SERIAL ULTRASOUND TO ESTIMATE FETAL GROWTH CURVES IN SOUTHERN TAMANDUA (*TAMANDUA TETRADACTYLA*)

Author(s): Rachel Thompson, D.V.M., Tiffany M. Wolf, D.V.M., Ph.D., Heather Robertson, D.V.M., Margarita Woc Colburn, D.V.M., Alexis Moreno, D.V.M., Anneke Moresco, D.V.M., Ph.D., Anne Elise Napier, D.V.M., and Sally A. Nofs, D.V.M.

Source: Journal of Zoo and Wildlife Medicine, 48(2):294-297.
Published By: American Association of Zoo Veterinarians
https://doi.org/10.1638/2016-0197R1.1
URL: http://www.bioone.org/doi/full/10.1638/2016-0197R1.1

Journal of Zoo and Wildlife Medicine 48(2): 294–297, 2017

SERIAL ULTRASOUND TO ESTIMATE FETAL GROWTH CURVES IN SOUTHERN TAMANDUA (*TAMANDUA TETRADACTYLA*)

Rachel Thompson, D.V.M., Tiffany M. Wolf, D.V.M., Ph.D., Heather Robertson, D.V.M., Margarita Woc Colburn, D.V.M., Alexis Moreno, D.V.M., Anneke Moresco, D.V.M., Ph.D., Anne Elise Napier, D.V.M., and Sally A. Nofs, D.V.M.

Abstract: From 2012 to 2015, 16 pregnancies were monitored by ultrasonography in nine tamanduas (*Tamandua tetradactyla*) housed in three zoological facilities. Sonographic measurements were recorded to establish fetal growth curves using thoracic and skull landmarks described for giant anteaters *(Myrmecophaga tridactyla)*. All pregnancies resulted in the uncomplicated delivery of healthy offspring, thus gestational development was considered normal. These data may be used as a reference for normal fetal development with potential for estimating parturition date in the absence of breeding data.
Key words: Anteater, fetal growth, reproduction, *Tamandua tetradactyla*, ultrasound, Xenarthra.

INTRODUCTION

Knowledge about reproduction in the primarily nocturnal southern tamandua (*Tamandua tetradactyla*) is poorly documented. Published gestation periods range from 130 to 190 days with a mean of approximately 160 days.[1,2,3,5] Macro- and microscopic features of the nongravid reproductive tract have been described.[8] Urine and serum hormone concentrations and vaginal cytology have been evaluated to estimate estrous cycle of 42–44 ± 4 days with estrus lasting 7 to 12 days.[4,5] In this species, vaginal bloody discharge occurs at the end of the luteal phase and estrus can be expected approximately 3 wk after first bleeding.[5] Serosanguinous discharge can be used as an indicator of ovarian activity. Few resources detail normal gestation for tamandua and these are based on breeding observations and not timing of ovulation.[1,2,3,5] Ultrasonic fetal thoracic diameter measurement has been described as a reliable predictor of parturition date in giant anteaters (*Myrmecophaga tridactyla*).[7]

Sixteen tamandua pregnancies from nine females housed in four zoological facilities were monitored by ultrasound. Measurements were recorded to establish fetal growth curves using thoracic and skull landmarks. These data were gathered as a reference for normal fetal development with potential for estimating parturition date in the absence of breeding data.

MATERIALS AND METHODS

From 2012 to 2016, animals were selected for inclusion in the study based on good health, active reproductive status, and ability to be monitored serially over time without the need for anesthesia. All dams were considered to have normal health status during gestation and all pregnancies resulted in uncomplicated delivery of healthy offspring, thus fetal development was considered normal. Dams ranged from 2 to 11 yr of age with four animals having estimated birth dates. Data represented both primiparous and multiparous dams. Regular transabdominal ultrasound was performed under behavioral control using positive reinforcement to confirm and monitor pregnancy.

Animals were positioned in bipedal standing position with forelimbs elevated to allow sonographers access to the ventral abdomen for probe placement (Fig. 1). Ultrasound machines varied by institution, but included Ibex (EI Medical, Loveland, Colorado 80537, USA), LOGIQ e (Sound Technologies, Carlsbad, California 92008, USA), and Sonoscape S8 (International Diagnostic Devices, Las Vegas, Nevada 89103, USA). Frequencies at which scans were performed varied from 4.0 to 12 MHz using linear

From the Minnesota Zoological Garden, 13000 Zoo Boulevard, Apple Valley, Minnesota 55124, USA (Thompson); Veterinary Population Medicine Department, University of Minnesota, 1365 Gortner Avenue, St. Paul, Minnesota 55108, USA (Wolf); Nashville Zoo at Grassmere, 3777 Nolensville Pike, Nashville, Tennessee 37211, USA (Robertson, Woc Colburn); Reid Park Zoo, 3400 East Zoo Court, Tucson, Arizona 85716, USA (Moreno); Denver Zoo, 2300 Steele Street, Denver, Colorado 80205, USA (Moresco); Ultrasound Solutions, 12134 Old Hickory Boulevard, Hermitage, Tennessee 37076, USA (Napier); Potter Park Zoo, 1301 South Pennsylvania Avenue, Lansing, Michigan 48912, USA (Nofs). Correspondence should be directed to Dr. Thompson (Rachel.Thompson@state.mn.us).

Figure 1. Positioning of the southern tamandua (*Tamandua tetradactyla*) for ultrasonic evaluation of pregnancy. The individual is placed into a bipedal standing position with behavioral enrichment. Photo credit: Denver Zoo.

or curved probes that corresponded to the ultrasound machine manufacturer.

Measurements were made of thoracic diameter at the level of the heart (TD), maximal heart diameter (HD), biparietal skull diameter (SD),

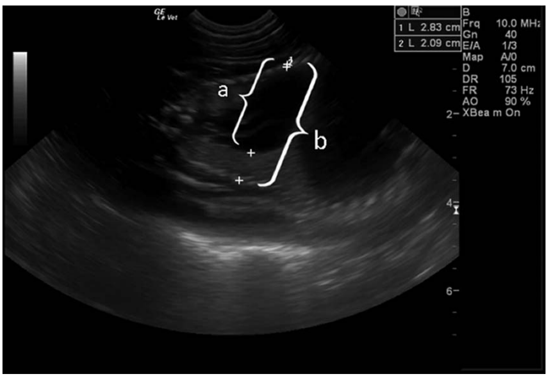

Figure 2. Ultrasonographic view of the fetal thorax for measurement of (**a**) maximal heart diameter and (**b**) thoracic diameter at the level of the heart in a southern tamandua (*Tamandua tetradactyla*). Cross points represent the locations of measurements. Photo credit: Minnesota Zoological Garden

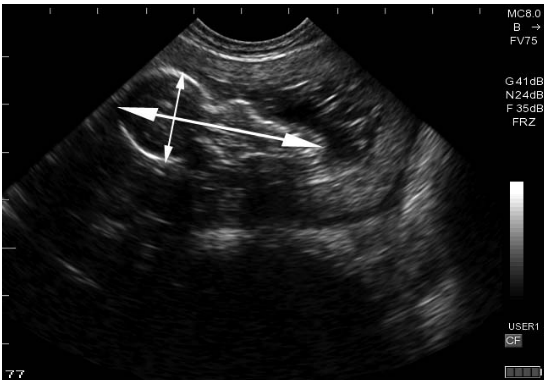

Figure 3. Ultrasonographic view of the fetal skull for measurement of biparietal skull diameter and skull length in a southern tamandua (*Tamandua tetradactyla*). The arrows represent the locations at which measurements were taken. Photo credit: Denver Zoo.

skull length (SL), and heart rate (HR) (Figs. 2, 3). It was not possible to document all measurements at every scan for each individual. Voluntary compliance by the patient determined the length of each session, which typically lasted 10 to15 min, and detail of scan obtained.

Summary statistics of each measurement were generated (Excel, Microsoft Office Professional Plus 2013, Microsoft Corporation, Redmond, Washington 98052, USA). To estimate growth curves for TD, SD, and SL, linear mixed models were created (SAS 9.4, Windows SAS Institute Inc., Cary, North Carolina 27513, USA). In these models, the number of days to parturition was modeled as the predictor variable, random intercepts and slopes were specified, and repeated sampling in the dam was modeled using an unstructured covariance structure. As sparse data for HD precluded model convergence and parameter estimation under this design, this growth curve model differed from previous models in the elimination of the random slope specification from the model.

RESULTS

Sixteen pregnancies were documented in nine individuals during the study period. The earliest reported fetal detection was 18 weeks (126 days) prior to parturition with an average first date of sonographic examination at 97 $\pm$ 14 days prior to parturition. Thoracic diameter was the most reliably obtained metric ($n = 101$) from 13 births in seven animals. Biparietal skull diameter ($n = 83$) from 14 births in eight animals, and SL ($n = 51$) from 13 births in eight animals were less commonly recorded in all stages of gestation; and HD ($n = 50$) from nine births in five animals was the

Table 1. Results of the linear mixed models for estimating fetal growth over time to parturition. The predicted slope represents the average increase in body measurement per day of gestation. For example, on average, thoracic diameter increases 0.04 cm each day of gestation.

Metric	Predicted slope	Standard error	t value	*Pr* > |t|[a]
Thoracic diameter	0.04	0.003	13.38	<0.0001
Heart diameter	0.02	0.002	12.91	<0.0001
Biparietal skull diameter	0.03	0.002	15.11	<0.0001
Skull length	0.06	0.007	7.83	0.0001

[a] *Pr* > |t| is the statistical test of significance (i.e. *P*-value). A *P*-value less than 0.05 is considered significant.

least reliably acquired fetal measurement. All growth metrics were significantly and positively associated with time to parturition, as expected. All models, with the exception of the SL growth curve model ($X^2 = 6.97$, df = 3, $P = 0.0727$), were significantly better than the null model, and estimated growth coefficients from each model were calculated (Table 1) and depicted graphically (Fig. 4). Fetal heart rate was detected 8–98 days prior to birth and averaged 144.7 ± 18.6 beats/min ($n = 62$). Reported average birth weight was 386.6 ± 53.9 grams ($n = 9$).

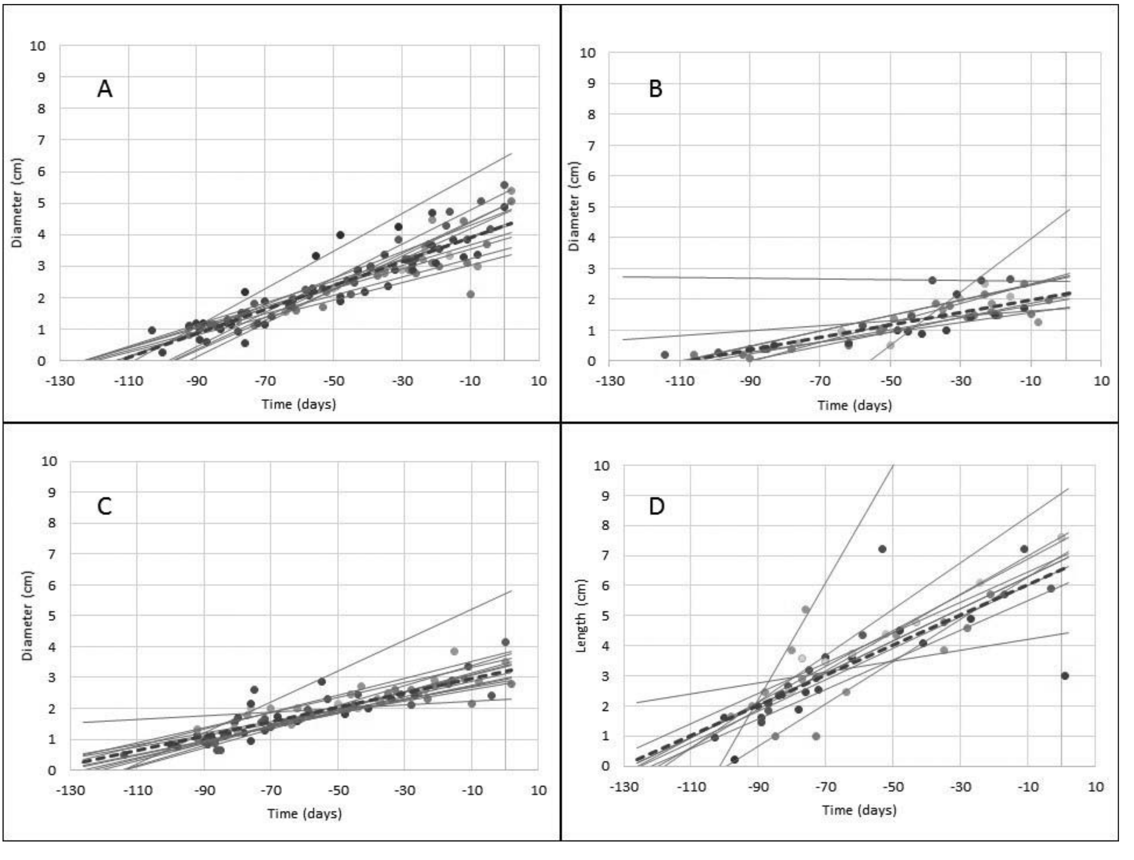

Figure 4. Fetal growth curves for the southern tamandua (*Tamandua tetradactyla*). Each panel depicts the estimated individual slopes, representing change in growth over time, for each of the 16 pregnancies monitored in this study. The dots represent individual measurements taken during pregnancy and the dashed lines represent the predicted growth curves estimated from the mixed models. The growth curve for thoracic diameter (**A**), measured at the level of the heart, was estimated from 13 births in seven dams ($n = 101$ measurements). The growth curve for heart diameter (**B**), measured at the widest expansion, was estimated from 9 births in five dams ($n = 50$ measurements). Biparietal skull diameter (**C**) growth was estimated from 14 births in eight dams ($n = 83$ measurements). Skull length (**D**) growth was estimated from 13 births in eight dams ($n = 51$ measurements).

DISCUSSION

This study presents the largest dataset on fetal measurements in tamandua published to date. This data provides growth curves that can be used as a tool to estimate dates of parturition based on single measurements. Presented here are growth curves for four measurements: TD, HD, SD, and SL. Femur and humerus lengths were originally targeted as additional fetal growth metrics; however due to fetal positioning, these measurements were frequently not possible to obtain. All growth curves had inherent variability and estimating date of parturition using multiple metrics seemed to increase confidence in predicted parturition date or date range as compared with a single prenatal assessment. Breeding pairs generally were housed together for long periods of time without observation, so specific breeding dates were typically unknown. Therefore, growth curves were developed retrospectively based on date of parturition rather than breeding date.

Challenges encountered include variation in transducer placement, patient positioning, and clinician technique for standardized measurements. Although clinicians were provided with a list of metrics and pictures, the scans were not performed and interpreted by a single clinician; therefore, interobserver variability is likely to have contributed to some of the variability in the measurements in the combined dataset. Evaluation of images by a single clinician may reduce such variation. However, the factors that might account for some of the variation in fetal measurements in this study would realistically be encountered in the monitoring of future pregnancies across zoological institutions, thus must be considered.

Not all measurements were recorded for each individual at every scan. This may be related to patient compliance, fetal position, or fetal size. Clinicians noted that in the earlier part of pregnancy some measurements were more difficult to obtain due to the lack of mineralization (e.g., skull measurements) and because of ventral flexion of the head by the fetus. Towards the latter part of pregnancy, the fetus also may be in a position that is not conducive to obtaining measurements. Because the fetus is large relative to the uterine space and can occupy 75% of the fundic area of the uterine lumen,[6] fetal motion is reduced and position is less likely to change during an examination. Similarly, heart rate may be less challenging to find later in pregnancy while in early gestation the heart is smaller so fetal or maternal motion make it difficult to keep it in view long enough to determine the heart rate.

Thoracic diameter at the heart level was most consistently documented, so it may be the most easily obtained metric of fetal growth. Combining thoracic diameter with the other metrics may increase confidence in predicted parturition date when limited to a single prenatal assessment. It is noted that few measurements were collected more than 100 days before parturition, which suggests that even with closely monitored animals a gap exists in the current knowledge of early gestational development in the tamandua. However, fetal growth curves resulting from this study may be useful as a reference for normal fetal development, with potential for estimating date of parturition in the absence of precise breeding data.

Acknowledgments: The authors would like to thank the animal husbandry staff at the Minnesota Zoo, Denver Zoo, Nashville Zoo, and Reid Park Zoo who facilitated the daily care and training of tamanduas for data collection for this study.

LITERATURE CITED

1. Divers BJ. Edentata. In: Fowler ME (ed.). Zoo and wild animal medicine. 2nd ed. Philadelphia (PA): WB Saunders; 1986. p. 628.

2. Eisenberg JF, Redford KH. Mammals of the Neotropics, the Central Neotropics, Volume 3, Ecuador, Peru, Bolivia, Brazil. Chicago (IL): University of Chicago Press; 1999. p. 94.

3. Gillespie D. Xenarthra: edentate (anteaters, armadillos, sloths). In: Fowler ME and Miller RE (eds.). Zoo and wild animal medicine. 5th ed. St. Louis (MO): Saunders; 2003.p. 397–407.

4. Hay MA, Bellum AC, Brown JL, Goodrowe KL. Reproductive patterns in tamandua (*Tamandua tetradactyla*). J. Zoo Wildl. Med. 1994;25(2):248–258.

5. Kusuda S, Endoh T, Tanaka H, Adachi I, Doi O, Kimura J. Relationship between gonadal steroid hormones and vulvar bleeding in southern tamandua, *Tamandua tetradactyla*. Zoo Biol. 2011;30(2); 212–217.

6. Mess AM, Favaron PO, Pfarrer C, Osmann C, Melo APF, Rodrigues RF, Ambrosio CE, Bevilacqua E, Miglino MA. Placentation in the anteaters *Myrmecophaga tridactyla* and *Tamandua tetradactyla* (Eutheria, Xenarthra). Reprod Biol Endocrinol. 2012;10(102):1–7.

7. Napier AE, Nofs SA, Boedeker NC, Howard L. Serial ultrasonographic evaluation of the non-gravid and gravid uterus of the giant anteater (*Myrmecophaga tridactyla*) for determination of pregnancy, estimation of parturition date, and breeding management. In: Proc Am Assoc Zoo Vet; 2009. p. 806–812.

Accepted for publication 1 March 2017