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Prediction of gestational age by ultrasonic fetometry in llamas (*Lama glama*) and alpacas (*Lama pacos*)

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Abstract

Fetal biparietal diameter (BPD) and thorax height (TH) were measured by ultrasound during intrauterine growth in pregnant llamas (*Lama glama*) and alpacas (*Lama pacos*). The goal was to establish representative curves that allows estimation of gestational age (GA) from real-time ultrasonic measurements of these fetal structures at any stage of gestation. Llamas and alpacas were mated under controlled conditions. Ultrasound exams were conducted to determine pregnancy status 1 month later. Measurements of fetal BPD and TH were conducted from the second month of pregnancy until term. Observation and assessment of fetal TH was difficult during the last 3 months of pregnancy, specially in llamas. Regression curves were calculated from the data as a function of GA, with the best fit represented by the following equations: llama GA $=$ (BPD $-$ 0.002399)43.02293, $r = 0.98$, $P < 0.001$; llama GA $=$ (TH $-$ 0.07137)46.94485, $r = 0.95$, $P < 0.001$; alpaca GA $=$ (BPD $-$ 0.11376)47.23287, $r = 0.98$, $P < 0.001$; alpaca GA $=$ (TH $-$ 0.36436)52.87663, $r = 0.96$, $P < 0.001$, where GA was measured in days and BPD and TH in centimeters. Results indicate that ultrasonic measurement of these fetal biometric variables constitute a valuable tool to estimate GA at any stage of pregnancy in these domestic South American camelids.

Keywords: Fetal biometry; Llama; Alpaca; Pregnancy; Ultrasound

1. Introduction

Breeding of domestic South American camelids, llamas (*Lama glama*) and alpacas (*Lama pacos*), has acquired great relevance, and today these species have an important productive and economic role in farming activities.

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Like traditional livestock, the success of South American camelid breeding, depends on adequate reproductive management. Llamas and alpacas are induced ovulating species and have low reproductive rates in comparison with spontaneous ovulating ruminants, especially considering the large number of matings required to obtain pregnancy (Novoa, 1970; Fernández-Baca, 1993; Ullrich, 1996). This unfavorable characteristic results from a long sexual receptive period, in which the ovaries contain follicles of different diameters and developmental stages, allowing some times to anovulatory matings or delayed ovulations (Bravo, 1994; Smith et al., 1994). Thus, the prolonged mating periods to obtain pregnancy, where females can be inseminated many times by the males, compromises the knowledge of gestational age and the probable date of parturition.

Use of ultrasound to diagnose early pregnancy in llamas and alpacas has been previously reported (Bourke et al., 1992; Mialot and Villemain, 1994; Parraguez et al., 1997). Although, this procedure has been shown to be efficient during the first month after a fertile mating, there is no consistent data to assess camelid pregnancies later in gestation.

In addition to livestock and pet interest, during recent years domestic South American camelids are being used as an animal model to study fetal and maternal physiological adaptations to low oxygen pressure environments (Moraga et al., 1996; Blanco et al., 1997; Llanos et al., 1998; Riquelme et al., 1998; Giussani et al., 1996, 1999). Since protocols used require fetuses of known gestational age and normal developments, ultrasonic fetometry has been of invaluable importance. In the available literature there are only three reports of fetal growth during pregnancy in domestic South American camelids. However, these studies do not consider the entire pregnancy period (Haibel and Fung, 1991; Iason et al., 1993; Mialot and Villemain, 1994).

The goal of the present study was to describe in llamas and alpacas, the fetal biparietal diameter and thorax height during intrauterine growth and to establish representative curves that can allow estimation of gestational age from measurement of these fetal structures by real-time ultrasound.

2. Material and methods

Pregnant llamas ($n = 10$) and alpacas ($n = 10$), ranging from 4 to 6 years of age were used. Animals were healthy and had at least one normal pregnancy and parturition before inclusion in this study. Llamas and alpacas were maintained as independent groups in different pens with free access to food and water. Matings were done by controlled daily introduction of known fertile males to the females. Each female was separated immediately after an effective mating (with penis introduction in the female genitalia and intercourse duration for over 12 min) and no exposure to the male was repeated. Pregnancy was established by transrectal ultrasound examination 25–30 days after mating.

From day 30 until day 60 of gestation, pregnant females were examined by ultrasound once a week, and at 15-day intervals until parturition occurred. Exams consisted in measurements of fetal biparietal diameter (BPD) and fetal thorax height (TH). There was specific rationale for choosing these biometric characteristics. First, fetal head and thorax are easily to observe and define during all the gestation. Second, measurement of DBP is one of the most effective ways to determine stage of pregnancy in humans (Sabbagha, 1987), and has the best correlation with gestational age in different species, including ruminants (White et al., 1985; Kahn, 1989; Aiumlamai et al., 1992; Parraguez et al., 2000) and non-human primates (Nyland et al., 1984; Corradini et al., 1998). Thirdly, there is a high correlation between thorax circumference and gestational age in humans and other primates. However, as the ultrasound equipment for veterinary use does not always easily facilitate the measurement of circumference, a linear thorax measurement was preferred, considering in addition the high predictive value of the TH obtained in a previous study in fetal capuchin monkeys (Corradini et al., 1998).

BPD was measured transversal to the occipitofrontal axis, choosing the largest and clearest images, by setting the calipers on the external side of temporoparietal bones. TH was measured transversal to the sagital axis, as the distance between the sternum and vertebral column, by setting the calipers on the external surface of the bones and crossing the middle of the heart. Exams were conducted using a real-time B-mode ultrasound apparatus and a 5 MHz linear probe. Transrectal or transabdominal evaluations were alternatively used, depending on the pregnancy state and fetal position. Measurements were taken with internal calipers and images were recorded with a videographic printer connected to the ultrasound apparatus.

Predictive regression curves for each variable as a function of the gestational age were calculated by the best adjustment of the experimental data to a theoretical function. Length of gestation and body weight at birth are presented as averages and corresponding standard deviations ($X \pm$ S.D.M.).

3. Results

Animals were not subjected to any unnecessary suffering, and as indicated in a previous work (Parraguez et al., 1997), no distress or rectal bleeding was observed during or after ultrasound examination. All the females had normal gestations and parturitions. Average length of pregnancy periods were 352.6 ± 5.9 and 339.7 ± 12.0 days for llamas and alpacas, respectively. Body weight of the newborn was 12.3 ± 1.8 kg in llamas and 6.9 ± 0.8 kg in alpacas.

From the beginning of the study the fetuses were clearly observed, both in llamas and alpacas, appearing as an echoic mass located in the basal zone of the gestational sac. During the second month of gestation, definition of the different parts of the fetuses was difficult, however, observation of the heart beat allowed for definition of the head and thorax (Fig. 1).

Between 30 to 70 days of pregnancy, it was difficult to take the measurements as described in Section 2. Thus, the BPD during this period of pregnancy was measured as the greatest head diameter, perpendicular to the major axis of the embryo, without considering skull axis or bony structures. Similarly, TH was measured as the distance between the ventral and dorsal border of the thoracic cavity, crossing the middle of the heart, because it was not always possible to identify the sternum or vertebral column.

BPD measurements were conducted transrectally until the sixth month of pregnancy. Subsequently, the pregnant uterus dropped into the abdominal cavity, thus transrectal and transabdominal methods of evaluation were used after this occurred. From 11 months until delivery, the fetal head was located in the pelvic cavity, with the transrectal method being

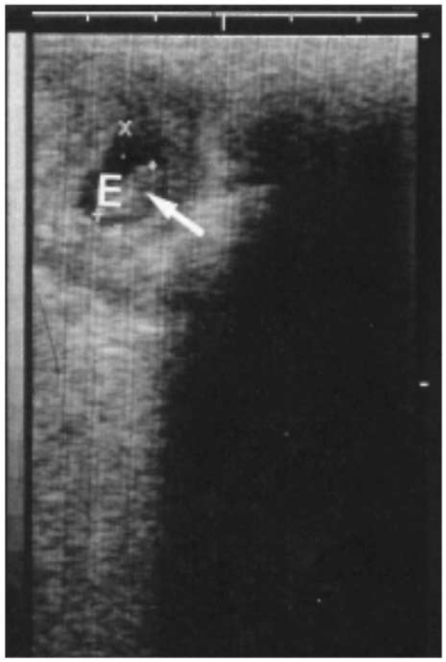

Fig. 1. Echographic image of pregnant uterus in a alpaca at 34 days of gestation. The embryo (E) is located on the basal zone of the gestational sac. The arrow indicate the position of the heart: ×, dorsoventral limits of the gestational sac; +, longitudinal axis of the embryo. Scale 1:1.16.

used after this occurred. In the case of TH, measurements were done through the transrectal route until the fifth month of pregnancy. From this time until term, TH measurements were conducted transabdominally.

3.1. Llamas

A total of 132 fetal BPD and 79 fetal TH measurements were obtained. Linear regression curves (Figs. 2 and 3) were calculated from the data as a function of gestational age. Best fitted functions were

$$\mathrm{GA} = (\mathrm{BPD} - 0.002399)\,43.02293, \qquad r = 0.98, \quad P < 0.001$$

$$\mathrm{GA} = (\mathrm{TH} - 0.07137)\,46.94485, \qquad r = 0.95, \quad P < 0.001$$

where GA was measured in days and BPD and TH in centimeters.

3.2. Alpacas

During the entire gestational period, 126 fetal BPD and 111 fetal TH measurements were obtained. The corresponding regression curves were also calculated from measurements of

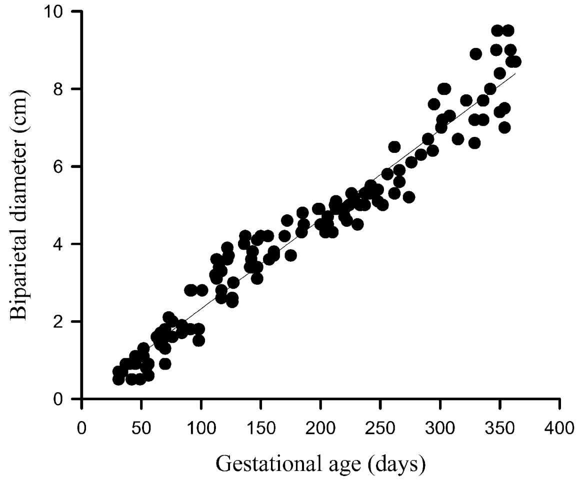

Fig. 2. Regression curve (line) of the fetal BPD at different gestational ages. Points represent individual ultrasonic measurements ($n = 132$) obtained from 10 pregnant llamas.

the variables (Figs. 4 and 5). Data were best fitted to the following functions:

$$\mathrm{GA} = (\mathrm{BPD} - 0.11376)\,47.23287, \quad r = 0.98, \quad P < 0.001$$

$$\mathrm{GA} = (\mathrm{TH} - 0.36436)\,52.87663, \quad r = 0.96, \quad P < 0.001$$

where GA was measured in days and BPD and TH in centimeters.

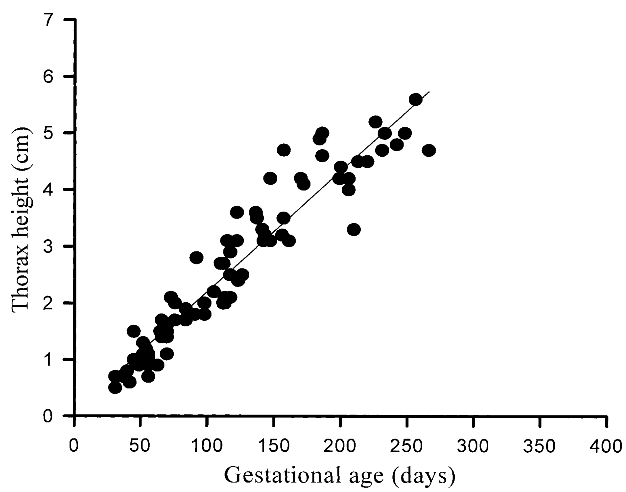

Fig. 3. Regression curve (line) of the fetal TH at different gestational ages. Points represent individual ultrasonic measurements ($n = 79$) obtained from 10 pregnant llamas.

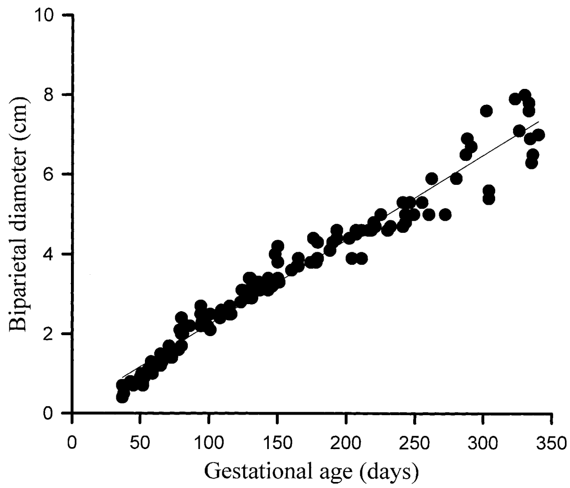

Fig. 4. Regression curve (line) of the fetal BPD at different gestational ages. Points represent individual ultrasonic measurements ($n = 126$) obtained from 10 pregnant alpacas.

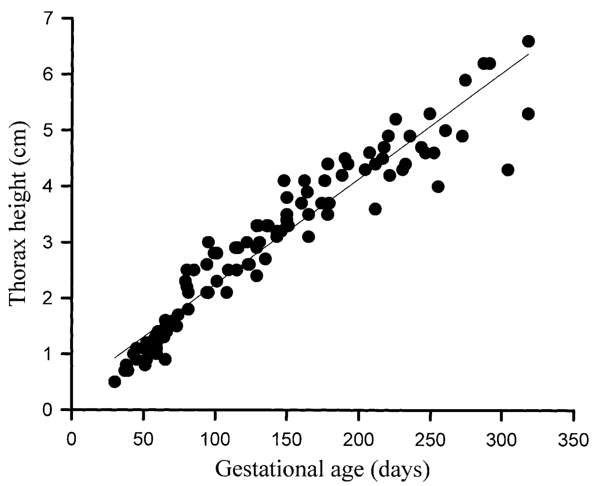

Fig. 5. Regression curve (line) of the fetal TH at different gestational ages. Points represent individual ultrasonic measurements ($n = 111$) obtained from 10 pregnant alpacas.

Representative images of fetal BPD and TH from llamas and alpacas at different gestational ages are presented in Figs. 6–9.

4. Discussion

All pregnancies resulted in normal deliveries and healthy offsprings. Indeed, the continued manipulation, as a consequence of ultrasonography, seems to not be of significant

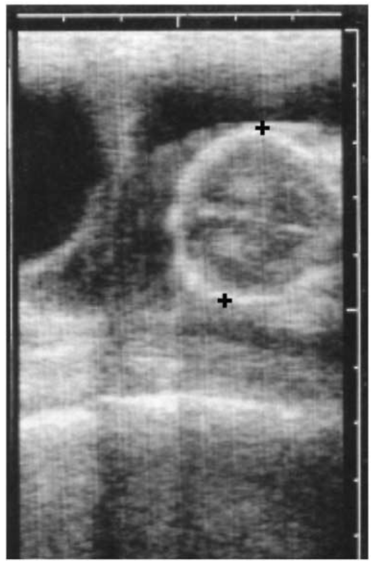

Fig. 6. Echographic image of the head in a llama fetus of 120 days of gestational age: +, limits of the biparietal diameter. Scale 1:1.03.

Fig. 7. Echographic image of the thorax in a llama fetus of 200 days of gestational age. The heart (H) it is observed at the right side as an anechoic structure: +, limits of the thorax height. Scale 1:1.

Fig. 8. Echographic image of the head in a alpaca fetus of 170 days of gestational age: +, limits of the biparietal diameter. Scale 1:1.03.

Fig. 9. Echographic image of the thorax in a alpaca fetus of 50 days of gestational age: +, limits of the thorax height. Scale 1:1.08.

disturbance to the pregnancy of females in domestic South American camelids. Gestation length and weight at birth, both in llamas and alpacas were of normal ranges (San Martín et al., 1968; Franklin, 1982; Novoa, 1991; Raggi et al., 1997).

Results of this study allowed for generation of predictive equations of gestational age from fetal BPD and TH measurements in pregnant llamas and alpacas. Although, during development of the protocol there were some inconveniences in obtaining fetal measurements, the resultant functions were reliable predictors of gestational age, as indicated by the highly significant correlation coefficients (between 0.95 and 0.98).

Although the proposed protocol for the study considered taking measurements of the variables once a week during the first month of pregnancy and every 15 days from the second month until delivery, this was not possible due to some practical difficulties presented from the middle of gestation. This resulted in an unequal distribution of the data, where more than 50% correspond to observations made during the first 5 months of pregnancy. Certainly, the greater frequency of measurements during the initial months of pregnancy contributed to these differences. However, the fetal and uterine changes at this gestational period are the primary explanatory factor. The pregnant uterus drops into the abdominal cavity after 4 months of pregnancy (Bravo and Varela, 1993), which explains the complications of taking transrectal measurements after this time. Furthermore, the fetal head and thorax are usually located distant from the pelvic cavity, a situation that in the case of the fetal head is reversed near term.

Both in llamas and alpacas, the best representative functions of gestational age were obtained from BPD measurements, due to large number of measurements of this variable during all gestational periods, in contrast with fetal TH. Therefore, considering both the predictive value for gestational age and the ability to obtain the measurements of fetal BPD during all gestational ages, this variable is of great practical value. This observation is consistent with those reported in other species of ruminants (Kahn, 1989; Aiumlamai et al., 1992) and primates (Shimizu, 1988; Herring et al., 1991; Corradini et al., 1998).

Although in the case of llamas, the best adjusted function of fetal BPD was linear, the distribution of experimental data indicated an exponential tendency after 150 days of pregnancy, a tendency also observed by Iason et al. (1993) in a study of the fetal llama BPD between days 84 and 271 of pregnancy. As a consequence of this observation, an exponential regression was calculated for fetal BPD in llamas (not shown), however, the correlation coefficient was less than a linear function (0.8 versus 0.98, respectively), so to estimate gestational age the linear equation is preferred.

In a partial ultrasound study of fetal growth in llamas, a linear regression function was obtained from fetal BPD measurements and the corresponding gestational age (Haibel and Fung, 1991). Although these authors do not consider two initial and three last months of pregnancy, there are very few differences in gestational ages obtained when predictive calculations are done using the equation of Haibel and Fung (1991) or the equation reported in the present work. In contrast, greater differences exist when comparisons are made with another study in llamas (Iason et al., 1993). It is important to emphasize a considerable underestimation of the fetal measurements in later gestation in this latter study. A probable explanation for these differences is that the authors used only the transabdominal method to take fetal measurements, a method that in our experience and in that reported by Mialot and Villemain (1994), results in less precision of the images in contrast with the transrectal method.

In the case of fetal llama TH, although the predictive value of the resultant function was also greater, obtaining measurements of TH was more difficult than that of BPD at any stage of gestation. Being critical after 8 months of pregnancy, measurement of this fetal variable was, therefore, a less practical value. Previously reported results from fetal TH measurements between 3 and 6 months of pregnancy (Iason et al., 1993), indicated notable differences with our results, overestimating the size of the variable at any stage of gestation. As was discussed for BPD, a possible explanation for differences is the use of transabdominal method by the authors.

For fetal alpacas, the best representative curve of BPD as a function of gestational age was also of linear shape. Similar to that observed in llamas, there was an apparent exponential distribution of the data after 150 days of pregnancy, but a function of this type provided for a lesser correlation coefficient. Mialot and Villemain (1994) reported fetal BPD ultrasound measurements in alpacas between 6 and 8 months of pregnancy. They did not calculate a predictive equation, but BPD measurements reported coincided with measurements obtained from the equation reported in the present research.

Measurement of fetal TH in alpacas was less difficult than in llamas, as suggested by the amount of data obtained. The most important problems in taking measurements appeared after 8 months of gestation, being critical near term when it was not possible to observe clearly the fetal thorax. Data were also best fitted to a linear function, this variable having a greater predictive value than in llamas. Mialot and Villemain (1994) have shown some measurements of fetal thorax diameter in alpacas between 140 and 230 days of gestation. Only the first measurements (140 days of gestation) are consistent with the results reported in the present study. All the subsequent data overestimate progressively the characteristic as gestation advances. Although the authors had used a 5.0 MHz linear probe, alternatively by transrectal or transabdominal methods, as in the present study, a probable explanation for the differences may be the anatomical position in which the measurements were taken. Authors do not describe how the thorax diameter was measured, however, the greater thorax size in comparison with the present results suggests that measurements could have been obtained in a more caudal position, nearest to abdomen, where the thorax amplitude is larger than at the central core position. With the fetal TH size estimation, it is very important to define clearly the anatomical position in which measurement should be performed, because of the obvious cranium–caudal differences. Anatomical position is also considered in the biometry of adult camelids, when body weight is estimated through measurement of thoracic perimeter (León et al., 1989).

Comparing the growth patterns of DBP and TH between llamas and alpacas, it is noteworthy that, although the slopes of growth curves are different, at early stages of gestation there are no remarkable differences in size. However, after 150 days of gestation a greater increase in size is observed in llamas in both fetal DBP and TH, explaining the evident differences in size and weight at parturition.

Finally, it is important to consider that ultrasonic fetal biometry constitutes a valuable method for pregnancy diagnosis and the state of pregnancy, as well as fetal well being, growth and development, characteristics that can allow for improvement of the reproductive management and contribute to fetal physiology research in South American camelids.

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