

INFERTILITY IN THE FEMALE LAMOID

Ahmed Tibary¹ DMV, PhD, Dip. ACT and David Pugh² DVM, MS, Dip. ACT and ACVN

¹College of Veterinary Medicine, Washington State University, Pullman, WA

²College of Veterinary Medicine, Auburn University, Auburn, AL

Reproductive management and medicine is most likely the single most common veterinary call in alpaca and llama practice. This is due in part to the difficulty for most breeders to understand the intricacies of reproductive physiology in this species. In addition, the long pregnancy length, the constraint to breed at specific times of the year in order to avoid winter and summer and the desire to breed to outside males make female reproductive evaluation of paramount importance for the success of an operation.

Common complaints in the female lamoid infertility

The primary complaints in lamoids infertility are: repeat breeding (75.6%), Early pregnancy loss (18.3%) visible abnormalities of the genitalia (4.9%) and continuously rejecting the male (2.4%)[31, 34]. Arriving at a precise diagnosis of the cause of infertility requires a thorough evaluation that should include history and physical examination as well as a complete evaluation of the reproductive organs[37]. In many cases diagnosis of the cause of infertility may require monitoring the female over at least one reproductive cycle (from follicular growth to mating and pregnancy diagnosis). The objectives would be to answer the following questions: what is the expertise of the breeder? Is the male fertile? Does the females have a normal genitalia? Is the female ovulating? Judicious choice of examination technique and interpretation allows obtaining an accurate diagnosis (Tables 1 and 2) [34].

Breeding soundness evaluation of the female lamoid

History and physical examination: General and reproductive history should cover all parameters that may have an impact on reproductive performance including, age, type of animal, and breeding management system. A history of previous illnesses and treatments is important because of the possible effect on reproductive performance and helps identify females with high-risk pregnancies. Complete clinical evaluation of the female, including body condition, should be done before any manipulation of the genital tract.

Female behavior provides a relatively accurate and inexpensive means for pregnancy diagnosis (spitting)[32]. The behaviors most indicative of reproductive status are spitting and attempting to escape, which are consistent with the presence of a corpus luteum[18]. Receptivity on the other hand is not well correlated with ovarian status[18, 26]. Absence of rejection of the male (spitting) strongly suggests non-pregnancy[4].

Per rectum evaluation of the reproductive tract: *Per rectum* palpation of the genitalia is an essential part of the examination of the female. Rectal tears or colonic injuries can result from the procedure if done by non-trained persons[20]. Lubricant containing a local anesthetic helps to reduce straining. Epidural anesthesia provides relaxation of the anal sphincter but will not inhibit rectal peristaltic activity[6].

The cervix is difficult to differentiate from the uterine body by palpation. However its posterior orifice can be felt per rectum if the uterus is contracted. The uterus is “T” or “Y” shaped with the horns curling slightly downward and backward at times. There is no intercornual ligament. Uterine tone and edema increase during the follicular phase and become maximal in the presence of a mature follicle. The uterus is flaccid and thin-walled during the luteal phase and pregnancy[27].

Ovarian structures (corpus luteum, follicles) are palpable in llamas. The ovaries are very small and sometimes difficult to identify by palpation in cases of hypoplasia due to nutritional or genetic factors. The ovary is difficult to locate in cases of adhesions to the surrounding tissue, ovarian bursa, and uterine tube. Enlargement of the ovary is generally due to neoplasm or cystic formations[27].

Increased size of the uterus in the absence of pregnancy can be due to pyometra or mucometra. Palpation of the uterine horn can reveal some localized pathological processes such as peri or intra-uterine abscesses, peri or intra uterine cysts and peri-uterine adhesions. Other types of abnormal uterine content that can be felt by palpation per rectum are fetal parts - especially bones in case of maceration, mummification, foreign bodies and neoplastic changes[27, 28].

Ultrasonography of the female genital tract: Transrectal ultrasonography of the genital tract is performed using a 7.5 MHz or 5 MHz linear transducer. For alpacas, the transducer is mounted on a handle to allow manipulation without inserting the hand in the rectum. Ultrasonography allows a more precise evaluation of follicular and luteal activity within the ovary. In the early stage of the follicular wave, the ovary appears elliptical with several small (2 to 5 mm in diameter) follicles disposed along the periphery in a diadem fashion. Dominance is established when follicular diameter reaches 6 mm. The dominant follicle continues to grow steadily until it reaches its maximal size of 9 to 14 mm in llamas and 8 to 12 mm in alpacas [2, 9]. Visualization of the corpus luteum after mating, is possible within 3 days[2]. The corpus luteum is less echogenic than the ovarian stroma. It appears as a protruding round structure, sometimes with a central cavity. In other cases, the mature corpus luteum has a dense, hyperechoic central area in the shape of a star. The diameter of the mature corpus luteum varies between 11 and 13 mm.

Pathological changes of the ovaries detected by ultrasonography include cystic changes (anovulatory or hemorrhagic follicles) inflammation, and tumors. Hypoplastic ovaries are very small and the condition is suspected if ovaries cannot be visualized. In camelidae, development of large anovulatory hemorrhagic follicles is common in the absence of ovulation-inducing stimulus. Peri-ovarian cysts are not rare and should be differentiated from enlarged oviducts. Ovarian inflammation (oophoritis) is suspected when the ovarian size is normal or slightly increased and does not show clear delineation from the rest of the tissue. Ovarian tumors are suspected when the ovary is enlarged and show an abnormal échotexture with no clear follicular

dynamic. The ultrasonographic appearance of the ovary depends on the type of neoplasm.

During the follicular phase, the uterus is contracted and the uterine horns are straight. The echotexture of the uterus is usually heterogeneous and shows increased edema of endometrial folds. During the luteal phase, the uterus is homogeneous with a medium degree of echogenicity. Abnormalities can be of two types: abnormal uterine echotexture (endometritis, metritis, neoplasm) and abnormal uterine content (pyometra, mummification, maceration, embryonic death)[27, 28]. Evaluation of the uterine wall can be facilitated by ultrasonography while the uterus is being flushed.

Vulva and vaginal examination: The vulva is inspected for any discharge and /or lesions and its size and conformation are evaluated. The size of the vulva in camelidae is relatively small compared to other species. Females presenting increased size and edema of the vulva should be examined for recent parturition or abortion. Abnormal size and position of the vulva in maiden females may suggest presence of congenital problems or intersexuality. Intersexed animals present ambiguous external genitalia sometimes with a rudimentary penis. The distance from the anal sphincter to the vulvar opening is usually increased and the animal shows urinary difficulties[38]. Copious vaginal discharge beyond 7 days postpartum is almost always pathological. Vaginal discharge is suspected upon observation of dried material on the ventral aspect of the tail or by matting of the wool around the tail and perineal area.

Digital examination of the vestibulo-vaginal area should be performed on all maiden females (persistent hymen and segmental aplasia) and females with pyometra or hydrometra (vestibular or vaginal adhesions). Open-sided specula are helpful if a biopsy punch or a culture swab needs to be guided into the cervix. The most frequent abnormalities encountered in the vagina and cervix are inflammations or adhesions. Vaginal adhesions are a frequent cause of pyometra and inability to copulate [33]. The most frequent development abnormality of the cervix in camelidae is the presence of a double cervix[33]. Thorough vaginal and cervical evaluation requires sometimes the use of a flexible endoscope.

Uterine cytology and culture: Endometritis is a common cause of infertility in camelidae. It is confirmed by uterine cytology or culture. Samples should be taken from the uterine cavity using a double guarded swab. Swabs are examined routinely for aerobic and anaerobic bacteria, ureaplasma, mycoplasma, and for fungus [29]. The bacteria responsible for endometritis in camelids are essentially those found in the equine and bovine species. Uterine culture may be negative in many cases of chronic endometritis.

Other examination procedures

Uterine biopsy: Uterine biopsy is used to detect inflammatory, degenerative, or neoplastic changes in the endometrium[19]. The technique is similar to that used in the equine. The cervix can be opened by administration of estradiol (1 to 2 mg) 12 to 24 before biopsy. Evaluation of the specimen should be done by a person familiar with the normal histology of the camelid endometrium. Degenerative changes are mainly due to the presence of peri-glandular or peri-vascular fibrosis. In severe cases, nesting with cystic dilation of the endometrial glands or

lymphatic cysts are observed. A classification of lamoid endometrial biopsies has been proposed[19].

Laparoscopy and exploratory laparotomy: Laparoscopy is an invaluable technique for the confirmation of lesions suspected by ultrasonography or palpation (ovarian hypoplasia, hydrosalpinx, segmental aplasia, peri-uterine adhesions...), particularly in alpacas [7, 25, 33-35]. Selected cases for these procedures include alpacas with suspected ovarian hypoplasia, ovarian masses, hydrosalpinx, pyosalpinx, ovarian and uterine adhesion, segmental aplasia of the uterus and uterine tubes. Laparotomy is indicated for oviductal flushing to verify the patency of the uterine tube.

Hysteroscopy: Hysteroscopy is performed using a flexible endoscope. The cervical canal and uterine cavity can be evaluated for the presence of adhesions, cystic dilation or abnormal content. Targeted biopsy can be performed in combination with hysteroscopy [30, 34].

Hormonal evaluation: Determination of progesterone levels in blood is probably the most widely used hormone assay in camelidae[1, 24]. Progesterone levels above 1.5 ng/ml indicate the presence of a functional corpus luteum or luteinized anovulatory follicle. The assay is commonly used to determine occurrence of ovulation in bred females. Pregnancy is suspected if progesterone level remains high in a second sample taken 2 weeks after breeding. This method of pregnancy diagnosis is relatively precise if breeding history is accurate. Plasma estrogens levels above 10 pg/ml in plasma indicate presence of follicular activity.

Cytogenetic evaluation: All camelidae have the same number of chromosomes ($n=74$). Abnormal karyotype has been associated with different forms of reproductive problems in camelidae [31, 34, 38]. Cytogenetic studies should be considered when external sexual characteristics are ambiguous or when there is extreme aplasia of the ovary or genitalia.

Causes of infertility in the female lamoid

Congenital abnormalities: Abnormalities of the vagina and vestibulum reported in camelidae include segmental aplasia, persistent hymen, vaginal stricture and presence of vaginal septum. These anomalies should be suspected when there is a difficulty in penile penetration. Abnormalities of the external genitalia can arise from cytogenetic abnormalities. Ambiguous external genitalia with a 10cm long prepuce like structure was described in a case of XX sex reversal in a llama with urinary incontinence[38] The animal was genotypically female but the gonads were composed entirely of testicular tissue. Post-mortem examination showed a genitalia with cystic distension of the apical and of uterine tube, uterine horns. The cervix was not discernible and the vagina ended at the hymen [38]. Intersex, Freemartinism, and Sry-negative XX sex reversal have been reported in llamas with anomalies of the vulva and clitoris[13, 15, 16].

Double cervix with some degree of didelphia (double vagina) has been described in llamas and alpacas[5, 21, 31, 34]. The major complaints are infertility with a persistent vaginal discharge or repeat breeding. Diagnosis of the condition is made after vaginal examination using a speculum and confirmed by direct examination of the genitalia after ovario-hysterectomy.

Ovarian hypoplasia was found in 16.8% on postmortem examination of 155 infertile alpaca females[21]. Monitoring ovarian activity by ultrasonography every 2 to 3 days for 10 days allows diagnosis of such disorder. Follicular development may be seen in some females but the follicles fail to develop to an ovulatory size[10].

The most common congenital abnormalities of the uterus in camelidae are segmental aplasia, uterus unicornis and infantilism[21, 34]. Segmental aplasia can affect any part of the genitalia from the vestibulum to the oviduct. Aplasia of the posterior part of the tubular genitalia is usually associated to muco or pyometra. Uterus unicornis may be diagnosed by palpation, ultrasound or hysteroscopy. Pregnancy is possible, but breeding of animals presenting such a condition is strongly discouraged because of the risk of genetic transmission of the abnormality [14, 17, 34].

Common acquired abnormalities leading to infertility

Conditions involving the ovaries: Persistent corpora lutea are very rare in the female camelidae. However, the condition has been suspected in llamas on the basis of prolonged periods of high progesteronemia without pregnancy[3, 36]. Many of these conditions may not be truly “persistent corpora lutea” but rather persistent luteinized hemorrhagic follicle[36]. These structure may respond to PGF2 α but injection may need to be repeated.

Cystic conditions of the ovaries have been described in llamas and alpacas[10, 21]. The term cystic ovaries may not always apply to camelidae because a large proportion (30 to 40%) of non-mated females develop some form of follicular cyst [36]. The role of ovarian cysts as a cause of infertility is not well known. An incidence of 4.7% was reported in a random sample of slaughtered alpacas, while a higher frequency of 8.3% was reported in specimens collected from infertile animals[21]. There are few studies dealing with the endocrinology of cystic conditions of the ovaries. Animals with cystic follicles (> 12 mm) show a decrease in estrogen secretion[10].

Failure of ovulation is a common problem in lamoids [10, 23]. Ovulation failure may be due to inadequate LH release after copulation [10]. Assessment of ovulation by ultrasonography or progesterone assay should be an integral part of the reproductive management of the female.

Conditions affecting the cervix and uterus: Uterine infection is the most common acquired reproductive problem resulting in infertility[29]. Uterine infection should be suspected in animals that have a history of repeat breeding or early embryonic death following at least one normal pregnancy. Diagnosis is confirmed by the results of clinical examination of the animal (vaginal discharge, uterine wall thickening, presence of intrauterine fluid). Diagnosis is confirmed by uterine culture, uterine cytology and eventually uterine biopsy. Treatment approaches are similar to those described for the equine and consist of uterine flushing followed by daily infusion of antibiotics of choice for 3 to 5 days. Treated animals should be sexually rested for 2 weeks. Minimizing number of mating and breeding during the period of the follicular wave most likely to result in ovulation improves fertility and reduces chance of recurrent endometritis [29].

Cervicitis is due to overbreeding, injuries during parturition or gynaecological manipulations. Cervicitis is usually associated to uterine infections and vaginal mucopurulent or bloody discharge. Other acquired anomalies of the cervix include, cervical adhesions or lacerations resulting from a complication of birth or excessive trauma during manipulation.

Endometriosis and Early embryonic death: Early embryonic death is commonly reported in lamoids. Incidence of early embryonic death in these species can be as high as 57.8% [4, 11, 22]. Most of the early embryonic loss occurs before day 45. Some of the possible etiologies of embryonic death in camelidae include genetic or environmental factors, systemic diseases, corpus luteum dysfunction and uterine pathology such as infection or fibrosis. Deficiencies in vitamins A, E or selenium have been incriminated in increased early embryo loss. Increased early embryonic death has been associated to early postpartum breeding (before 3 weeks) [8]. Reduced ovulation rate and embryonic viability may be seen in females in a negative energy balance due to heavy milk production.

Uncommon acquired abnormalities leading to infertility

Oophoritis: Inflammation of the ovaries may be due to an extension of a peritonitis or perimetritis. It is characterized by loss of follicular activity because of the presence of adhesions between the ovarian surface and the surrounding tissues including the ovarian bursa, uterine tube and sometimes extending to include some intestinal loops.

Salpingitis, hydrosalpinx, pyosalpinx: The most common disorders of the uterine tube in camelidae are inflammations or pyosalpinx or hydrosalpinx [21, 34]. The enlarged uterine tube can be visualized by ultrasonography. In severe cases, the ovary and ovarian bursa may be involved and adhere to each other. Mucosal cysts and micro-abscesses are reported but relatively rare. Diagnosis of these affections requires endoscopic evaluation of the uterine tube papillae and/or laparoscopy. Prognosis for all these affections is poor if bilateral and guarded if unilateral.

Ovarian tumors: Ovarian tumors have been reported in infertile Lamoids and most are teratomas[21]. A case of bilateral teratoma has been described recently in an alpaca. Unilateral ovariectomy followed by partial ovariectomy has been successful in removing the tumor and restoring fertility [12].

Pyometra: This condition has been reported in lamoids[31, 34]. Pyometra with an open cervix and vaginal discharge are observed primarily in the puerperium period and is due to a postpartum complication (retained placenta, dystocia, uterine prolapse) resulting in delayed involution due to infection and accumulation of fluid. Closed pyometra is usually associated with cervical adhesions or prolonged progesterone therapy.

Severe acquired abnormalities leading to sterility

Uterine diseases: Other reported diseases of the uterus include uterine cysts, uterine abscesses, peri-uterine adhesions, polyps and uterine neoplasm[21] [17, 19, 28, 34]. Periuterine adhesions are suspected in llamas if difficulty is encountered when attempting to retract the uterus by palpation per rectum. These adhesions are usually a consequence of peritonitis and may be

originate from post-surgical complications. Uterine neoplasia (adenocarcinoma) has been described in the llama[19].

Complete vaginal or cervical adhesions: All diagnosed cases of pyometra seen by one of authors (Tibary) were associated to either cervical or vaginal adhesions or to prolonged progesterone treatments.

**Reference list available upon request, please send self-addressed, stamped envelope to A. Tibary, Dept. veterinary Clinical Sciences, WSU, Pullman, WA 99164-6610*

Table 1: Reproductive disorders by organ diagnosed over a 20 year period in female Lamoids presented for infertility and method used to suspect (SM = suspicion method) and confirm (CM = confirmation method) the diagnosis (adapted from Tibary et al 2001[34])

Diagnosis	N	SM	CM
<i>Vulva and Vagina</i>			
Ambiguous gender	2	External	Cytogenetics, post-mortem
Enlarged clitoris	4	External examination	
Abnormal vulva	3	External examination	
Vaginal adhesions	3	Vaginoscopy	Videoendoscopy
Vaginitis	1	Vaginoscopy	
Double vagina	2	Vaginoscopy	Videoendoscopy
Persistent Hymen	4	Digital palpation	Videoendoscopy
<i>Cervix</i>			
Cervicitis	5	Vaginoscopy	
Double cervix	2	Vaginoscopy	Videoendoscopy
Cervical adhesions	3	Manual examination	Videoendoscopy
<i>Uterus</i>			
Segmental aplasia uterus	3	Ultrasonography	Laparoscopy*
Endometrial abscess	1	Ultrasonography	Videoendoscopy
Cystic formation	2	Ultrasonography	Videoendoscopy
Endometrial fibrosis	18	Ultrasonography	Biopsy
Endometritis	46	History, vaginoscopy	Culture, cytology, biopsy
Intestinal-uterine adhesions	1	Palpation	Laparoscopy
Mucometra - Pyometra	5	Palpation	Ultrasonography
Mummification	0	Palpation	Laparotomy
<i>Uterine tube</i>			
Segmental aplasia	1	All failed	Laparoscopy
Hydrosalpinx/ Pyosalpinx	1	Ultrasonography	Laparoscopy
Salpingitis	3	All failed	Laparoscopy / laparotomy
<i>Ovary and Ovarian bursa</i>			
Ovarian hypoplasia	22	Ultrasonography	Laparoscopy
Hemorrhagic follicles/cysts	20	Ultrasonography	Ultrasonography
Ovulation failure	8	History	Endocrinology
Persistent luteal structures	8	Ultrasonography	Endocrinology
Ovarian adhesions	1	Ultrasonography	Laparotomy
Ovarian tumor	1	Palpation, ultrasonography	Laparotomy
Ovario-bursal adhesions	1	Palpation, ultrasonography	laparotomy

Table 2: Major complaints and value of specific examination procedures in determination of the major causes of infertility in camelids (from Tibary et al 2001 [34]).

Condition to be considered	Major complaint*	Primary method of diagnosis	Confirmation method
Maiden Females			
Pregnancy	1	History, ultrasound	Ultrasound
Immature female	1, 3	History, physical examination	Ultrasound
Persistent hymen	1	Vaginal examination	Videoendoscopy
Ovarian hypoplasia	1, 2	Ultrasound	Endocrinology, Laparoscopy
Segmental aplasia	1, 2	Ultrasound	Laparoscopy/Laparotomy
Ovulation failure	1, 2	Ultrasound	Endocrinology
Behavioural problems	1	Physical, ultrasound	Normal genitalia, normal endocrinology
Intersex	1, 4	Physical	Laparoscopy, Cytogenetics
Multiparous females			
Pregnancy			
Anovulatory/hemorrhagic follicles	1	Ultrasound	Progesterone
Luteal activity/persistent corpus luteum	1	Ultrasound	Progesterone
Ovarian inactivity	1	Ultrasound	Endocrinology, Laparoscopy
Failure of ovulation	1	Ultrasound	Endocrinology
Ovarian adhesions/ abscess	1, 2	Ultrasound	Laparoscopy
Vaginal/cervical adhesion	1	Vaginoscopy	Videoendoscopy
Endometritis	2, 3, 4	Vaginoscopy, uterine culture and cytology	Uterine biopsy
Endometrial fibrosis	2, 3	History, ultrasonography	Uterine biopsy
Salpingitis	2	Ultrasonography	Laparoscopy, laparotomy
Uterine adhesions	2,3	Ultrasonography	Hysteroscopy
Systemic diseases	3	History, physical examination	Serology, hematology
Poor body condition	1, 2, 3, 4	Physical examination	
Vaginal conformation	4	Physical examination	

*Major Complaints: Inability to breed (1), Repeat breeding (2), Pregnancy loss (3), abnormal external genitalia (4)