

## Application of Ultrasonography for Early Pregnancy Diagnosis in Alpacas (*Lama pacos*)

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**Abstract:** Early and accurate diagnosis of pregnancy is important for effective management of food and fiber producing species including alpacas. In this study, the utility of real-time ultrasonography to diagnose early pregnancy in alpacas at various stages of gestation was assessed. Alpaca females were examined for pregnancy status using a real-time B-mode ultrasound scanner and 10 MHz transrectal probe at day 7-10, 12-15, 17-20, 30-40 and 50-60 post mating (n = 20 alpacas per time point). Positive pregnancy diagnosis was based on detection of presence of an non echogenic vesicle or conceptus and confirmation of accuracy of diagnosis for each alpaca determined based on non return to estrus and examination of birth records at end of gestation. Signs of pregnancy (small fluid filled vesicle) were detected as early as 7 days post mating in a small proportion of animals and accuracy of diagnosis (pregnant or open) reached 100% by days 17-20 post mating. Overall accuracies at 7-10, 12-15, 17-20, 30-40 and 50-60 days post mating were 72, 80, 100, 100, 100 and 100%, respectively. The results indicate that transrectal ultrasonography is a reliable technique for early pregnancy diagnosis as early as days 17-20 post mating and may prove a useful tool to increase efficiency of reproductive management in alpacas.

**Key words:** Alpacas, early pregnancy, ultrasonography, fiber producing species, estrus

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### INTRODUCTION

Use of ultrasonography has revolutionized the medical and industrial fields. Ultrasonography was first used as a medical diagnostic aid in the 1940s. The first veterinary application, the detection of ovine pregnancy was reported in 1966 (King, 2006). Since then, improvements in imaging quality combined with an increased awareness of the benefits of ultrasound as a clinical tool have led to its widespread use in the veterinary field for reproductive management and other applications (King, 2006).

Use of ultrasound as a tool for reproductive diagnosis/management has been demonstrated in numerous species of domestic farm animals such as cattle (Braun and Sicher, 2006; Hanzen *et al.*, 2000), pigs (Kauffold and Althouse, 2007; Mayor *et al.*, 2005), horses (Ginther *et al.*, 2009; Gorecka *et al.*, 2005), goats (Simoes *et al.*, 2005; De Bulnes *et al.*, 1999; Amer, 2010) and camelids (Vyas and Sahani, 2000; Vyas *et al.*, 2004; Skidmore and Adams, 2000). Widespread applications of

ultrasound for reproductive management include monitoring follicular status, prediction of breeding time, evaluation of corpus luteum status, estimation of stage of the oestrous cycle, diagnosis of ovarian irregularities and pathologies, detection of early pregnancy and multiple gestation pregnancies, fetal sexing, diagnosis of embryonic death and evaluation of uterine pathologies in females and evaluation of testes and accessory sex glands (Lemma *et al.*, 2006) in males. Application of ultrasonography as a reproductive management tool is most prevalent in large farm animals including horses and cattle.

While ultrasound has been widely applied to reproductive management in horses and cattle, reports of use of ultrasonography for reproductive management in domestic camelids such as the alpaca are more limited. The gross anatomy of the female genital organs and follicle wave characteristics have been described in alpacas (Vyas and Sahani, 2000; Skidmore and Adams, 2000; Adams *et al.*, 1989; Hoffman and Baum, 2006; Vaughan *et al.*, 2004; Bravo *et al.*, 1995). Use of

ultrasound to diagnose early pregnancy in llamas and alpacas has been earlier evaluated from days 7-60 (Parraguez *et al.*, 1997; Bourke *et al.*, 1992; Mialot and Villemain, 1994) but numbers of animals assessed and variation in equipment utilized have limited conclusions on earliest stages of accurate pregnancy diagnosis in the alpaca. Earlier studies of pregnancy diagnosis in alpacas utilized lower resolution ultrasound probes which may impact ability to reliably diagnose pregnancy at early stages (Hanzen *et al.*, 2000; Parraguez *et al.*, 1997; Del Campo *et al.*, 1995). The objective of this study was to determine the utility of ultrasonography for pregnancy diagnosis in alpacas at several stages of early pregnancy (7-60 days after mating) using a 10.0 MHz transrectal probe.

## MATERIALS AND METHODS

**Animals:** Housing and care of animals for use in described experiments were conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals <http://www.cioms.ch/frame1985texts/guidelines.htm>. The study was conducted at the alpaca central farm of Shanxi Agricultural University which is located in Jinzhong City, Shanxi Province, China, located 112°46'N and 37°41'E with an altitude of about 860 m. The region lies in a temperate zone with average annual precipitation of 503.7 mm (range 380-770 mm), average daily temperature of 6.3-10.7°C and mean frost free period of 150 days.

**Ultrasonography:** Pregnancy testing was initiated beginning on day 7 post-mating. The ultrasonographic images were obtained with a real-time B-mode ultrasound scanner (HONDA, HS-1500, Japan), connected to a transrectal 10 MHz linear probe. The transducer was manipulated externally and consisted of a standard linear probe as used for transrectal examinations in cattle with a stiff polyethylene tube (35 cm long, 15 mm O.D.) connected. The tube was covered by a sanitary plastic sheath. The transducer was lubricated with liquid paraffin and introduced into the rectum until the bladder or uterine horns were identifiable and then moved along the dorsal surface of the reproductive tract. Ultrasound images denoting presence or absence of embryonic vesicle or conceptus were recorded electronically and in experiment records. The transducer was cleaned with an antiseptic solution between examinations. The duration of ultrasonography exams ranged from 1-2 min per female. All examinations were performed by the same operator.

The pregnancy diagnosis was performed at five stages: 7-10, 12-15, 17-20, 30-40 and 50-60 days after

mating. Pregnancy diagnosis was performed over an approximately 1 year period. Final confirmation of positive pregnancy diagnosis was based on birth of offspring at expected time (averaged 345±2.9 days) and examination of breeding records to confirm absence of return to estrus and subsequent mating.

On the basis of the final birth rate data, the pregnancy diagnosis results for each animal at each time point post mating were classified as follows: correct positive pregnancy diagnosis, incorrect positive pregnancy diagnosis, correct non pregnant diagnosis and incorrect non-pregnant diagnosis. From these values, the sensitivity [(No. of correct positive pregnancy diagnoses/No. of correct positive pregnancy diagnoses+No. of incorrect positive non-pregnancy diagnoses)×100], specificity [(No. of correct non-pregnant diagnoses/No. of correct non-pregnant diagnoses+No. of incorrect positive pregnancy diagnoses)×100], positive predictive value [(No. of correct positive pregnancy diagnoses/No. of correct positive pregnancy diagnoses+No. of incorrect positive pregnancy diagnoses)×100] and negative predictive value [(No. of correct non pregnant diagnoses/No. of correct non pregnant diagnoses+No. of incorrect non-pregnant diagnoses)×100] of ultrasound based pregnancy diagnosis in above study for each stage of pregnancy were calculated as described by Szenci *et al.* (1993). Overall accuracy was defined as [No. of correct positive pregnancy diagnoses+No. of correct non-pregnant diagnoses/total numbers of animals examined].

## RESULTS AND DISCUSSION

**Accuracy of ultrasonographic imaging for pregnancy diagnosis using 10 MHz probe:** Table 1 shows the results of pregnancy diagnoses made at various time intervals after mating. The earliest pregnancy diagnosis was made on post-mating days 7-10 at which time overall accuracy was 55%. At day 12-15, accuracy was 85.7% for pregnant females and 66.7% for non-pregnant females. At post-mating day 17-20 and later stages examined, accuracy of diagnosis of pregnant and open animals was 100%. The sensitivity of this method of pregnancy diagnosis was 35.7% at 7-10 days, increasing to 100% at 17-20 days. Specificity was 50% at 7-10 days and increased to 100% at 17-20 days and later stages.

**Specific observations during ultrasonographic examinations:** Positive pregnancy diagnosis was defined as detection of the presence of a conceptus or non-echogenic vesicle at time of ultrasonographic evaluation. Signs of pregnancy were observed at 7-10 days post-mating in 5 out of the 20 animals mated

(Table 1). The embryonic vesicle could be observed as a non-echoic accumulation of fluid in the lumen of the uterine horn (Fig. 1A). At 12-15 days post mating, elongation of the non-echoic area was noted and the vesicle appeared as a spherical shape in a transverse view of the uterine horns (Fig. 1B).

Based on detection of an embryo proper, the earliest pregnancy diagnosis was made on pregnancy day 17. Between days 17 and 23 the embryo proper could be observed as an echogenic structure within the embryonic vesicle, separated from the uterine cavity by the amniotic membrane which appeared as a very narrow, hypo-echoic line (Fig. 1C and D). By gestation day 30, the full diameter of the embryo proper could be seen and fetal heart beat was detectable. At this stage, the chorioallantois

expanded to occupy the whole extent of the uterine horn and there appeared an obvious separation within the amniotic cavity (Fig. 1E).

By day 40, the volume of the fetus had increased dramatically and no separation of the amniotic cavity was visible. At this stage, fetal ossification in the thorax, head and limb buds were detected and different parts of the fetuses were very distinct in ultrasound images (Fig. 1F).

A number of methods are available for pregnancy diagnosis in alpacas. Pregnancy diagnosis can be done by external palpation (ballotement) at 8 months of gestation (Sumar, 1996) but the late stage of detection leaves this method most suited for detection and management of pregnant females in the last trimester and is not appropriate for use in breeding decisions. Vasectomised males used to detect oestrus 20 or more days after an earlier service are reliable for detection of non-pregnant females but not all females that reject the male are necessarily pregnant (Sumar, 1996). Likewise, serum progesterone measurements do not give a pregnancy specific indicator and cannot be readily done on farm. Use of ultrasonography remains the most practical approach for early pregnancy detection in alpacas. Alpacas become accustomed to this procedure after two or three sessions. It takes minimal time to diagnose an animal and minimal cost is involved after purchase of the machine. However, reports of the efficacy of ultrasonography in pregnancy diagnosis in alpacas are confounded by inconsistencies in nature and sensitivity of equipment utilized and by limited numbers of animals

Table 1: Early pregnancy diagnosis on days 7-60 post-mating in alpacas females by real-time transrectal ultrasonography

Evaluation	Days of pregnancy (days)				
	7-10	12-15	17-20	30-40	50-60
No. of correct pregnancy diagnosis	5.0	12.0	15	15	15
No. of incorrect pregnancy diagnosis	3.0	2.0	0	0	0
No. of correct non-pregnant diagnosis	3.0	4.0	5	5	5
No. of incorrect non-pregnant diagnosis	9.0	2.0	0	0	0
Sensitivity rate (%)	35.7	85.7	100	100	100
Specificity rate (%)	50.0	66.7	100	100	100
Positive predictive value (%)	62.5	85.7	100	100	100
Negative predictive value (%)	25.0	66.7	100	100	100
Overall accuracy (%)	55.0	80.0	100	100	100

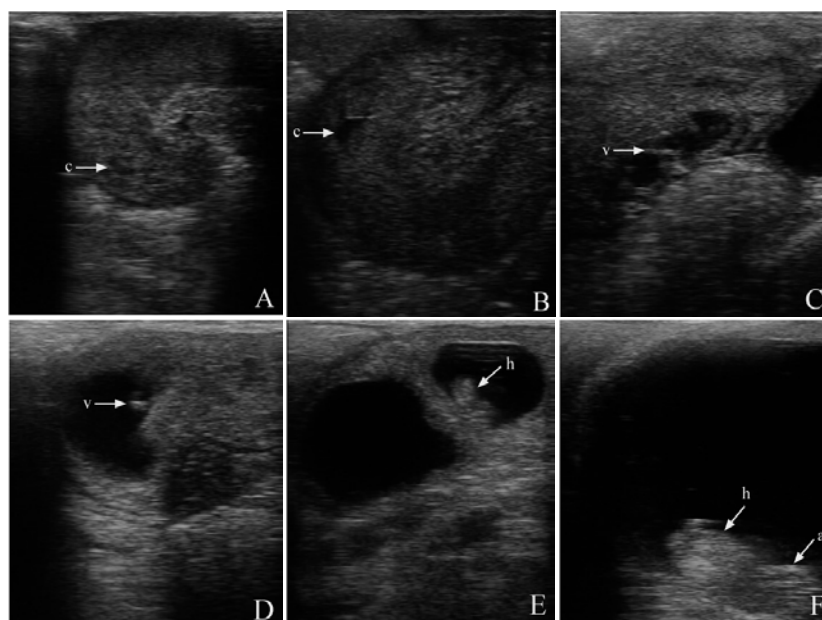


Fig. 1: Ultrasonographic images of the uterine horn in pregnant alpacas at various days of pregnancy: A) day 8, B) day 15, C) day 17, D) day 21, E) day 35 and F) day 50 (c: conceptus; v: vesicle; h: head; a: abdomen)

assessed. Results of the current studies clearly established the efficacy of ultrasonography for early pregnancy diagnosis and indicate very high accuracy of detection of pregnant and non-pregnant animals beginning on days 17-20.

Presumptive early pregnancy diagnosis is based on the detection of a discrete, non-echoic structure within the uterine lumen (Szenci *et al.*, 1993; Brown, 2000). In the alpaca, signs of fluid accumulation were observed as early as day 7 and 10 of pregnancy. Subsequently, by ultrasound there appeared elongation of the non-echoic area (vesicle) in the uterine horn which further confirmed the potential pregnancy but detection of embryo proper was difficult until day 17 at which time detection of pregnant and non pregnant animals reached 100%. The effectiveness of presumptive pregnancy diagnosis prior to day 17 may be limited by the presence of small amounts of intrauterine fluid in non-pregnant females. As described for cattle, this fluid is indistinguishable from the embryonic vesicle (Bravo *et al.*, 1995). Detection of fluid accumulation independent of successful pregnancy establishment likely accounts for the lower negative predictive values observed on days 7-10 and 12-15 in the current studies.

Results of current studies are generally consistent with other reports on transrectal pregnancy diagnosis in llamas and alpacas using ultrasonography. In earlier studies, transabdominal ultrasonography using either a 5 or 3.5 MHz transducer, enabled detection and measurement of the llama fetus from approximately 60 days of gestation onward (Haibel and Fung, 1991). However, the transrectal approach using a 5 or 7.5 MHz transducer allowed much earlier detection of the conceptus. Under optimal conditions, the embryonic vesicle (fluid) was first detected on days 8-15 (day 0 = ovulation), the embryo proper was first detected on days 18-24 and the embryonic heartbeat was first detected on day 21-27 in llamas (Del Campo *et al.*, 1995). In alpacas, the embryonic vesicle was first detected on days 12-14, the embryo proper was detected on day 19 and the embryo heartbeat was detected on days 25-27 after the first breeding (Del Campo *et al.*, 1995). Other researchers have detected early pregnancy between 20 and 100 days after mating in alpacas and llamas (Mialot and Villemain, 1994). Parraguez *et al.* (1997) reported pregnancy diagnosis in alpacas could be carried out from 9 days after mating with 100% accuracy at day 23. In the case of llamas, the test could be carried out from 7 days after mating with 100% accuracy at 34 day (Parraguez *et al.*, 1997). However, as mentioned earlier, great care should be undertaken in interpreting validity of early pregnancy diagnoses based on fluid accumulation in the uterus in the absence of a notable embryo proper.

## CONCLUSION

The findings demonstrate that transrectal ultrasonography is an accurate tool for early pregnancy diagnosis in alpacas, beginning on days 17-20. The only obvious limitation of such technology is the cost of equipment. Furthermore, no obvious advantage of pregnancy diagnosis using a 10 MHz probe was observed in current studies relative to earlier reports using a 5 or 7.5 MHz probe. Use of ultrasonography holds advantages over traditional procedures for pregnancy diagnosis (e.g., estrous detection at ~20 days post mating) in terms of accuracy and stress on the animals and can make for more effective use of procedures for synchronized breeding (Ratto *et al.*, 2003) along with facilitation of grouping and better management of animals during early pregnancy.

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