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Does the conceptus of the viviparous lizard *Barisia imbricata imbricata* participates in the regulation of progesterone production and the control of luteolysis?

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ABSTRACT

It is generally accepted that progesterone is necessary to maintain gestation; however, the mechanisms that control the production of this steroid remain unknown. The corpus luteum has been assigned a central role in the maintenance of gestation based on its capacity to produce progesterone. A pseudopregnancy model was performed in a viviparous lizard, *Barisia imbricata imbricata*, to determine whether the absence of embryos would affect the pattern of progesterone production or the corpus luteum histology. Blood samples were obtained prior to ovulation and at 8, 16, and 24 weeks after ovulation (pseudopregnant and pregnant lizards), as well as one day after parturition (pregnant lizards) or 32 weeks after ovulation (pseudopregnant lizards). The corpus luteum was surgically removed one day after blood samples were obtained. Blood aliquots from nongravid females were obtained at similar timepoints. We found a significant reduction in plasma progesterone concentrations at 24 and 32 weeks post-ovulation in pseudopregnant lizards compared with those observed at similar times in intact pregnant lizards, whereas the progesterone levels in non-gestant lizards remained significantly lower than in either pseudopregnant or pregnant lizards. Moreover, we observed that the histological appearance of the corpus luteum from pseudogestational females (obtained 24 and 32 weeks post-ovulation) differed from the corpora lutea from lizards in late gestation and intact parturient lizards. These observations suggest that the conceptus participates in the regulation of progesterone production in late gestation and also in luteolysis control.

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1. Introduction

Viviparity is a reproductive strategy utilised by many species in most major clades of vertebrates, (Angelini and Ghiara, 1984; Rothchild, 2003). Several phylogenetic analyses indicate that this trait evolved independently more than 100 times within Squamata, a frequency greater than that of all other vertebrate clades (Blackburn, 1995; Wang and Evans, 2011). Paleontological and embryological evidence indicates that oviparity in reptiles represents a primitive reproductive strategy that led to viviparity (Blackburn, 1982; Ghiara et al., 1987). During the transition to viviparity, anatomical and physiological modifications affected the gestant females and *conceptuses*. These modifications included the following: (1) extension of the lifespan of the corpus luteum (CL), (2) enlargement of the secretion pattern of progesterone (P_4), (3) increased uterine vascularity, (4) reduction in the number, size, and activity levels of the uterine glands, (5) loss of calcareous shell, and (6) placental development (Blackburn, 1998; Callard et al., 1992; Guillette, 1993, 1995 Guillette and Jones, 1985; Wourms and Callard, 1992).

The need for P_4 to sustain gestation is well established; no species has yet been discovered that does not require the steroid for the maintenance of pregnancy (Guillette et al., 1985, 1989; Bourne et al., 1986; Xavier, 1987; Callard et al., 1992; Martínez-Torres et al., 2003). In mammals, several lines of evidence show that the P_4 is crucial in the maintenance of gestation (Graham and Clarke, 1997). The major physiological role of this hormone during pregnancy in this vertebrates, is the maintenance of pregnancy by the promotion of uterine growth and the suppression of myometrial contractility (Yaron, 1972, 1985; Graham and Clarke, 1997; Mulac-Jericevic and Conneely, 2004). In reptiles, P_4 has been detected in gravid females in all stages of pregnancy (for review, see Xavier, 1987) and, although the profiles of this hormone vary dramatically among viviparous squamate species (Jones and Baxter, 1991; Xavier, 1987), several studies agree that the P_4 may play a central role in embryo retention during gravidity and in the evolution of viviparity (Callard et al., 1992; Guillette, 1985; Shine and Guillette, 1988; Yaron, 1985; Martínez-Torres et al., 2010).

On the other hand, there are studies that show, that in the majority of viviparous lizards, the CL is a principal source of P_4 during gestation (for review see Xavier, 1987). This finding has led several authors to assign a central role in the maintenance of gestation and the evolution of viviparity to this gland. However, several lines of indirect experimental evidence suggest that viviparous squamata possess a secondary source of P_4. In *Lacerta vivipara*, Dauphin-Villemant and Xavier (1985) and Dauphin-Villemant et al. (1990) observed an *in vitro* increase in the adrenal activity and production of P_4 during gestation. In *Chalcides chalcides*, *Sceloporus jarrovi* and *Barisia imbricata*, it has been observed that the placenta is capable of producing (Guarino et al., 1998; Martínez-Torres et al., 2006a) and metabolising P_4 (Painter and Moore, 2005). Moreover, lutectomy or ovariectomy in early pregnancy does not completely eliminate the plasma P_4 in the viviparous lizards *Tiliqua rugosa* (Fergusson and Bradshaw, 1991) and *B. i. imbricata* (Martínez-Torres et al., 2010) or in viviparous snakes (*Thamnophis sirtalis*, Highfill and Mead, 1975).

Participation of the *conceptus* in the prevention of luteal tissue regression and in the regulation of P_4 production by CL has been widely documented in several mammalian species (Bazer and Roberts, 1983; Gordon and Net, 1988; Gordon et al., 2000; Zeleznick and Clifford, 2006). A similar phenomenon may occur in reptiles. Xavier et al. (1989) found indirect evidence that suggest that the *conceptus* of *L. vivipara* could participate in the regulation of P_4 production and CL activity.

B. i. imbricata is a Mexican viviparous temperate lizard, commonly known as the scorpion or alligator Popocatepetl lizard, which exhibits autumnal reproduction (Guillette and Casas-Andreu, 1987; Martínez-Torres et al., 2003). Oogenesis occurs during summer and fall, and ovulation occurs during November or early December (Martínez-Torres et al., 2006a). Mating takes place several weeks before ovulation occurs (Martínez-Torres, unpublished data). In this species, gestation occurs throughout the winter months and most of the spring (from late November or early December until late May or early June) (Martínez-Torres et al., 2003). The CL develops during the first third of pregnancy, and luteolysis occurs during the remaining months. Four sequential stages have been identified during luteal development and three stages during luteal regression (Martínez-Torres et al., 2003). It has been observed that there is a positive correlation between the P_4 plasma levels and the histological appearance and histochemical activity of Δ^{5-4}-isomerase-3β hydroxy steroid dehydrogenase in the luteal tissue (Martínez-Torres et al., 2003) throughout gestation. These observations suggest that the CL is the major source of P_4 during pregnancy in *B. i. imbricata*. However, in this lizard, it was recently observed that ablation of the CL in early pregnancy provoked a significant reduction in P_4 plasma levels during the first half of pregnancy without inducing abortion but resulting in abnormal parturition (Martínez-Torres et al., 2010).

Despite the importance that several researchers have assigned to P_4 in the maintenance of gestation and the evolution of reptilian viviparity, the mechanisms that modulate the production of this hormone (from corpus luteum or an extra-ovarian source) remain unknown. In this paper, a pseudopregnancy model was used to determine whether the absence of a *conceptus* modifies the plasma P_4 levels and the appearance of the CL in the viviparous temperate lizard *B. i. imbricata*.

2. Materials and methods

2.1. Animals

Adult females (113 ± 36 mm snout-vent length and 24.6 ± 5.7 g body weight) of *B. i. imbricata* were collected after the mating period but before ovulation (the last week of October and first week of November in 2007, 2008 and 2010) from Cuautitlán, México State (19° 37′N, 99° 11′W; 2253 m altitude). Females were toe-clipped for individual identification. On the day of capture, all lizards collected were transported to the laboratory and submitted for

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