

Contributions to the Study of Oviparity–Viviparity Transition: Placental Structures of *Liolaemus elongatus* (Squamata: Liolaemidae)

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ABSTRACT *Liolaemus elongatus* (Liolaemidae) is a viviparous, mainly lecithotrophic species with placental structures specialized for uptake of oxygen and inorganic nutrient transport. An allantoplacenta and an omphaloplacenta are present during early embryonic stages (25–28) and there is a moderate distension of the uterine wall and major glandular activity in the uterine mucosa and submucosa compared with nonpregnant females. The uterine epithelium increases in height, first as a growth in the height of some dispersed cells localized in all regions of the placenta, and later as groups of cells localized in the periembryonic and central-abembryonic regions. At embryonic stage 39, the allantoplacenta reaches its maximum extension around the yolk sac. Omphaloplacenta is restricted to the abembryonic zone, the yolk cleft limiting the newly formed isolated vitelline mass. At more advanced embryonic stages (39–42), the blood supply to the allantoplacenta's periembryonic zone increases, matching the profuse allantoic vascularization. At embryonic stage 42, a secondary cleft opens in the main vitelline mass, above the first yolk cleft, and allantoic blood vessels enter into this secondary cleft. This secondary cleft subdivides the vitelline mass into a large embryonic region connected to a much smaller abembryonic region. In *L. elongatus* most nutritional resources seem to be provided by the yolk that remains attached to the newborn for 2 or 3 days as an external supply. The embryo's wet weight doubles the weight of the decrease observed in vitelline mass. But the dry wet diminishes, evidencing the importance of the exchange of water and inorganic nutrients. *J. Morphol.* 269:865–874, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: placentas; lecithotrophy; placentotrophy; oviparity–viviparity transition; Liolaemidae

Reptiles provide an excellent model for the evolution of viviparity because both oviparous and viviparous sister taxa can be studied. The amniote egg allowed the embryo's independence from aquatic environments. The extraembryonic membranes of these eggs generate elaborate vascular systems for transferring oxygen, water, and nutrients to the embryo, while eliminating metabolic wastes. These vascular systems support the development of reptilian and mammalian placentas, with their wide range of structural arrangements (Gilbert, 1994).

Oviparity in lizards is the ancestral and more common reproductive mode, but viviparity evolved about a hundred times in Squamata (Packard et al., 1977; Blackburn, 1982; Shine, 1985) and at least three times in the lizard genus *Liolaemus* (Schulte et al., 2000). Considering the comprehensive review of Blackburn (1994a,b) regarding the concept of viviparity, the question arises as to the effective physiological exchanges needed to produce living young, rather than eggs. Functional placentas have been proposed in those squamates that retain the eggshell during gestation. For example, Lemus and Duvauchelle (1966) observed the simultaneous existence of an eggshell and a placental structure in *Liolaemus tenuis tenuis*, accompanied by noticeable thinning and abundant vascularization of the uterine wall, structural arrangements later depicted by Stewart and Blackburn (1988). In vertebrate development, the role of the egg envelopes, i.e., a fibrous vitelline membrane (in mammals called the zona pellucida, *sensu* Gilbert, 2005) and eggshell, is of utmost importance. The loss of these structures in mammalian blastocysts may contribute to a new pattern in the development of the extraembryonic membranes as it occurs in some species (Gilbert, 1994).

In the uterine cavity of members of the subclass Lepidosauria, all eggshells are secreted in a similar way. In Lepidosauria, the structure of the eggshell includes an inner boundary layer, fibrous mesh, and calcium carbonate (Packard and DeMarco, 1991). Eggshell in some species is a continuous structure, whereas in others it appears fragmented.

Contract grant sponsor: Universidad Nacional del Comahue; Contract grant number: PIP 5625; Contact grant sponsor: Agencia Nacional de Promoción de Ciencia y Tecnología; Contract grant number: PICT 98: 04867.

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Published online 19 May 2008 in
Wiley InterScience (www.interscience.wiley.com)
DOI: 10.1002/jmor.10632

Nevertheless, just in some species eggshell integrity is conserved throughout pregnancy (Guillette et al., 1980). In the evolution of the lepidosaurian order Squamata, the eggshell and extraembryonic membranes are deemed conservative characters (Stewart, 1993). With ~1,200 viviparous species, Squamata presents an extraordinary opportunity for a better understanding of the reproductive and evolutionary diversity of placentation, even though only 3% of the viviparous species have been studied (Blackburn, 1993a). The extraembryonic membranes (chorioallantoic membrane and the bilaminar omphalopleure) are homologous to their mammalian counterparts (Mossman, 1937). However, placentary structures are convergent. The chorioallantoic membrane seems to be a plesiomorphic character in Squamata, as it is also present in Chelonians, Crocodylia, Aves, and Mammalia. In contrast, the formation of the isolated vitelline mass seems to be an autapomorphy, present in oviparous as well as some viviparous Squamata. The development and variation of the epithelia associated with the isolated vitelline mass of viviparous species led Stewart (1993) to consider all of these structures as placental specializations and derived characters.

The epithelia of the chorioallantoic membrane are very similar in many, but not all, oviparous and viviparous reptiles. Also, it has been proposed that the chorioallantoic placenta allows respiratory exchanges and uptake of small quantities of nutrients (Lemus, 1967; Blackburn, 1993a). Differing from some Eutheria and from most of the other oviparous amniotes, Squamata exhibit a bilaminar and avascular omphalopleure during almost the entire embryonic development (Hughes, 1993). Also in Squamata, an abembryonic "isolated vitelline mass" is formed by entry of the extraembryonic mesoderm into the yolk sac. The permanence, complexity, and variations of these structures support the conjecture that, phylogenetically, in viviparous Squamata the isolated vitelline mass and the omphaloplacenta mark the initial structural locus of the yolk sac placental specialization (Stewart, 1993). In sum, it can be stated that the structure of the omphaloplacenta supports the hypothesis that extraembryonic tissues underwent a selection that optimized histotrophic interchanges (Stewart, 1985, 1993).

The genus *Liolaemus* could be considered a model to study the structural and functional diversity of placentas and the transition from oviparity to viviparity. *Liolaemus* includes almost equal numbers of oviparous and viviparous species (Donoso Barros, 1966; Cei, 1986, 1993; Schulte et al., 2000) and presents significant advantages for experimental work: gravid females survive multiple surgeries, their eggs successfully develop in terraria, and the embryos can be cultured and experimentally manipulated (Lemus and Duvauchole, 1966; Lemus, 1967; Lemus and Badínez, 1967; Lemus

and Wacyk, 1969; Leyton et al., 1980; Lemus et al., 1981). From a biogeographic point of view, *Liolaemus* species inhabit biotopes of several kinds, in which the individuals undergo climatic constraints via short activity seasons and long hibernation periods (Ibargüengoytia and Cussac, 1998, 1999). With ~160 species, the genus is distributed from the highlands of Perú and Bolivia to Tierra del Fuego and from the Pacific islands to Brazil, over topographies ranging from close to sea level up to 5,000 m, and showing a broad latitudinal, altitudinal, and climatic distribution (Cei, 1986; Schulte et al., 2000). The purpose of our research was to acquire basic data on the structure of *Liolaemus elongatus* placentation and correlated developmental changes, to contribute to understanding the variation in reptilian reproductive structures, and to advocate the possible use of this species as an experimental model in the study of vertebrate developmental biology.

MATERIALS AND METHODS

Females of *Liolaemus elongatus* (Kosłowsky, 1896; $N = 11$) were caught by noose from September 1994 to February 2001 in San Carlos de Bariloche (39° to 41.5° S and 70.5° to 71.6° W, between 500 and 1,800 m high) and Esquel (43° S and 71° W; at 769 m high), Argentine Republic. The lizards were immediately killed by intraperitoneal administration of sodium thiopental, fixed in Bouin's solution for 24 h, and preserved in 70% ethanol. The uteri were removed and dehydrated in an ethanol series and embedded in Paraplast. Samples from pregnant female uteri were kept 72 h in 1% celloidin (or parlodion) methylbenzene solution during dehydration. Sections of 4–7 μm were stained with Masson trichrome, Hematoxylin-eosin, or periodic acid-Schiff (PAS, Martoja and Martoja Pierson, 1970).

Wet weight of embryos, yolk sacs, and extraembryonic membranes were recorded (± 0.1 mg) from ethanol preserved samples. Dry weight was recorded when a constant weight was reached after a dehydration process at 60°C.

Egg laying and hatching behavior were photographically recorded (two females and eight newly hatched juveniles) in a glass open-topped terrarium of 117 \times 40 \times 50 cm outfitted with a photographic digital camera (Sony, MVC FD73) and a video camera (Panasonic 3.000). The uteri of nonpregnant females were characterized following Ibargüengoytia and Cussac (1998) as: uterus Type 1—threadlike oviduct typical of juveniles; uterus Type 2—with medium size folds spreading all over the uterus, typical of vitellogenic females; and uterus Type 3—with bigger folds, indicative of recent parturition. Uteri were divided into three zones, namely, infundibular, mid, and vaginal. In pregnant females, only the mid zone occupied by the embryo was analyzed.

The height of the mucosa and submucosa from each zone was measured in nonpregnant females, by taking 10 transverse sections, one every 70 μm . The average of six random base-to-top measures was recorded for each section. In pregnant females, measurements were taken on transverse sections (90° with respect to the embryonic-abembryonic plane) at four sites: embryonic, abembryonic, and two periembryonic zones. Visible-light microscopy (Olympus BX40) and image analyzer (Image Pro Plus) were used. Because of the low number of individuals, the observed tendencies were not treated statistically.

To ascribe embryonic development to the developmental stages described by Lemus and Duvauchole (1966) and Lemus et al. (1981) for *Liolaemus tenuis tenuis*, we considered somite number, limb development, and the following distances: 1) apex-mesencephalon to frontal process, 2) apex-mesencephalon

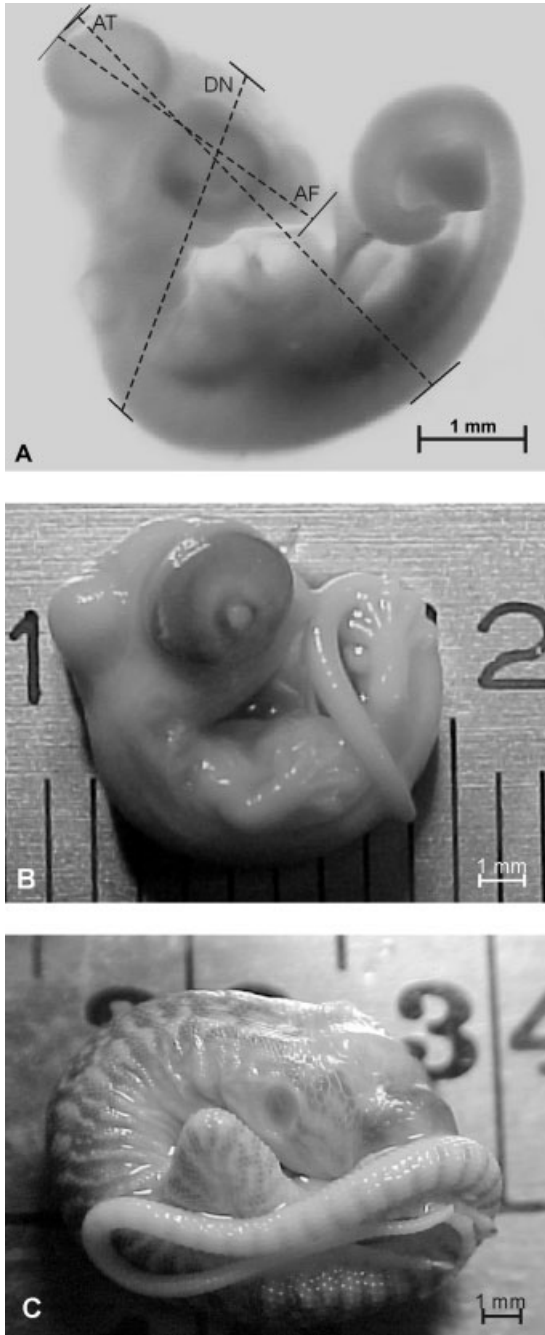


Fig. 1. *Liolaemus elongatus* embryos. **A:** Stage 25–26. **B:** Stage 39. **C:** Stage 42. AF, distances of apex-mesencephalon to frontal process; AT, apex-mesencephalon to posterior trunk flexure; DN, apex-diencephalon to apex-cervical or nuchal flexure.

to posterior trunk flexure, and 3) apex-diencephalon to apex-cervical or nuchal flexure (Fig. 1A).

RESULTS

The embryo developmental stages 25–26, 27–28, 39, and 42 are described in Table 1 and in Figure 1.

Egg-laying was registered in captivity in two females that laid four eggs each. The females buried the eggs. The embryos moved actively inside the egg and broke the eggshell (Fig. 2A) probably with the egg tooth (Fig. 2B,C) and helped with their legs, between 15 min and 12 h after egg laying. Newborn lizards begin immediately to move across the terrarium, carrying a small yolk sac (Crocco, 2001), which was lost within a period of a few hours to 3 days.

The wet weight of the *Liolaemus elongatus* embryo almost duplicates the decrease of the yolk's wet weight during the development (Table 2). The gains in the embryo's wet weight cannot come from the losses in the yolk's wet weight. Accordingly, the increase in embryo's dry weight is much lesser than the decrease of yolk's dry weight.

Uterine Changes

The reproductive tract of *Liolaemus elongatus* does not exhibit differences from the general description for Iguania (Ceï, 1986). The uterine wall exhibits the typical histological organization in mucosa, submucosa, muscularis, and adventitia. The epithelium of the mucosa is simple and varies from columnar to squamous, with ciliated and nonciliated secretory cells and is subtended by a lamina propria. The submucosa shows glandular acines and a variable vascular supply. Smooth muscle fibers characterize the muscularis. These fibers form two layers: an inner circular layer and an outer longitudinal one. The adventitia is inconspicuous and is covered by the mesothelium. The oviduct changes from a threadlike and fold-less condition in the nonvitellogenic females—to regularly folded in the vitellogenic females—to highly enlarged and unfolded in pregnant females—to deeply and irregularly folded in postpartum females.

The heights of both the mucosa and the submucosa vary in the same female depending on the zone of the uterus and on the female reproductive condition (Table 3). In vitellogenic females, the uterine transverse section is small and the height of the mucosa increases at the infundibular zone, showing a great number of shell glands. In pregnant females the height of the mucosa and submucosa decreases. In postpartum females, the uterine mucosa shows the same height as in vitellogenic females, but there is a noticeable enlargement of the submucosa with many shell glands.

In pregnant females with 25–26 stage embryos, we observed scattered cuboidal uterine epithelial cells in the abembryonic and periembryonic zones (Fig. 3A). At stages 27–28 both mucosa and submucosa are thinner than at stages 25–26. The cuboidal uterine epithelial cells are organized in patches in the abembryonic and periembryonic zones and contain chromophobic vacuoles (Fig.

TABLE 1. Morphometric characterization of the embryonic developmental stages

Embryo stage	25–26	27–28	39	42
Somite pairs	46	52	+76	+90
A–F distance	2.65	2.68	5.98	11.26
A–T distance	4.29	4.18	8.17	—
D–N distance	3.13	2.90	6.99	—
AF/AT ratio	0.62	0.64	0.73	—
Pharyngeal arches	3	4		
Limbs development	Hind < Fore buds	Hind = Fore buds	5 digits and interdigital membranes	Fully formed and pigmented
Isolated yolk mass	—	—	At the abembryonic pole of the egg	Like 39 stage
Egg position (in relation to maternal body)	Ventrolateral (abembryonic pole laying under the mesometrium)	Ventrolateral (embryonic pole lies under the mesometrium)	Like 27–28 stage	Like 27–28 stage

A–F, apex mesencephalon to frontal process; A–T, apex mesencephalon to posterior trunk flexure; D–N, apex diencephalon to apex cervical or nuchal flexure. Distances are in mm.

3B). From stage 39 to stage 42, the uterine mucosa remains thin, but the submucosa enlarges, with a greater height at stage 42. The cuboidal cells disappear at stage 39. In the submucosa, there is a thickening, mainly determined by the variation in the proportion of blood vessels and acinar glands. This variation occurs according to the composition and function of each placenta.

Placental Types

A thin, fibrous eggshell of $10.9 \pm 4.5 \mu\text{m}$ average thickness is found in every placental arrangement, under the maternal tissue and covering the fetal ones (Fig. 2A).

Uteri With 25–26 Stage Embryo

Chorioallantoic placenta (sensu Harrison and Weekes, 1925, in Stewart, 1993). This type of placenta is associated with all embryos between stages 25 and 42 and it extends from the embryonic to the periembryonic zone. It attains maximum extension at stage 39. The uterine layer is composed of a simple epithelium of squamous cells with rounded nuclei, and a submucosa with large vessels and large acinar glands.

The chorioallantoic membrane (CAM) forms the fetal component. It is composed by an allantoic splanchnopleure in contact with the chorionic avascular somatopleure. The allantoic splanchnopleure has a flat endodermal epithelium, varying from simple to multistratified layers, covered by a scarce mesenchyme with conspicuous mesodermal vascularization. Nevertheless, in the allantoic zone associated with the amniotic sac, this epithelium becomes cuboidal and its vascularization is reduced. The chorionic avascular somatopleure, composed of a simple squamous epithelium and undifferentiated mesodermal cells, is closely related to the allantois.

Omphaloplacenta (sensu Stewart, 1993).

This kind of placenta occurs between stages 25 and 42, progressively diminishing from periembryonic to abembryonic zones while the chorioallantoic placenta shortens, reaching the minimum extension in stage 39.

The uterine layer consists of a simple epithelium of cuboidal cells with a rounded apical zone arranged in groups or separated by squamous cells, over the whole region of the placenta. The submucosa shows numerous acinar glands and scarce small blood vessels between them. Almost the whole layer exhibits the thickness of a simple acinar gland (Fig. 3A).

The fetal component is composed of a squamous ectoderm arranged below the thin vitelline endoderm (bilaminar omphalopleure).

Uteri With 27–28 Stage Embryo

Chorioallantoic placenta. The uterine submucosa mostly includes blood vessels and the acinar glands are absent. The fetal component does not differ from that of stages 25–26 described earlier.

Omphaloplacenta. In the periembryonic and central-abembryonic zones, the uterine epithelium exhibits isolated patches of high cells surrounded by squamous cells. High cells appear cuboidal to columnar, exhibit basal nuclei and a distinct apical zone containing chromophobic vacuoles (Crocco, 2001). This apical aspect is flat (Fig. 3B). Neither the submucosa nor the embryonic components of this placenta show differences from those of stages 25–26 (Fig. 3C).

Uteri With 39-Stage Embryo

Chorioallantoic placenta. The uterine submucosa is characterized by abundant blood vessels, mainly concentrated in the periembryonic zone, as well as scarce acinar glands. The fetal component

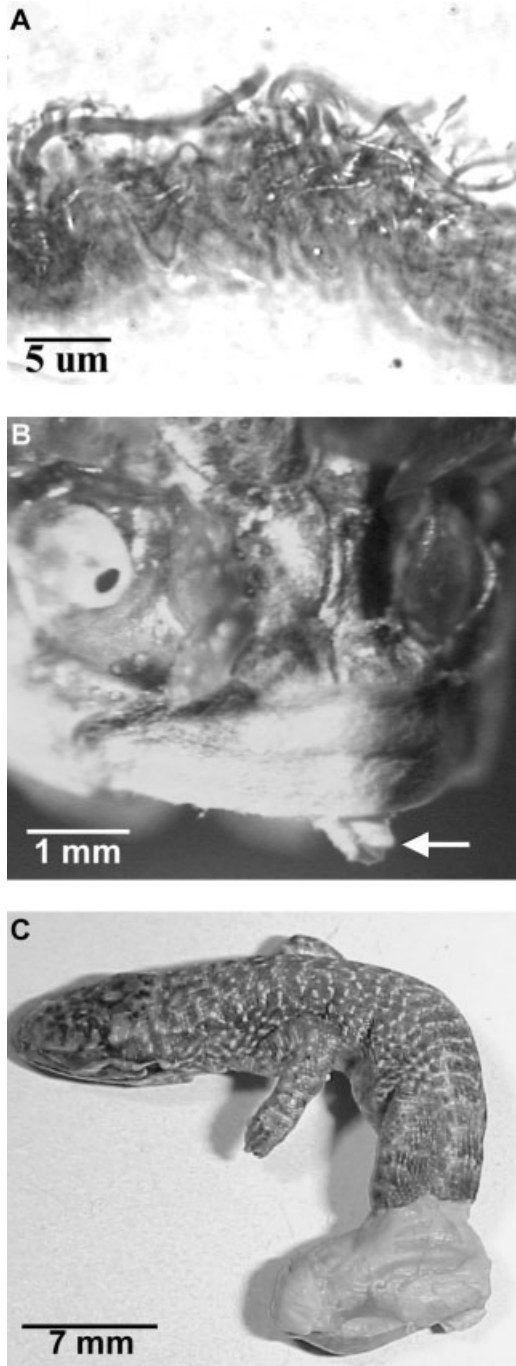


Fig. 2. *Liolaemus elongatus*. **A:** Detail of the eggshell. **B:** Detail of the egg tooth (arrow). **C:** Embryo coming out of the egg envelopes.

does not differ from that of previous stages (Fig. 3D).

Omphaloplacenta. In contrast with the preceding stages, a simple layer of squamous epithelial cells appears in the uterus, whereas in the submucosa some large blood vessels are present. The acinar glands determine the thickness of this stratum.

The fetal component does not differ from that of stages 25–26.

Yolk sac organization. The yolk cleft can be seen in the abembryonic zone as a narrow cavity, coated with a sheet of cuboidal intravitelline mesodermal cells. Under the yolk cleft, there is a non-vascularized vitelline zone, the isolated vitelline mass (Fig. 4A).

Uteri With 42-Stage Embryo

Chorioallantoaplacenta. There are no differences in this type of placenta compared with the previous stage.

Omphaloplacenta. The uterine layer shows a simple epithelium of squamous cells and abundant acinar glands together with large and small blood vessels in the submucosa. The acinar glands, along with blood vessels, determine the thickness of this stratum. The fetal component does not differ from that of stages 25–26.

Yolk sac organization. A secondary cleft with allantoic blood vessels is present (Fig. 4B). The secondary cleft separates two connected regions in the main vitelline mass: the embryonic and the abembryonic region (Fig. 4C,D).

DISCUSSION

The freshly fertilized eggs of squamate reptiles have a large vitelline mass surrounded by vitelline membrane, the zona pellucida, and the eggshell. In viviparous lizards, the eggshell is thin, translucent, and lacks calcium deposits and the embryo's development is highly advanced at the time of egg laying or parturition (Packard et al., 1977; Packard and Packard, 1980; Blackburn, 1982; Shine, 1983). Development of the *Liolaemus elongatus* embryo occurs in the mid-uterine zones but the uterus expands so much that one could also say that development extends to take place even about the limit of both mid- and infundibular zones. Sibs develop at the same or very similar rate. In *L. elongatus* the vitelline envelope and the eggshell of embryos are retained in the uterus. Females lay shelled eggs that enclose a juvenile, a yolk sac and extraembryonic membranes. The eggs hatch from a few minutes to hours following egg-laying as a result of the egg tooth and of the embryo's movements within the egg. The results show that, even though there are intermediate features between oviparity-viviparity, as egg laying and the egg tooth, the *L. elongatus* mode of reproduction coincides with the category of viviparous species proposed by Blackburn (1993a, 1994a, 1994b). In species of the genus *Sceloporus* (Stewart and Thompson, 1993) as in *Liolaemus* species (Ibar-güengoytía et al., 2001) the viviparous reproductive mode coexists with the oviparous mode. In the same way, both modes are present in populations

TABLE 2. Embryo, yolk, extraembryonic membranes, and total wet and dry weight

Stage	Embryo weight (mg)		Yolk weight (mg)		Extraembryonic membranes (mg)		Total weight including eggshell (mg)	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
25–26	8	2	590	303	—	13	>598	318
27–28	16	2	580	286	—	5	>596	293
39	96	—	538	—	—	—	>634	—
42	378	42	378	187	—	7	>756	236
Newly laid eggs	—	138	—	4	—	13	1168	155
	—	154	—	8	—	0	1348	162
	—	163	—	8	—	5	1016	176

Each line corresponds to one individual. The symbol — indicates that it was not weighed.

of *Lacerta vivipara* (Stewart et al., 2004; Heulin et al., 2005), and *Lerista bougainvillii* (Qualls, 1996).

Placentas and Uterine Changes

In reptiles, there are placentas with diverse degrees of contact between the fetuses and the mother, with reduction in the eggshell structure and in the yolk sac quantity, and with an augmentation of nutrients (Thompson et al., 1999). However, comparative studies in closely related oviparous and viviparous squamate species (Guillette and Jones, 1985; Stewart et al., 2004) showed no differences in the topographic layout of extraembryonic membranes, despite the vast disparity between the external environment and maternal tissues. During the reproductive cycle, morphological changes in the uterus of *Liolaemus elongatus* take place in the mucosa and the submucosa, diminishing the distance between maternal and fetal vessels and reducing the height of uterine epithelia in pregnant females. During pregnancy, the earliest stages (25 and 26) resembled the uteri of nonpregnant females. But, successive developmental stages (27–42) show a progressive distension of

the uterine mucosa. During the later stages, major glandular activity, an increase in the blood supply, and an allantoic vascularization, also occur.

Omphaloplacenta

The bilaminar omphalopleure is a major exchange site in Marsupialia (Tyndale-Biscoe and Renfree, 1987) and in Squamata the ectodermal epithelium of this structure is specialized (Blackburn, 1993a). The isolated vitelline mass and yolk cleft of Squamata are unique among amniotes. During stages 25–28 of *Liolaemus elongatus* embryos, the height of some uterine cells increases from smooth to cuboidal and the uterine cells show chromophobic vacuoles typical of secretory activity. This increment in cell height initially occurs in the whole omphaloplacenta and, later, only in the periblastotic and central-abembryonic regions.

Allantoplacenta

In agreement with the observations of Stewart (1993), we found that the outer allantoic membrane of *Liolaemus elongatus* fuses with the cho-

TABLE 3. Height of mucosa and submucosa in infundibular, mid, and vaginal zones of non-vitellogenic, vitellogenic, pregnant, and postpartum females of *L. elongatus*

Female reproductive condition	Zone/Stage	Mucosa thickness ($\mu\text{m} \pm$ coronal variance)	Submucosa thickness ($\mu\text{m} \pm$ coronal variance)
Non vitellogenic ($N = 1$)	Infundibular	8.3 ± 1.87	26.1 ± 4.24
	Mid	10.9 ± 3.67	38.8 ± 4.98
	Vaginal	8.4 ± 2.46	23.5 ± 3.83
Vitellogenic ($N = 1$)	Infundibular	16.6 ± 2.78	26.3 ± 5.32
	Mid	9.4 ± 3.45	38.4 ± 5.36
	Vaginal	12.2 ± 3.02	43.9 ± 6.24
Pregnant ($N = 4$)	25–26	9.5 ± 2.26	27.4 ± 5.85
	27–28	6.5 ± 2.09	13.8 ± 4.41
	39	4.8 ± 1.56	17.3 ± 4.86
	42	5.3 ± 1.47	20.9 ± 6.81
Post partum ($N = 1$)	Infundibular	12.5 ± 3.45	102.0 ± 14.47
	Mid	10.4 ± 3.27	84.2 ± 12.76
	Vaginal	9.6 ± 2.65	70.0 ± 9.84

In pregnant females, developmental stages 25–26, 27–28, 39, and 42. Coronal variance means the variation in the uterus thickness in different points of its circumference.

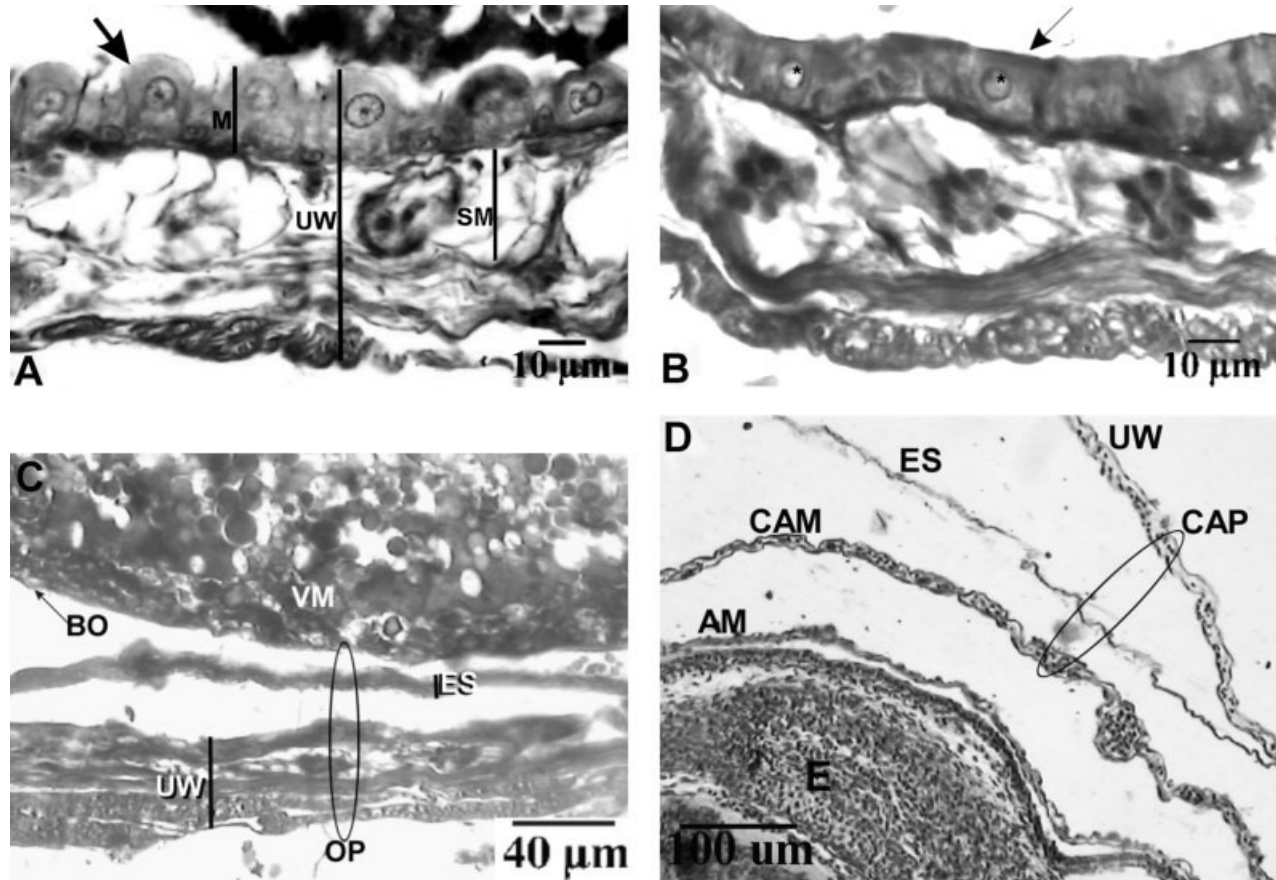


Fig. 3. Placental types of *Liolaemus elongatus*. The separation between membranes is a technique artifact. **A, B:** Characteristics of omphaloplacenta of *L. elongatus*. Arrows show cuboidal epithelial cells in the uterus of pregnant female with embryo at stage 25–26 and 27–28, respectively. Asterisk (*) marks chromophobic vacuoles. **C:** Omphaloplacenta of 27–28 stage embryo. **D:** Chorioallantoic placenta of 39-stage embryo. UW, uterine wall; M, mucosa; SM, submucosa; BO, bilaminar omphalopleure; OP, omphaloplacenta; VM, vitelline mass; ES, eggshell; E, embryo; AM, amnion; CAM, chorioallantoic membrane; CAP, chorioallantoic placenta.

tion, dorsal to the embryo, forming the richly vascularized chorioallantoic membrane. While the isolated vitelline mass is forming, the chorioallantoic membrane apposes the uterus, forming the chorioallantoic placenta, or allantoplacenta (Stewart, 1985, 1990, 1993). We confirm Crocco's (2001) observation that in *L. elongatus* the allantoplacental formation of stages 25–28 occurs before the yolk cleft—isolated vitelline mass structures are formed. The primary yolk cleft is present at stage 39. The sheets of this yolk cleft (primary or original cleft) appear toward the end of the development (stage 42). Ontogeny of *L. elongatus* strongly resembles the one described by Stewart et al. (2004) for oviparous and viviparous *Lacerta vivipara*. The last authors also found omphalomesenteric blood vessels, associated with a deep fold that penetrates the yolk parallel to the isolated vitelline mass. This fold partially subdivides the vitelline mass into a large embryonic region and a much smaller abembryonic region, and in the division zone a secondary cleft is formed. In *L. elongatus*

(stage 42), we also found a secondary cleft with allantoic blood vessels, and both regions in the main vitelline mass. In many Squamata, the isolated vitelline mass diminishes in size and as a result the fetal and maternal vessels approximate each other (Hoffman, 1970; Stewart and Thompson, 1994). In *L. elongatus*, the newly hatched individuals carry a small yolk sac during their first 2 or 3 days of life. The same occurs in *Chalcides chalcides* (Blackburn, 1993b), *C. ocellatus* (Badir, 1955), *Hoplodactylus maculatus* (Boyd, 1942), and in *Gerrhonotus coeruleus* (Stewart and Castillo, 1984). However, we cannot specify whether in the newborns the remnant yolk sac is a part of the isolated vitelline mass, or either a part of the abembryonic region of the main vitelline mass.

Maternal-Fetal Nutrient Exchanges

Viviparous Squamata include mainly lecithotrophic (Stewart and Castillo, 1984) and placentotrophic species (Blackburn et al., 1984) and both

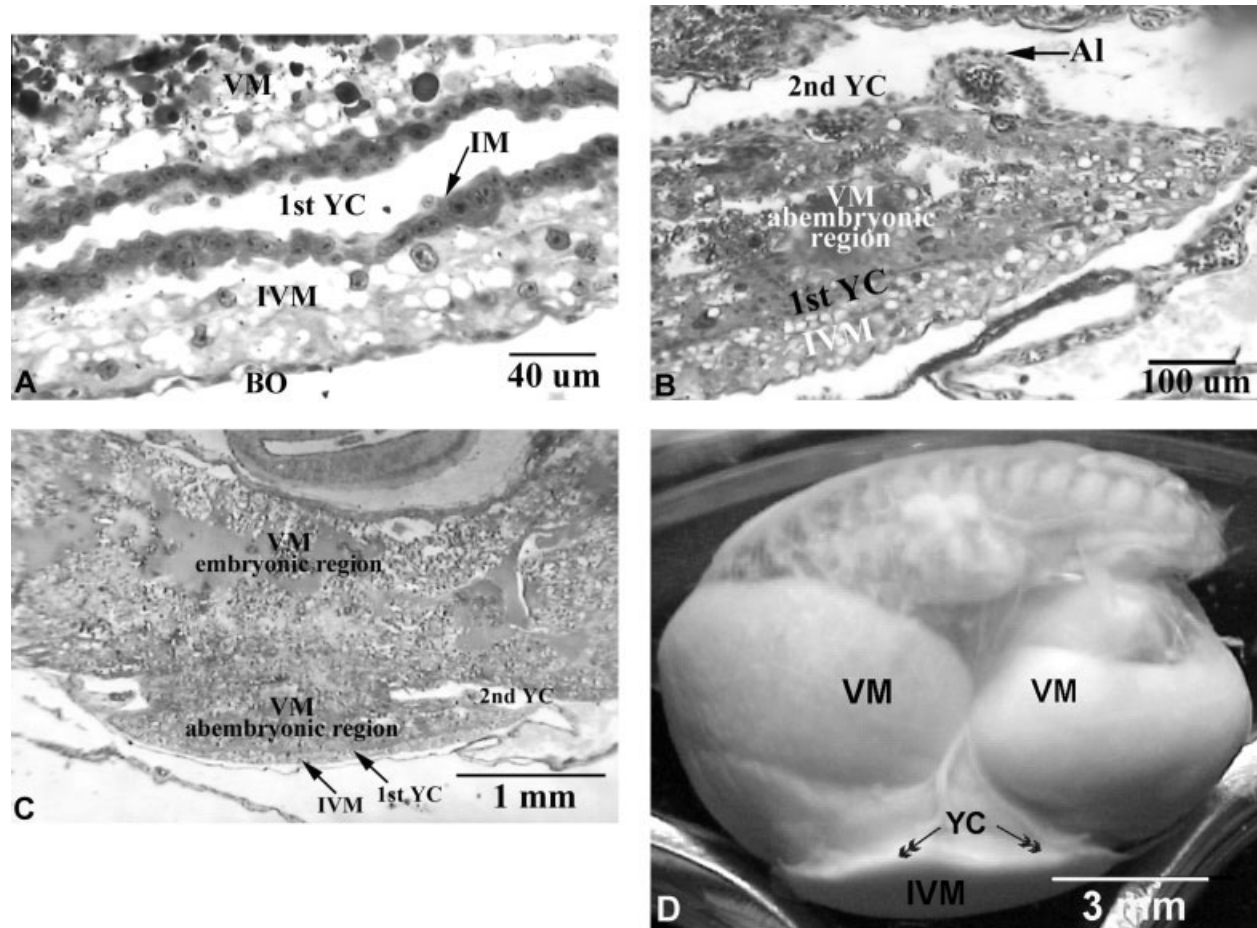


Fig. 4. Characteristics of vitelline mass of *Liolaemus elongatus*. **A:** Yolk cleft of 39-stage embryo. **B:** Detail of allantois entering into the secondary yolk cleft of 42-stage embryo. **C:** Stereoscopic view of the vitelline mass. **D:** View of egg with a 42 stage embryo. VM, vitelline mass; 1st YC, first yolk cleft; IM, intravitelline mesoderm; IVM, isolated vitelline mass; BO, bilaminar omphalopleura; 2nd YC, secondary yolk cleft; Al, allantois.

types of embryonic nutrition occur in most species, but differ quantitatively (Thompson, 1981, 1982; Stewart, 1989). In some mainly lecithotrophic species there is also a facultative exchange of organic and inorganic substances (Swain and Jones, 2000). The *Liolaemus elongatus* placenta seems mainly to transport water and salts by the cylindrical cells present in the omphaloplacenta of the uterine epithelia. Nevertheless, the methods utilized in this study do not allow us to eliminate the presence of any organic transport by these cells.

Placental Functions

Allantoplacental functions. The allantoplacenta of *Liolaemus elongatus* persists until parturition and expands, embracing almost all the yolk sac with a strongly vascularized allantoic mesoderm. The vascularization, the apposition of highly vascularized tissues, and the spread throughout development, strongly suggest a respiratory function throughout incubation. Development of the

chorioallantoic membrane is similar in oviparous snake embryos, which consistently increase their fetal oxygen dependence throughout development, together with the vast increase of their embryonic mass. The chorioallantoic membrane performs gas exchange in oviparous amniote eggs (Stewart, 1985, 1990; Baxter, 1987). The thick eggshell of oviparous reptiles reduces diffusion (Packard et al., 1977) and thin eggshells, such as those of *L. elongatus*, could be a prerequisite for intrauterine development.

Four types of allantoplacenta have been described in Squamata, with increasing structural complexity (Blackburn, 1992, 1993a; Stewart and Thompson, 1994; Jerez and Ramírez-Pinilla, 2001). The allantoplacenta of *Liolaemus elongatus* corresponds to type I, the most common in Squamata, with minor modifications from oviparous species, reduction of the fibrous eggshell and complete intrauterine development (Weekes, 1935 in Yaron, 1985). The high vascularization of the squamate type I allantoplacenta was described ultrastructur-

ally by Blackburn et al. (2002) in the garter snakes *Thamnophis ordinoides* and *T. sirtalis*, and might allow transfer of small quantities of organic and inorganic nutrients. In some species, this also probably relates to differences in oxygen affinity between fetal and maternal blood (Blackburn, 1993a). Maternal-fetal transfer of labeled ions or amino acids has been demonstrated in Squamata (Hoffman, 1970; Yaron, 1977). Quantitative analysis in *Thamnophis ordinoides* and *Virginia striatula* showed that placental transfer of inorganic nutrients involves more than simple permeability (Stewart, 1989, 1992; Stewart et al., 1990). Also in *V. striatula*, a predominantly lecithotrophic viviparous snake, ultrastructural characteristics indicate that the allantoic placenta is a place for histotrophic nutrient supply (Stewart and Brasch, 2003).

Omphaloplacental functions. In *Liolaemus elongatus*, the wet mass of the embryo increases nearly twice the decrease of the vitelline mass during the same period (Table 2). This strongly suggests that there is a nutrient exchange, in the addition to the vitelline supply. However, the embryo's increment in dry weight is smaller than the reduction of vitelline mass. Although we deem evidences not enough to ascertain nutrient exchanges other than the vitelline, we find that the comparison of total, wet and dry weights shows the importance of water exchange during development, used, for example, for conversion from yolk (lipoprotein) to cytoplasm.

Transfer of amino acids or proteins to the embryo through the omphaloplacenta was found by Hoffman (1970) in *Thamnophis sirtalis*, and histotrophic exchange through the omphalallantoic placenta was also found in *Virginia striatula* (Stewart and Brasch, 2003). We also observe that the increase in embryo's dry weight is smaller than the decrease of yolk's dry weight. Although the dry weight data do not allow the inference of organic transport, possible secretion toward the bilaminar omphalopleure could be inferred, from the increasingly high vacuolization and the displacement of the nucleus toward the basal region of the modified cells in the uterine epithelia of *L. elongatus*. Stewart (1989) observed that, in a mainly lecithotrophic species, placental transfer of organic compounds, if present, is not comparable to yolk metabolism. The transfer of inorganic compounds, instead, is a general characteristic that occurs in a facultative way, at least for calcium, sodium, and water. *Liolaemus elongatus* is, therefore, a mainly lecithotrophic lizard with placental structures adapted to oxygen, water and perhaps inorganic uptake.

ACKNOWLEDGMENTS

We are grateful to Prof. David Lemus for help and advice in the identification and understanding

of placental structures and Prof. James Stewart and Prof. John Krenz for their valuable reading of the draft.

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