Measurement of foetal size by ultrasonography and progesterone concentrations in pregnant alpacas.

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Abstract

Forty seven pregnant alpacas were bled every 2 weeks from mating until parturition. Bloods were analyzed for progesterone using a commercial assay kit (DPC). Thirty-seven pregnant alpacas were scanned at 28 day intervals over pregnancy using a 3.5mHz trans-abdominal probe, while a further 21 pregnant alpacas were scanned with both a trans-abdominal probe and a 5mHz trans-rectal probe. Measurements of foetal growth characteristics were attempted on crown-rump (CR), biparietal diameter (BPD), head length (HL), chest width (CW), and heart size (HS). Analysis was carried out using linear regression of foetal age on foetal measurements. The trans-rectal probe was suitable for foetal measurements on days 30-87, and the trans-abdominal on days 41-237. There were no differences for any traits between the two probes for the regression equations of foetal age on foetal measurement (r^2 were CW 83%, HS 80%, HD 74%, HL 69%, CR 53%).

Progesterone concentration in the plasma remained over 2ng/ml throughout gestation.

Keywords Alpaca, progesterone, ultrasonography, foetus.

INTRODUCTION

With the arrival of alpacas into New Zealand, the situation has arisen due to the practice of running males with females continuously, where breeders have pregnant female alpacas of unknown gestational age. Real-time ultrasonography is widely used for pregnancy diagnosis in various species and measurements of foetal growth have been used for assessment of chronological age of the foetus (Haibel & Perkins, 1989; Haibel & Fung, 1991; Wilson & Bingham, 1990). Trans-rectal probes have been used successfully in the first months of pregnancy to measure biparietal diameter, crown rump, uterine vesicle size, and placentomes in sheep, goats, cows, and deer while trans-abdominal probes have been used later in the pregnancy when the uterus descends into the abdominal cavity (Curran *et al.*, 1986; Wilson & Bingham, 1990; Haibel & Fung, 1991; Reichle & Heibel, 1991). Studies on llamas have used trans-abdominal probes from day 60 onwards, but there have been no reports on ageing the foetus of alpacas or the use of trans-rectal probes at an early gestational age (Haibel & Perkins, 1989; Alarcon *et al.*, 1990).

A few reports are published on pregnancy progesterone levels in llamas (Adam *et al.*,1989; Leon *et al.*, 1991), but there is no published information on alpacas. This study investigates the use of trans-rectal and trans-abdominal probes for ageing alpaca foetuses based on foetal size and measures the progesterone concentrations over pregnancy.

MATERIALS AND METHODS

A flock of 47 alpacas were mated in autumn and spring 1990, and autumn 1991. The alpacas were scanned with trans-rectal and trans-abdominal ultrasound probes at 28 day intervals from day 20 to 240 of pregnancy.

From 20 to 85 days an Aloka Ultrasound with a 5mHz rectal probe in a rigid stainless steel extender was used. The animal was restrained in a padded narrow raceway, and the lubricated probe was inserted. Many of the alpacas sat down but this did not interfere with the scanning. From day 40 to 240 a Tokyo Keiki Ultrasound with a 3.5mHz trans-abdominal probe was used. The animal was restrained whilst standing against a padded wall, and the probe was positioned on the right inguinal area.

Each scan attempt was limited to 3 minutes to prevent undue stress on the animal. All scans were performed by one experienced operator and recorded on videotape using a Sony Handycam. Measurements and analyses were carried out later by one technician and measurements were recorded only from scans where sharp definition and good foetal orientation was apparent. Measurements were made of biparietal diameter (BPD), crown-rump (CR), chest width at the caudal end of the ribcage (CW), head length including nose (HL), maximum heart diameter (H), and chest depth from the spine to the caudal end of the sternum (CD).

Blood samples for progesterone where taken fortnightly from the jugular vein into 10ml sodium heparinised tubes. The frozen plasma samples were stored for later analysis using a Coat-a-Count Assay Kit (Diagnostic Products Corporation). Inter-assay and intra-assay coefficients of variation were 13.7% and 8.6% respectively over the working range of 1.0ng to 20ng per tube.

Statistical analyses were carried out on data using SAS. Linear regression was used to calculate relationships.

RESULTS

The trans-rectal probe distinguishes the vacuole of pregnancy in the alpaca as early as day 20 with the conceptus becoming visible with obvious heartbeats between days 20 and 30 (Table 1). Up to day 30 the foetus lay along the uterine wall but after day 30 it appeared suspended in the uterine body and foetal measurements could be taken. Measurements with the trans-rectal probe could be made up to day 87 after which the foetus had moved too far forward into the abdominal cavity. The trans-abdominal probe

could be used as early as 40 days and foetal measurements were possible up to day 237. As foetal age increased (ie greater than day 120) only the head or thorax and abdomen could be viewed in one image but not both together. When both probes could be used the trans-rectal probe gave a clearer and sharper image than the trans-abdominal probe. The presence of a number of physical characters of the foetus could be used to give some estimate of the gestational age of the foetus (Table 1). Of these characters the most readily identifiable were the heartbeat at 30 days, body at 40 days, limb buds at 50 days and vertebrae at 70 days.

TABLE 1 Chronological sequence of ultrasonographic features appearing in the developing alpaca foetus, observed with trans-rectal and trans-abdominal probes.

Feature	Day or Pregnancy					
	20	30	40	50	60	70
Vacuole of Pregnancy	*	*	*	*	*	*
Foetus		*	*	*	*	*
Heartbeat		*	*	*	*	*
Skull			*	*	*	*
Body			*	*	*	*
Foetal movement			*	*	*	*
Ribs				*	*	*
Limb buds				*	*	*
Vertebrae						*

* 20-70 days trans-rectal probe
40-70 days trans-abdominal probe.

Difficulties were experienced in obtaining images which could be measured over the duration of pregnancy. The foetus was often either too small or too large, or inappropriately orientated for a good measurement to be taken. The rapid movement of the foetus in the uterus after day 60 also lead to difficulties in getting measurable scans.

CD was only measured once out of 192 trans-abdominal scans. The most frequent measurements with the trans-rectal probe were CR, BPD and CW (Table 2). Most of these measurements (78-87%) were obtained over the range of days 40 to 70. For the trans-abdominal probe the most frequent measurements were BPD and CW and most of the measurements were made over the ranges of 60-150 days and 90-200 days respectively.

TABLE 2 Percentage frequency of measurable traits for all scans of alpaca foetuses when using trans-rectal (TR) and trans-abdominal (TA) probes.

Type of probe	Crown-rump	Biparietal diameter	Chest width	Heart	Head length
TR (94)	24 (23)	20 (19)	24 (23)	14(13)	4 (4)
TA (192)	5 (9)	26 (50)	24 (46)	8(15)	5 (10)

Number of observations are in parentheses.

For all foetal measurements made with both probes the relationships between foetal measurements and gestational age were linear. Since there were no significant differences in regressions when measurements were made with trans-rectal or trans-abdominal probes the data from both probes were pooled for each trait measured. The range of sizes for each trait measured was large especially for CR and CW (Table 3). All the regressions were significant ($P<0.001$) with CW giving the best relationship between gestational age and foetal measurement.

Progesterone concentrations were high (4.4ng/ml) by day 25 after mating and then gradually declined to 2.6ng/ml by day 175 (Figure 1). This was followed by an increase to 4.4ng/ml by day 235. The pre-parturient decline in progesterone concentrations started after day 285 and decreased to less than 2ng/ml over 25 days. Throughout pregnancy progesterone concentrations of all alpacas remained above 2ng/ml. All non-pregnant animals had a progesterone level below 0.5ng/ml. The mean (±SD) duration of pregnancy on 33 alpacas was 340.8 ± 8.38 days with a range of 314-362 days. All births were observed to occur in daylight hours between 0700 hours to 1430 hours with a median of 1100 hours.

TABLE 3 Regression equations for prediction of age of alpaca foetuses when measurements were made on foetal images from ultrasonography scans using trans-rectal and trans-abdominal probes.

Parameter x(mm)	No. of Observations	Regression for age calculation (days)	Range for measurements (mm)	r2
Crown-rump	32	34.31 + 0.41 X	10 - 160	0.67
Biparietal diameter	69	1.14 + 3.89 X	12 - 45	0.81
Chest width	69	18.52 + 2.73 X	8 - 80	0.88
Heart	28	16.72 + 9.15 X	4 - 26	0.83
Head length	14	-14.03 + 2.98 X	25 -70	0.81

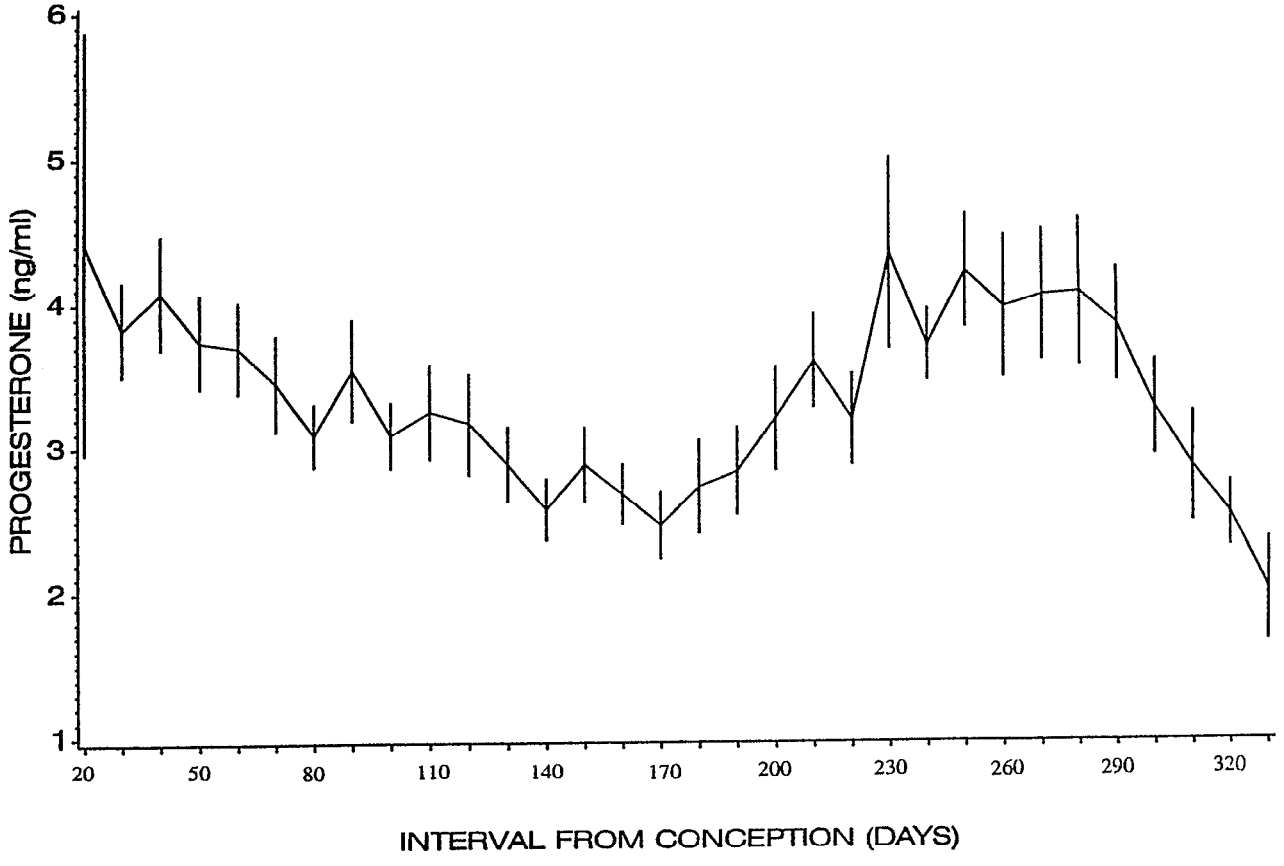
DISCUSSION

Measurement of foetal traits from images provided by ultrasonography with either trans-rectal or trans-abdominal probes can be used to accurately estimate foetal age of alpacas. Haibel and Fung (1991) obtained higher r^2 for their correlations between BPD and chronological age in llamas using a trans-abdominal probe but the mean of 3 measurements was used in their analysis. The frequency of finding suitable images to measure was low, but similar to that found by Wilson and Bingham (1990) for deer using a trans-rectal probe. Their most frequent measured trait (80%) was for placentome diameter but alpacas do not have placentomes. The main problems were the rapid movement of the foetus in the uterus and the difficulties of obtaining the foetus in the right orientation for good measurements to be taken. The frequency of measurable images would increase if more time was allowed, if the scanning was confined to the period of pregnancy when the measurable traits are more readily obtained, and if the time was spent concentrating on getting good images of the best traits to measure. Foetal measurement with the trans-rectal probe should be confined to days 40 to 70 of gestation and concentrated on the CW, BPD or CR measurements. For the trans-abdominal probe, scanning should be confined to 60-200 days and concentrating on CW and BPD measurements. While the trans-rectal probe gives clearer and sharper images, the trans-abdominal probe is easier to manipulate to get a better orientation of the foetus for producing measurable images.

The appearances of physical features of the foetus could be used as a guide to foetal age. Generally the features appear about 10 days earlier than found in the deer (Bingham *et al.*, 1990), the exception being vertebrae which appeared 10 days later than in the deer.

The progesterone concentrations in the alpaca of 2 to 5ng/ml from 25 to 320 days of gestation were similar to the values found for llama by Leon *et al.*, (1990) but lower than the values of 5-9ng/ml found in llama by Adam *et al.*, (1989). Values of greater than 2ng/ml of progesterone can be used to diagnose pregnancy in the alpaca. The decline in progesterone concentra-

FIG 1 Mean progesterone concentrations over the duration of pregnancy in alpacas. The bars indicate the standard deviations about the means.



tions from day 25 to day 175 was not observed in the llama although Leon *et al.*, (1990) did find a small decline at about day 126. In the alpaca the decline in progesterone concentrations could reflect a reduced output of progesterone from the corpus luteum while the subsequent increase could reflect increased output from the placenta.

In conclusion therefore, the pregnancy status of female alpaca could be assessed with a random blood sample for progesterone concentration. Estimation of foetal age could then be determined by using one or both types of ultrasound probe.

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