Use of fetal biometry to determine fetal age in late pregnancy in llamas

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Abstract

Sixty-three pregnant llamas of known breeding date were used in this study. Forty-six of them were submitted to surgery between 186 and 320 days of gestation (52–91% of average gestation period, respectively). Under general anesthesia their fetuses were exteriorized and fetal weight (W), biparietal diameter (BPD) and femoral (F), tarsus-hoof (T-H), tibial (T)) and fronto-occipital (F-O) length were determined. Additionally, the same variables were determined on 16 newborn llamas. The weight was measured in kg and the length in cm. All the collected data was entered into a spreadsheet and different regression analyses as a function of gestational age (GA) were assessed. The best fit equations and their correlation for linear regression were the following: $GA = 169.448 + 16.66^{*}W$, $r = 0.99$; $GA = -51.713 + 44.77^{*}BPD$, $r = 0.88$; $GA = -72.139 + 39.48^{*}F\text{-}O$, $r = 0.71$; $GA = 39.304 + 8.35^{*}T\text{-}H$, $r = 0.97$; $GA = 91.276 + 8.23^{*}T$, $r = 0.86$; $GA = 102.029 + 9.94^{*}F$, $r = 0.91$. For multiple regression, the dependent variable GA can be predicted by the following equation: $GA = 67.462 + 11.163^{*}W + 20.297^{*}BPD$. Results of the present study indicated measured variables to be highly correlated with GA. This could be useful on daily basis in clinical examination of the neonates, in assessment of fetal growth and well being with cesarean sections, in the determination of GA in late gestation abortions, and in perinatal and reproductive research in the llama.

Keywords: Fetometry; South american camelids; Newborn; Fetal growth; Pregnancy

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1. Introduction

In the last decade, the interest in Domestic South American camelids (DSAC) has increased in different areas, such as meat and fiber industry (Raggi, 2000), as company animals and in research (Herrera et al., 2000; Llanos et al., 1998). The llama (*Lama glama*) and alpaca (*L. pacos*) are species in which ovulation is induced by the mating stimulus. They present low fertility rates in comparison to other domestic ruminants (Fernández-Baca, 1993; Wright y cols, 1998). In addition, female DSAC are uniparous and have long gestational periods of approximately 347 days (Brown, 2000). These characteristics make good reproductive management necessary to improve productive efficiency.

Ultrasonographic techniques allow determination of early pregnancy and the gestational age (GA) in these animals (Bourke et al., 1992; Parraguez et al., 1997). They are also very helpful in the mating and calving planning. In contrast, this technique loses efficiency in late gestation, because the fetus shifts and drops into the abdominal cavity and then it is not easily accessible via ultrasonography, and to the large size of its corporal structures (Parraguez et al., 1997). Eventually, rectal palpation could be useful for the diagnosis and evaluation of gestation (Alarcón et al., 1990). For this purpose, there are some prerequisites to consider, such as hand size of a skilled examiner and size of the animal. Generally only larger animals can be rectally palpated (Fowler, 1989). Although, a subjective measurement, the experienced professional can estimate the size of fetal structures when making rectal palpation.

Some previous studies made relatively good approximations for determining GA using fetal biometry, but in none of these studies has there been used this procedure during late gestation by measuring fetal weight (W) and body proportions (Gazitúa et al., 2001; Haibel and Fung, 1991; Iason et al., 1993; Mialot and Villemain, 1994). Nowadays it is very important to have these approaches to make an adequate diagnosis of fetal age in obstetric examinations, abortion and parturition where the breeding date is unknown. This could be particularly useful for many DSAC breeders in South America, where males and females are maintained in the same yard all year long with no mating programs.

The aim of the present study, was to develop procedures to estimate fetal age through correlation of W, biparietal diameter (BPD) and femoral (F), tarsus-hoof (T-H), tibial (T) and fronto-occipital (F-O) length with GA, and calculate the equations and parameters which inter-relate these variables in late-gestation llamas.

2. Materials and methods

2.1. Use of animals

Pregnant llamas ($n = 63$) with known breeding dates from the University of Chile farm at Rinconada de Maipú, 39°29′34.4″ Lat S; 70°49′24.5″ long W, at 580 m above sea level were used in the present study. They were mated at a known date and their pregnancy was diagnosed at day 20 after mating. The gestation was monitored by ultrasonography (Aloka, Echo chamera SSO-210 DXII) using a linear 5 MHz trans-rectal probe at 2 month intervals until the date of surgery or parturition. Upon arrival in Santiago, (585 m above sea level), the llamas were housed in an open yard with access to food and water ad libitum.

Pregnant llamas ($n = 46$) were submitted to a cesarean section and their fetuses used for physiological studies between 186 and 320 days of GA. Sixteen newborn llamas were weighed and their dimensions measured at birth. All animals included in this study were clinically healthy.

2.2. Surgical preparation

Maternal and fetal surgeries were conducted using well-established techniques previously described in detail (Benavides et al., 1989; Llanos et al., 1995). Briefly, following food and water deprivation for 24 h, the maternal llamas were pre-medicated with atropine (1 mg, i.m., Atropina Sulfato, Laboratorio Chile®, Santiago, Chile) and prepared under general anesthesia (5–7 mg kg^{-1} sodium thiopentone, Tiopental Sódico, Laboratorio Biosano SA®, Santiago, Chile for induction and 0.5–2% halothane in 50:50 O_2 and N_2O for maintenance). Following a midline laparotomy, a cesarean section was conducted and the fetus used for physiological studies. At this time, the length of the femur (F), T-H and tibia (T) were determined. After these data were collected, the fetal BPD and F-O length were determined and the fetus was weighed.

During surgery, all llamas were constantly hydrated with a warm intravenous solution of 0.9% NaCl, at a rate of 15–20 ml kg^{-1} h^{-1} to compensate for fluid loss during the procedures. At the end of the surgery, ampicillin (500 mg; Ampicilina, Laboratorio Best Pharma®, Santiago, Chile) and gentamicin (80 mg; Gentamicina Sulfato, Laboratorio Biosano SA®, Santiago, Chile) were given intravascular. The newborn dimensions were taken during the first 6 h of birth, and they were the same measurements obtained in the fetuses.

All experimental protocols were reviewed and approved by the Faculty of Medicine Ethics Committee of the University of Chile. Furthermore, animal care procedures experimentation was conducted in accordance with The Guide for the Care and Use of Laboratory Animals of the National Research Council (1996).

2.3. Measurements and calculations

The length measurements were determined using a Vernier caliper. F was considered as the maximum length between the greater trochanter of the femur and the distal extreme of the lateral condyle (external condyle). T was considered as the maximum length between the T lateral condyle and the lateral maleolus. In the same way, T-H was measured as the maximum length between the calcanean tuberosity and the distal extreme of the hoof with the foot in extension position (Fig. 1a). BPD was measured as the distance between the extreme borders of the parietal bones, perpendicular to the F-O axis. In addition, F-O was established as the distance between the occipital prominence and the midline fronto-nasal junction (Fig. 1b). The fetal W was determined using a pediatric balance (Seca III, Germany).

2.4. Statistical analysis

A simple regression analysis was assessed by Spearman rank order correlation and used to correlate each variable as a function of GA. Differences where $P < 0.01$ were regarded as significant. A multiple forward stepwise regression analysis was used with GA as dependent

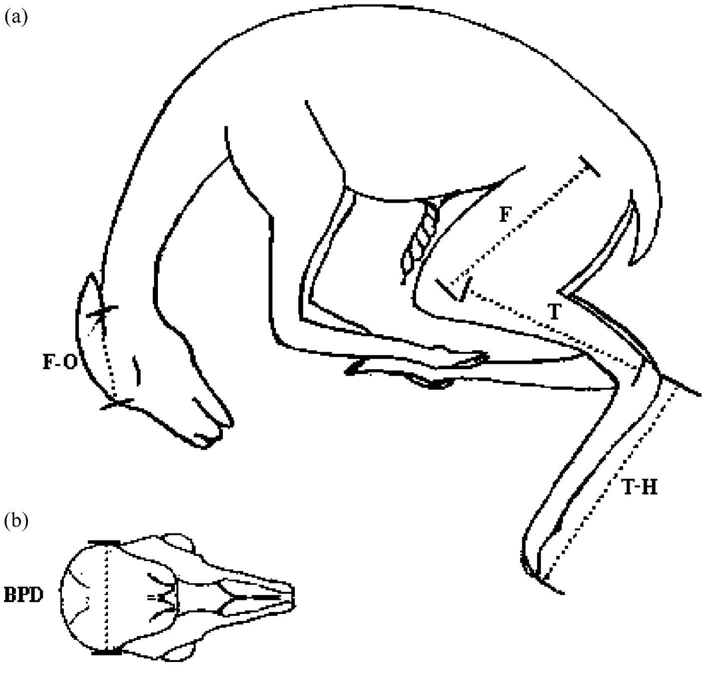

Fig. 1. Length measurements taken in the fetus and the newborn llama: (a) lateral view of the animal; (b) dorsal view of the head; BPD, biparietal diameter; F-O, fronto-occipital length; F, femoral length; T, tibial length; T-H, tarsus-hoof length.

variable. The 95% limits of confidence for each function were calculated. The regression lines predict theoretical days after mating. Gestational length and weight in newborn llamas were expressed as averages and standard error of the mean.

3. Results

3.1. Ultrasonographic and birth data

Ultrasonographic examinations showed the gestational sac and the embryo at 20 days of gestation. All females studied had normal initial gestations and those who gave birth had normal deliveries, except for two llamas that presented a positional dystocia. The 63 female llamas weighed $107 + 3$ kg, after the cesarean section or after giving birth. From the 63 pregnant llamas, 46 fetuses were measured between 186 and 320 days of gestation. Fourteen llamas gave birth to live newborns at $342 + 4$ days of gestation at birth and $10.32 + 0.3$ kg of weight. All these animals were used for the study. There was only one abortion of unknown cause at 273 days and two positional dystocias where the *cria* died. The dystocic stillborn was included in the collected data, but the abortion was not considered. All the measurements were taken in 28 of the 46 fetuses and in 10 of the 14 newly born llamas.

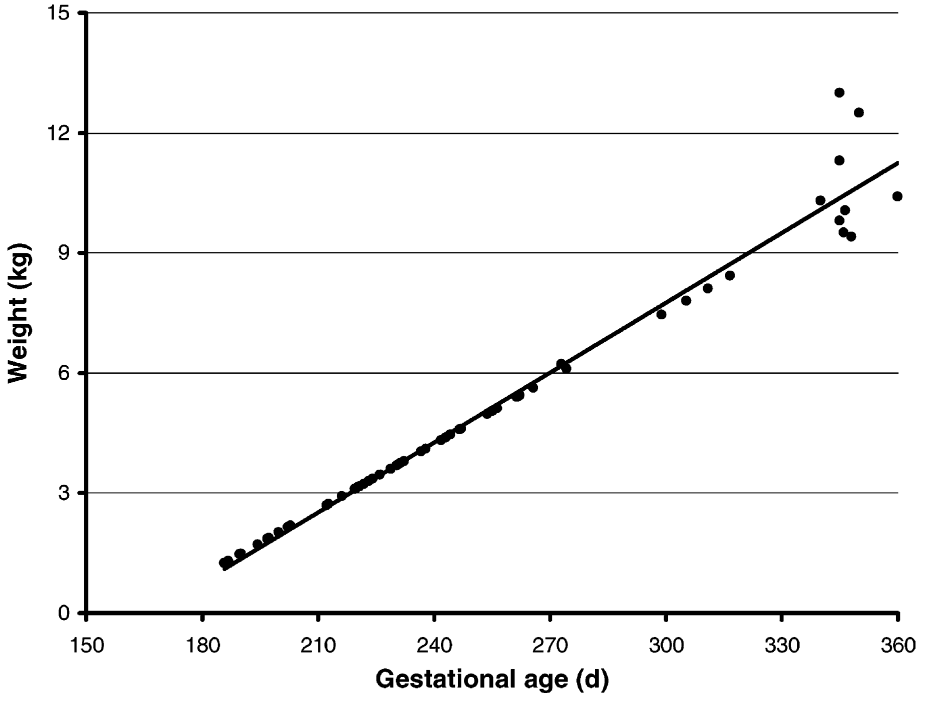

Fig. 2. Regression curve of fetal weight in the llama during the last half of pregnancy. Points represent individual measurements from 62 llamas; $r = 0.99$, $n = 62$, $P < 0.001$.

3.2. Weight

A total of 62 animals were weighed. With the collected data, linear regression curves were calculated as function of GA (Fig. 2). The best function and correlation was: $GA = 169.448 + 16.66^{*}W$, $r = 0.99$.

3.3. Femoral length

This variable was measured in 37 animals. Linear regression curves were calculated as function of GA. The best function and correlation was: $GA = 102.029 + 9.94^{*}F$, $r = 0.91$.

3.4. Tibial length

A total of 47 animals were used for this measurement. With the collected data, linear regression curves were calculated as function of GA. The best function and correlation was: $GA = 91.276 + 8.23^{*}T$, $r = 0.86$.

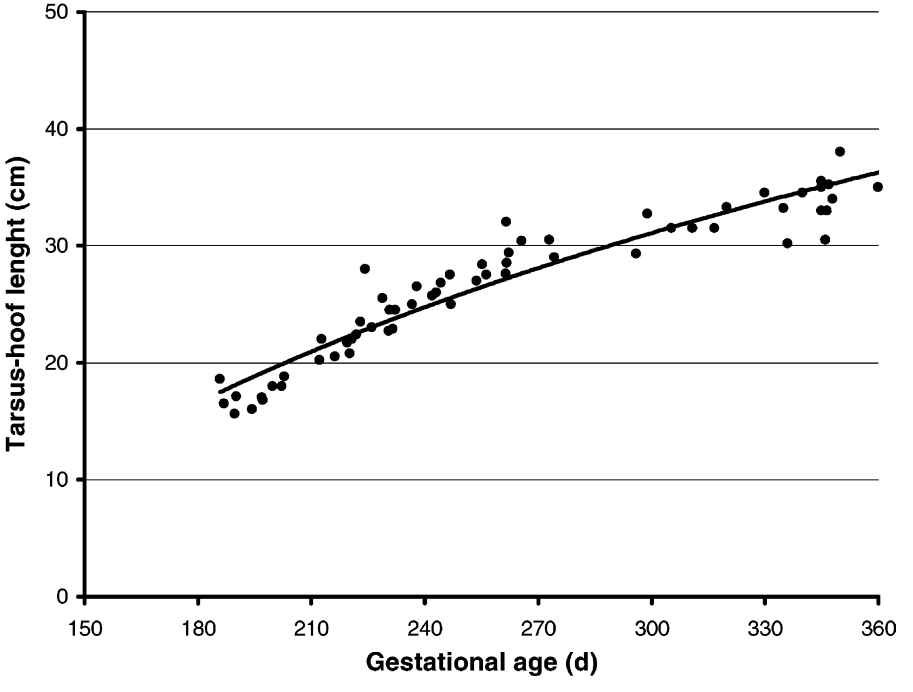

Fig. 3. Regression curve of fetal tarsus-hoof length in the llama during the last half of pregnancy. Points represent individual measurements from 62 llamas; $r = 0.97$, $n = 62$, $P < 0.001$.

3.5. Tarsus-hoof length

A total of 62 animals were used for this measurement. With the collected data, linear regression curves were calculated as function of GA (Fig. 3). The function with the best fit and correlation was: GA = 39.304 + 8.35*T-H, $r = 0.97$.

3.6. Biparietal diameter

The BPD was determined in 43 animals. With the collected data, linear regression curves were calculated as function of GA (Fig. 4). The function with the best fit and correlation was: GA = −51.713 + 44.77*BPD, $r = 0.88$.

3.7. Fronto-occipital diameter

A total of 30 animals were measured. With the collected data, linear regression curves were calculated as function of GA. The function with the best fit and correlation was: GA = −72.139 + 39.48*F-O, $r = 0.71$.

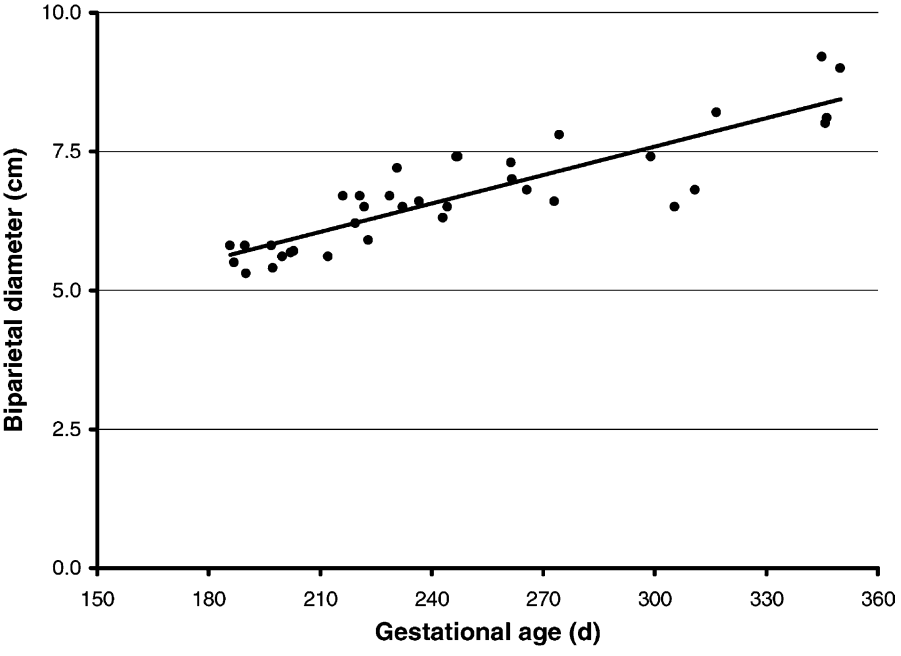

Fig. 4. Regression curve of fetal biparietal diameter in the llama during the last half of pregnancy. Points represent individual measurements from 43 llamas; $r = 0.88$, $n = 43$, $P < 0.001$.

3.8. *Multiple regression analysis*

All of the collected measurements were analyzed with GA as dependent variable. The GA can be predicted from a linear combination of the independent variables with the following function: $\mathrm{GA} = 67.462 + 11.163^{*}\mathrm{W} + 20.297^{*}\mathrm{BPD}$, $P < 0.001$. The variables F, T, T-H and F-O did not significantly add to the ability of the equation to predict GA and were not included in the final equation.

4. Discussion

Results allowed the generation of predictive equations for GAs from fetal measurements in the llama that were never taken before. Moreover, this is the first report of fetal biometry with direct measurements in llamas, and has proved to be a very useful tool for estimating GA. The high correlation between the measured variables and GA indicates that the regression estimation could be used for evaluation of ages of aborted fetuses, premature births and term newborns, as well as in research and cesarean sections.

Consistent with previous publications, in the present study the gestations were diagnosed as early as 20 days after mating (Bourke et al., 1992; Parraguez et al., 1997). These initial gestations were detected as an anechogenic area that identifies the gestational sac. Moreover, after day 20 an echogenic mass could be observed in the base of this sac, presumably the embryo.

Although, some of the births were precocious, the offspring were viable in all of them and they developed as llamas would be typically expected to do. Considering the number of animals studied, GA at term agreed with data reported in previous publications (Brown, 2000; Fernández-Baca, 1993; Gazitúa et al., 2001). In contrast, the birth weight was slightly less than described in these previous reports. However, in the present study, the non-pregnant adult llamas weighed less as compared with the weights described by Fowler (range 108–200 kg for adult females; Fowler, 1989).

Previous studies are consistent with the BPD and GA in the present study (Gazitúa et al., 2001; Haibel and Fung, 1991; Iason et al., 1993; Mialot and Villemain, 1994), but three of these reports are for measurements in the first half of pregnancy, with no data in later gestation. However, Gazitúa et al. (2001) developed linear equations that describe this relationship throughout pregnancy with high correlations being reported. But, the measurements in these previous reports were performed by ultrasonography, with all the inherent uncertainties in the use of this technique (Harrington and Campbell, 1993). Nevertheless, although there were significant differences between the present and the previous study during the last trimester, the average BPD at birth that was reported in the two studies was very similar. Previous reports (Gazitúa et al., 2001) described that BPD measurements were conducted trans-abdominally between the sixth and eleventh month of pregnancy. A probable explanation of these differences is that in previous reports trans-abdominal ultrasonography was used for fetal measurements, a method that results in less precision of the images as compared with the trans-rectal method of evaluation (Gazitúa et al., 2001; Mialot and Villeman, 1994).

The DSAC could be potentially used as livestock in other areas where the population would accept their meat. Moreover, the expansion on the use of these animals in farming activities is sustained by the excellent adaptation that they have to different climatic zones (Latorre, 1997; Raggi and Ferrando, 1998). The extensive management of llamas will be possible when the reproductive performance of this species is improved, therefore, there is a necessity to have improved technologies for productive and reproductive management in this species.

5. Conclusion

Fetometry is an appropriate and useful technique to determinate the GA, especially when having the weight, BPD and T-H length data. The results of the present study were obtained for maternal non-pregnant llamas ranging in weight from 85 to 152 kg, therefore, the formulae from the present study is useful in female llamas with similar weights. Unfortunately, in spite of the precision of these measurements, fetometry is practically impossible without invasive techniques, but can be very valuable in aborted llamas, in a rapid assessment of GA at birth or in research in llama reproduction and development.

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