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Case Report

Viviparity in Snakes – Histological Study of the Relationship Between Fetus, Fetal Membranes and Oviduct in Emerald Tree boa (*Corallus caninus*)

Key words

viviparity;
snakes;
placenta;
histology;
immunohistochemistry;
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Abstract: Viviparity is an important reproductive mode in reptiles from an evolutionary perspective. Viviparous reproduction is associated with certain physiological changes, probably in response to inadequate environmental conditions for egg development. Unlike in oviparous species, embryos remain and develop in the oviduct until birth. In order for the developing embryo to exchange respiratory gasses, water, and food, a placenta is required, which consists of fetal membranes that interact with the maternal oviduct. About 20% of squamates (snakes and lizards) are viviparous, but the morphology of the snake placenta has been studied only in the subfamilies Thamnophiinae and Hydrophiinae. Our objective was to study the structure of the placental layers and fetus *in situ* in the maternal oviduct of a 6-year-old Emerald tree boa (*Corallus caninus*). Five fertilized and three unfertilized slugs were found in the uterus during *post mortem* examination. The average mass of the slug with the fetus (48 mm length x 26 width) was 55–65 g and that of the unfertilized slug was 15–35 g. The fetal membranes and two fetuses were examined by light microscopy. Multiple projections of the tissue samples were made and cut into 5 µm thick paraffin tissue sections, which were stained with Haematoxylin-eosin, Toluidine blue, Goldner's Trichrome and assessed immunohistochemically with monoclonal antibodies for cytokeratin. The morphology of the fetal membranes was described and found to have an anatomy similar to that of most squamates: a type I allantoplacenta. The structure of the oviduct and of the fertilized and unfertilized slug was described. This case report provides a better understanding of placental morphology in boids and expands the spectrum of viviparous squamate species described.

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Introduction

Reptiles have evolved numerous modes of reproduction to ensure the survivability of their offspring in the habitats they inhabit. Most reproduce by egg-laying, known as oviparity, in which the eggshell and membranes protect the developing embryo from environmental influences outside the mother's body. However, about 20% of squamate species (snakes and lizards) have evolved to retain the embryo in the oviduct until development is complete and give birth to live, fully functional young. This form of parity, termed viviparity, probably evolved from egg-laying species because climatic conditions were insufficient for egg development (1, 2).

The structure of the placenta in viviparous species, as well as nutrient uptake, varies according to the feeding mode of the fetus. These can be divided into lecithotrophy in which nutrients for embryonic development come primarily from the yolk, and matrotrophy, in which the female provides nutrients through the placenta or a functionally homologous trait (3).

In both cases, the placenta is required for the uptake of nutrients from the yolk and for gas exchange (4, 5). The extraembryonic membranes that contribute to placentation are complex. The placenta is formed by the attachment of extraembryonic membranes (chorioallantois) and tissues of the maternal fallopian tube (oviduct, uterus). The chorioallantois surrounds most of the embryo, while the omphallantois forms the ventral wall, which consists of a bilaminar omphalopleure and an omphalallantois membrane (6), aligned with the chorioviteline membrane (7). The chorioallantois is the only vascular membrane thought to be involved in gas exchange. It is connected to the uterus and forms the allantoplacenta. The types of allantoplacenta in squamates are classified according to differently organized morphotypes defined by i) the degree of folding between uterine and chorionic tissues and ii) differences at the maternal-fetal interface. Type I allantoplacenta is known to be the simplest and most common form. It is dependent on nutrient uptake from the yolk, the so-called "leicitotrophic viviparity". The maternal-fetal interface consists of a vascularized chorioallantois with squamous epithelium (in some species also remnants of the shell membrane) and uterine tissue. In the allantoplacenta type II, in which the shell is lost earlier, the luminal surface of the uterus is elevated in shallow ridges and consists of capillaries and a very thin layer of uterine epithelium. The chorionic epithelium is more cuboidal. The type III allantoplacenta is described as a placentoma in which an elliptical area of folded maternal and fetal tissue is located at the mesometrial pole of the uterus ventral to the great uterine vessels. The IV type allantoplacenta is also described as a placentoma located below the uterine artery and vein. Distinct villous folds of the uterine endometrium radiate outward and project deeply into an invagination of the chorioallantois, making separation of these tissues difficult (7, 8).

Viviparous (life-bearing) snakes are an important alternative to traditional mammalian models for studying placental structure, function, and development. Understanding of placental morphology in snakes is based on a handful of publications covering only a small fraction of viviparous species. More data on placental morphology are available from lizard species (6, 9). However, the independent evolutionary origin limits the comparability of the two groups. Although there are some similarities, there are also numerous differences, particularly in the chorioallantoic portion of the placenta, that warrant further investigation of placentation in snakes.

In the present study we examined the histological characteristics of the placental membranes of gravid Emerald tree boa (*Corallus caninus*), as well as the oviduct and yolk characteristics. Our aim was to identify the type of placenta and describe its histological characteristics using various histological, histochemical and immunohistochemical stains.

Materials and methods

Material and sample processing

The samples of a 6-year-old Emerald tree boa (*Corallus caninus*) for gross and histological examination were obtained *post mortem* from Golob d.o.o., Clinic for small, wild, and exotic animals, Muta Slovenia, under permit number U34443-6/2917/2, as animal by-products according to Regulation (EC) No. 1069/2009. Two deceased embryos with their surrounding fetal membranes and one slug were fixed in 10% buffered formalin, and routinely embedded in paraffin using a Leica TP1020 automated tissue processor (Leica Biosystems, Buffalo Grove, USA). The tissue samples were then cut into 5 µm thick tissue sections at 50-µm intervals using a Leica SM 2000R microtome (Leica Biosystems, Nußloch, Germany).

Histological and immunohistochemical preparation

After deparaffinization, sections were stained with Haematoxylin-eosin (HE), Toluidine blue, Goldner's Trichrome as well as immunohistochemically stained with anti-human cytokeratin and examined under a light microscope.

Briefly, samples were deparaffinized (2 x 5 minutes) in xylene substitute (Neo-Clear™ Xylene Substitute, Merck Millipore) and rehydrated in decreasing concentrations of ethanol (100% 2 x 5 minutes, 96% 5 minutes, 75% 5 minutes) and distilled water (2 x 5 minutes). Subsequently, samples were either stained with haematoxylin (Merck) (2 minutes), washed under running water (20 minutes), stained with eosin (1–2 minutes) and washed in distilled water (5 minutes), or stained with Toluidine blue solution (7 minutes) and washed three times in distilled water (5 minutes).

The samples were also stained with Goldner's Trichrome stain (Masson-Goldner staining kit, Merck, Darmstadt, Germany) according to the standard procedure. Briefly, after initial deparaffinization and rehydration, nuclei were stained with Weigert's iron haematoxylin for 2 minutes. Samples were washed under tap water for 10 minutes then rinsed in 1% acetic acid for 30 seconds, stained in azophloxin solution for 10 minutes, and subsequently rinsed in 1% acetic acid for 30 seconds. Samples were then incubated in tungstophosphoric acid orange G solution for 1 minute, rinsed with 1% acetic acid for 30 seconds, incubated in light green SF solution for 2 minutes, and finally rinsed in 1% acetic acid for 30 seconds.

After dehydration with increasing concentration of ethanol (75% about 5 minutes, 96% 1 x about 5 minutes, 100% of 2 x 5 minutes) clearing of the samples was performed in xylene substitute (Neo-Clear™ Xylene Substitute, Merck Millipore) (3 x 5 minutes). Finally, a drop of Neo-Mount medium™ -anhydrous mounting medium (Merck Millipore) was added to each tissue sample and the sample was covered with a cover slide. The samples were then air dried for approximately 30 minutes. The samples were stored in the dark prior to analysis.

In addition, paraffin-embedded tissue sections were stained by immunohistochemical procedure using an anti-human cytokeratin antibody (1: 100, CK MNF 116, Dako, Glostrup, Denmark) for immunolabelling of epithelial cells. For immunohistochemistry, deparaffinized and rehydrated tissue sections were unmasked by boiling the slides in citrate buffer (pH 6.0) for 20 minutes in a microwave oven (for immunolabeling of cytokeratin). Incubation with the primary antibodies lasted for one hour at room temperature in a humid chamber. The rest of the immunohistochemical procedure was performed according to a previously described protocol (10). A Nikon Microphot-FXA microscope equipped with a DS-Fi1 camera and NIS Elements imaging software (NIS Elements D.32; Nikon Instruments Europe B.V., Badhoevedorp, The Netherlands) was used for histological examination.

Results and discussion

In our case, five embryos and three unfertilized slugs were collected from an adult female Emerald tree boa (*Corallus caninus*). Figure 1A shows the species in a very typical loop position on the branch. The average size of the embryo with surrounding membranes and yolk was 48 x 26 mm and the mass was 55–65 g. The unfertilized eggs were lighter, and their mass was 15–35 g. Female Emerald tree boas reach sexual maturity at about 4–5 years of age. Their gestation lasts about 7 months, and they give birth to 7–10 live, fully developed young (11). In our study, embryos were

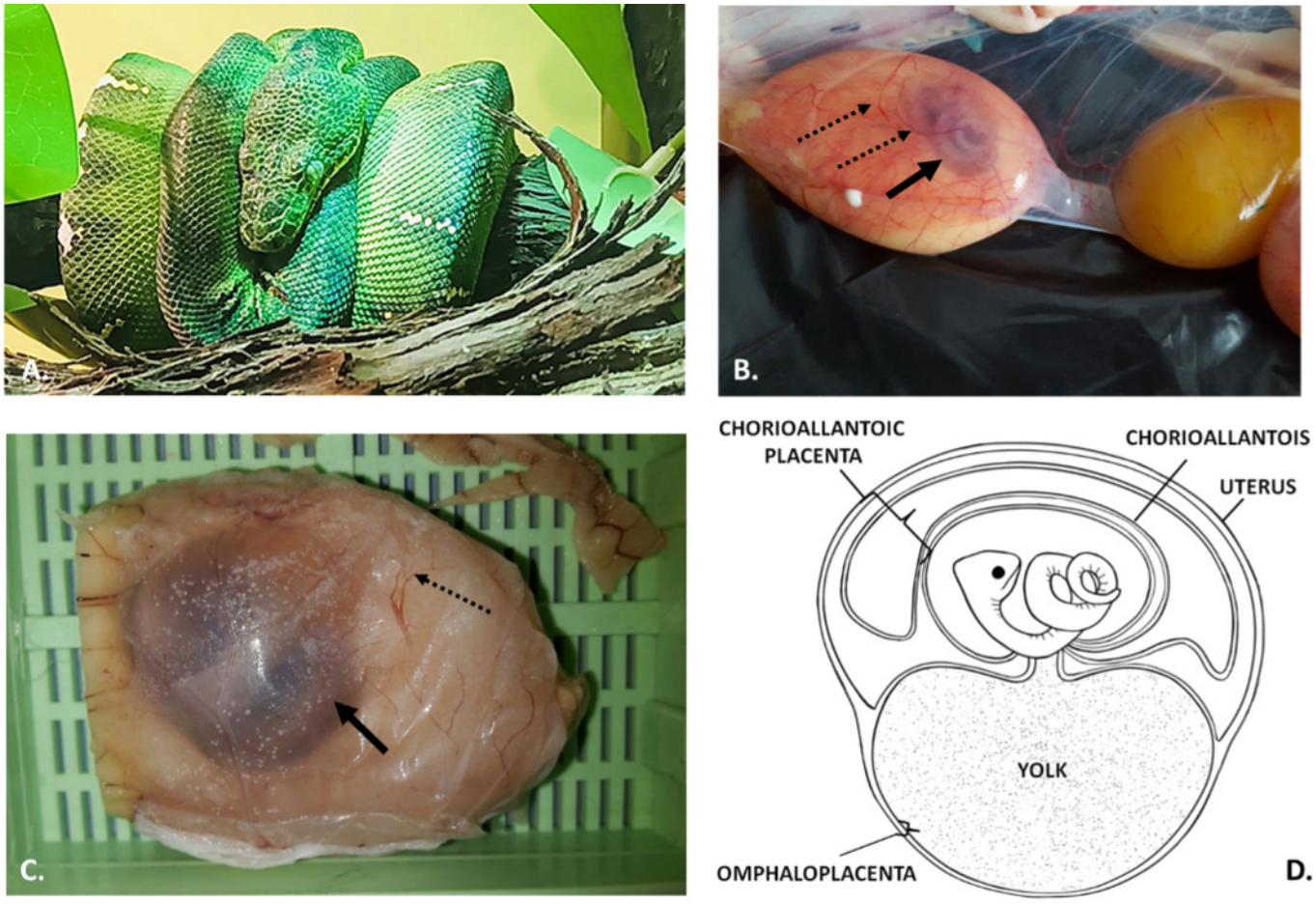

Figure 1: Adult specimen and fetuses of *Corallus caninus*

A. Adult female Emerald tree boa (*Corralus caninus*) in a coiled loop on a branch (Photo: Freja Katarina Dvojmoč). B. Developing embryo (bold arrow) of *Corallus caninus* in the oviduct, vascularization (dotted arrow) (Photo: Zlatko Golob). D. A whole formalin-fixed and paraffin-embedded *Corallus caninus* embryo (bold arrow) with preserved fetal membranes and well seen vascularization (dotted arrows) in Tissue-Tek Mega-Cassette system (size 40 x 25 x 10 mm) (Photo: Valentina Kubale). D. The position of the snake embryo in the uterus and the two types of placental contact; chorioallantoic and omphaloallantoic placenta (designed by Pia Cigler, adapted from Blackbourn DG (4) and Bavdek SV et. al. (12).

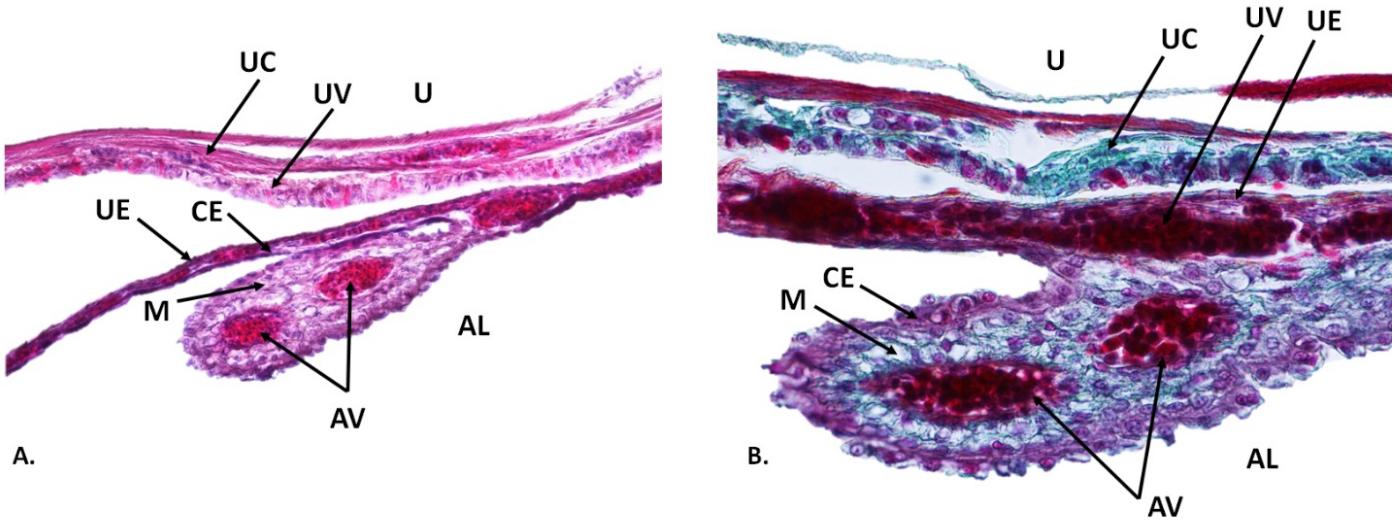

Figure 2: Representative histological characteristics of chorioallantoic placenta

The chorioallantois was observed as thin vascularized membrane, next to uterus. AL, allantoic lumen, AV, allantoic blood vessel, M, mesenchyme, CE, chorionic epithelium, U, uterus, UE, uterine epithelium, UV, uterine vessel, UC, uterine connective tissue (A. 200× magnification, Haematoxylin-eosin; B. 400× magnification, Goldner's Trichrome).

observed on the upper surface of the yolk sac in a coiled position, with a highly vascularized chorioallantois (Figure 1B). Because the embryo was preserved in its original position, it was difficult to estimate the length of the embryo. However, we were able to estimate the diameter of the embryo in the coiled form, which was approximately 1 cm (Figure 1C). The embryos described in this case were at an early stage of development. The position of the snake embryo in the uterus and the type of placental contact (chorioallantoic and omphalloalantoic placenta) are shown in Figure 1D.

Light microscopic examination of the fetal membranes in the region of the chorioallantoic placenta revealed that the chorionic epithelium consists of thin unilaminar squamous epithelium (Figure 2A, B). In the following layer of the trophectoderm, a highly vascularized area with an extensive network of allantois capillaries was observed, located near the uterus in the mesoderm and stained green with Goldner's Trichrome (Figure 2B). No lamina propria or allantois connective tissue was identified. The chorionic epithelium interacted with the uterine epithelium, a very thin, unilaminar squamous epithelium comparable to the chorionic epithelium (Figure 2A, B). The chorioallantois formed multiple folds in the numerous areas above the fetus, most likely important for enhanced gas exchange through enlarged areas of mesenchymal tissue with blood vessels in the allantois (Figure 3). In addition to Haematoxylin-eosin staining and Goldner's Trichrome, an immunohistochemical approach showed that the epithelial cells of the chorioallantois folds strongly expressed cytokeratin MNF116 (Fig. 3B). The broad-spectrum cytokeratin marker which was used recognizes the basic and some acidic keratins. Schematic representations of the chorioallantois are shown in Figure 3D. No shell membrane was observed between the highly vascularized fetal and maternal epithelia.

The omphaloplacenta consisted of an omphalopleure with a layer of thin squamous epithelium and a layer of simple cuboidal epithelium, and a yolk splanchnopleure. The yolk splanchnopleure consisted of squamous epithelium and a highly vascularized area. The omphalopleure and yolk splanchnopleure formed the lining of the yolk cleft. The yolk splanchnopleure surrounded the yolk with numerous yolk vesicles (Figure 4 and 5). Immediately adjacent to it, the embryo was surrounded by the amnion (Figure 4) and further superficially by the allantois. The yolk splanchnopleure and the allantois formed a yolk sac cleft. The morphology of placenta examined was consistent with a type I allantoplacenta.

The oviduct is divided into four distinct regions in snakes, namely infundibulum, uterine tube, uterus and vagina. In our study we examined the infundibulum, uterine tube, and uterus. In the infundibulum, we observed finger-like irregular folds of the mucosal layer that protruded anteriorly. The epithelium was a cuboidal epithelium that was cilliated in some places and was not cilliated toward the uterine tube. The wall of the infundibulum was thin. The uterine tube area was similar to the infundibulum. However, the wall, which consisted of lamina propria, tunica muscularis, and partially visible tunica serosa, was much thicker (Figure 6). The uterus consisted of three histological layers: the luminal epithelium, the subepithelial lamina propria, and the uterine muscularis. The luminal epithelium consisted of cuboidal to slightly columnar, non-cilliated cells that strongly expressed cytokeratin by immunohistochemistry. The underlying lamina propria of the uterus consisted of dense, irregular tissue composed of fibroblasts in a matrix of irregularly arranged

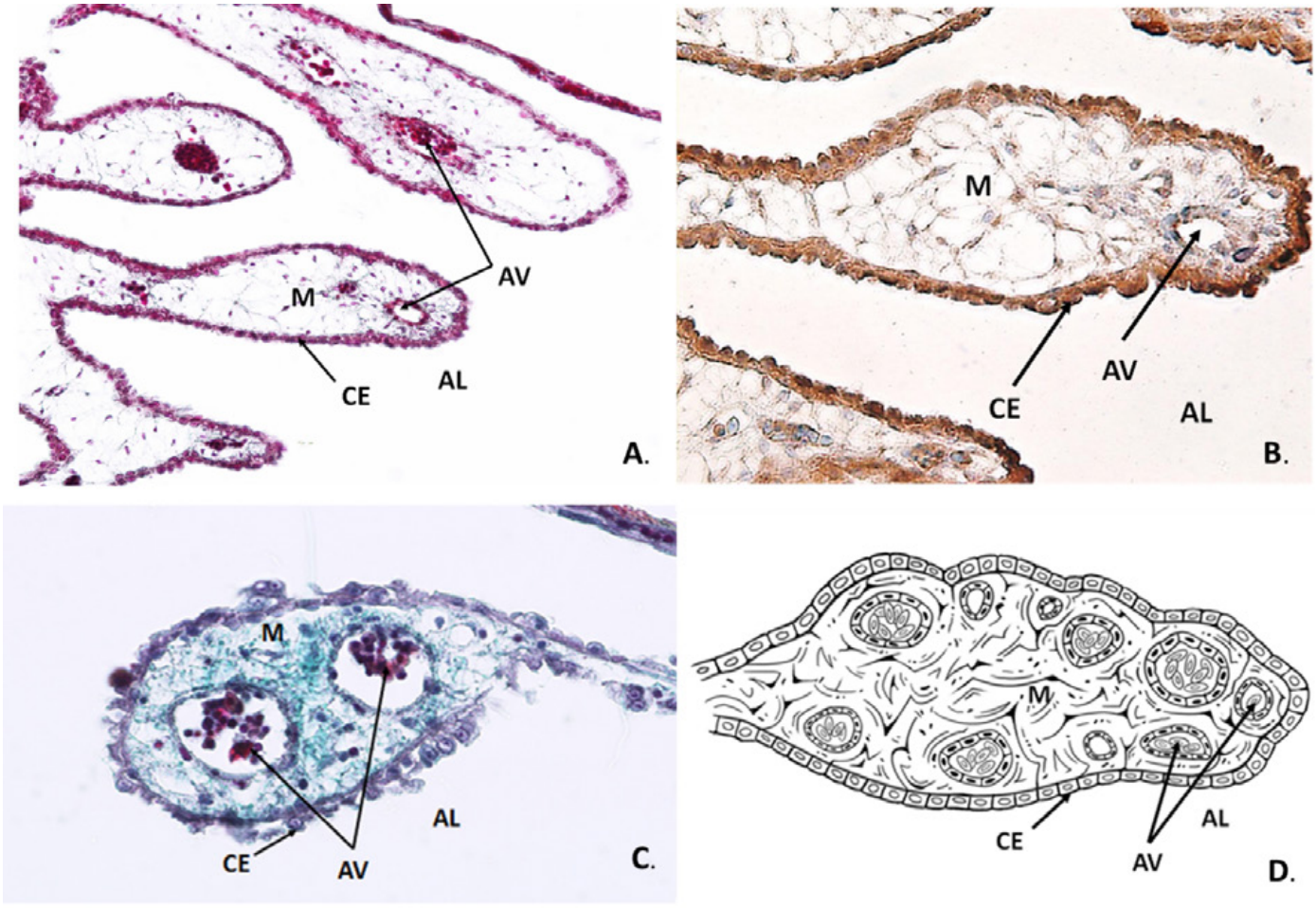

Figure 3: Histological characteristics of chorioallantoic placenta, enlargement of the surface of the fetal membranes

Several folds of chorioallantois were observed in the numerous parts above the fetus, having important role in improved gas exchange through enlarged areas of mesenchymal tissue with blood vessels in the allantois. AL, allantois lumen, AV, allantois blood vessel, M, mesenchyme, CE, chorionic epithelium. A. 200× magnification, Haematoxylin-eosin, B. Cytokeratin immunohistochemistry for cytokeratin labelled epithelial cells and confirmed their origin. 400× magnification, mouse monoclonal anti-cytokeratin MNF 116 antibody, horseradish peroxidase-labelled polymer (EnVision + Kit), counterstained with Mayer's haematoxylin; C. 400× magnification, Goldner's Trichrome, D. Schematic representation of chorioallantois, designed by Pia Cigler).

collagen fibers, with vessels of various sizes (ranging from capillaries to other small vessels) scattered among them. Vascularity was modest, typical of early to mid-gestation. This layer also contained tubular shell glands with a simple cuboidal epithelium surrounding a small central lumen. The glands in the lamina propria were stained blue with Toluidine blue, indicating their activity, but did not express cytokeratins. They were not numerous and did not appear to be very active. The tunica muscularis was organized into 2 layers: an inner circular layer and an outer longitudinal layer of smooth muscle cells. The simple squamous cells of the visceral peritoneum are located outside the tunica muscularis (Figure 7).

Furthermore, as fertilized and unfertilized slugs were harvested, we have examined both microscopically (Figure 8). The Haematoxylin-eosin and Goldner's Trichrome stainings showed distinguishing yolk structures between fertilized and unfertilized yolk. Both yolks were distinctive already at low magnification. In the fertilized eggs, they were partly surrounded by a light eosinophilic fibrous shell membrane. The yolk supporting developing embryos was less densely packed and had small to medium sized spherical yolk granules that are strongly stained with eosinophilic stain and a moderate amount of amorphous substance. The unfertilized yolk contained round spaces, that resembled lipid vesicles, were the same size as yolk granules and contained dense, darker eosinophilic amorphous material. Individual yolk droplets were rarely seen, and eosinophilic compounds were absent. In fertilized slug, stained with Goldner's Trichrome, the amorphous material was stained green (Figure 8B).

Lecitotrophy is the predominant form of viviparity described in reptiles. There are numerous morphological differences between histotrophic and lecitotrophic species, but placental changes are not the only indicator of the degree of nutrient transfer between mother and embryo. Boids are also snakes, known for their viviparity, but the placental anatomy of boids has not been described in detail. Emerald tree boa embryos, like those of most snakes, are known to develop by lecitotrophy. Similar placental anatomy has been

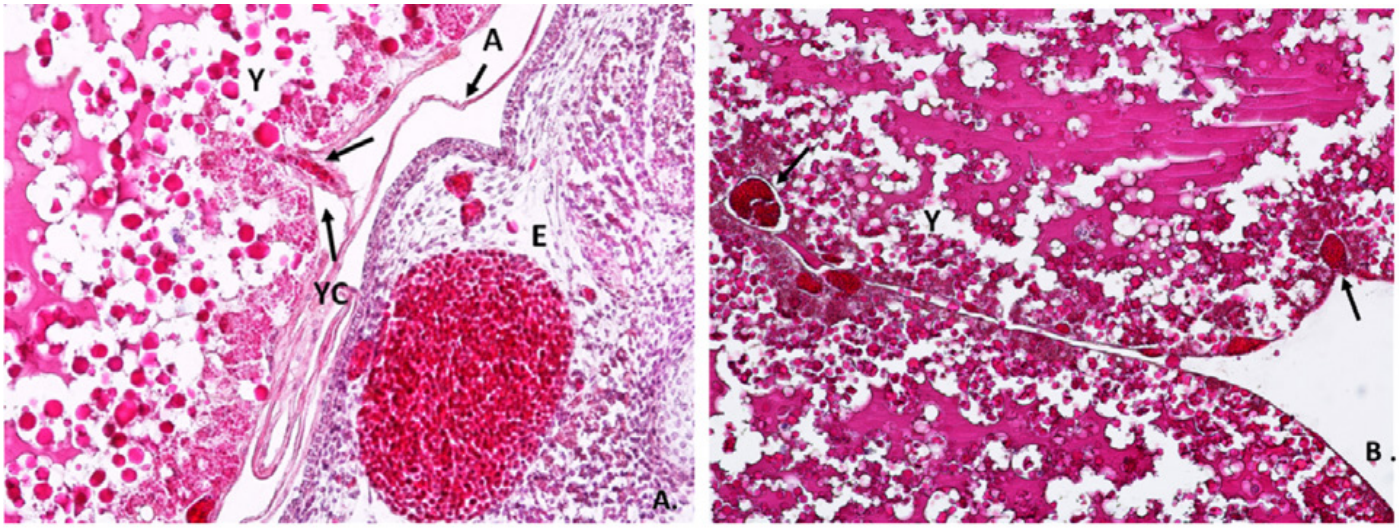

Figure 4: Representative histological characteristics of amnion

The embryo was surrounded by the amnion and superficially by the allantois, next to which was the yolk splanchnopleure. Between embryo and egg yolk is a yolk cleft. (Y, yolk vesicle, YC, yolk cleft, A, amnion, E, embryo, egg yolk vessels are indicated by arrows (100× magnification, Haematoxylin-eosin).

described in other snake species including numerous thamnophine snakes such as Da Kay´s brown snake (*Storeria dekayi*) (13) and the common garter snake (*Thamnophis sirtalis*) (Hoffman, 1970), as well as the rough earth snake (*Virginia striatula*) (9) and *Dieurostus dussumierii* (14).

Much of the research on viviparity in squamates has focused on lizards, particularly those in the family Scincaidae (6, 15). Although comparisons can be made between them and snakes, the difference in evolutionary origin limits direct comparability. A type I allantoplacenta has been described in most lizard species with lecitotrophic viviparity (16).

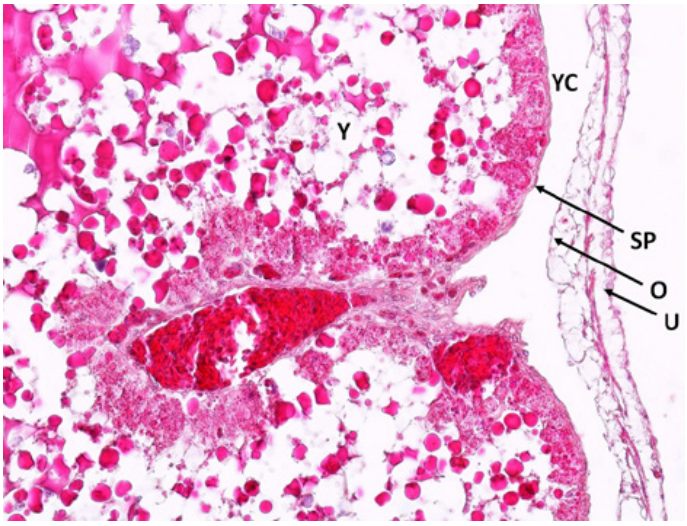

Figure 5: Representative histological characteristics of omphaloplacenta

The omphaloplacenta consisted of an omphalopleure with a layer of thin squamous epithelium, a layer of simple cuboidal epithelium and a yolk splanchnopleure. The yolk splanchnopleure consisted of squamous epithelium and a highly vascularized area. The omphalopleure and yolk splanchnopleure formed the lining of the yolk cleft. The yolk splanchnopleure surrounded the yolk with numerous yolk vesicles. U, uterus, O, omphalopleure, SP, yolk sac splanchnopleure, Y, yolk vesicle, YC, yolk cleft (100× magnification, Haematoxylin-eosin).

The placenta of viviparous snakes, as in lizards, is a temporary organ that develops during pregnancy to facilitate gas and nutrient exchange between the mother and her developing offspring. It is composed of the chorionic layer and the allantoic layer. The chorionic layer is the outer layer and is connected to the mother's uterine wall. It is composed of cells that secrete a variety of hormones and proteins that facilitate gas and nutrient exchange and protect the embryo from the mother's immune system. The allantoic layer is the inner layer that is directly connected to the developing offspring. This layer consists of a highly vascularized network of blood vessels that facilitate the exchange of oxygen and nutrients from the mother to the developing offspring (3).

The developmental morphology of chorioallantoic membranes has been studied in only two species of Galloanserae (17, 18). Some similarities were noted, but more of these species are oviparous than viviparous, so the structure was usually described and associated with the eggshell, which was not observed in our case. It is known that the chorioallantoic membrane of egg-laying reptiles forms a vascular interface with the eggshell. The eggshell of the oviparous species contains calcium, mainly in the form of calcium carbonate. The calcium is extracted from the chorioallantoic membrane and mobilized to contribute to the nutrition of the embryo (19). Eggshell calcium is thought to have been a source of embryonic nutrition in the early developmental stage of Sauropsida. It is known that there are calcium-transporting cells in the chorioallantoic membrane of corn snakes (19). This specialization of the chorioallantoic membrane to calcium uptake was not observed in our case, nor was the eggshell. The chorioallantoic membrane plays an important role in mobilizing calcium from the eggshell

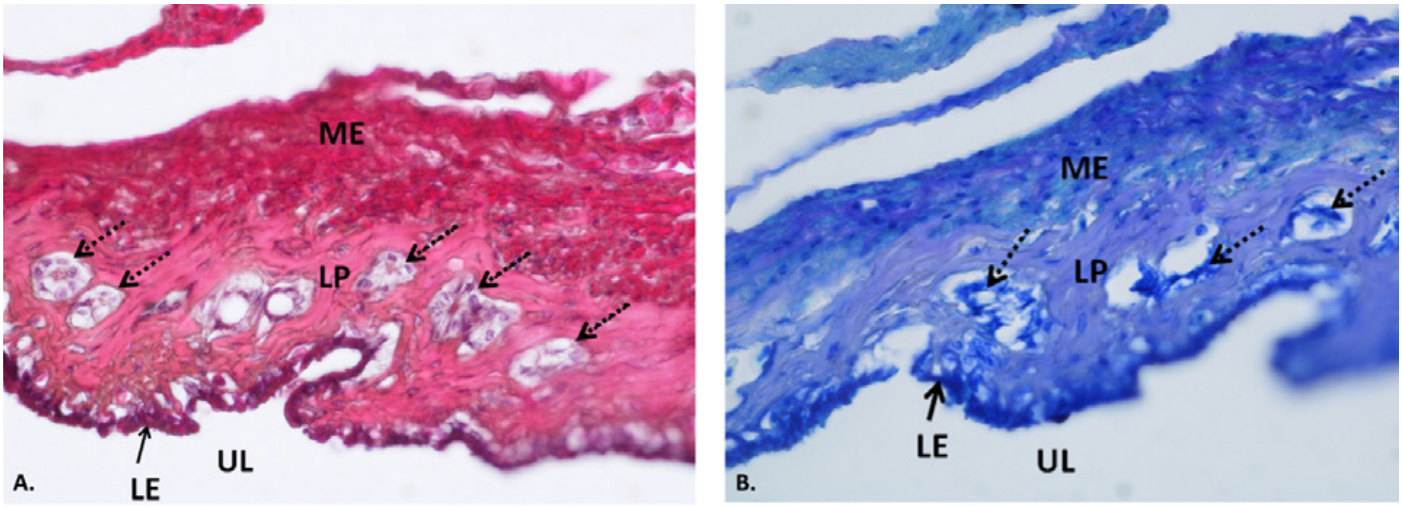

Figure 6: Representative histological characteristics of maternal component – oviduct (uterine tube)

A. 200× magnification, Haematoxylin-eosin. B. 400× magnification, Toluidine blue staining. UL, uterine lumen, LE, lamina epithelialis, LP, lamina propria, ME, muscularis externa, glands (dotted arrowheads).

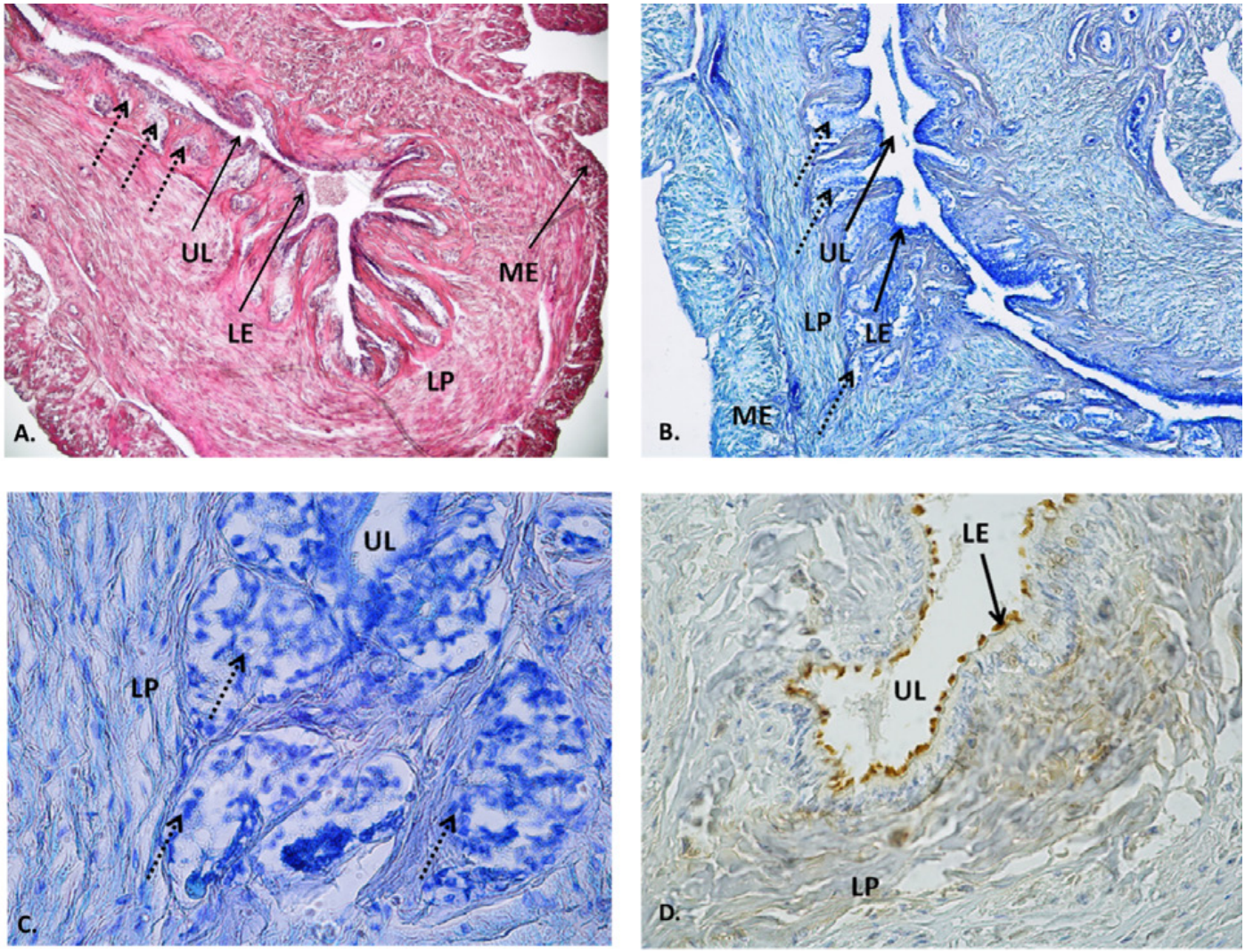

Figure 7: Representative and histological characteristics of maternal component – oviduct (uterus)

UL, uterine lumen, LE, lamina epithelialis, LP, lamina propria, ME, muscularis externa, glands (dotted arrows). A. 100× magnification, haematoxylin eosin; B. 100× magnification, Toluidine blue staining; C. 200× magnification, Toluidine blue staining; D. Immunohistochemistry for cytokeratin shows strong expression of cystokeratins in luminal epithelium. 200× magnification, mouse monoclonal anti-cytokeratin MNF 116 antibody, horseradish peroxidase-labelled polymer (EnVision + Kit), counterstained with Mayer's haematoxylin.

of egg-laying species or directly, as in our case, from the uterus, as known from other viviparous species (20–22). Some studies compare the structure of the chorioallantoic membrane to that of birds, but not much is known about the morphology and function of the chorioallantoic membrane in turtles, crocodiles, and tuatara.

In most viviparous reptiles, the first part of the oviduct (infundibulum and uterine tube) consists of a thinner wall with ciliary to non-ciliary epithelium and finger-like irregular folds in the mucosal layer. These irregular folds probably allow the infundibulum to expand as the eggs pass through (6). Uterine wall consists of three layers: an outer layer of longitudinal muscle fibers, a middle layer of circular muscle fibers, and an inner layer of simple columnar epithelium. This epithelial layer is particularly important because it facilitates the exchange of nutrients, hormones, and waste products between the mother and her developing embryos.

The uterine wall of viviparous reptiles also contains glands that produce secretions that contribute to the nutrition of the embryos and provide them with hormones. The glands are located in the lamina propria of the uterine wall and are surrounded by a thin layer of connective tissue. The secretions produced by these glands are then distributed throughout the uterus by contractions of the muscle fibers in the middle layer of the uterine wall. The epithelial layer of the uterine wall is also responsible for the formation of the chorion in viviparous reptiles. These glands are more present in active in oviparous species and contain secretory granules (23).

Comparing the structure of the uterus with other described species, many similarities can be observed, but also differences that depend on the type of placenta (13, 24, 25).

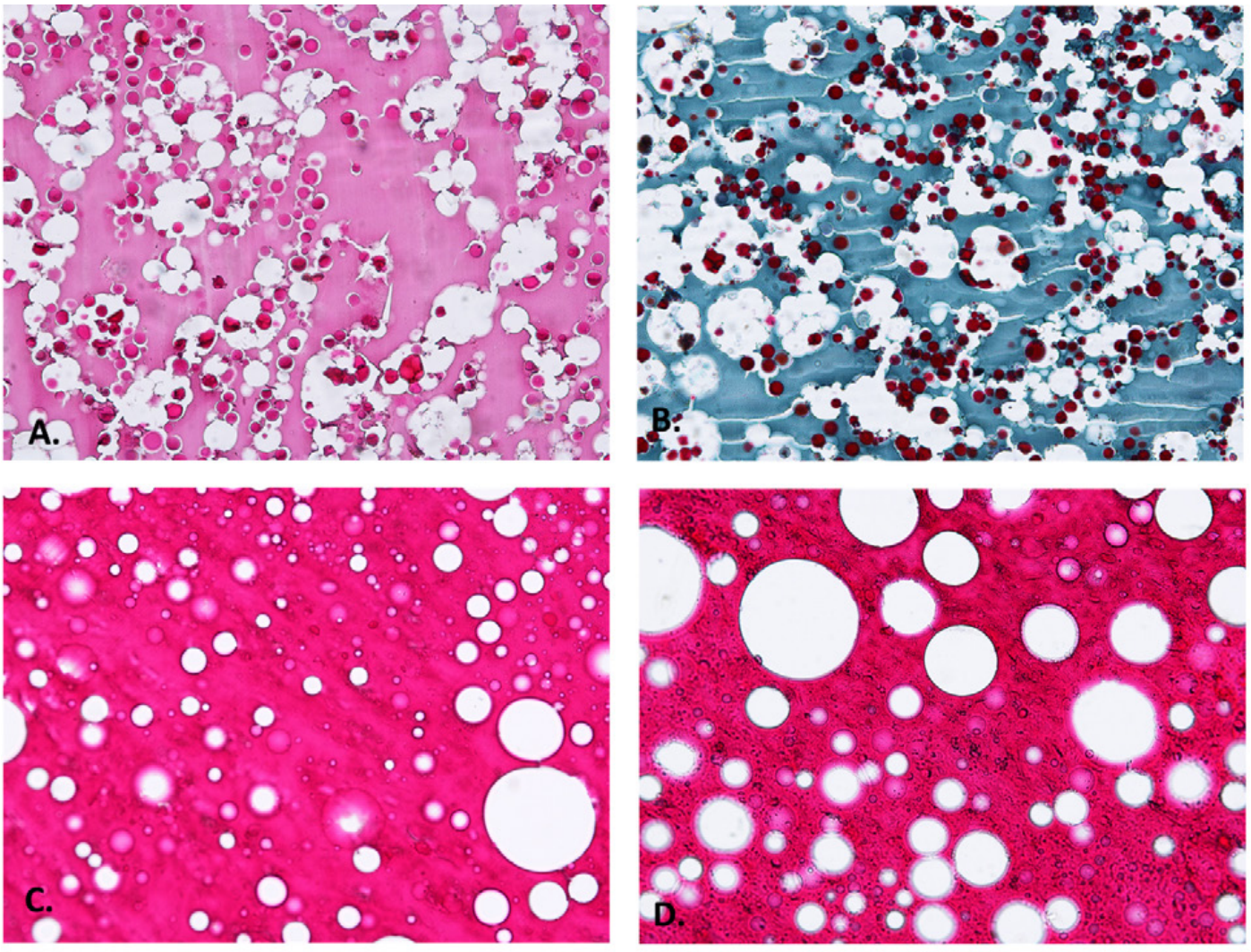

Figure 8: Histological characteristics of fertilized and non-fertilized yolk vesicles

Microscopic examination of the slugs revealed differences in yolk droplet structures between fertilized and non-fertilized yolk. The yolk supporting developing embryos was less densely packed, light eosinophilic and amorphous, while the unfertilized yolk was dark eosinophilic and contained many lipid vesicles. A. Fertilized yolk vesicle, 200× magnification, haematoxylin eosin. B. Fertilized yolk vesicle, 200× magnification, Goldner's Trichrome. C. and D. Non-fertilized yolk vesicle, 200× and 400× magnification, Haematoxylin-eosin.

Structures called "yolk sacs" are responsible for nourishing the developing embryo. The yolk sacs are located in the uterus and are connected to the embryo by a yolk stalk. The yolk stalk consists of a network of blood vessels that transports nutrients from the yolk sac to the embryo. The yolk stalk is composed of a network of blood vessels, that transports the nutrients from the yolk sac to the embryo. The embryo also receives oxygen and other substances from the mother's bloodstream through the placenta. The yolk sac also plays an important role in the development of the embryo's organs, including the liver and intestines (26). The yolk sac structure in viviparous reptiles is unique among vertebrates. It is the only reproductive system in which the embryo is nourished and developed internally, rather than externally. It is believed that this structure evolved to protect the embryo from the harsh environment of the outside world. As a result, viviparous reptiles have a better chance of survival and more successful reproduction than their egg-laying counterparts. Egg development is nonsynchronous, so intact eggs can be observed without signs of fertilization. Unfertilized slugs are known to be reabsorbed in the oviduct, which is beneficial in viviparous snakes and lizards to adapt reproduction to environmental conditions in this manner while minimizing the physical stress and loss of nutrients that these eggs represent (25, 27). Reabsorption is thought to occur through digestion and phagocytosis, but we have not yet observed higher numbers of phagocytes in our case report. In our case, the unfertilized eggs showed no signs of thinning or rupture at the mesometrial pole.

In conclusion, the placenta of viviparous snakes plays an important role in the development of the offspring. It helps to provide the developing offspring with the nutrients and gasses necessary for growth and development and protects them from harmful environmental factors. Emerald tree boa embryos develop by lecitotrophic viviparity using a type I allantoplacenta. However, because only embryos at early stages of development were available for study, further investigation is needed to better understand the dynamics of uterine and placental structure in emerald tree boas and boids in general throughout gestation.

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The authors declare no conflict of interest.

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Viviparnost pri kačah – histološka povezava plodu, plodovih ovojev in jajcevoda pri pasjeglavem udavu (*Corallus caninus*)

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Izvleček: Viviparnost (živorodnost) je z evolucijskega vidika pomemben način razmnoževanja pri plazilcih. Takšen način razmnoževanja je povezan z določenimi fiziološkimi spremembami, verjetno kot odziv na neustrezne okolijske razmere za razvoj jajc. Za razliko od oviparih vrst se zarodki do rojstva razvijajo in se zadržijo v jajcevodu. Razvijajoči se zarodek potrebuje način za izmenjavo dihalnih plinov, vode in hrane. Za to potrebuje placento, ki jo sestavljajo plodove membrane in jajcevod matere. Približno 20 odstotkov luskarjev (*Squamata*) (kuščarjev in kač) je živorodnih, morfologijo placente pa so proučevali predvsem pri rodovih Thamnophiinae in Hydrophinae. Namen naše raziskave je bil preučiti strukturo placente in situ v jajcevodu 6-letne samice pasjeglavega udava (*Corallus caninus*). Pri sekciji smo našli 5 oplojenih in 3 neoplojena jajca. Povprečna masa jajca s plodom (48 mm dolžine x 26 širine) je bila 55–65 g, neoplojenega jajca pa 15–35 g. Fetalne membrane in dva ploda pasjeglavega udava smo pregledali s pomočjo svetlobne mikroskopije. Narejenih je bilo več 5 µm debelih parafinskih tkivih rezin, ki so bile obarvane s hematoksilinom in eozinom, toluidinskim modrilom, trikromnim barvanjem po Goldnerju ter s pomočjo imunohistokemičnega barvanja citokeratina in dezmina. Opazovali smo položaj in strukturo plodovih ovojev *in situ*. Morfologija plodovih ovojev je pokazala, da pri pasjeglavem udavu najdemo tip I alantoplacente. Opisali smo tudi strukturo jajcevoda, oplojenega in neoplojenega jajca. Raziskava je omogočila boljše razumevanje morfologije placente pri kačah in razširila spekter opisanih živorodnih vrst luskarjev.

Ključne besede: živorodnost; kače; placenta; histologija; imunohistokemija; *Corallus caninus*