter radii (Figure 6a), specifically in nonreproductive adults, and in these cases, the lumina of the oviducts about the central axis of the coils when rotating around the central axes. In midsagittal section, the lumina of the oviducts exhibit a waveform morphology (Figure 6b). In cases of more pronounced coiling (larger radii), the gross appearance of the oviducts exhibits major coil segments,

whereas a more straight gross appearance is observed with less pronounced coil segments (shorter radii; compared Figures 5b and 6b). Pregnant females and juveniles do not exhibit any coiling at the utero-vaginal junctions; in these cases, the lumina of the oviducts and inner layers form a straight tube.

3.3 | Variation in oviduct coiling visibility

Variation in coiling is apparent when comparing fresh and fixed tissues. When fresh tissue is observed, the coils are clearly visible in some individuals, even through the parietal serosa (Figure 7a). In other specimens, the coil is only vaguely visible (Figure 7b) and is only perceptible after excision of the parietal serosa. In some individuals, coiling of the caudal portions of the oviducts was not apparent. In these cases, the utero-vaginal junction is wider and has a smooth appearance, independent of what is observed through the parietal serosa (Figure 7c) or after dissection of the parietal serosa (Figure 7d).

Coils that are macroscopically visible in fresh tissues are difficult to observe after fixation in 10% buffered formalin because the visceral serosa and the outer longitudinal muscular layer become opaque (Figure 8a). In fresh tissues where coiling is vaguely visible, after fixation, the visceral serosa and the outer longitudinal muscular layer become opaque and

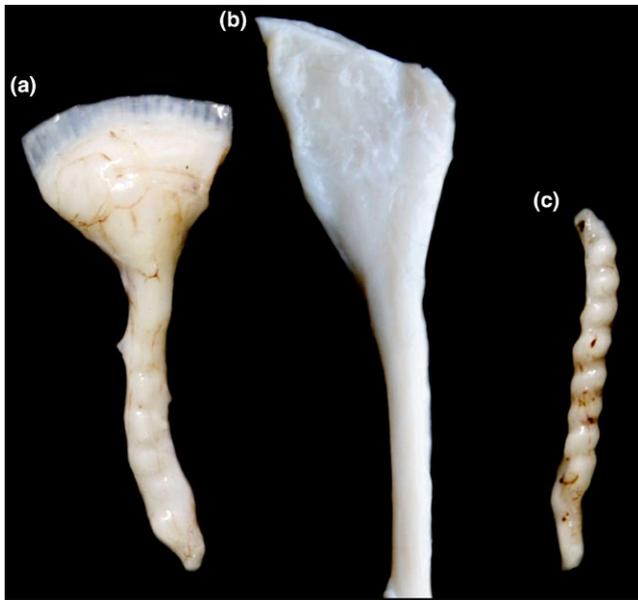


FIGURE 8 Utero-vaginal junction with *pronounced* and *discreet* coiling fixed in 10% formalin. (a) Nondissected utero-vaginal junction of a female snake with *pronounced coiling* of the caudal portions of the oviduct. (b) Nondissected utero-vaginal junction of a female snake with *discreet coiling* of the caudal portions of the oviduct. (c) *Pronounced coiling* segment of the same oviduct as (a) with the serosa and part of the outer longitudinal muscle layer removed. Note that the outer layers of the oviduct become opaque after fixation, making it difficult to see the coil in (a) and (b)

completely mask the existence of coils (Figure 8b). When the serosa and the outer longitudinal muscle layer are removed, and only the inner layers forming the coils are maintained, coiling is visible even after fixation (Figure 8c).

3.4 | Classification of oviduct coiling

Based on our results, we suggest a new classification scheme of what was originally termed UMT, one that is consistent with gross and microscopic morphology (Table 1). *Pronounced coil* is easily visible through the serosal layers, with inner layer morphology formed by a large coil of the mucosa. Whether oviduct tissues are fresh or fixed, the

outermost layers possess a waveform morphology (Figure 9a). *Discreet coil* is represented by a more difficult gross visualization because the serosal linings and longitudinal layer of the muscularis externa are fairly straight, independent of coiling of the mucosa and circular layer of the muscularis externa (Figure 9b). This type of coiling is only easily observed in fresh tissues or in fixed tissues with the serosal membranes and outer longitudinal muscle layer removed. *Absent coil* is represented by no coiling appearance of any layers of the oviduct. Whether in fresh, fixed or dissected preparation, the oviducts appear straight under close examination (Figure 9c).

4 | DISCUSSION

The name uterine muscular twisting (Almeida-Santos & Salomão, 2002) was given to the twisted gross appearance of the utero-vaginal junction in snakes; however, the morphological examination that we provide demonstrates that the “twisting” appearance is not an accurate assessment of the morphological condition when examined with multiple levels of microscopy. The oviduct does not rotate on its own axis, a morphology that a true twisting tube requires. A true twisting of the oviduct is most likely not viable as pointed out by Siegel and Sever (2006) because of vascularization and ligaments of the oviduct. We have shown that the ligaments and vascularity of the oviduct do not prevent the formation of what was traditionally termed UMT because the formation is not the result of twisting of the oviduct or even of the uterine musculature. The formation occurs only in the innermost layers of the utero-vaginal junction. When contracted, the inner circular muscle of the muscularis externa, together with the mucosa, is organized into a coiling pattern. The outer longitudinal muscle layer and the visceral serosa do not participate in the helical morphology.

From our morphological examination, we infer that the inner circular muscle layer of the oviduct in the utero-vaginal region possesses muscle fibres in a coiled arrangement and not in a circular pattern, similar to that suggested for the intestine by Carey (1921). When the circular muscle layer is relaxed, the oviduct appears straight (*absent coil*). When contracted, the fibres would rotate the inner circular muscle layer

TABLE 1 Suggested classification of coiling at the utero-vaginal junction

Condition	Appearance of Lumen in Midsagittal Section	Visibility	
		Fresh tissue	Postfixed tissue
Pronounced coil	Two columns of cross sections (coil with long radius)	Easily observable through serosal membranes	Vaguely visible through serosal membranes
Discreet coil	Waveform (coil with short radius)	Only visible after excision of serosal membranes	Not visible without removal of serosa and longitudinal muscle
Absent coil	Straight (no coil)	Nonexistent, smooth appearance	Nonexistent, smooth appearance

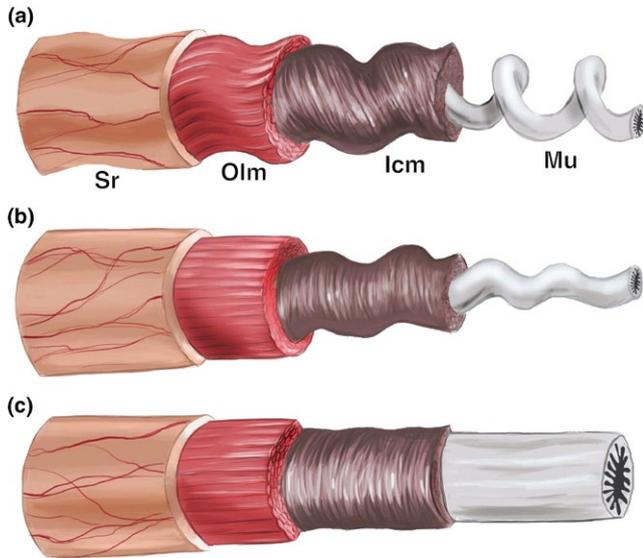


FIGURE 9 Illustration of the layers that make up *pronounced*, *discreet* and *absent* coil of the caudal portion of the oviduct. (a) *Pronounced coil*; note that the strong contraction of the inner circular muscle layer results in a long radius coil of the mucosa and makes the outer layers (serosa and outer longitudinal muscle layer) possess a rippling appearance. (b) *Discreet coil*; note the slightly contracted inner circular muscle layer resulting in the formation of a coil with a short/absent radius without modifying the structure of the outer layers. (c) *Coil absent*; note the well-relaxed inner circular muscle layer with all layers of the utero-vaginal junction in a straight shape and a larger diameter of the mucosa

which would result in a coil (*pronounced* or *discreet coil*, depending on intensity of contraction). Imagine lengthening and shortening of a Slinky®. We assume that the outer longitudinal muscle fibres, working together with the inner circular muscle, would contract during coil formation to allow for the vast shortening of the oviduct during coil formation.

Reports of the existence of female snakes with what was originally termed UMT are quite controversial. Ludwig and Rahn (1943) reported the existence of a coiled tube in the vagina (probably similar to the coil described herein) as a permanent feature of the oviduct in *Crotalus viridis*. Nilson and Andr n (1982) referred to the structure as being temporary, and since then, several authors have reported a coiling morphology of the caudal oviduct as being a seasonal structure in a variety of species (e.g., Almeida-Santos & Salom o, 1997, 2002; Yamanouye et al., 2004; Almeida-Santos, 2005; Andr n & Nilson, 1987; Barros et al., 2012, 2014a, 2014b; Stille, Madsen, & Niklasson, 1986). In some cases, this structure was described as nonexistent (Siegel & Sever, 2006), even in species that were clearly described as possessing coiling of the caudal oviduct (Almeida-Santos & Salom o, 2002). Although we have only looked at one species in great detail, we assume that contractions of the caudal oviduct in all snakes previously pinpointed to possess UMT

most likely possess coiling of the caudal oviduct, and not a twisting. Based on our findings of variation in caudal oviduct morphology with reproductive condition, we agree with previous authors that contractions of the caudal oviduct causing a coiling morphology are seasonal; however, no in vivo examination of the caudal oviduct has been conducted on the same female snake, of any species, over time.

Most of the previous reports on existence or absence of what was originally termed UMT in the literature are based on the observation of this structure without the aid of advanced optical instrumentation. Our results show that the perception of the existence of the coil of the caudal oviduct in *Crotalus durissus* is influenced by a series of factors, such as the following: the presence of the parietal serosa, opacity of the parietal serosa and outer longitudinal muscular layer, fixation of tissue and degree of coil formation (most likely influenced by degree of circular and longitudinal muscle contractions). Furthermore, previous histological studies of the utero-vaginal region of snake oviducts that intended to examine UMT often only made transversal sections (e.g., Almeida-Santos & Salom o, 1997; Almeida-Santos, 2005; Barros et al., 2012, 2014b). It is nearly impossible to histologically visualize the coiling morphology of the caudal oviduct without sagittal sections, as demonstrated herein.

No work until now reported the existence of different degrees of UMT/coiling existence in snakes. Previous investigators only mention the existence or absence of this structure (e.g., Almeida-Santos & Salom o, 1997, 2002; Almeida-Santos, 2005; Barros et al., 2012, 2014a, 2014b; Yamanouye et al., 2004). We observed that the length of the radius of the coil can vary in *Crotalus durissus*. A coil formed by a longer radius probably intensifies the appearance of the coil through the outermost layers of the utero-vaginal junction, resulting in a coil that is more easily visible macroscopically. In cases where the coil is formed by a shorter radius, the external walls of the oviducts remain more straight in appearance, which makes it difficult to externally view this morphology, especially if the serosal linings are not excised. The *pronounced coil*, *discreet coil* and *absent coil* classification scheme will minimize future conflict in the literature regarding the existence of coil morphology, facilitating the identification of this structure in future studies.

Although the phrase “uterine muscular twisting” is not morphologically correct, at least for *Crotalus durissus*, its extensive presence in the literature (e.g., Almeida-Santos & Salom o, 2002; Almeida-Santos, 2005; Aldridge, Jellen, Siegel, & Wisniewski, 2011; Barros et al., 2012, 2014a, 2014b; Siegel & Sever, 2006; Yamanouye et al., 2004) will undoubtedly result in UMT adoption by future authors. However, we suggest “uterine muscular coiling” to subsequent authors so that terminology is more consistent with the morphology that we provided here. We hope the results presented here facilitate future studies on oviduct morphology/physiology in snakes.

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