Effects of luteectomy in early pregnancy on the maintenance of gestation and plasma progesterone concentrations in the viviparous temperate lizard Barisia imbricata imbricata

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Abstract

Background: Several studies have shown that the corpus luteum is the principal source of progesterone during the gravidity period in reptiles; however, its participation in the maintenance of gestation in the viviparous squamata is in dispute. The effects of ovariectomy or luteectomy vary according to the species and the time at which the procedure is performed. In this paper, we describe the effects of luteectomy during early pregnancy on the maintenance of gestation and progesterone concentrations in the temperate Mexican viviparous lizard Barisia imbricata imbricata.

Methods: Twenty-four lizards were subjected to three different treatments: luteectomy, sham luteectomy or non-surgical treatment, and blood samples were obtained before and after surgical treatment at different stages of gestation to determine the effects of luteectomy on the maintenance of gestation and progesterone concentrations.

Results: Spontaneous abortion was not observed in any of the females. However, luteectomy provoked abnormal parturition and a significant reduction in the number of young born alive. Parturition was normal in untreated females as well as those submitted to sham luteectomy. The surgical treatment also caused a significant reduction in progesterone concentrations in luteectomised females during early and middle gestation. However, no significant differences in hormone concentrations were observed among the three groups during late gestation or immediately post-parturition.

Conclusions: Our observations indicate that the presence of the corpus luteum is not necessary for the maintenance of gestation, but that it does participate in parturition control. Moreover, the corpus luteum of the viviparous lizard B. i. imbricata produces progesterone, at least during the first half of pregnancy, and that an extra-ovarian source of progesterone must maintain gestation in the absence of luteal tissue.
Luteectomy effect on the maintenance of pregnancy

The week after ultrasound scanning, the lizards in the luteectomy and sham luteectomy groups were anaesthetised with ether and a ventrolateral incision was performed. In the females submitted to luteectomy, the ovaries were exposed, all CL were surgically removed from each ovary and the total number of CL was registered. One CL of each female was fixed in 10% buffered formalin and processed for routine histology. Lizards submitted to sham luteectomy were treated identically, but the CL were not removed and only the numbers of CL were registered. When the surgical treatments were completed, the lizards were sutured and each female was deposited in an individual terrarium in our laboratory with free access to water and food (mealworms of Tenebrio, wax worms of Galleria mellonella, domestic crickets of Achaeta spp. and grasshoppers) for three days following surgery to allow for their recovery. On the day of the surgery, each lizard received an injection of 5000 U. I. of penicillin G and on the following two days, they received 500 μg streptomycin sulfate (I. M.). The group of intact lizards did not receive surgical treatment, but were given the antibiotics. After the recuperation period, all females were housed in individual terrariums (30 × 50 × 30 cm) and kept throughout the pregnancy period in the greenhouse of the UMF-FES Iztacala UNAM (19° 36' N, 98.5° 11’ W and 2240 m altitude). The lizards had unrestricted access to water and food and were maintained at a temperature and natural photoperiod throughout the experiment. The terrariums were scrutinised daily from the day of surgery until the time of parturition to detect early embryo expulsion or non-viable eggs (see definitions below). The dates of abortions, expulsion of non-viable eggs and parturition as well as the number of birth products (live young, stillborn embryos or nonviable eggs) were registered. We also recorded whether parturition was normal or abnormal. Each lizard was dissected three weeks after the birth of the young. In luteectomised females, we verified that the CL was missing in the ovary and that the uterus was devoid of embryos or nonviable eggs. In the
sham-luteectomised lizards, the uteri were reviewed in the same way. In intact lizards, the number of CL was recorded in addition to the state of the uterus. Finally, because oophagy has been reported to occur in this species [22], the stomachs of all lizards were also examined to determine whether the females ate the embryos or nonviable eggs.

We defined criteria to assess the effects of deluteinisation on the maintenance of gestation in B. i. imbricata according to those proposed by Panigel [13] for Lacerta vivipara and Callard et al [15], for Sceloporus cyanogenys: Abortion was diagnosed if A) the embryo or foetus (alive or dead) was expelled after surgical treatment (luteectomy or sham luteectomy) but before reaching stage 40 of development or B) if the expulsion of the embryo or foetus occurred in intact females (maintained in the same environmental conditions as the females submitted to surgery) during any period of development before stage 40. Normal parturition was diagnosed if the offspring had completed embryonic development (e. g., reached stage 40) and were expelled alive with the physiological capacity for life within 48 h from the first expulsion of young. Abnormal parturition was diagnosed if one of several scenarios were observed: A) premature parturition: if the young was expelled after reaching stage 40 of development, but at the time of expulsion, the foetus presented yolk sac residue (premature young) B) dissociate parturition: if all young completed embryonic development but were expelled over a period of more than 48 hours; C) delayed parturition: if the young completed embryonic development but were expelled dead; or D) if at the surgical inspection or autopsy performed 21 days after the first young was born embryos (alive or dead) were found within the uterus.

Corpus luteum histology
Fixed corpora lutea were washed in running water, dehydrated in an alcohol gradient, cleared in xylene and embedded in paraplast. Histological sections (7 μm) were cut and stained using Harri’s haematoxylin and eosin [23]. The sections were examined by microscopy to determine the stage of development according to Martínez-Torres et al., [20].

Effects of luteectomy on progesterone concentrations
About 2-3 hours before surgery (luteectomy or sham luteectomy), a blood sample of 200 ± 10 μl was obtained from each female by intracardiac puncture with a heparinised syringe [20]. Blood samples were also obtained at 24 hours as well as 8 (early gestation), 16 (middle gestation) and 24 weeks (late gestation) after surgical treatment (luteectomy or sham luteectomy) and one day after parturition (immediate postpartum). Intact females were bled to the same volumes and at the same times as lizards in other treatment groups. The blood was centrifuged immediately after collection, and the plasma was decanted and frozen at -40°C until the P₄ radioimmunoassay was performed, according to the methods of Martínez-Torres et al. [20]. All aliquots were obtained between 9.00 and 12.00 hours.

Radioimmunoassay
Plasma progesterone concentrations were quantified using a commercial kit (Coat-A-Count Progesterone, Diagnostic Products Corporation, Ca 90045). The assay was performed in duplicate using 50 μl of plasma without prior dilution or extraction. ¹²⁵I-labelled progesterone was supplied as the reactive tracer. The antiserum was specific for progesterone. Steroids showing cross-reactivity (relative to progesterone, 100%) were androstenediol (non-detectable), corticosterone (0.9%), cortisol (0.03%), 11-deoxycorticosterone (2.2%), 20α-dihydroprogesterone (0.2%), estradiol (non-detectable), 17α-hydroxyprogesterone (3.4%), 5β-pregnan-3α-ol-20-one (0.05%), 5α-pregnan-3,20-dione (3.2%), pregnenolone (0.1%), and testosterone (0.1%). Inter- and intra-assay coefficients of variation were 7.9 and 7.6%, respectively. The sensitivity of the assay was 0.02 ng/ml. The values were obtained using GAMBYT software.

Statistics
To determine differences in litter size and number of offspring born alive as well as to compare the number of CL among the treatment groups, we used a one-way analysis of variance (ANOVA). A two-way ANOVA for repeated measures was used to determine significant changes in P₄ concentrations. Post hoc tests were carried out using the Tukey method to determine differences; the significance level was set at p < 0.05 [24]. All statistical analyses were performed with SigmaStat software (version 3.5 for Windows).

Results
Corpora lutea obtained from luteectomised lizards showed, upon histological examination, according to Martínez-Torres et al. [20], histologic characteristics of active immature glands, except for two CL that resembled mature glands. These results agree with Martínez-Torres et al. [20]. Microscopic analyses also showed that the CL were in several stages of luteal development. The CL of one lizard were in stage I, whereas the CL of four lizards were in stage II, two were in stage III, and two were in stage IV. Corpora lutea were constituted by the luteal cell mass surrounded by the theca interna and the theca externa. In the luteal cell mass, we observed some cells with light nuclei, with one, two or three nucleoli and acidophilic

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cytoplasm as well as others with condensed chromatin. These characteristics, according to Martínez-Torres et al. [20] and Saidapur [25], indicate that the luteal tissues were actively synthesizing steroids. A cavity was present in the luteal cell mass in all extirpated CL, except in the CL that were in stage IV. No CL showed any degenerative characteristics (luteolysis). We did not observe significant differences in CL number among the three treatment groups [luteectomy: 14.1 ± 1.6, sham luteectomy: 15.5 ± 2.6, intact lizards: 14.5 ± 2.3; F(2, 21) = 8.73, p > 0.41].

Effects of luteectomy on the maintenance of gestation

We observed no abortion in any females; however, all luteectomised females showed abnormal parturition (Table 1). Significant differences in clutch size were not observed among luteectomised, sham-luteectomised and intact lizards [luteectomy: 11.8 ± 2.7, sham-luteectomy: 14.1 ± 3.0, intact: 13.8 ± 2.5; F(2, 21) = 1.63, p > 0.219, Table 1], although a statistically significant reduction was observed in the number of young born alive from luteectomised lizards [luteectomy: 9.8 ± 2.3, sham-luteectomy: 13.1 ± 2.6, intact: 13.6 ± 2.4; F(2, 21) = 1.63, p < 0.05].

Delivery of offspring from luteectomised females occurred 26-30 weeks after surgical treatment and lasted for 5-19 days for each female. The newborns were fully developed (stage 40 according to Deffaure and Hubert [26]). However, some stillborn and live-born young were surrounded by extraembryonic membranes and yolk sacs containing vitelus (Table 1). All premature young that were born alive died the same day. We also observed that luteectomised females expelled non-viable eggs during parturition. Parturition in the sham-luteectomised lizards initiated 27 weeks after surgery in three lizards, 28 weeks in two lizards and 30 weeks in two lizards. Five sham-luteectomised lizards showed normal parturition while the remaining females exhibited abnormal parturition. Normal parturition was observed in all but one intact pregnant lizard. Parturition in the intact lizards occurred 27 weeks after the other lizards were subjected to surgery in two lizards, 29 weeks in three lizards, 30 weeks in one lizard and 31 weeks in two lizards. When parturition was normal in the intact and the sham-luteectomised lizards, all offspring were expelled in just one day.

All females were submitted to laparotomy 21 days after the expulsion of the first offspring in order to examine the ovary, uterus and stomach. Only one luteectomised female had retained young in her uterus. We observed that one deluteinised lizard ate one premature embryo and another ate one nonviable egg. The uteri of sham luteectomised and intact lizards

| Table 1 Effects of luteectomy on several parameters of the gestation period of the viviparous lizard Barisia imbricata imbricata |
| --- |
| Treatment | Abnormal parturition | Mean of corpora lutea | Total number of birth products | Mean of viable young born | Mean of stillborn and premature young | Mean of non-viable eggs |
| Luteectomy (n = 9) | 9 | 141 ± 16 (n = 127) | 118.2 ± 27 (n = 107) | 141.1 ± 3.0 (n = 108) | 0.8 ± 0.3 (n = 10 9.34%) | 1.2 ± 1.4 (n = 6 5.6%) |
| Sham luteectomy (n = 7) | 2 | 15.5 ± 2.6 (n = 128) | 141.1 ± 3.0 (n = 99) | 13.1 ± 2.6 (n = 92) | 0.3 ± 0.4 (n = 4 3.08%) | 0.6 ± 1.2 (n = 4 4.04%) |
| None (n = 8) | 1 | 145 ± 23 (n = 116) | 138 ± 25 (n = 111) | 13.4 ± 0.4 (n = 109 98.1%) | 0.09 ± 0.03 (n = 1 0.9%) | 0.09 ± 0.03 (n = 1 0.9%) |

* indicates significant minor with respect to b and c at p < 0.05.

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were devoid of embryos. However, we detected a nonviable egg in the stomach of one sham-luteectomised lizard and one fully developed embryo in another sham luteectomised lizard. Finally, there was a nonviable egg in the stomach of one intact female, and one young still-born was observed in another.

Effects of luteectomy on progesterone concentrations

The patterns of P₄ concentrations in luteectomised, sham-luteectomised and intact females are shown in Figure 1. We found that luteectomy provoked a significant reduction in plasma P₄ concentrations when values in the early and middle stages of gestation were compared in sham-luteectomised [F(2, 5) = 15.2, p < 0.001] and intact lizards [F(2, 5) = 19.1, p < 0.001] (Fig. 1, Table 2). We did not observe any significant differences between the intact control and the sham-luteectomised lizards throughout the stages of gestation [F(2, 5) = 4.41, p = 0.38] (Fig. 1, Table 2).

Progesterone concentrations prior to surgery were similar in all three groups [luteectomised: 1.22 ± 0.14 ng/ml, sham-luteectomised 1.01 (0.16 ng/ml, intact

![Figure 1](http://www.rbej.com/content/8/1/19)

Figure 1 Effect of luteectomy on the progesterone plasma concentration in viviparous lizard Barisia imbricate. Patterns of progesterone plasma concentrations in luteectomised, sham luteectomised and intact pregnant females of the viviparous lizard Barisia imbricata imbricata. BS: before surgery, W-AS: weeks after surgery, I-PP: immediately post-partum. ² is significantly higher with respect to remaining times of gestation of luteectomised lizards group at p < 0.01; ² is significantly higher with respect to remaining times of gestation of sham luteectomised lizards group at p < 0.01; ² is significantly higher with respect to remaining times of gestation of intact lizard group at p < 0.01; ² is significantly less with respect to a and d at p < 0.01; ² is significantly less with respect to b and e at p < 0.01; ² is significantly less with respect to c at p < 0.01.

| Treatment                  | before surgery | 24 h after surgery | 8 weeks after surgery | 16 weeks after surgery | 24 weeks after surgery | Immediate postpartum |
|----------------------------|----------------|-------------------|-----------------------|------------------------|------------------------|----------------------|
| Luteectomised              | 1.22 ± 0.14 (n = 9) | 0.48 ± 0.15 (n = 9) | 0.55 ± 0.14 (n = 8) | 0.76 ± 0.15 (n = 9) | 0.46 ± 0.16 (n = 7) | 0.47 ± 0.16 (n = 7) |
| Sham luteectomised         | 1.01 ± 0.16 (n = 7) | 1.01 ± 0.16 (n = 7) | 1.85 ± 0.16* (n = 7) | 1.12 ± 0.18 (n = 6) | 0.64 ± 0.19 (n = 5) | 0.27 ± 0.17 (n = 7) |
| Intact control             | 1.13 ± 0.15 (n = 8) | 1.04 ± 0.15* (n = 8) | 2.13 ± 0.15** (n = 8) | 1.33 ± 0.16 (n = 7) | 0.67 ± 0.19 (n = 5) | 0.38 ± 0.16 (n = 8) |

1 values are in ng/ml.

n = number of blood samples used.

² ** = Peak of P₄ levels; the means was significantly higher at p < 0.01 with respect to remaining times of pregnancy examined (whiting to the same group).

² is significantly less with respect to a and d at p < 0.01.

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² is significantly less with respect to * at p < 0.01.
females 1.13 (0.15 ng/ml) (Table 2). In luteectomised lizards, P₄ concentrations were significantly diminished within 24 hours following surgery. However, in sham-luteectomised and intact control lizards, P₄ concentrations were similar to those recorded before surgery and significantly higher than the corresponding values from the luteectomised females [luteectomy: 0.48 ± 0.15 ng/ml, sham-luteectomy: 1.01 ± 0.16 ng/ml, intact lizards 1.04 ± 0.15 ng/ml; luteectomy vs. sham luteectomy: p < 0.047, luteectomy vs. control intact p < 0.031] (Fig. 1, Table 2). In luteectomised lizards, low concentrations of P₄ were maintained at eight weeks after surgery (0.55 ± 0.16 ng/ml, sham-luteectomy: 1.01 ± 0.16 ng/ml, intact lizards 2.13 ± 0.15 ng/ml). These values were significantly different than those detected in luteectomised lizards at the same time point [luteectomy vs. sham luteectomy: p < 0.001, luteectomy vs. intact control: p < 0.001]. However, at mid-gestation, P₄ concentrations had diminished in the sham-luteectomised and intact lizards. In the luteectomised lizards, the P₄ concentrations 16 weeks post-luteectomy were also low [0.76 ± 0.15 ng/ml, and we determined that they were not significantly different from those of sham-luteectomised females [1.12 ± 0.18, p = 0.2], but were indeed different than the concentrations observed in intact lizards [1.33 ± 0.16 ng/ml, p = 0.037]. In late gestation and immediately post-parturition, P₄ concentrations were further diminished in all lizards, and significant differences were not observed among the three groups (Fig. 1, Table 2).

**Discussion**

Several researchers have suggested that the CL plays a central role in maintaining the gestation and the evolution of reptilian viviparity, given its capacity for P₄ production [27-31]. Moreover, several authors have reported evidence suggesting that this gland is the major source of P₄ during pregnancy in many species of viviparous lizards and snakes [1,20,32,33]. However, a number of experimental studies in several species of viviparous squamata have shown that while the deluteinisation causes significantly diminished P₄ concentrations, in no species thus far investigated does this treatment completely eliminate P₄ in the plasm [32,34]. In addition, the deluteinisation produces varying responses depending on the species and time of gestation at which the surgery is performed [12,30]. We removed the CL during early pregnancy of *B. i. imbricata* and we observed a significant reduction in P₄ after luteectomy, but hormone concentrations never fell below the limit of detection. Moreover, we observed no abortions; however, all luteectomised lizards showed abnormal parturition. We also observed that histological examination of the extirpated luteal tissues exhibited active endocrine glands midway through the maturation process, in accordance with results from Martínez-Torres et al. [20] and Saidapur [25]. These observations indirectly show that the CL continues to participate in the production of P₄ in *B. i. imbricata*. We think that these results also suggest that the primary importance of this gland has been overshadowed due to the emergence of extraluteal structures (i.e., glandula adrenal), during the evolution of viviparity, capable of producing this steroid to maintain gestation.

Luteectomy in the lizard *Tiliqua rugosa* [31,32] and in the garter snake *Thamnophis elegans* [33,34] produced results similar to those observed in our study. Such observations led at several researchers [31-34] at the same conclusion regarding an extra-ovarian supply of P₄, and they suggest the adrenal gland (AG) as a candidate source. Highfill and Mead [33] found that adenlectomy reduces P₄ concentrations to a non-detectable state in non-pregnant snakes. Likewise, in *Lacerta vivipara*, Dauphin-Villemant and Xavier [35] and Dauphin-Villemant et al., [36] observed in *vitro* and in *vivo* an increase in the adrenal activity and production of P₄ during gestation. These observations support the idea that the AG may participate in the maintenance of gestation in viviparous lizards. This may hold true for *B. i. imbricata*, though no studies have yet examined AG activity during gestation.

Other authors have suggested that atretic vitellogenic follicles (AVFs) may be an important ovarian source of P₄ [37,38]. Villagran-Santa Cruz [37] observed an increase in the number and volume of AVFs in the second half of gestation in *S. mucronatus*, coinciding with the degeneration of the CL. Moreover, Guillette et al. [38] showed a positive correlation between plasma P₄ concentrations and AVFs number as well as the presence of placenta chorioallantois (PCA) in the second part of gestation in *S. jarrovi*. In our study, we observed that three females submitted to luteectomy and two females submitted to sham-luteectomy did not contain any AVFs. This observation rejects the possibility that AVFs participate in the maintenance of gestation in this lizard species. Several studies have shown that the PCA of some lizard species (*Chalcides chalcides*, [39]; *Sceloporus jarrovi*, [40]) have endocrine capacity. Guarino et al. [39] and Painter and Moore [40] observed that the PCA is an important source of P₄ in late pregnancy and that it coincides with CL regression in these species. It is unknown whether the PCA of *B. i. imbricata* embryos have the capacity for P₄ production. However, Martinez-
Martinez-Torres et al. [21] recently found that the omphalo-placental residue of new born of B. i. imbricata, stained positive for Δ5-4 3β-HSD activity. This observation suggests that this structure is capable of metabolising or producing P4. However, we do not know the stage of gestation and/or embryonic development when omphalo-placenta acquire this ability. Similarly, we do not know which embryonic stage corresponds to the time of deluteinisation. We expected the AG to be the secondary source of P4 in B. i. imbricata (as claimed by other researchers for other lizard species), at least during early gestation, because we observed that the embryos of the lizards whose CL were in initial stages I and II were in the cicatriclea stage (segmentation or gastrulation, according to Defaure and Hubert [26]). Therefore, these young embryos have neither a placenta nor extra-embryonic membranes. However, we do not discard the possibility that the placenta may participate in the production or the metabolism of this hormone at some time during gestation.

Parturition has been widely studied in several mammalian species (for review, see [41,42]); however, few papers have addressed this process in reptiles (for review, see [13,34,12,43,44]). Nevertheless, several authors agree that the stimulation of uterine contraction associated with parturition involves a complex interaction between several hormones steroids (P4 and estradiol 17β), arginine-vasotocine (AVT) and prostaglandins [45,46]. Further evidence suggests that the CL may influence parturition in squamata since luteectomy provokes abnormal parturition in some species (Lacerta vivipara, [13], S. cyanogenys [15], T. sirtalis, [32]). In B. i. imbricata we found a similar situation. The expulsion of premature, stillborn young and dissociated parturition by luteectomised alligator lizards suggests that hormones from the CL (P4, estradiol 17β or other hormones) participate in the control of parturition. In the absence of CL, the uterine contraction may be perturbed and therefore, the delivery of all puppets occurred over a prolonged period of time (5-19 day). Moreover, these results show the importance of the CL in the modulation of contractile activity of the myometrium of B. i. imbricata.

According to Jones and Guillette [2], luteal steroids modulate the response of oviducal muscles to AVT, especially in late pregnancy. Moreover, Ferguson and Bradshaw [32] observed that the circulating plasma concentrations of AVT increase in pregnant Tiliqua rugosa lizards as the time of parturition approaches and that plasma P4 concentrations decline and CL degeneration proceed concomitant with this AVT elevation. In a previous paper, Martínez-Torres et al. [20] showed that in B. i. imbricata, plasma P4 concentrations are higher in early pregnancy and then drop gradually throughout gestation and that there is a positive correlation between P4 concentrations and the histological activity of the CL. In this study, we observed a similar situation in sham-luteectomised and intact pregnant lizards. In contrast, we saw a significant reduction in P4 concentrations after surgery in luteectomised lizards, and these low levels were maintained for the rest of gestation. However, we found that the P4 values during late gestation of luteectomised lizards were not significantly different than the concentrations determined in sham-luteectomised and intact lizards. These data suggest two conclusions: A) that the quiescent uterus necessary for embryo retention may be maintained by low levels of P4 and B) that the reduction of P4 in the first half of gestation and/or the absence of other hormones from the CL (e.g., estradiol 17β) might alter uterine sensitivity to neurohypoophyseal hormones (e.g., AVT), which consequently might cause abnormal parturition. Further studies are needed to determine how other progesterone, lutal hormone and arginine vasotocine levels fluctuate throughout gestation, in order to define the role of the CL in the maintenance of gestation.

Guillette and Casas-Andrew [19] notified that gestation in B. i. imbricata lasts for approximately six months. However, in our study, we observed that gestation could last for as long as seven months, since parturition occurred in all females between 27 and 31 weeks after that the presence of oviducal eggs was confirmed by ultrasound scanning. Precise determination of the duration of gestation is very important, as it would inform the study of the hormonal changes associated with the maintenance of gestation as well as the mechanism that controls parturition.

Conclusions
In accordance with all observations described above, we arrived at the following conclusions: A) the CL of B. i. imbricata is capable of P4 production, B) a secondary extra-ovarian source of P4 capable of maintaining gestation must exist, C) the CL participates in the modulation of parturition, and D) given the data obtained in our study and that reported by other authors, we presume that the level of CL participation in the maintenance of gestation in viviparous lizards differs across species.

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Authors' contributions

MMT and LMF conceived, designed and coordinated the study. MMT and MEHC collected all lizards and blood samples, and performed surgical procedures. JALD carried out all tissue processing, histological analysis and ultrasound scanning and participated in the analysis and discussion of the results. GOL and MCL carried out the radioimmunoassays and critical revisions of the manuscript. All co-authors provided inputs during final manuscript preparation. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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References

1. Xavier F: Functional morphology and regulation in the corpus luteum. Hormones and Reproduction in Fishes, Amphibians and Reptiles New York and London: Plenum PressNorris DO, Jones RE 1987, 523-562.
2. Jones RE, Guilleltte JL Jr: Hormonal control of oviposition and parturition in lizards. Herpetologica 1982, 38:80-93.
3. Moll EO, Legler JM: The life story of a neotropical slider turtle, Pseudemys scripta (Schoepf) in Panama. Bull Los Angeles Mus Nat Hist Sci 1971, 11:1-102.
4. Cyrus RV, Mahmoud IY, Klicka J: Functional morphology and regulation in the corpus luteum. J Morphol 1985, 184:85-98.
5. Barisia imbricata imbricata during pregnancy of the viviparous temperate lizard Barisia imbricata imbricata (Reptilia, Anguidae) después del nacimiento. Bol Soc Hist Mex 2006, 51:401-406.
6. Defaure JP, Hubert J: Table de développement du lézard vivipare Lacerta (Zootoca) vivipara Jacquin. Archiv Anat Mircrosc Morphol Exp 1961, 20:29-52.
7. Medawar PB: Some immunological and endocrinological problems raised by the evolution of viability in vertebrates. Symp Soc Exp Biol 1953, 7:320-338.
8. Shine R, Guilleltte JL Jr: The evolution of viability in reptiles: a physiological model and its ecological consequences. J Theor Biol 1988, 132:43-50.
9. Callard IP, Fileti LA, Perez LE, Sorbera LA, Giannouku G, Kostlerman LL, Tsang P, McCracken JA: Role of the corpus luteum and progesterone in the evolution of vertebrate viviparity. Amer Zool 1992, 32:264-275.
10. Yaron Z: Reptilian placentation and gestation: structure, function and endocrine control. Biology of the Reptilia New York: John Wiley and SonsGans C, Billet F 1985, 15:527-603.
11. Bourne AR: Progesterone like activity in the plasma of the viviparous skink, Trachydactylus rugosus (stump-tailed lizard). Proceeding of the Melbourne Herpetological Symposium: 19-21 May 1980, Melbourne Chris B Banks, Angus A Martin 1981, 17:24-27.
12. Freeman B, Badhshah SD: Plasma arginine vasotocin, progesterone and luteal development during pregnancy in the viviparous lizard Tiliqua rugosa. Gen Comp Endocrinol 1991, 82:140-151.
13. Higlhill DR, Mead RA: Sources and levels of progesterone during pregnancy in the garter snake, Thamnophis elegans. Gen Comp Endocrinol 1975, 27:389-400.
14. Higlhill DR, Mead RA: Function of corpora lutea of pregnancy in the viviparous garter snake, Thamnophis elegans. Gen Comp Endocrinol 1975, 27:401-407.
15. Dauphin-Villemant C, Xavier F: In vitro steroid biosynthesis by adrenal gland of the female Lacerta vivipara Jacquin: The metabolism of exogenous precursors. Gen Comp Endocrinol 1985, 58:1-9.
16. Dauphin-Villemant C, Leboulenger F, Vauduy H: Adrenal activity in the female lizard Lacerta vivipara Jacquin during artificial hibernation. Gen Comp Endocrinol 1990, 79:201-214.
17. Villagrán-Santa Cruz M: Desarrollo embrionario placentación y su relación con el cuerpo lúteo y la atresia follicular en Sceloporus muconatus and Sceloporus graciosus, (Sauria, Iguanidae). Ph D Thesis Facultad de Ciencias, UNAM 1989.
Chalcides chalcides (Reptilia, Squamata). Gen Comp Endocrinol 1998, 111:261-270.

40. Painter DL, Moore MC. Steroid hormone metabolism by the chorioallantoic placenta of the mountain spiny lizard Sceloporus jarrovi as a possible mechanism for buffering maternal-fetal hormone exchange. Physiol Biochem ZooL 2005, 78:364-372.

41. Challis JRG, Olson DM. Parturition. The Physiology of Reproduction Ltd, New York: Raben PressKnobil F, Neil J 1988, 2177-2216.

42. Olson DM, Ammann C. Role of the prostaglandins in labour and prostaglandin receptor inhibitors in the prevention of preterm labour. Front Biosci 2007, 12:1529-34, Review.

43. Guillette JL Jr, Dubois DH, Cree A. Prostaglandins, oviductal function, and parturient behaviour in nonmammalian vertebrates. Am J Physiol-Regulatory Integrative and Comp Physiol 1991, 260:R854-R861.

44. Atkins N, Jones SM, Guillette LJ Jr. Timing of parturition in two species of viviparous lizard: influences of β-adrenergic stimulation and temperature upon uterine responses to arginine vasotocin (AVT). J Comp Physiol [B] 2006, 176:783-792.

45. Guillette JL Jr, Gross TS, Matter JH, Palmer BD. Arginine vasotocin-induced prostaglandin synthesis in vitro by the reproductive tract of the viviparous lizard Sceloporus jarrovi. Prostaglandins 1990, 37:39-51.

46. Cree A, Guillette JL Jr. Effect of β-adrenergic stimulation on uterine contraction in response to arginine vasotocin and prostaglandin F2α in the gecko Haplodactylus maculatus. Biol Reprod 1991, 44:599-610.

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