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Ultrasonic measurements of second and third trimester fetuses to predict gestational age and date of parturition in captive and wild spotted hyenas *Crocuta crocuta*

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Abstract

Parturition in spotted hyenas (*Crocuta crocuta*) is a fascinating event to witness, as females of this species are highly masculinized and give birth through a penis-like clitoris. Furthermore, shortly after birth, a high rate of aggression occurs between littermates that can sometimes end in siblicide. To study these events thoroughly, an accurate estimate of the date of parturition is necessary. To this end, we performed transabdominal ultrasounds every 20–30 days in five captive spotted hyenas of known gestational age, beginning approximately 30 days after mating. We measured the femur length (FL), abdominal circumference (AC), and biparietal diameter (BPD) of eight fetuses from Days 42 to 100 of their 110 days of gestation. FL proved to be the most effective measurement, as it correlated well with gestational age and was easy to obtain consistently. The relationship between estimated gestational age (EGA) and FL is described by the equation: [EGA $= 37.3 + (14.0 \times$ FL)]. AC also correlated well with EGA, but was more difficult to measure than FL. Measuring BPD became increasingly difficult as pregnancies advanced beyond 70 days of gestation. Because gestational age is often not known in captive and free-ranging spotted hyenas, measuring fetal FL ultrasonographically is a rapid and reliable way to determine an approximate date of parturition. This technique proved invaluable when used to track and monitor a free-ranging spotted hyena during the days just before and after parturition.

Keywords: Spotted hyenas; *Crocuta crocuta*; Ultrasound; Fetus

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1. Introduction

Female spotted hyenas (*Crocuta crocuta*) are highly masculinized, as evident by the absence of an external vagina and the presence of a penis-like clitoris, through which the female copulates, voids, and gives birth [1–4]. This virilization has been associated with the peculiar hormonal milieu of these animals during sexual differentiation, i.e. high levels of androgens throughout gestation [5]. Giving birth to relatively large (1.1–1.6 kg) young after 110 days of gestation [6] complicates parturition in these remarkably virilized females. This masculinization of the external genitalia carries considerable reproductive costs, as spotted hyenas are thought to experience high rates of fetal death and maternal mortality [7]. However, detailed observations and descriptions of parturition in spotted hyenas are rare [8], particularly in the wild [9,10]. As such, the reproductive biology of the spotted hyena has been of interest to several groups of investigators who study these animals in the wild and in captivity. Spotted hyenas are also commonly displayed in zoos around the world, and maintenance of these zoo populations frequently depends on successful breeding programs.

The usual litter size in spotted hyenas is two, but singletons are a regular occurrence and triplets have been reported in captive and free-ranging animals [11]. The high rate of fetal death, especially in primiparous females, may result in fetal number and litter size being unequal. This inequality may also arise from siblicide among littermates at an early age, with subsequent ingestion of the victim by the mother, other members of the clan, or by scavengers [12,13]. These events, if not witnessed by observers, would lead to an underestimation of reproductive effort. Thus, accurately predicting the date of parturition can help researchers focus their efforts to carefully observe pregnant females around this critical period of time when litter reduction often occurs. Determining fetal number can be accomplished by a cursory ultrasonic exam, but accurately estimating gestational age is also essential, and requires a more detailed study. If a reduction in litter size is observed, knowing where and when parturition occurred may help to establish the time and cause of death, and to determine which cub died.

Observing the actual birth of a hyena cub also requires knowledge of the expected date of parturition. However, in many instances mating is not observed, and pregnancies in both wild and captive animals may not be detected, if detected at all, until the female shows overt signs of pregnancy, such as increased abdominal girth. Under such conditions, an estimate of gestational age by ultrasonography can help investigators determine when free-ranging animals should be closely monitored and when captive animals should be confined to afford the observation of parturition.

Gestational age has been estimated during the second and third trimesters by ultrasonic fetometry in many species of domestic and nondomestic mammals [14]. Measurement of the fetal biparietal diameter (BPD) has been commonly used in a number of ungulate species [15–18], but BPD can be difficult to measure during the latter part of pregnancy [17], as accurate measurements require proper orientation of the ultrasound probe to identifiable landmarks within the brain. Fetal position and ossification of the cranium can make identification of these landmarks difficult. Additionally, decreases in the volume of amniotic fluid toward the end of pregnancy can make clear visualization of the fetal head difficult.

As part of the research and breeding program at the Spotted Hyena Project of the University of California, Berkeley, real-time ultrasound has been used for several years to detect and monitor the pregnancies of spotted hyenas. Until recently, measurements were limited to the crown-rump length (CRL) of the fetus, which proved satisfactory for dating pregnancies during the first trimester. However, measurement of CRL becomes impractical and technically difficult because of increased fetal size when gestational age advances beyond 40–50 days. We now limit the use of the CRL to early gestation. We report here on the accurate estimation of gestational age in the spotted hyena during the second and third trimester by ultrasonographic measurement of fetal BPD, abdominal circumference (AC), and femur length (FL). We compare these three measurements for accuracy and consistency, and describe the use of FL measurement to estimate gestational age and predict the date of parturition in a free-ranging spotted hyena.

2. Materials and methods

2.1. Animals

Five pregnant spotted hyenas were studied at the Field Station for the Study of Behavior, Ecology, and Reproduction at the University of California, Berkeley. Animals had been born and raised at the Berkeley field station. Eight fetuses, from two singleton and three twin gestations, were monitored by serial ultrasonography starting at Days 34–42 post-mating. The date of mating was known for 3 of the 5 females. Measuring CRL on the fetuses of these three females on Days 34, 37, and 42 allowed us to estimate the gestational age, and thereby the date of mating for the two females in which mating was not directly observed.

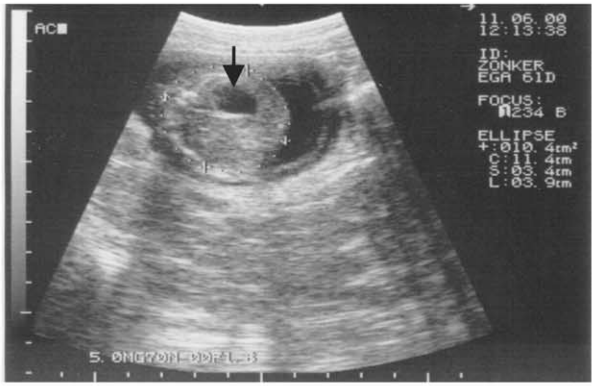
In captivity, all sonograms were performed transabdominally by a single investigator using a real-time Aloka SSD-500 scanner with a 5 MHz curved-array transducer. Prior to each examination, animals were immobilized with an intramuscular injection of Ketaset (4–6 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (1 mg/kg; Phoenix Scientific, Inc., St. Joseph, MO) administered by a blow-dart. Animals were scanned in the supine position after removing fur from the lower abdomen with electric clippers.

A free-ranging spotted hyena in the Masai Mara of Kenya underwent transabdominal ultrasonography using a real-time Hitachi EUB-405 scanner with a 5 MHz curved-array transducer. This animal was immobilized with an intramuscular injection of Telazol (2.5 mg/kg; Wildlife Pharmaceuticals, Fort Collins, CO) administered by dart gun.

2.2. Ultrasonography

We scanned captive females every 20–30 days throughout pregnancy, beginning with the measurement of CRL on Days 34–42 post-mating. We attempted to measure fetal BPD, AC, and FL on each occasion (12 exams total) from Days 42 to 100 post-mating. The number of viable fetuses was determined by presence of fetal cardiac activity. We measured FL by placing electronic calipers at the proximal (greater trochanter) and distal edge of the femoral shaft, as described by O'Brien and Queenan [19], Fig. 1A. We measured AC at a cross-sectional plane that contained the stomach and the umbilical vein

(A)



(B)

(C)

Fig. 1. Ultrasonic images of spotted hyena fetuses showing orientation for measurement of femur length (A), abdominal circumference (B), and biparietal diameter (C). Fetal stomach "bubble" is evident as a hypoechoic region within the image of the fetal abdomen (arrow in B). Cavum septum pallucidum (CSP) is identified within the cranium (C). Image axes are in 1 cm increments.

within the fetal liver [20]. Electronic calipers were placed at opposite edges of the abdomen, from the surface overlying the vertebral column across to the ventral surface; an elliptical function then circumscribed the abdomen and calculated AC, Fig. 1B. BPD was measured from the outer surface of the distal calvarium to the inner surface of the proximal calvarium, but only when the plane containing the cavum septum pellucidum could be identified, as described by Nyberg [21], Fig. 1C.

A single ultrasound was carried out on the free-ranging spotted hyena in Kenya. Fetal number was confirmed by fetal cardiac activity, and FL was measured as above. Gestational age was estimated by an equation derived from FL measurements in captive hyenas of known gestational age.

2.3. Statistical analysis

The relationships between estimated gestational age (EGA) and each of the ultrasonographic measurements (FL, AC, and BPD) were plotted as linear regressions and expressed as straight-line equations using Statview 5.0 (SAS Institute Inc., Cary, NC). Plotting residuals against observed gestational age supported the linear relationship between each of the three biometric variables and gestational age.

3. Results

An equation, [EGA $= 28.40 + (2.94 \times$ CRL)] ($r^2 = 0.994$, $P = 0.003$), derived from the measurement of CRL in the three females, for which the date of mating was known, enabled us to accurately estimate gestational age in the two females that had not been observed mating. The estimated date of mating calculated from the measurement of CRL agreed with an estimated date based on the observed date of parturition by ± 1 day.

We successfully obtained a measurement of FL and AC in all exams we attempted at 48 days or more post-mating. We were able to measure BPD slightly earlier in gestation, when CRL could also be measured accurately (42 days post-mating). However, we were unable to see the intracranial structures required for an accurate measurement of BPD in 6 of 12 ultrasounds performed between Days 42 and 100 post-mating. This typically occurred after 70 days of gestation. In one instance we were able to measure BPD in one twin, but not the other. FLs were highly correlated between twins ($r = 0.991$, $P < 0.0001$), as were the measurements for AC ($r = 0.991$, $P < 0.0001$); this relationship could not be adequately explored for BPD.

The relationship between EGA and FL can be described by the equation: [EGA $= 37.3 + (14.0 \times$ FL)], ($r^2 = 0.987$, $P < 0.0001$; Fig. 2A). The equation for estimating gestational age by AC was similarly effective at predicting gestational age: [EGA $= 30.7 + (2.6 \times$ AC)], ($r^2 = 0.927$, $P < 0.0001$; Fig. 2B). The linear regression equation for estimating gestational age by BPD actually had the highest coefficient of determination: [EGA $= 22.8 + (16.8 \times$ BPD)], ($r^2 = 0.997$, $P < 0.0001$; Fig. 2C), but this was effectively limited to the second trimester, when BPD could be consistently measured. After Day 70 of pregnancy, gestational age could be estimated by measuring either FL or AC, but finding the appropriate landmarks for measuring FL was easier and faster. For

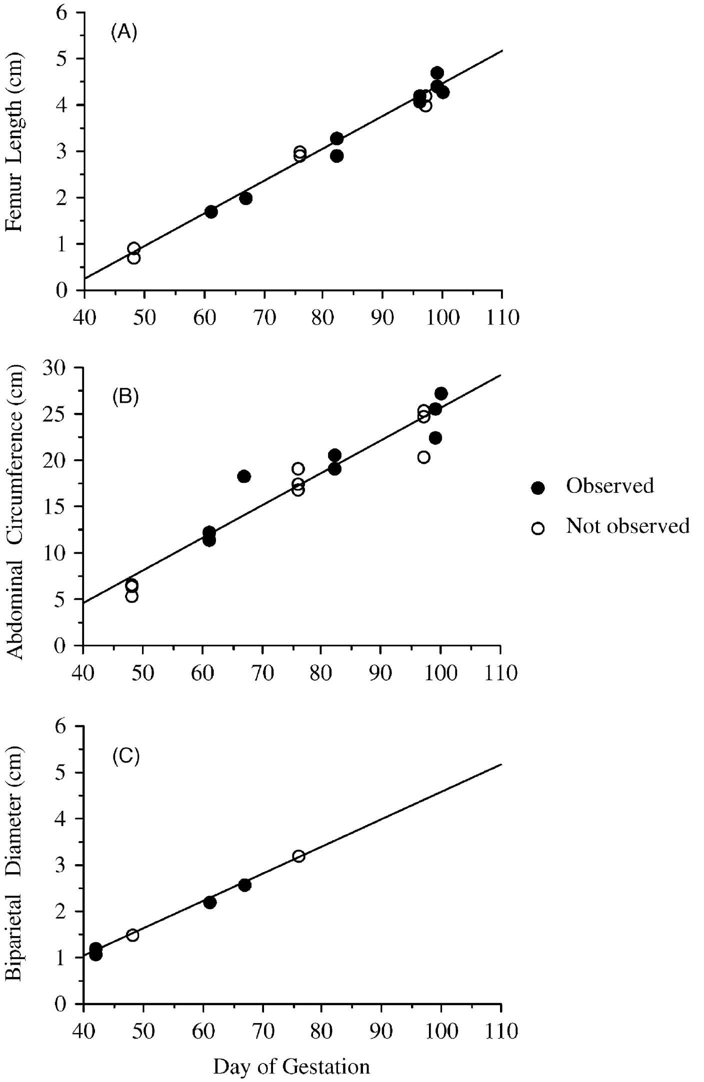


Fig. 2. Linear regression models used to calculate estimated gestational age by measurement of femur length (A), abdominal circumference (B), and biparietal diameter (C). Data points are from spotted hyenas for whom the date of mating was either directly observed (filled circles), or calculated by measuring crown rump length during the first trimester (open circles). Note: BPD measurements are limited to the period between 42 and 76 days post-conception. Owing to the reduction in amniotic fluid volume and the ossification of the cranium, appropriate landmarks could not be visualized later in gestation.

example, in one twin gestation at an EGA of 96 days, we rapidly measured FL to expedite the preparation for a Cesarean delivery, which was carried out as part of a separate study.

In the wild spotted hyena, gestational age was estimated at 65 days post-conception by measurement of fetal FL by real-time ultrasound. Knowing an approximate date of parturition in this hyena proved to be useful, as tracking efforts intensified as the estimated date of parturition approached. Parturition occurred within 1 week of the date predicted by ultrasound.

4. Discussion

Fetometry by real-time ultrasound during the second and third trimesters of pregnancy in the spotted hyena can accurately estimate gestational age and help predict the expected date of parturition. If carefully measured after identification of appropriate anatomical landmarks, the three measurements used in the present study, FL, AC, and BPD, were similarly effective in estimating gestational age. However, FL has advantages over the two other measures, especially BPD, as this measurement was difficult to obtain in late gestation. Haibel and Fung [17] had similar difficulties measuring BPD during late gestation in the llama (*Lama glama*). Additionally, the high correlation in FL between twins means gestational age can be accurately estimated by measuring FL in a single twin. This can reduce the time necessary to perform the examination, minimizing the risks associated with anesthesia.

The technique to measure FL was taught to a novice ultrasonographer during a single session and was then successfully used several months later to estimate gestational age in a wild spotted hyena in Kenya. Further attempts to predict an approximate date of parturition in the field may prove more accurate in the future, as this initial field trial of the FL technique occurred after only three of five captive hyenas had been scanned. Inclusion of results from two more pregnant females led to an improvement in the correlation coefficient between FL and EGA. Nevertheless, use of the FL technique in the field allowed us to intensify the monitoring of this pregnant female during a narrow window of time around the expected date of parturition. As a result, the natal burrow was discovered, litter size was compared to fetal number, and the activity of the mother and her offspring was observed. Interestingly, litter reduction appears to have occurred, as two viable fetuses were seen by ultrasonography on Day 65 of gestation, but only a single cub was ever seen. Litter reduction may have occurred anytime between Day 65 of gestation and 1 month post-parturition. Siblicide, as well as predation, infection, and starvation has to be included among the possible explanations for litter reduction. Fetal death is a less likely explanation, as this multiparous female successfully gave birth in the previous year. In captive spotted hyenas, the fetal death rate at parturition is high (61%) in primiparous females, but this outcome is much lower (7%) in multiparous females [7].

Frank and Glickman [8] described a captive hyena pregnancy that was undetected until late in gestation. Parturition was not monitored and the mother was found with a partially eaten newborn cub. Unfortunately, the cause and time of death could not be determined. Thus, an opportunity to gather additional information regarding reproduction in spotted hyenas was lost. Moreover, a number of pregnant hyenas have experienced potentially lethal complications associated with the birth of relatively large cubs [7,8]. Close monitoring around the expected date of parturition can facilitate early intervention by veterinarians [8].

In summary, the fetal femur in the spotted hyena can be easily visualized by real-time ultrasonography throughout the second and third trimesters, and the technique to measure

FL is relatively easy to teach and learn. The use of diagnostic ultrasonography has been extended to the study of pregnancy and parturition in a nondomestic carnivore, both in the wild and in captivity. As ultrasound devices have become increasingly more portable, their use and application in the field should continue to grow and include more species [22]. The ability to accurately estimate the date of parturition in wild animals has practical applications in conservation management. Endangered species are often closely monitored, and some situations (e.g. capture for radio-collar placement or relocation) lend themselves to the use of ultrasound for detection of pregnancies. When pregnancy is detected in an endangered or threatened animal, and the date of parturition can be accurately estimated, insightful management decisions can be made on behalf of that animal and her unborn offspring.

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