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Comparison of Noninvasive Methods for the Evaluation of Female Reproductive Condition in a Large Viviparous Lizard, *Tiliqua nigrolutea*

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We compared the diagnostic value of three noninvasive methods (radiography, ultrasound, and palpation) of evaluating reproductive status in females of a large viviparous skink, the blotched blue-tongued lizard (*Tiliqua nigrolutea*). Radiography could be used to identify postovulatory females during the periovulatory period. However, in early to mid-gestation, radiography did not allow differentiation of the relatively homogenous structures within the abdomen. In late gestation, once calcification of the embryonic skeleton had occurred, gestation could be diagnosed and embryos counted. Ultrasound allowed differentiation between preovulatory, postovulatory, and nonreproductive females during the periovulatory period. Ultrasound also allowed visualisation of follicles during the early and middle stages of gestation. However, the level of operator experience, and the misdiagnosis of artefacts limited the accuracy of diagnosis. In the later stages of gestation, ultrasound did allow the detection of identifiable features of the developing foetal unit (fluid-filled foetal membranes, independent movement of the embryo, and visualisation of the heartbeat). Colour Doppler imaging could be used to visualise embryonic blood flow. Ultrasonography was not useful in detecting the number of embryos present. Palpation during the periovulatory period could be used to detect follicles and determine whether ovulation had occurred. However, during the remainder of the gestation period, palpation was unable to differentiate reproductive condition. This study provides a basis for the diagnosis of reproductive condition and problems in viviparous reptiles. Each of the diagnostic techniques is useful at different stages of gestation, and the use of a combined approach will give the most information throughout the reproductive cycle. Zoo Biol 21:253–268, 2002. © 2002 Wiley-Liss, Inc.

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INTRODUCTION

Blue-tongued skinks (such as *Tiliqua nigrolutea*, *T. rugosa*, and *T. scincoides*) are large (snout-vent length up to 370 mm), placid, viviparous lizards that are commonly kept for display purposes, and are also the subjects of research in Australia [Fergusson and Bradshaw, 1992; Bull et al., 1993; Edwards, 1999; Zuri and Bull, 2000; Edwards and Jones, 2001a, b; Atkins et al., 2002] and the United States [Graves and Halpern, 1991; Cooper, 2000]. Currently, the reproductive physiology and endocrinology of *T. nigrolutea* is being researched [Edwards, 1999; Edwards and Jones, 2001a, b; Atkins et al., 2002]. This species is an excellent model for reproductive studies as it is common throughout its distribution in southeastern Australia [Rawlinson, 1974], and adapts quickly to life in captivity. Comprehensive background information already exists about the timing of reproductive events in both sexes [Edwards, 1999; Edwards and Jones, 2001a, b]. Vitellogenesis commences in spring (October–November) and is followed by mating and ovulation (November); gestation lasts approximately 4–4½ months over summer (late November–April) [Edwards and Jones, 2001a]. Females give birth to between one and 15 young [Edwards, 1999]. Females of *T. nigrolutea* do not reproduce every year; thus, reproductively active as well as quiescent females are found concurrently in the population [Edwards and Jones, 2001a]. Despite these details, current research is hampered by the difficulty of accurately determining the reproductive status of individual females.

Analysis of steroid hormones can be used to distinguish reproductive from nonreproductive females of *T. nigrolutea*. Repeated blood sampling throughout the reproductive cycle and subsequent analysis for plasma progesterone (P4) produces a characteristic profile in pregnant female lizards [Edwards and Jones, 2001a]. However, this technique requires time and effort in the laboratory, as well as special facilities and staff. The technique is invasive, and several samples are generally necessary. In addition, only limited data is obtained. Information about the timing of ovulation is only available retrospectively, and the number and condition of developing embryos cannot be determined.

In this study, we aimed to compare the diagnostic value of three noninvasive methods of evaluating reproductive status (radiography, ultrasound, and palpation) in the large, viviparous skink *T. nigrolutea* from preovulation to parturition. Features of particular interest included the timing of ovulation, the number and stage of developing embryos, and an estimation of the timing of parturition. The results are discussed in relation to their possible applications to other reptilian species.

METHODS

Animal Husbandry

Female lizards were captured opportunistically by hand around Hobart (42°53′S, 147°19′E), Tasmania, Australia, during their active season (September–April). Captive animals were housed in roofed enclosures 1.9 × 3.4 × 2.1 m, which were wire-fronted, allowing access to UV light and a natural photoperiod. Direct sunlight and a 120-W globe as an additional heat source suspended 30 cm above the floor at the front of each cage provided a thermal gradient across which the lizards could thermoregulate. Bark and leaf litter, in which the animals could hide, were also provided. Mixed-sex groups of approximately five animals were maintained in each cage, so that females could mate. Lizards were fed three times weekly during the active season. Their diet consisted of tinned cat food (meat varieties), snails when available, and a selection of fresh fruits, including apple and banana. Water was available ad libitum. All procedures were approved by the University of Tasmania Animal Ethics Committee (approval no. A5521).

Imaging Protocol

Eight female *T. nigrolutea* were examined using the three methods of assessment as described below. Palpation and ultrasound were performed monthly. Due to concerns about repeated exposure of embryos to radiation, each animal was only radiographed three times during the period of gestation. A single animal, which retained an embryo (see Results), was radiographed a fourth time 1 week after all the other embryos had been delivered. To prevent bias in the results, all examinations were done so that the operators had no prior knowledge of previous examination results. To accomplish this, each animal was identified each month with a different number. A single male lizard was included in the group to act as a control. The lizards of this species show very little sexual dimorphism, and it was not readily apparent on external examination which animal was the male.

Radiography

All lizards were fasted for 24 hr prior to the procedure. All radiography was carried out with the animals in ventral recumbency, and gentle manual restraint was used when required. A Kodak Min R® (Eastman Kodak Co., Rochester, NY) film cassette designed for mammography was used to achieve good soft-tissue contrast and high resolution. Each lizard was placed directly on the cassette as the average tissue thickness was approximately 4 cm. Optimal soft-tissue contrast was achieved using a kVp of 60, mA of 300, and mAs of 2.0. The X-ray machine used was a Varian® Emerald 125 (Varian, Arlington Heights, IL).

Ultrasonography

All lizards were fasted for 24 hr prior to the procedure. Ultrasonography was performed using an Advanced Technology Laboratories Ultramark 9 system (Philips Medical Systems, Best, The Netherlands). A 5.0 MHz phased array transducer designed for Doppler echocardiography provided better detail at the small depths of tissue in these lizards (1.5–3 cm) than the standard 5.0 or 3.0 MHz transducers. The use of a 7.5 MHz transducer has been recommended [Silverman and Janssen, 1996], but this was not available to us. Use of a stand-off device did not improve resolution. Regardless of the transducer used, the clarity of the ultrasound images was less than expected. We suggest that this was due to scattering of the ultrasound by the lizards' scaly integument. Each lizard was placed in dorsal recumbency and manually restrained. Acoustic coupling gel was liberally applied to the abdomen. Examination of each lizard's abdomen followed a standardised routine. The vertebral column was used as a distinctive landmark located along the midline, and the body wall could be located to each side of this point. Each side of the animal was then examined from the pelvis proximally until the air-filled lungs obscured the image. When embryonic movements were detected, colour Doppler imaging was used to confirm cardiac function. When the animals were relaxed, good images could be obtained; however, when the animals tensed, the lungs expanded and obscured the underlying organs. Gentle handling and patience were required for a successful examination. In addition, it was noted that the duration of each examination decreased, and confidence in the technique increased, with increasing operator experience. The procedure was recorded on VHS videotape and still images were subsequently captured using WinTV2K Version 2.24® (Hauppauge Computer Works, Hauppauge, NY). The images were converted to an 8-bit greyscale and contrast was adjusted digitally.

Palpation

Lizards were manually restrained and the abdomen was palpated by using the thumbs to support the dorsal body wall and running the forefingers along each side of the abdomen. Moderate pressure was required to overcome the abdominal musculature; however, we were careful not to exert excess pressure that might result in follicle or embryonic damage. Two operators (B.G. and J.G.) independently examined the lizards using this method.

RESULTS

Periovulation (November)

Radiography

Postovulatory females could be identified using radiographs; the large (approximately 2 cm diameter) homogenous follicles were visible laterally along the dorsal body wall (Fig. 1A). No preovulatory females were radiographed at this stage. Nonreproductive females (and the male) were diagnosed when no follicles were observed.

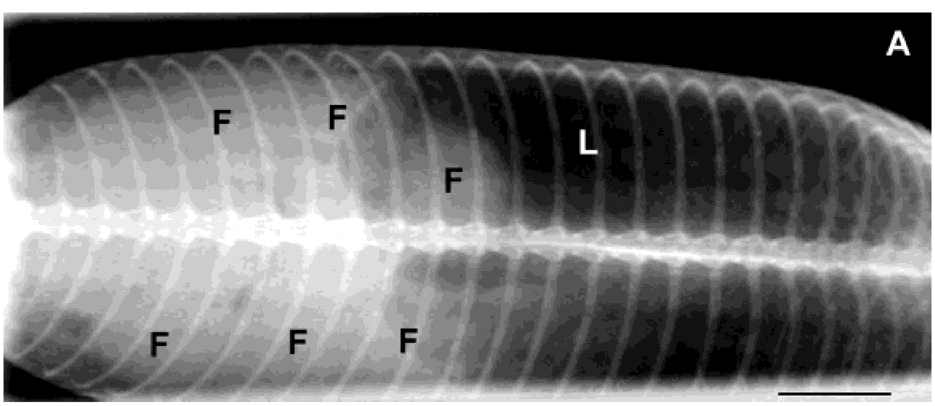


Fig. 1. Imaging appearance of reproductive structures in female *Tiliqua nigrolutea* during the period encompassing ovulation (November). **A:** Radiographic appearance of the body postovulation. Follicles (F) are arranged linearly in the oviduct. The lungs (L) appear as nonsymmetrical radio-lucent areas. Scale bar represents 20 mm. **B:** Ultrasound appearance of the ovary prior to ovulation. Follicles (F) are grouped together adjacent to the vertebral column (VC). Vertical scale bar measures distance in cm from transducer. **C:** Ultrasound appearance of an ovulated follicle (F) in the oviduct. Only a single follicle is visible in the image plane, located adjacent to the vertebral column (VC). Movement of the image plane along the body revealed more follicles arranged linearly in the oviduct. Vertical scale bar measures distance in cm from transducer.

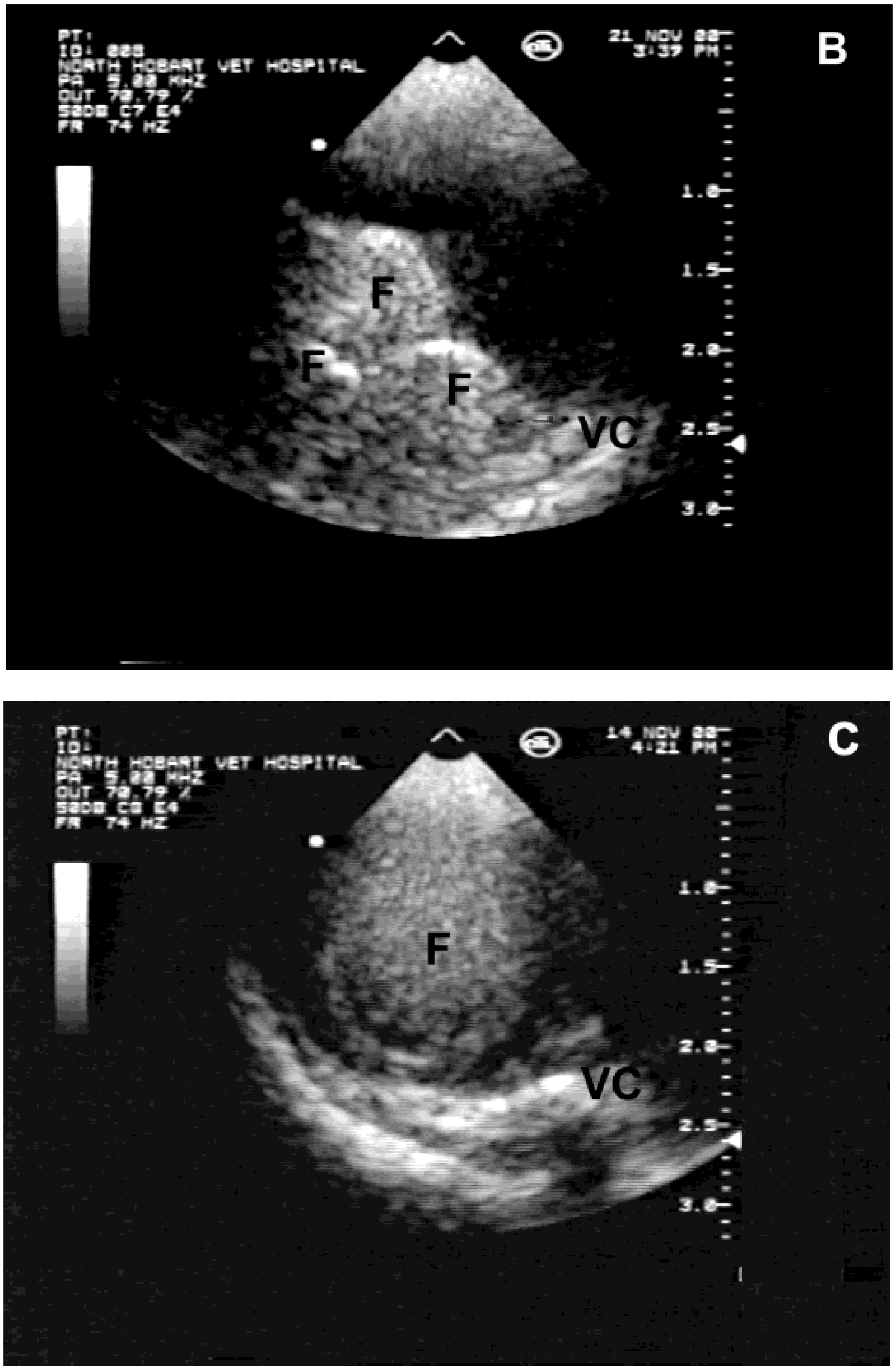


Fig. 1. Continued.

Ultrasound

It was possible to differentiate between preovulatory, postovulatory, and nonreproductive females using ultrasound. However, the accuracy of diagnosis was limited by the level of operator experience with the equipment and with misdiagnosis of artefacts. Preovulatory follicles were grouped together and positioned laterally, approximately halfway down the abdomen (Fig. 1B). Postovulatory follicles were not grouped and could be found laterally, directly against the dorsal body wall (Fig. 1C). Follicles (pre- and postovulatory) were visualised as large, homogenous, spherical-to-ovoid structures. Nonreproductive females (and the male) were diagnosed when no follicles were visualised.

Palpation

Follicles could easily be detected by palpation, and with practice it was possible to determine whether ovulation had occurred. Follicles could be felt as large spherical-ovoid structures that were highly mobile when rolled gently under the fingers. Prior to ovulation follicles were clustered together on each side approximately halfway along the length of the lizard. After ovulation, the follicles could be felt arranged linearly along the body. Caution was needed to avoid misdiagnosis when food items were felt within the gut. Nonreproductive females (and the male) were diagnosed when no follicles were felt.

Early Gestation (December and January)

Radiography

Gestation could not be diagnosed by radiography when the foetal unit became homogeneous with the other abdominal structures (Fig. 2A); that is, the distinctive shape and consistency of the follicles were lost.

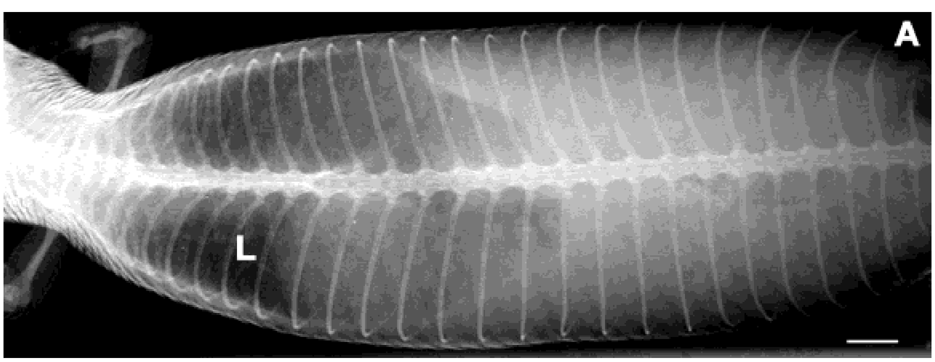


Fig. 2. Imaging appearance of reproductive structures in female *Tiliqua nigrolutea* in early gestation (December–January). **A:** Radiographic appearance of the body of an early gravid female. The only indication of gestation is a nonspecific reduction in the area comprising the lungs (L). Scale bar represents 20 mm. **B:** Ultrasound appearance of an early stage foetal unit (F) displaying no discernible embryonic structures. The foetal unit is still positioned next to the vertebral column (VC). Vertical scale bar measures distance in cm from transducer. **C:** Ultrasound appearance of the gut in cross section (G). This structure can be differentiated from foetal units by its position away from the vertebral column (which is not present in the image plane), and by advancing and retreating the image plane to reveal an elongated rather than ovoid-to-spherical structure. Vertical scale bar measures distance in cm from transducer.

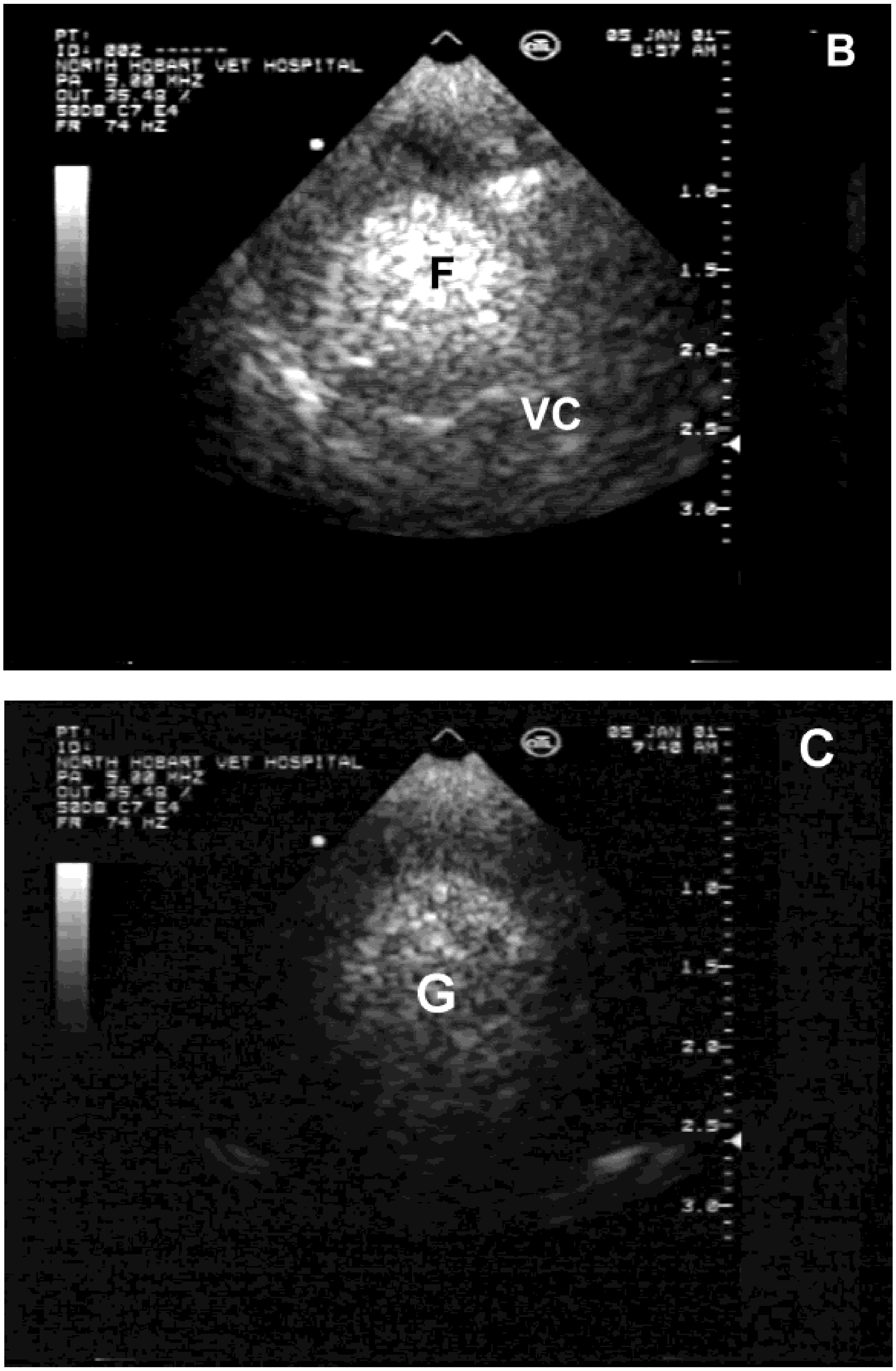


Fig. 2. Continued.

Ultrasound

Although it was possible to visualise follicles during the early stages of gestation, accuracy of diagnosis was limited. Follicles at this stage were relatively homogenous and there were no identifiable features that allowed definitive diagnosis (Fig. 2B). Caution was required to avoid misdiagnosis of images of gut cross-section (Fig. 2C).

Palpation

As embryos developed, palpation was no longer reliable as a diagnostic tool. The foetal unit was not homogenous, and identifiable structures could not be felt within the abdomen. Although a taut abdomen was highly suggestive of pregnancy, it was by no means an accurate indicator.

Mid-Gestation (Early February)

Radiography

Gestation could not be diagnosed using radiography because the relatively homogenous structures within the abdomen could not be distinguished.

Ultrasound

As gestation progressed, identifiable features developed, which increased the accuracy of ultrasonic diagnosis. The developing foetal units were found laterally, directly alongside the dorsal body wall. The fluid-filled foetal membranes could be seen as a hypoechoic sac surrounding the embryo (Fig. 3A). The most obvious indicator of gestation was independent movement of the embryo and visualisation of the heartbeat. Colour Doppler imaging could be used to visualise the blood flow to and from the heart. In some individuals, developing skeletal structures, such as the skull, were visible (Fig. 3B).

Palpation

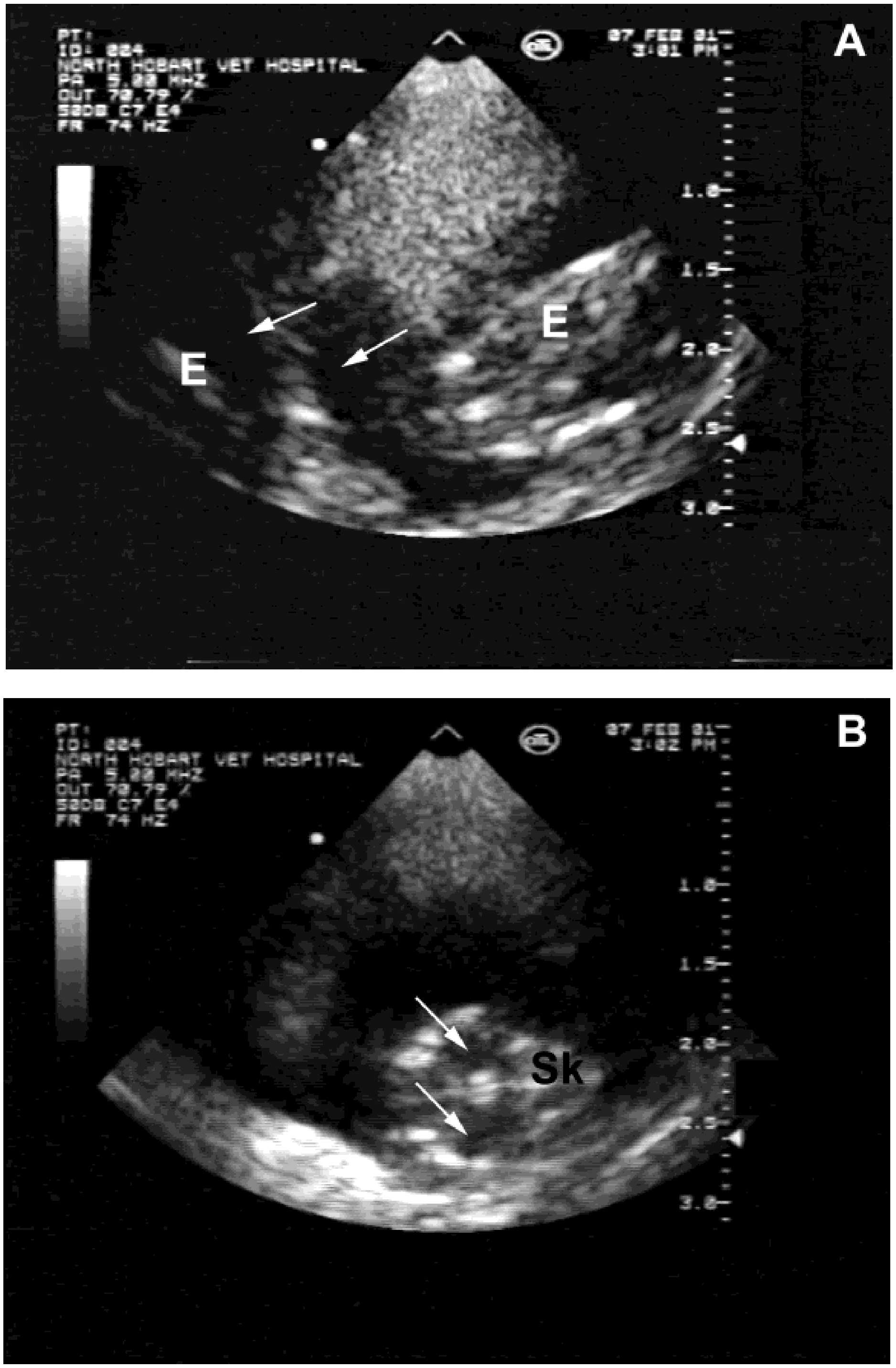
Reproductive condition could not be diagnosed using palpation.

Late Gestation (Late February and March)

Radiography

Once calcification of the embryonic skeleton occurred, late gestation could be diagnosed using radiographs. The skull and mandible of the embryos were visible, allowing the number of embryos to be counted (Fig. 4A). These counts correlated with the number of neonates at parturition. Radiography was used to predict retention of an embryo when the number of neonates delivered was one less than the embryos counted on radiographs. Radiography also confirmed the diagnosis of a retained embryo that was subsequently delivered in a state of advanced autolysis.

Fig. 3. Ultrasound appearance of embryonic structures in *Tiliqua nigrolutea* during mid-gestation (early February). Vertical scale bar measures distance in cm from the transducer. **A:** Two embryos (E) are visible as complex echo-dense structures surrounded by the echo-lucent foetal fluids and membranes (arrows). **B:** Appearance of the embryonic skull (Sk) in transverse section. The skeletal elements have a characteristic bilaterally symmetrical and echo-dense structure. The vitreous humour within each eye (arrows) is echo-lucent.


A
NORTH HOBART VET HOSPITAL
E
E
1.0
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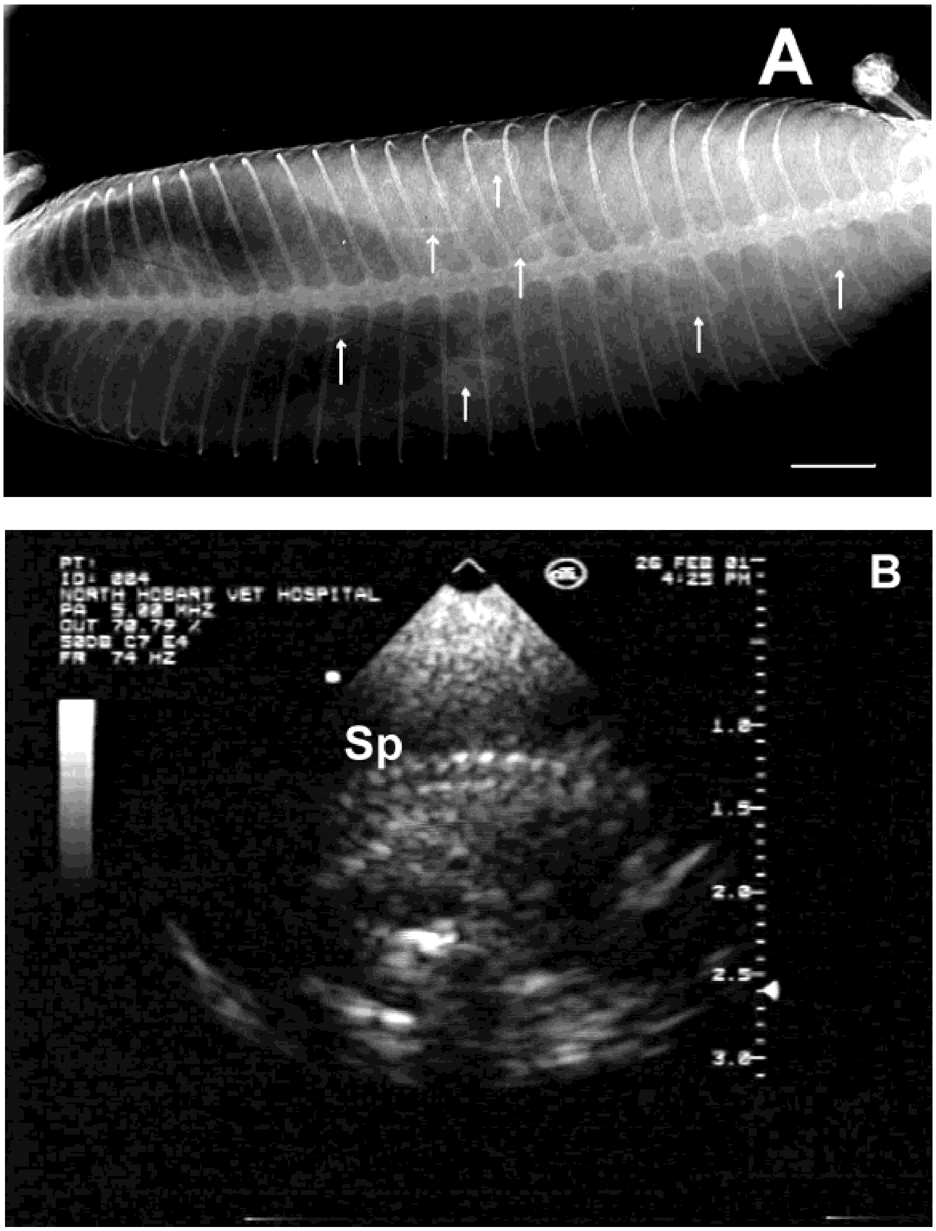

Fig. 4. Imaging appearance of reproductive structures in female *Tiliqua nigrolutea* in late gestation (late February–March). **A:** Radiographic appearance of the body in late gestation. Multiple embryos are visible as the skull and mandibles (arrows) calcify. Embryos extend cranially, reducing the available space for the lungs (L). Scale bar represents 20 mm. **B:** Ultrasound appearance of the embryonic spine (arrow), which is characterised by its echo-dense and segmented structure. Vertical scale bar measures distance in cm from transducer.

Ultrasound

In the very late stages of gestation, the hypoechoic sac (foetal membranes and fluids) surrounding the embryos was minimal. Pregnancy diagnosis relied on observing independent embryonic movement, heartbeat, and/or skeletal structures (particularly the spine) (Fig. 4B). Ultrasonography was not useful in detecting the number of embryos present, and observations were limited to grouping lizards into: 1) multiple embryos (more than four), 2) few embryos (two to four), 3) a single embryo, or 4) no embryos. Despite the excellent visualisation of embryonic movement and heartbeat, we failed to detect the single nonviable embryo from a clutch of four.

Palpation

Reproductive condition could not be diagnosed using palpation.

A summary of the usefulness and accuracy of each diagnostic mode for the different reproductive states in this viviparous lizard is presented in Tables 1 (radiography), 2 (ultrasound), and 3 (palpation). No procedure falsely identified the male as gravid; however, none of the described procedures were able to distinguish the male from the nonreproductive females.

DISCUSSION

Techniques that enable accurate evaluation of reproductive condition are potentially valuable in the captive management of reptiles and in research into their reproductive biology. For captive animals, accurate assessment of reproductive condition allows the optimal management of the animals to improve fertility and offspring survival. Ideally, a technique for the evaluation of reproductive state in viviparous reptiles should be able to identify when females are ready to mate and when matings have been successful; monitor the number, health, and development of embryos; and accurately predict the timing of parturition. It should also aid in the diagnosis of reproductive disorders (e.g., infertility, embryonic death, and dystocia); be carried out with minimal disruption to the animals; and have no side effects to the mother, offspring, or investigator. Additional benefits would include the ability to be carried out with minimal equipment and/or cost, and accuracy in the hands of inexperienced operators.

TABLE 1. Characteristic findings and relative usefulness of radiography for assessing reproductive condition of female *Tiliqua nigrolutea**

Reproductive state	Radiography	Usefulness
Non-reproductive (all months)	Diagnosis by exclusion. High chance of false negative diagnosis	Low
Preovulatory vitellogenesis	Not assessed	Not assessed
Postovulatory	Follicles in characteristic rows. May be obscured by abdominal viscera	Low
Mid gestation	Not distinguishable from non-reproductive	Nil
Late gestation, prior to foetal calcification	Not distinguishable from non-reproductive	Nil
Late gestation, post foetal calcification	Skull and mandibles visible first, followed by spine, limbs and ribs. Accurate quantitative counts possible	High

*Accuracy was not determined because of concerns about repeated exposure of embryos to radiation; not all lizards were radiographed at each stage.

TABLE 2. Characteristic findings, relative usefulness and accuracy of ultrasonography throughout the reproductive cycle of *Tiliqua nigrolutea*

Reproductive state	Ultrasound	Usefulness	Accuracy	
			Pregnant	Non-pregnant
Non-reproductive (all months)	Diagnosis by exclusion. Moderate chance of false negative diagnosis	Moderate		
Preovulatory vitellogenesis	Characteristic ovarian structure visible	High	NA[a]	NA[a]
Postovulatory	Characteristic follicular structures arranged in longitudinal rows	High	3/4[b]	NA[a]
Mid gestation	Follicles become progressively less distinct. High chance of false negative and false positive diagnosis	Moderate	3/4[b]	2/4
Late gestation, prior to foetal calcification	Characteristics visible include embryonic movement, foetal membrane sac, heartbeat and developing skeletal structures	High	4/4	4/4
Late gestation, post foetal calcification	Characteristics visible include embryonic movement, foetal membrane, sac, heartbeat and developiing skeletal structures	High	4/4	4/4

[a]Not assessed as females may have been vitellogenic but not ovulated, or may have ovulated and not been fertilised.
[b]The animal that was misdiagnosed was carrying only one embryo.

Although there are several methods currently available for the evaluation of reproductive state in reptiles, none fulfill all of the characteristics outlined above. Readily available noninvasive techniques for the evaluation of reproductive state include radiography, ultrasonography, and palpation. Clinical texts have documented the use of all three diagnostic methods [Silverman and Janssen, 1996; Schildger et al., 2000], but no detailed studies have been published comparing the techniques in

TABLE 3. Characteristic findings, relative usefulness and accuracy of palpation for assessing reproductive condition of female *Tiliqua nigrolutea*

Reproductive state	Ultrasound	Usefulness	Accuracy	
			Pregnant	Non-pregnant
Non-reproductive (all months)	Diagnosis by exclusion. High chance of false negative diagnosis	High		
Preovulatory vitellogenesis	Characteristic follicular development palpable on ovary	High	NA[a]	NA[a]
Postovulatory	Characteristic follicular structures arranged in longitudinal rows	High	3/4[b]	NA[a]
Mid gestation	Not distinguishable from non-reproductive	Nil	–	–
Late gestation, prior to foetal calcification	Not distinguishable from non-reproductive	Nil	–	–
Late gestation, post foetal calcification	Not distinguishable from non-reproductive, except by expanding girth and increasing body weight	Nil	–	–

[a]Not assessed as females may have been vitellogenic but not ovulated, or may have ovulated and not been fertilised.
[b]The animal that was misdiagnosed was carrying only one embryo.

reptiles. Our study showed that each of the diagnostic techniques is useful at different stages throughout gestation, and that a combined approach will give the most information throughout the reproductive cycle.

Radiography has been recommended for use in oviparous reptiles, as the high radiodensity of the eggshells allows easy visualisation. This is particularly valuable in chelonians, in which other techniques are limited by the plastron [Silverman and Janssen, 1996; Schildger et al., 2000]. However, for viviparous reptiles like *T. nigrolutea*, it is difficult to differentiate soft tissues using radiography, and embryonic development is not visible until calcification of the skeleton has occurred. Once calcification has occurred, radiography is accurate in diagnosing pregnancy, and the number of embryos can be determined by skull counts. An advantage of radiography is that it can be carried out with physical restraint only. Disadvantages of radiography include the fact that accurate information is only obtainable for a limited period of gestation, and there are concerns about repeated exposure of the mother, embryos, and investigators to radiation. There is no specific information about safe levels of radiation for developing embryos in reptiles. The use of radiography to assess preovulatory animals was not assessed in this study.

Ultrasonography has been recommended for the evaluation of reproductive state in lizards and snakes [Silverman and Janssen, 1996; Schildger et al., 2000]. It is safe for the mother, the embryos, and the investigator; it can be used repeatedly throughout the gestation period; and only manual restraint is required [DeNardo, 1996; Silverman and Janssen, 1996; Schildger et al., 2000]. However, the operators' knowledge of the species' anatomy, and their skill in controlling the equipment can limit the accuracy of ultrasound examination [Nyland et al., 1995]. Some reptile species are unsuited for ultrasound (e.g., Tuatara (*Sphenodon punctatus*)) due to the caudal continuation of the ribs down the length of the abdomen, which impedes the acoustic window [Cree et al., 1991].

Ultrasonography has been used to show ovarian abnormalities [Anderson et al., 1996], ovarian development prior to ovulation, and the linear arrangement of follicles in utero postovulation [DeNardo, 1996]. It is potentially the most useful tool for determining whether ovulation has occurred and, with refinement, can be used to assess the number and size of follicles. In *T. nigrolutea* (this study) and other viviparous species [Silverman and Janssen, 1996], the yolk dominates the ultrasonographic findings during early pregnancy until the embryo has grown large enough to be seen. The amniotic sac is visible as a small echo-lucent sphere on the periphery of the larger echogenic yolk mass [DeNardo, 1996]. False-negative assessment of gestation is possible if animals are not fasted, as the gut may obscure embryonic structures. False-positive assessment of gestation may occur, as some viviparous lizards retain infertile yolks for the full duration of gestation [Bull et al., 1993] (Edwards and Jones, unpublished data). Our accuracy and ability to identify other structures, such as the gut, improved markedly with experience. When we reviewed the footage of the ultrasound images retrospectively we were able to correctly identify pregnant from nonreproductive animals, suggesting that ultrasound can be useful in this period but requires greater operator skill and experience. However, ultrasound is unreliable in determining the number of embryos present because only a small section of the reproductive tract can be imaged at any one time [Mattoon and Nyland, 1995].

Later in gestation, development can be assessed by the appearance of the embryonic heart, ribs, and spine, and the viability of the embryos confirmed by visualising the embryonic heartbeat [DeNardo, 1996]. Ultrasound was able to identify embryonic structures well before calcification had occurred and radiology became useful. The ability to visualise independent embryonic movement and heartbeat allowed us to assess embryonic viability. Further investigation is needed to correlate these ultrasound findings with exact embryonic stage.

Palpation of the abdomen can be used to detect follicular development, ovulation, and pregnancy in reptiles [Cree et al., 1991; DeNardo, 1996; Wapstra and Swain, 2001]. Follicles are reported as firm, spherical structures that can be differentiated from the softer embryos. For palpation to be successful in large lizards, the abdomen must be relaxed. Palpation requires investigator experience but requires minimal equipment, disruption to the animal, and restraint. The disadvantages of palpation include the possibility of false-negative diagnoses and the risk of follicle or yolk rupture from aggressive palpation. Similar constraints to ultrasound occur in some species (e.g., tuatara) because their ribs extend along the abdominal cavity [Cree at al., 1991]. Palpation has been used to assess the number of embryos in small skinks [Olsson et al., 1993; Wapstra and Swain, 2001] but is unreliable in larger lizards and does not allow any assessment of embryonic development or viability [DeNardo, 1996]. Palpation was effective in our animals as they were docile and easily handled, but would be much less effective in tense or larger viviparous reptiles.

This study provides recommendations for techniques to be used in the evaluation of reproductive condition in large viviparous reptiles. It also provides a basis for the diagnosis of reproductive problems. Particularly in the later stages of gestation, the use of both radiography and ultrasound can determine the presence and viability of embryos. However, more work is needed to identify characteristic signs of embryonic distress or maternal uterine dysfunction. Future studies could compare the success of the techniques used here to other, more invasive methods, such as laparotomy and endoscopy. However, the latter involve repeated anaesthesia and surgical trauma, with their attendant risks to mother and offspring. They also entail major disruption to the mother's daily routine and, subsequently, to her basking routine. Such disruption will likely have some impact on the development of the offspring [Wapstra, 2000]. Laparotomy and endoscopy are also limited to observations of the ovary and external oviducts, and hence provide little information on embryonic development or viability.

Other recently developed noninvasive techniques that could be applied to the evaluation of reproductive state include computer tomography (CT) scanning and magnetic resonance imaging (MRI). These technologies are not yet readily or cheaply available but show promise as advanced and safe diagnostic techniques. These techniques were not available to our study. CT has poor resolution of soft tissues and is better suited to evaluation of bone and bony abnormalities [Krautwald-Junghanns et al., 1998; Romagnano et al., 1996]. As such, it may be useful in the later stages of gestation, when embryonic calcification has occurred. MRI provides detailed multiplanar images with excellent soft-tissue resolution, without the use of ionising radiation [Krautwald-Junghanns et al., 1998; Romagnano et al., 1996]. Therefore, MRI should be an excellent technique for evaluating pregnancy throughout the gestation period. However, a disadvantage of both MRI and CT scanning is that immobilisation of the mother is necessary for the duration of the scanning, which necessitates sedation or anaesthesia.

The use of faecal steroid concentrations is also being investigated as a noninvasive method of obtaining information about the reproductive state. However, in some species, including *T. nigrolutea*, plasma steroid concentrations are not reflected in faecal steroid concentrations [Atkins et al., 2002]. Also, the assays required are technically difficult and, particularly in lizard species, results may vary with diet and time of year [Atkins et al., 2002].

CONCLUSIONS

1. This study provides a basis for the diagnosis of reproductive condition and problems in viviparous reptiles. Each of the diagnostic techniques (radiography, ultrasound, and palpation) is useful at different stages throughout gestation, and the use of a combined approach will give the most information throughout the reproductive cycle.

2. Both radiography and ultrasound can determine the presence and viability of embryos, particularly in the later stages of gestation. However, more work is needed to identify characteristic signs of embryonic distress or maternal uterine dysfunction.

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