Theriogenology in Llamas and Alpacas

By Gregg P. Adams DVM, PhD, Diplomate ACT

Llamas and alpacas are domesticated species of South American camelids thought to have descended from wild species, the guanaco and the vicuna, respectively. Despite distinctive physical characteristics, the 4 species of South American camelids are genetically similar and can readily interbreed, producing fertile offspring. The similarity even extends to Old World camelids; crosses between male dromedary camels and female llamas have resulted in the birth of male and female “camas.” As a result of selection for meat and as beasts of burden, llamas are taller and heavier (40–45” at the withers, 280–400 lb) than alpacas (32–39”, 100–150 lbs), which have been selected, primarily, for their fleece. Two breeds of alpacas are recognized based on fleece characteristics, the Huacaya and the much less common Suri. Huacayas have pronounced crimp in their fiber giving them a characteristic fluffy or “cotton ball” appearance, whereas the Suri fleece hangs in long silky locks (Figure 1). Although not recognized as different breeds, 2 general llama types have also been described based on fleece characteristics, the heavy-wool (Chaku) and light-wool (Kara) types (Figure 1). This issue of Large Animal Rounds discusses the reproductive management of South American camelids.

The reproductive characteristics of llamas and alpacas are similar and the practitioner need not distinguish between the two in clinical management. Camelids are the only large domestic species that are induced ovulators. The term estrous cycle must be qualified when used in reference to induced ovulators, since a regular cyclic pattern of behavior does not occur as it does in spontaneous ovulators. The terms receptive vs nonreceptive and follicular phase vs luteal phase are more appropriate for communicating “cyclic” changes in camelids. Sexual behavior and copulation time is strikingly different from any other domestic species. As well, gestation is unusually long, and uterine anatomy, placentation, and birthing in camelids all have distinctly different characteristics than found in any other species.

**Sexual receptivity and mating behavior**

In the absence of copulatory stimulation, female llamas and alpacas display virtually constant sexual receptivity. The receptive female will assume the prone position (sternal recumbency) after a short period of pursuit by the male, or she may approach a male that is copulating with another female and adopt the prone position. Some receptive females may occasionally display mounting behavior with other females of the herd, although such behavior is much less common than in cattle. If the female is nonreceptive, rejection is shown by spitting at the male and running away. Males will attempt to mount receptive or nonreceptive females; their initial approach is indiscriminant. Copulation is remarkably prolonged (10 to 50 minutes) during which the male makes a guttural humming sound called “orgling”.

**Ultrasonography of the uterus and ovaries**

The gross and ultrasonographic appearances of the female reproductive organs are illustrated in Figure 2. The morphology of the ovary has greater similarity to that of a cow than of a mare. Ovarian structures are smaller than in cows (eg, ovulatory-sized follicle, 10 mm vs 16 mm, respectively; mature corpus luteum (CL), 13 mm vs 28 mm, respectively). Similar to the cow, but unlike the mare, ovarian follicles are arranged in a peripheral cortex and ovulation can take place at any spot on the surface of the ovary. The CL and large follicles protrude distinctly from the surface of the ovary in llamas and are readily palpable. By ultrasonography, corpora lutea have a characteristic spherical shape and are hypoechogenic (dark gray) relative to the surrounding tissues. A horizontal echogenic (light gray) area traversing the center of the CL is also a distinctive ultrasonographic feature. The ability to ultrasonographically recognize and monitor the development of the CL is fortuitous because it provides the ability to confirm ovulation and is an immediate method of “progesterone testing.”
The oviducts are long and tortuous, ending in open bursas that normally cover the ovary. The tip of the uterine horn is blunt and rounded, unlike in other ruminants where it tapers slowly toward the uterotubal junction. Accordingly, the oviduct of alpacas and llamas opens into the uterine horns via a small, raised papilla, which acts as a well-defined sphincter. Even under great pressure it is not possible to flush liquids from the uterus into the oviduct, but it is possible in the opposite direction. The uterus of the alpaca and llama is bicorneate, but the left uterine horn is slightly larger than the right, even in nullipara females (Figure 2).

In situ, the uterine horns curl ventrally and caudally, and the degree of curl is maximal during the luteal phase (progesterone dominance) and minimal during the follicular phase (estrogen dominance; Figure 3). The degree of uterine horn curl is much less than in other ruminants (e.g., sheep, goats, cattle) and in the excised tract, the uterine horns shrink and may not appear curled at all, but rather Y-shaped. The uterus echotexture (grain of the ultrasound image) changes from homogeneous light gray (smooth and uniform) during the luteal phase and early pregnancy, to heterogeneous and increasingly dark during the follicular phase (Figure 3). The body of the uterus is short (2 to 3 cm) and not clearly differentiated from the base of either horn by ultrasonography. Uterine tone, based on digital palpation, is maximal during the follicular phase and minimal during the luteal phase and early pregnancy, similar to cattle. The uterus is remarkably turgid during the follicular phase and longitudinal grooves can be palpated along its upper surface. A broad ligament attaches along the ventral aspect of the uterus such that the uterine body and horns are readily palpable per rectum.

The cervix has 2 or 3 irregular annular or spiral folds. By ultrasonography, the cervical folds appear as transverse echogenic bands, and are especially prominent during the luteal phase and pregnancy (Figures 2 and 3). Presumably, edema of the cervical folds during the follicular phase is responsible for the dark echotexture and indistinct appearance characteristic of this phase. The vagina is deceptively long and commonly exceeds 20 cm.

### Follicular dynamics

As induced ovulators, 3 naturally occurring reproductive states exist in llamas and alpacas:
- Nonovulatory
- Ovulatory, but not pregnant
- Pregnant

Ovarian follicle development follows a wave-like pattern (Figure 4) regardless of reproductive status (nonovulatory, ovulatory nonpregnant, or pregnant) or lactational status (lactating, non-lactating). A group of follicles begins to grow, synchronously, one of which continues to grow (dominant), while the others (subordinates) soon begin to regress. If ovulation is not induced, the dominant follicle eventually regresses, as well, and a new wave emerges to repeat the ovarian “cycle.” The interval between the emergence of successive waves of follicles is 15 to 20 days. Dominant follicles of successive waves are equally as likely to develop in the ipsilateral as in the contralateral ovary; i.e., they do not regularly alternate between ovaries.

### Ovulation

Female llamas and alpacas ovulate only after copulation or after the administration of hormones with luteinizing hormone (LH)-like activity. The mechanical stimulation of copulation (penile intromission, treading and clasping of the male’s legs on the back and sides of the female, and gutteral humming sounds emitted by the male) was originally thought to be responsible for inducing ovulation, but recent studies have documented that a protein in seminal plasma, referred to as ovulation-inducing factor (OIF), is responsible for eliciting ovulation. Circulating concentrations of LH begin to rise 15 minutes after copulation, peak at approximately 2 to 4 hours, and decrease to basal values by 6 hours.
appears that “cystic” follicles in llamas and alpacas, previously defined as any follicle ≥ 12 mm, may have been over-diagnosed and over-treated in the past. Based on ultrasound studies, the mean (± standard error of the mean [SEM]) maximum diameter of the dominant follicle is 12.1 ± 0.4 mm, and the range extends to 16 mm.12 Females that are not exposed to a male (ie, no sexual stimulation) frequently (16% of nonovulatory follicles) develop oversized follicles (≥ 25 mm in diameter). These oversized follicles were found to contain bloody fluid and were, therefore, termed hemorrhagic follicles.12 Hemorrhagic follicles may become very large (up to 35 mm) and persist for a prolonged period (weeks); however, they resolve spontaneously and do not disrupt ovarian function. Hemorrhagic follicles are not associated with infertility and treatment is not necessary.

Primary ovulation failure has been reported in females that have apparent normal follicle development, but do not ovulate after repeated copulation. The condition has not been systematically studied, but treatment with GnRH, LH, or human chorionic gonadotropin (hCG) has been used to overcome this condition with variable success.

Breeding schemes
Pasture breeding (one or more males with a group of females) is commonplace in South America. In some Peruvian pasture breeding programs, males are maintained at only 3% of after copulation. The interval from mating (or gonadotropin-releasing hormone [GnRH] or LH treatment) to ovulation is 30.0 ± 0.5 hours.7 The mean diameter of the ovulatory follicle on the day before ovulation is 10 mm (range, 7 to 14 mm). Females with small follicles (4–5 mm) or regressing dominant follicles will not ovulate after copulation, whereas females with follicles ≥ 7 mm that are in the growing phase will ovulate after copulation.8,9 Ovulation failure occurs in 5% to 10% of females with mature follicles and the incidence of spontaneous ovulation is 4%-8%.5

Luteal dynamics
CL diameter, assessed by transrectal ultrasonography, is correlated with plasma progesterone concentration (r=83%; P<0.0001; Figure 5).10 In nonpregnant females, maximum CL diameter and plasma progesterone concentration occur 8 days after mating, and luteolysis is associated with pulsatile release of prostaglandin F (PGF2α) from the uterus around 10 days after mating. In pregnant animals, luteal diameter and plasma progesterone continue to increase until a maximum, on approximately day 25. It should be emphasized, however, that elevated progesterone indicates the presence of a functional CL, it does not necessarily indicate pregnancy.

Ovarian irregularities
From examinations of reproductive tracts of animals sent to slaughter, about 15% of females exhibit some reproductive abnormality.11 Ovarian hypoplasia was the most common ovarian anomaly recorded; small ovaries (1 x 1.5 cm) and the absence of follicular development characterize this anomaly.

The existence of a condition similar to cystic ovarian degeneration, as described in cattle, remains equivocal in camelids. It appears that “cystic” follicles in llamas and alpacas, previously defined as any follicle ≥ 12 mm, may have been over-diagnosed and over-treated in the past. Based on ultrasound studies, the mean (± standard error of the mean [SEM]) maximum diameter of the dominant follicle is 12.1 ± 0.4 mm, and the range extends to 16 mm.12 Females that are not exposed to a male (ie, no sexual stimulation) frequently (16% of nonovulatory follicles) develop oversized follicles (≥ 25 mm in diameter). These oversized follicles were found to contain bloody fluid and were, therefore, termed hemorrhagic follicles.12 Hemorrhagic follicles may become very large (up to 35 mm) and persist for a prolonged period (weeks); however, they resolve spontaneously and do not disrupt ovarian function. Hemorrhagic follicles are not associated with infertility and treatment is not necessary.

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Breeding schemes
Pasture breeding (one or more males with a group of females) is commonplace in South America. In some Peruvian pasture breeding programs, males are maintained at only 3% of
the herd (one male per 33 females) and replaced by new males every week (alternating or rotary system). Up to 18 copulations per day are possible by males, particularly on the first day of presentation to females.

In a modified form of pasture breeding, the males are removed after 7 to 14 days and re-introduced 14 to 21 days later. Females that refuse mating after reintroduction are examined for pregnancy (eg, by transrectal ultrasonography). Modified pasture breeding programs allow more accurate record keeping and more rapid identification of problem animals.

Individual, or hand breeding requires the most intense management and record keeping, but provides early identification of females with reproductive problems. Males may be presented in-hand or set free in a paddock with an individual female. A receptive female will usually assume sternal recumbency quickly; however, behavior may vary considerably depending on age, experience, and relative dominance of the male and female. This ‘teasing’ procedure may be repeated daily to weekly until mating occurs. Teasing should be repeated at 1 week and again at 2 weeks after mating, and if receptive, the female may be mated again. Females that continue to breed over 3 such breeding cycles should be examined for abnormalities.

More than a single mating does not improve the pregnancy rate, if mating occurs when a mature viable dominant follicle is present. However, in a field setting where ovarian status is unknown, there is some evidence that 2 matings on the same day may increase the pregnancy rate. During the first 3 to 4 days post-mating, when the CL is forming and progesterone concentrations are low, most females remain receptive to the male, and over-mating during this period will result in endometritis (ie, intrauterine ejaculation) and lower pregnancy rates.

**Puberty and the postpartum period**

Onset of puberty ranges from as young as 10 months of age to 3 years of age, but a general rule of 65% of projected mature adult weight is often used as a guide. Most females are first bred between the age of 12 and 18 months.

To maintain an annual birthing rate, female llamas and alpacas must become pregnant within about 20 days of giving birth (gestation length, 345 days). The female is submissive immediately after birthing and will readily permit mating (apparent receptivity) up to 4 days after parturition; however, follicular growth and uterine involution are not sufficient for ovulation and the establishment of pregnancy. By 10 days postpartum, the diameter of the largest follicle is about 8 to 10 mm, and the uterus has involuted substantially. Assuming a normal puerperium, mating of females within 12 to 20 days after birthing is recommended.

**Synchronization and fixed-time breeding**

One may expect to find a follicle of ≥6 mm in one of the ovaries at any given time (Figure 4), but to determine whether the follicle is growing (viable) or regressing (dying) would require more than one examination. This determination is important for breeding management, since follicles that are immature (<6 mm) or over-mature (regressing) are not capable of ovulation. In a recent study on ovarian synchronization, the effects of treatment with estradiol plus progesterone (E/P), LH, or ultrasound-guided follicular ablation, were compared by monitoring follicular wave dynamics, ovulation, and pregnancy rate after fixed-time natural mating. The interval from treatment to the day on which the new dominant follicle reached 7 mm (large enough to ovulate) did not differ between the LH and follicle-ablation
groups (5.2±0.5 days and 5.0±0.5 days, respectively), but both were shorter and less variable than in the control group (8.4±2.0 days), while the E/P group (7.7±0.5 days) was intermediate. Ovulation rates after a single, fixed-time natural mating 10 to 12 days after treatment did not differ among groups, but the pregnancy rate was higher for synchronized llamas (76%) than for non-synchronized llamas (54%). The results demonstrated clearly that follicular wave emergence can be induced electively, and animals can be synchronized sufficiently to permit fixed-time insemination without the arduous task of testing behavioral receptivity. Treatment with LH (eg, 5 mg lutropin) or GnRH (eg, 50 µg cysteolin) 10 days before intended insemination is a simple and effective way to synchronize llamas and alpacas for prescheduled breeding management.

In females with a CL, treatment with prostaglandin is effective in inducing luteolysis and a return to sexual receptivity. Although dose and timing of prostaglandin treatment has not been systematically examined, luteolysis has been induced with 100 µg cloprostenol, intramuscularly (IM), in alpacas and 250 µg cloprostenol IM in llamas, when given 24 days after ovulation.14 The CL of pregnancy may be somewhat more resistant to prostaglandin, but luteolysis and abortion can be effectively induced at any stage of pregnancy with single or multiple injections of cloprostenol.13 Prostaglandin Fα toxicity and death have been reported in llamas following IM use of dinoprostr tromethamine, but no adverse reactions have been reported with fluprostenol or cloprostenol.14

**Pregnancy diagnosis**

Diagnosis of pregnancy in llamas and alpacas can be performed by indirect or direct means. Indirect methods usually involve assessment of sexual receptivity using a “teaser” male, or measurement of progesterone concentrations in blood or milk. Direct methods involve palpation, ballottement, or ultrasonography of the conceptus and associated fluid.

The early embryo resembles that of other ruminant species, but batching from the zona pellucida usually occurs before the embryo reaches the uterus. Though initially spherical (ie, at 57 days postmating), the hatched blastocyst quickly elongates into a delicate filamentous embryonic vesicle that occupies most of one uterine horn by 10 days postmating. Camelids have a diffuse chorioepithelial type of placenta, and, in virtually all pregnancies, the fetus occupies the left horn.16

**Sexual behavior and systemic progesterone**

Systemic concentrations of progesterone are inversely related to sexual receptivity. That is, females under the prevailing influence of progesterone are nonreceptive to sexual advances of a male. In nonmated females, follicular waves emerge at regular periodic intervals without interruption by ovulation or luteal gland development. Although no obvious relationship between circulating estrogen concentrations and the degree of sexual receptivity has been documented, it is clear that, in the absence of progesterone, female llamas and alpacas are receptive more-or-less continuously.

Based on the assumption that nonreceptivity is a reflection of elevated systemic progesterone, a teasing strategy may be used as an indirect indicator of pregnancy status. Ovulation occurs consistently 1 to 2 days (mean, 30 hours) after the first mating, but plasma progesterone does not rise significantly until 4 days after mating. Plasma progesterone concentration peaks 8 days after first mating and decreases rapidly thereafter in nonpregnant animals (Figure 5). In pregnant animals, plasma progesterone concentrations will remain elevated throughout gestation. In nonmated, nonovulatory females, plasma progesterone is usually ≤0.4 ng/mL. In pregnant females, it usually remains in excess of 2 ng/mL after day 12 postmating.16

Based on these observations, a diagnosis of ovulation may be made between 6 and 8 days after mating by:

- behavior assessment
- measurement of progesterone concentrations in blood or milk
- measurement of CL diameter

A diagnosis of nonpregnancy may be made by day 12 after mating by detecting behavioral receptivity or basal progesterone concentrations and a regressing CL. Behavior and progesterone-testing may be more appropriately referred to as methods of diagnosing nonpregnancy because luteal gland function may be elicited by factors other than pregnancy. Spontaneous ovulation has been reported in approximately 10% of females not exposed to a male and pathologic processes such as luteal cysts, embryo/fetal loss, as well as, pyometra or mucometra may result in a prolonged luteal phase and a false-positive diagnosis of pregnancy. Depending on the range of normal values established by the respective laboratory, progesterone values between 1 and 2 ng/mL are equivocal and warrant repeated measurement. While behavior and progesterone testing can be extremely useful management tools, they must be used strategically and only for presumptive diagnosis of pregnancy.

**Other endocrine indicators of pregnancy**

The use of estrone sulfate concentrations in blood or urine to diagnose pregnancy is highly dependent on the time of sampling; it peaks twice during pregnancy: at 21 days after mating and again during the last month of gestation.17 Measurement of serum relaxin concentration is a better indicator of pregnancy after the second month of gestation because it represents an interaction between mother and fetus (pregnancy-specific), and concentrations are elevated for the majority of gestation.17

**Palpation and ballottement**

Transrectal palpation can be used to detect pregnancy as early as 35 days postmating, based on marked asymmetry of the uterine horns (conceptus is invariably maintained in the left horn), fluid fluctuance and, in later gestation, palpation of fetal parts. The placenta has a diffuse epitheliochorial attachment; therefore, a fetal membrane slip, characteristic in cattle, cannot be felt. No specific reports on the use of transabdominal ballottement were found, but apparently some Peruvian herdsmen are quite skilled and it is used quite commonly after 8 months of gestation.16

**Diagnostic ultrasonography**

The transducer may be placed on the outside of the body and directed dorsally or obliquely across the abdomen (transabdominal approach) or placed within the rectum and directed ventrally (transrectal approach). The transabdominal approach is useful for diagnosing pregnancy after approximately 60 days; however, the fiber, skin, fat, and muscle of the body wall, and the depth of penetration...
required, causes attenuation of the ultrasound signal and the resolution of images is limited.16

The transrectal approach involves the insertion of a gloved
hand and transducer into the rectum. Since the reproductive
organs are located immediately beneath the rectum with very
little intervening tissue, the transrectal approach offers better
access and a better image than the transabdominal approach. A
clear image of the ovaries, uterus, and cervix can be achieved at
any time during pregnancy or nonpregnancy. Although the
transrectal approach is preferred, some llamas and alpaca owners,
as well as, their veterinarians are hesitant to use it. Indeed,
physical limitations (ie, relatively small rectum) may preclude this
approach in some animals. The author estimates that > 90% of
mature llamas and > 75% of mature alpacas are able to accom-
modate the transrectal approach (glove-size, 7 1/2). A rigid or
semi-rigid probe extension may be used to permit external
manipulation of an intrarectally placed transducer in animals too
small to accommodate the operator’s hand.

Serial examination of 60 llamas revealed no difference in the
proportion of left-sided versus right-sided ovolutions and the
evolved embryonic vesicle could be detected in any segment of
either uterine horn ipsilateral or contralateral to the side of ovu-
lation.15 After day 20, however, the embryo appeared to become
fixed in place and was most frequently observed in the middle
portion of the left uterine horn, where development continued.
After 26 days of pregnancy, the developing embryo/fetus was
invariably observed in the left uterine horn.

The embryonic vesicle is first detected as an irregular
collection of fluid as early as 12 days after first mating (2 to 4
mm in diameter) and the beating heart of the embryo may be
detected by 26 days postmating. In ultrasound studies of alpacas,
the embryonic vesicle was detected in 50% of pregnant alpacas at
12 to 15 days postmating, and 100% at 16 to 23 days postmat-
ing.17 A reasonable time for initial pregnancy diagnosis, therefore,
is about 20 days after mating, depending on examination condi-
tions and the experience of the clinician.

Summary

The reproductive characteristics of llamas and alpacas are
similar, and clinical management need not distinguish between the
two. Camelids are the only large domestic species that are induced
ovulators. Sexual behaviour and copulation time is strikingly dif-
ferent from any other domestic species. In addition, gestation is
unusually long and uterine anatomy, placentaion, and birthing
distinctly differ from any other species.

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Dr. Adams has stated that he has no disclosures to announce in associa-
tion with the contents of this issue.

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Publications Post 8401032303

This publication is made possible by an educational grant from
Schering-Plough-Canada Inc.

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