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TECHNICAL REPORT

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Ultrasonographic and radiographic evaluation of gestation in golden lanceheads (*Bothrops insularis*) in ex situ breeding programs

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Abstract

The golden lancehead (*Bothrops insularis*) is a critically endangered snake endemic to Queimada Grande Island, southeastern Brazil. Captive breeding programs are vital for the conservation of this species. This study evaluates the gestation of two females golden lanceheads using radiography and ultrasonography. The first female was collected on the island while reproductively active (late vitellogenesis or early pregnancy) and kept in captivity. This female gave birth to five neonates after an 8-month gestation period, which is longer than estimates based on specimens preserved in museums. The second female copulated in captivity in July and probably ovulated in October. In this case, no embryonic development was detected, and the female deposited 14 undeveloped eggs approximately 6 months after ovulation.

KEYWORDS

captive breeding, diagnostic imaging, endangered species, reproduction

1 | INTRODUCTION

The golden lancehead pitviper, *Bothrops insularis*, is endemic to Queimada Grande Island, São Paulo state, southeastern Brazil (Amaral, 1921; Marques et al., 2002). This species exhibits many ecological and morphological peculiarities compared to its closest mainland relative, the common lancehead, *Bothrops jararaca* (Marques et al., 2002). For example, adult golden lanceheads feed primarily on migratory birds, whereas adult common lanceheads feed primarily on rodents (Marques et al., 2002).

Reproduction is seasonal. Mating occurs from autumn to winter, vitellogenesis from late summer to spring, ovulation/fertilization in early spring, and females are pregnant from mid-spring to mid-autumn (Marques et al., 2013). Gestation length is unknown, but the appearance of the first pregnant females in October and the first births in February (Marques et al., 2013) suggests that gestation lasts 4–5 months. Litter size averages seven offspring (Almeida-Santos & Salomão, 2002; Hoge et al., 1959). The reproductive frequency varies annually but seems lower than that in *B. jararaca*, probably reflecting a divergence in resource availability (Marques et al., 2013).

Golden lanceheads are classified as critically endangered in the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN, 2012). The most recent population estimates indicate some stability or a slight decline

(Abrahão et al., 2021; Martins et al., 2008). However, several intrinsic and extrinsic factors may increase the vulnerability to extinction of this insular pitviper. One of the greatest barriers to the successful ex situ reproduction of reptiles is the lack of knowledge of their biology and ecology, especially reproductive biology (Barros et al., 2020). Thus, understanding reproduction in captivity is crucial for attempts to reintroduce the species into the wild as a conservation strategy (Zacariotti & Guimarães, 2010).

Diagnostic imaging is a powerful tool for monitoring the reproductive activity of captive snakes (Canny, 1998; Funk, 2002; Garcia & Almeida-Santos, 2021; Gnudi et al., 2009; Silverman, 2006; Stahl, 2002). Ultrasonography is a safe and noninvasive diagnostic technique that can be used to monitor reproductive events such as gestation and embryonic development (Garcia & Almeida-Santos, 2021). Radiography is a popular imaging technique in reptiles widely used to detect eggs or reproductive disorders (Gumpenberger, 2017; Pees, 2010; Silverman, 2006).

In this study, we evaluate the gestation of two captive golden lancehead females using ultrasonography and radiography.

2 | MATERIALS AND METHODS

2.1 | General procedures

We evaluated two females golden lanceheads using ultrasonography and radiography. The snakes were safely immobilized in a transparent acrylic tube, where they remained during the examinations. For the ultrasonographic examinations, we applied a layer of conductive gel to the snake's skin and scanned the lateral and ventral regions of the snake's body. The gallbladder was used as a marker (Garcia & Almeida-Santos, 2021). The length and the width of at least one oviductal egg were measured. For the radiographic examinations, the laterolateral and dorsoventral regions of the caudal third of the snakes' bodies were examined. The liver and lungs were used as markers.

2.2 | Case 1

A golden lancehead female (870 mm body size and 440 g body mass) was found on Queimada Grande Island on September 15, 2019. Abdominal palpation revealed reproductive activity (either vitellogenesis or pregnancy), and the female was brought to the Laboratory of Ecology and Evolution of the Butantan Institute (São Paulo, southeastern Brazil). The snake (ID 271843) was kept individually in a plastic box (56 × 38 × 37 cm) and housed in a temperature- and humidity-controlled room. During the study period, room temperature averaged 24°C in spring (October–December), 24.8°C in summer (January–March), and 20.5°C in autumn (April–May). Ultrasounds were performed monthly between October 2019 and March 2020 (Figure 1a–f), and radiography was performed once in March 2020 (Figure 1h). All examinations were performed at the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo (Brazil). Ultrasounds were performed using portable devices (Titan, Sonosite, Bothell, Washington, United States of America and MyLab™30Gold VET, Esaote) with a linear transducer of 5–12 MHz. Radiography was taken in a laterolateral and a dorsoventral projection using a direct digital radiography system (DX-D 40C, AGFA Healthcare).

2.3 | Case 2

A female (ID 5775; 1022 mm body size and 550 g body mass) and a male (ID 5776; 933 mm body size and 150 g body mass) were collected in 2011 on Queimada Grande Island and taken to the Vital Brazil Institute (Niterói, southeastern Brazil). The snakes were kept individually in plastic boxes (70 × 40 × 50 cm) housed in a temperature- and humidity-controlled room. Between March and July 2015, the female and male were placed together in a plastic box (100 × 100 × 60 cm). Copulation was observed on July 14, 2015. To assess embryonic development, radiographic and ultrasonographic examinations were performed between October 29, 2015 and February 15, 2016 (Figure 2a–d). The ultrasonographic examination was conducted in the Veterinary Hospital of the Fluminense Federal University (Niterói, Brazil) using a portable ultrasound device (MyLab™30Gold VET, Esaote) with a linear transducer of 5–12 MHz. Radiography was performed in a dorsoventral projection at the PCA—Veterinary Diagnostic Center (Niterói, Brazil) using an indirect digital radiography system (CR 30X, AGFA Healthcare).

3 | RESULTS

3.1 | Case 1

On October 15, 2019, the ultrasound examination revealed rounded (1.77 × 1.33 cm), juxtaposed, heterogeneous, and hyperechoic yolk masses (without discernible embryos) with hypoechoic halos and internal contents (Figure 1a). On November 25, 2019, the yolk masses were more elongated (3.29 × 1.25 cm), heterogeneous and hypoechoic, and the halos were hyperechoic (Figure 1b). Embryonic discs (hyperechoic) became noticeable, but no embryo was seen (Figure 1b). On December 17, 2019, the yolk masses were still elongated (3.46 × 1.32 cm), heterogeneous and hyperechoic, and the halos and embryonic discs were still hyperechoic (Figure 1c). On January 20, 2020, the yolk masses kept their elongated (3.67 × 1.62 cm), heterogeneous, and hyperechoic appearance, but the halos and embryonic discs became hypoechoic (Figure 1d). On February 17, 2020, the eggs were still elongated (3.47 × 1.84 cm), heterogeneous, and hyperechoic (Figure 1e). Visible fetuses showed early bone formation, with a hyperechoic appearance, and substantial amounts of yolk were still noticed (Figure 1e). On March 12, 2020,

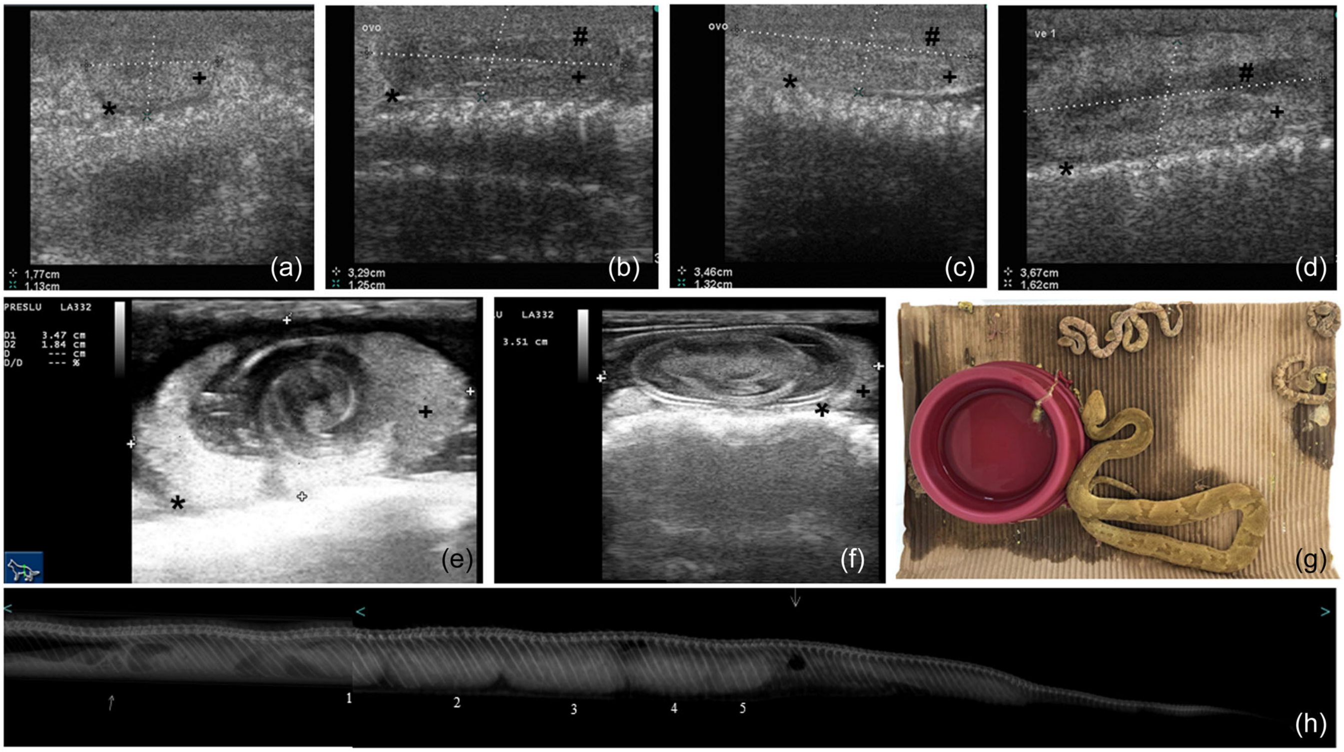

FIGURE 1 Gestation (a–f, h) and parturition (g) in a captive golden lancehead, *Bothrops insularis* (case 1). Ultrasound images (a–f): (a) A rounded (1.77 × 1.33 cm), heterogeneous, hyperechoic yolk mass, with hypoechoic halo and internal content (October 15, 2019). (b) An elongated (3.29 × 1.25 cm), heterogeneous, and hypoechoic yolk mass with hyperechoic halo and embryonic disc (November 25, 2019). (c) An elongated (3.46 × 1.32 cm), heterogeneous, and hyperechoic yolk mass with hyperechoic halo and embryonic disc (December 17, 2020). (d) An elongated (3.67 × 1.62 cm), heterogeneous, and hyperechoic yolk mass with hypoechoic halo and embryonic disc (January 20, 2020). (e) An elongated (3.47 × 1.84 cm), heterogeneous, and hyperechoic egg containing an embryo showing early bone formation with a hyperechoic appearance (February 17, 2020). (f) An elongated (3.51 cm) and heterogeneous egg with little yolk and a fetus showing fully formed bone structure (March 12, 2020). (g) A postparturient female with five neonates (May 18, 2020). (h) Radiography of the caudal third of the female body showing five well-delineated, radiopaque eggs and faint bone structures (March 12, 2020). Yolk mass (+). Halo (*). Embryonic disc (#). [Color figure can be viewed at wileyonlinelibrary.com]

the elongated eggs (3.51 cm) were heterogeneous, the fetuses had fully formed bone structures, and the eggs contained less yolk (Figure 1f). The radiographic examination conducted on the same day indicated five well-delineated, radiopaque eggs and faint fetal bone structures (Figure 1h). On May 18, 2020, the female gave birth to five neonates (Figure 1g) after an 8-month gestation period (October to May). Offspring size ranged from 245 to 280 mm, and offspring mass ranged from 7 to 9 g.

3.2 | Case 2

The radiographs performed on October 29, 2015, December 9, 2015, and February 15, 2016 showed 14 round, juxtaposed, radiopaque oviductal yolk masses with soft tissue radiopacity (Figure 2a–c). In late February, the ultrasonographic examination revealed heterogeneous and hypoechoic oviductal yolk masses (2.27 × 0.96 cm), with mixed echogenicity and hyperechoic linear structure, suggesting undeveloped embryos (Figure 2d). On April 4, 2016, the female deposited 14 undeveloped eggs (Figure 2e).

4 | DISCUSSION AND CONCLUSION

A previous study based on museum specimens collected in the wild found that the first pregnant female golden lancehead appear in October, and the first parturitions occur in February (Marques et al., 2013). Thus, it was believed that gestation lasts 4–5 months. Our findings do not support this inference because the gestation described in case 1 lasted about 8 months. A potential explanation for this difference is that lower temperatures in captivity may have prolonged the gestation of the captive female. However, the mean temperature in captivity was 1°C higher than the mean air temperature on Queimada Grande Island in spring (24.0 vs. 23.0°C, respectively) and similar in summer (24.8 vs. 24.6°C) and autumn (both 20.5°C; see Bovo et al., 2012 for air temperatures on Queimada Grande Island). Despite the similarity in air temperature, the physical restrictions imposed by captivity limit opportunities for thermoregulation, which may affect gestation length. Poor captive conditions may also restrict an animal's physiological requirements, thus affecting gestation length. Further studies are needed to determine the factors affecting gestation length in captive golden lanceheads.

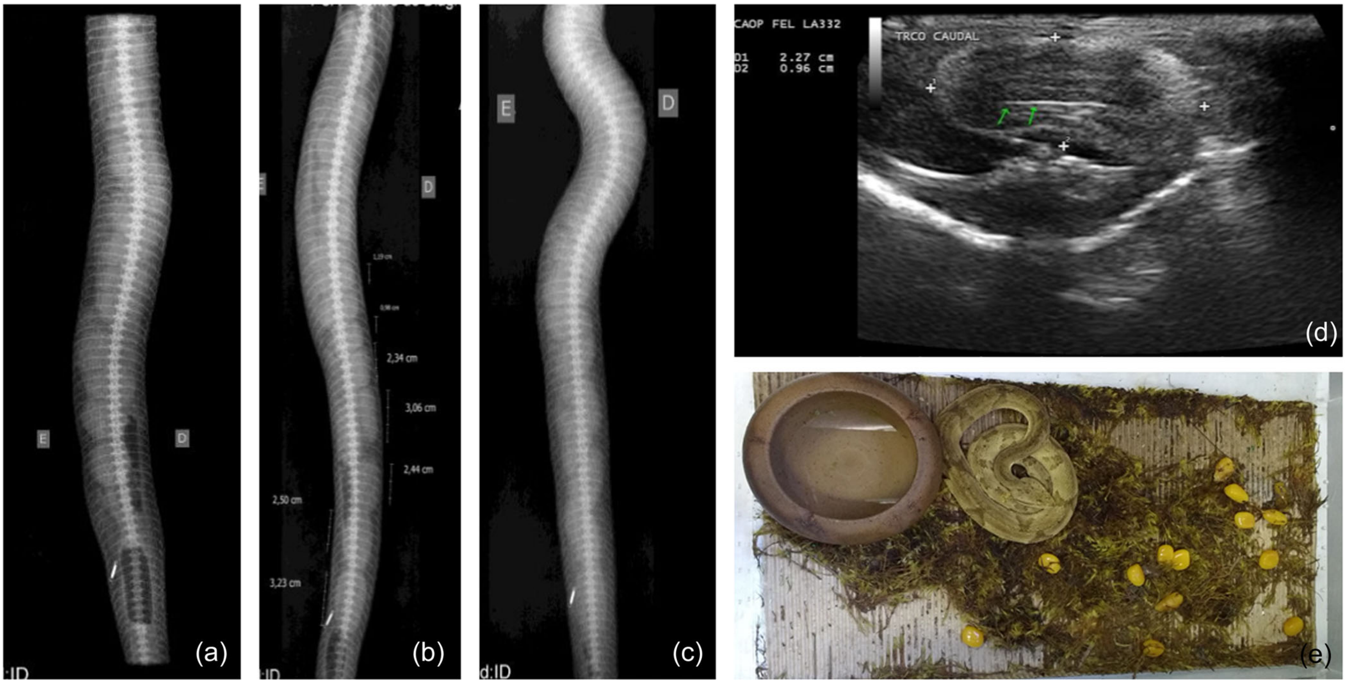

FIGURE 2 Oviductal yolk masses (a–d) and deposition of undeveloped eggs (e) in a captive golden lancehead, *Bothrops insularis* (case 2). (a–c) Radiographic images of the caudal third of the female's body showing 14 rounded, juxtaposed, radiopaque oviductal yolk masses with soft tissues radiopacity on October 29, 2015 (a), December 9, 2015 (b), and February 15, 2016 (c). (d) Ultrasound image of a heterogeneous and hypoechoic yolk mass (2.27 × 0.96 cm), with mixed echogenicity and hyperechoic linear structure, suggesting bone formation (February 15, 2016). (e) A postparturient female with 14 undeveloped eggs (April 4, 2016). [Color figure can be viewed at wileyonlinelibrary.com]

The high incidence of undeveloped eggs reported in case 2 is a common phenomenon in free-ranging and captive golden lanceheads, probably due to fertilization failure or embryonic death in early and mid-pregnancy (Hoge et al., 1959; Marques et al., 2013). It is unknown why this phenomenon occurs and whether the incidence of undeveloped eggs differs between free-ranging and captive females. Besides the tendency of golden lanceheads to produce large numbers of undeveloped eggs, radiographic examinations performed during gestation may also have negatively affected the embryonic development described in case 2. According to Garcia and Feliciano (2015), the deleterious effects of ionizing radiation are greatest early in embryonic development. Radiographic examination is most appropriate in late pregnancy, when all organs and bone structures are formed (Gartrell et al., 2002). Radiography also helps estimate gestational age and litter size, but it is inadequate to determine whether the fetus is alive or ready to be born (Garcia & Feliciano, 2015). Ultrasound examination is more suitable for assessing the viability of fetuses and monitoring their development (Garcia & Almeida-Santos, 2021).

In golden lanceheads, courtship and mating occur from autumn to winter (Amaral, 1921; Amorim et al., 2019; Marques et al., 2013). Ovulation and fertilization occur from early spring, females are pregnant from spring to early autumn, and parturitions occur from mid-summer to early autumn (Marques et al., 2013). Therefore, our observations on the timing of mating, ovulation, pregnancy, and parturition are consistent with previous studies. This report demonstrates the importance of diagnostic imaging for monitoring the reproductive activity of endangered species in captivity.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

The data that support the findings of this study (Ultrasonographic and radiographic evaluation of gestation in golden lanceheads (*Bothrops insularis*) in ex situ breeding programs) are available upon request to the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

This study was conducted under the approval of the Animal Ethics Committee of the Butantan Institute (approval number 2732151216) and the Biodiversity Authorization and Information System (SISBIO license 49360-1).

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