

Reproductive Ultrasound Examination in Early Pregnancy in Alpacas

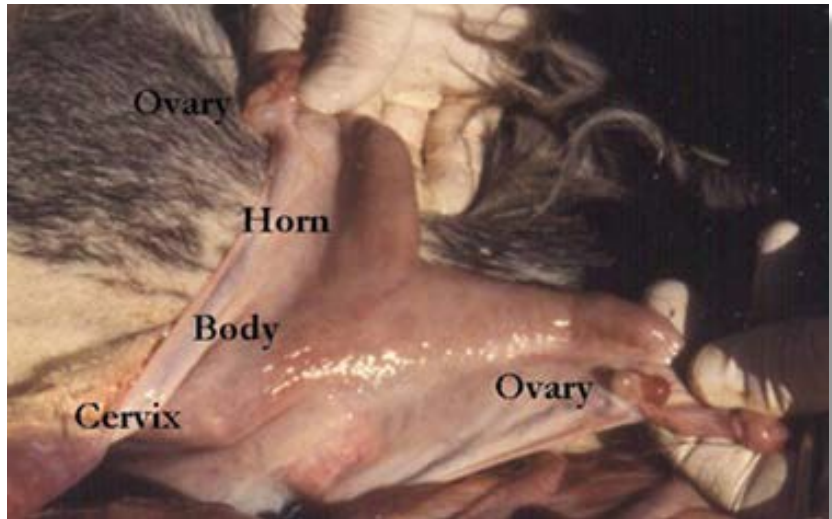
Stephen R. Purdy, DVM srpurdy@nunoaproject.org
(A study in progress at Nunoa Project's North American Camelid Studies Program, Belchertown, MA)

Female Alpaca Reproductive Ultrasound Examinations

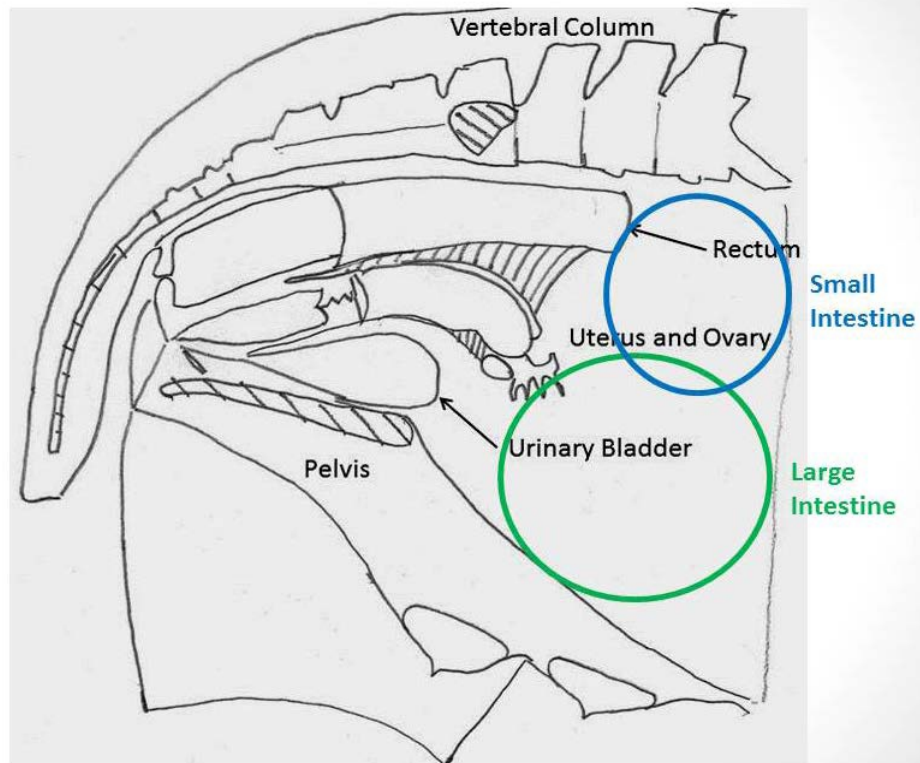
Internal/Transrectal Exam used for: non-pregnant uterus and ovaries at any time, pregnancies from 10+ days (most accurate at 20+ days). Good throughout gestation depending on probe length and frequency. Better tolerated by females than a transabdominal exam. The fetus and most of the fetal fluid will be lost from view in mid-gestation, but uterine horns can be found

Uterine and Ovarian Anatomy

Note that horns are stretched out in this picture. In the live animal they are most often adjacent to each other, draped over the urinary bladder, and lying on top of and among the large and small intestines.



Right lateral view of caudal abdomen showing rectum, uterus and ovaries, urinary bladder and location of intestinal tract for internal and external exams.



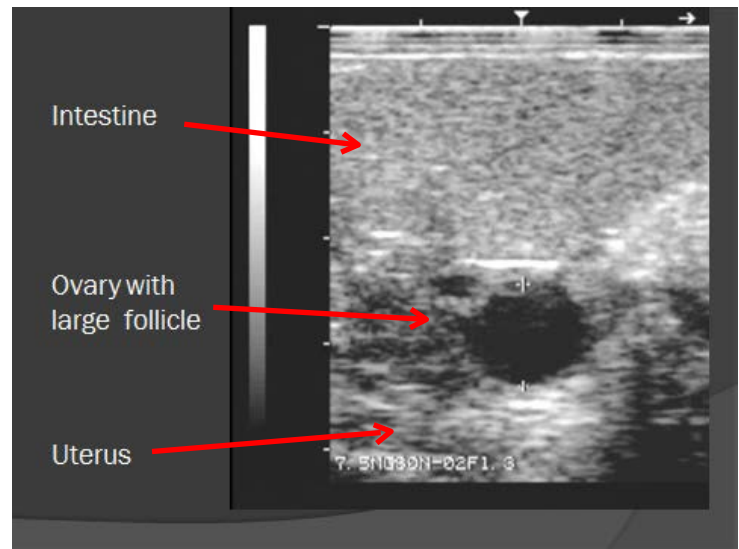
Systematic Transrectal Examination of the Female Reproductive Tract

Then ultrasound probe is inserted in rectum after removing feces with two fingers and inserting 30 cc of water-soluble lubricant. The starting reference **point is the urinary bladder** on the ventral midline. The uterus is draped on and over the bladder at the rostral end. The probe window is pointed straight down and swept gently and slowly back and forth to locate both uterine horns (usually within 2 inches of each other). If any resistance is felt when advancing the probe slowly, the examination is stopped. Then entire uterus is scanned to the horn tips if possible. Ovaries are found near the uterine horns, usually above or below them, not beyond them. They can also be found adjacent to the bladder.

Scanning Alpaca Ovaries and Uterus

Ovaries are approximately 1.2 mm across minimum to 2.8 mm long max. They may be difficult to identify after 60 days of pregnancy as they are displaced downwards with the weight of the advancing pregnancy.

1. **Ovarian Follicles** (black circles) are characteristically arranged along the periphery of the ovary. Large follicles and the corpus luteum protrude distinctly from the ovarian surface. Ovulation is detected approximately 1 to 2 days after a single mating. Follicles ≥ 6 mm in diameter can ovulate and result in pregnancy.



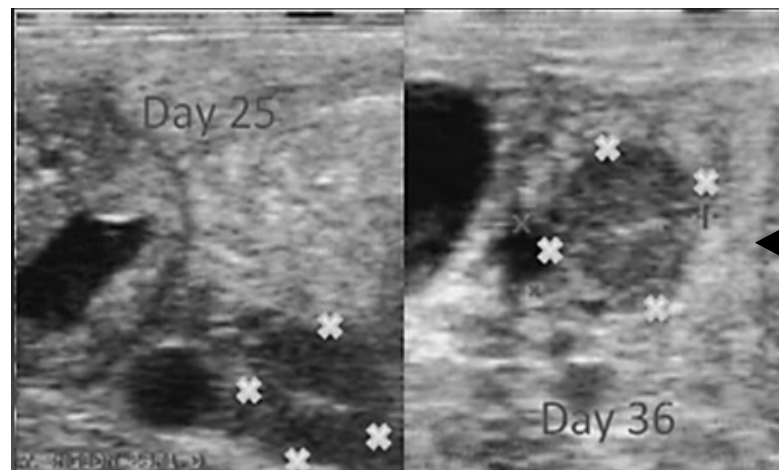
Corpus Luteum (CL) Dynamics

The CL is seen on ultrasound at 3 to 4 days post breeding if ovulation occurred and persists throughout pregnancy. **Multiple ovulations result in multiple CLs.** A transitory corpus hemorrhagicum (CH) is seen 1 -2 days post breeding with ovulation. Regression of the CL in the absence of pregnancy can be immediate (a few days) or delayed (1 week+), or until the female ovulates again on occasion. **Normally receptive behavior is seen in 10 -12 days after breeding if the ovulation did not result in a pregnancy.** The interval is extended if a pregnancy was established but subsequently lost even though it may not have been detected by ultrasound.

Ultrasound appearance of the CL

The CL protrudes distinctly from the ovarian surface. The mature CL is 11-14 mm in diameter and is characteristically fuzzy black (hypoechoic) with a white (hyperechoic) horizontal line in the center (arrow on figure below).

CLs inside Xs; Circular ovarian Follicles to the immediate left; Embryonic Vesicle to the far left in both images (irregular shape at day 25, smoother on cross section at day 36).

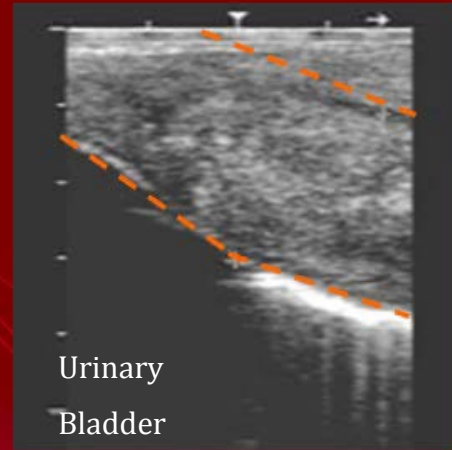


Uterine Horns

Horns are mottled black and white appearance, very distinct from the intestinal tract, and lack peristaltic

contractions. They are located just above and beyond the urinary bladder (black) on transrectalexam. Horns are often found adjacent to each other, but one can be below the other. The ultrasound probe can push the horns to either side during the exam since they are not suspended in place from above. Both horns are equal in diameter as the pregnancy spreads within both horns as it progresses. Horn diameter is 15 to 25 mm on cross section for the non-pregnant uterus depending on age and number of past pregnancies.

Uterine horn as imaged by transrectal ultrasound with a 7.5 MHz linear probe



Pregnancy Examinations

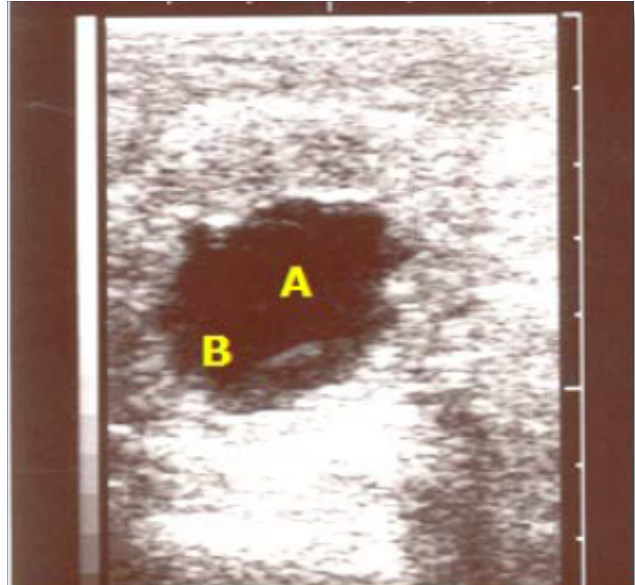
Transrectal ultrasound exams are useful for pregnancy diagnosis in early pregnancy as early as 7 to 12 days post breeding but are more accurate at 18 - 20+ days. They are well tolerated by most alpacas. One should look proximal to the urinary bladder as the probe is advanced. Embryonic and fetal fluids are seen as black in color. Look for the elongated, irregular shape of the early embryo. It changes to a curved outline later in the embryonic stage. The conceptus is termed an embryo until 40 days when the yolk sac is gone; thereafter a fetus being nourished through the placenta.



Pictures taken 2 minutes apart in the same location in the uterine horn illustrating embryo mobility and deformation of the embryonic vesicle at day 20 of pregnancy.

25-day Pregnancy Ultrasound Scan

*A = embryonic fluid
B = embryo proper
(10 mm divisions)*



Implantation of the Embryo and Development of Placentation (after Olivera, 2003)

Trophoblast cells of the embryo adhere to the uterine epithelium and subsequently interact to establish the epitheliochorial placentation in the alpaca. On day 15, embryonic blastocysts are completely free within the uterine lumen, with **implantation starting around day 20**; deformation of the uterus and movement of the embryonic vesicle is seen.

Implantation sites occur most commonly in the left uterine horn, but the expanded embryonic vesicle also occupies large extensions of the right horn by day 30. **Day 30 of gestation is the starting point of placental formation** which is completed at approximately day 40.

Appearance of Reproductive Structures and Timing of Pregnancy Events

Day of Pregnancy	Event Detected by Ultrasonography
2	Corpus hemorrhagicum
4	Corpus luteum
7	Earliest pregnancy diagnosis
18-20	Routine pregnancy diagnosis and best time to scan for twin conceptions
25	Embryo proper and embryonic heartbeat
30	Start of placental formation; difficult to identify twins after this date
40	End of embryonic stage; start of fetal stage
40	Stomach first seen
50	Cephalization and head nods
60	Orbit and long bones of limbs first seen
70	Ossification of cervical vertebrae

(Melle, Purdy, et al, 2013: 5 females followed from breeding to pregnancy termination- Ultrasound, fetal dissection, and computer aided tomography (CAT Scan) measurements; Brunsten, Purdy, et al, 2011- ultrasound only

Early Embryonic Death (EED) in Alpacas

Potential causes include external factors such as stress due to changes in location or social environment. It is best to wait to make changes until after 40 days when critical events such as descent of the embryo into the uterus and transition from the yolk sac to the chorioallantoic placentation have occurred. Other causes of EED are malnutrition, climate extremes, and sire effects. Some sires may be associated with a higher incidence of EED. Maternal factors include hormone deficiencies and imbalances. Progesterone is cited but not proven. Other causes are a poor uterine environment (endometritis, endometrial fibrosis) and advanced age. Embryonic factors such as genetic and embryonic abnormalities are also suspected. It is most efficient for genetic progression to eliminate bad embryos early so that the female has a chance to become pregnant again within a short time. Twin conceptions can also progress to EED.

Past Data from the NACSP herd showed a range of 40 to 57% EED. The most recent data from the herd (September 2013- May 2018) revealed an **average of approximately 20% EED**. This was not consistent in females and therefore not likely to be a result of endometritis or hormone deficiencies. To date the rate is not increasing with the increasing age of the female.

Twinning in Alpacas

Twin conceptions occur as the result of double ovulations and result in formation of 2 CLs. They can be identified best between 15 to 30 days of pregnancy with careful ultrasound scanning of both uterine horns. Possible outcomes of twin conceptions include: reduction to a singleton pregnancy by approximately 40 days, death of both embryos early, abortion of both fetuses in the 3rd trimester due to placental insufficiency, or birth of twins (rate is extremely low). These pregnancies can be followed with ultrasound to monitor the most common outcome which is reduction to a singleton pregnancy. Most often one embryo will continue to increase in size while the other decreases and disappears. The NACSP herd (11 females) September 2014 to May 2018 demonstrated a **34% incidence of twin conceptions**. They were not consistent with particular females. 67% of twins reverted to singleton pregnancies; 33% ended as early embryonic deaths of both.

Should you worry about diagnosing twins and bad outcomes from twin conceptions? In an informal survey of approximately 700 reported alpaca births on multiple farms in the northeastern US there were no aborted twins, and only 1 set of twins born with problems in both crias (a common occurrence). The incidence of problem twin conceptions therefore is very low.

Ultrasound image of 20-day twin

embryonic vesicles (black fluid)

