Reproductive failure in female camelids

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Introduction

Diagnosis of the cause of reproductive failure in female camelids is a challenging task because of the numerous peculiarities of the reproductive anatomy and physiology in these species. The practitioner is often asked to investigate reproductive failure not only on the individual basis but also on the herd basis. This requires a thorough understanding of breeding management and a mastery of a wide range of clinical techniques for reproductive function investigation. In the present paper we discuss the main complaints, diagnostic and therapeutic approach in infertility in female South American camelids (SAC) as well as camels with a primary focus on individual animal.

Keywords: Infertility, ultrasonograhy, repeat breeding, early embryonic death

Reproductive management and common complaints in reproduction

Reproductive failure case definition relies on three main aspects: 1) the type of complaint, 2) signalment, and 3) a thorough breeding history. Availability of accurate individual health and reproduction records is often a problem in large herds of SAC and in camels. Client education on record keeping is of paramount importance. Although many SAC breeders have computerized record systems, exact breeding dates and techniques used for diagnosis of pregnancy are often not precise. In addition, most "in-hand" mating is based on receptive behavior and not on specific ovarian follicular status. Most breeders use behavioral signs for pregnancy diagnosis ("spiting off" in SAC and "tail curling" in camels) at 14 days after mating. A small proportion of SAC breeders uses progesterone assay for determination of pregnancy status in addition to the"spitting off" test. When used, progesterone levels are generally determined at 21 days after mating. An increase in diagnostic errors has been noted by the authors in recent years as more and more breeders have ventured into the use of sonograms and ultrasonography without veterinary supervision.

Herd reproductive efficiency may be assessed by annual pregnancy rate, abortion rate, birthing rate, distribution of births in the season, and weaning rate. These calculations are extremely valuable for the investigation of herd problems.[1] Client education should emphasize proper nutritional management, prophylactic treatments (especially vaccination and deworming programs), and male selection in order to maximize fertility.

For individual animals, reproductive failure is defined based on number of unsuccessful mating cycles. Females should be mated only once per week based on strong receptive behavior. If the female is not spitting off the male after a maximum of three matings to a proven male, she should be presented to the veterinarian for a breeding soundness examination. This will ensure an early diagnosis of the cause of infertility and reduce the risk of further complications or jeopardy to the animal welfare.[2]

In our referral practice, the most common complaints in 2435 SAC females examined over the last 15 years were repeat breeding (76%), recurrent early pregnancy loss (18%), continuous spitting off the male (4%) and observed abnormalities of the vulva and perineum (2%).[3]

The repeat breeding complaints include different manifestations, from breeding continuously (every day or every other day), breeding every week or every two weeks. This is due to the variety of breeding management techniques used by different breeders. Early pregnancy loss is generally defined as return to receptivity following one positive pregnancy diagnosis based either on behavior or progesterone level.

Repeat breeding syndrome can be due to many factors, some of which are pathological, whereas others are management errors. The pathological factors involved in repeat breeding include all conditions that may affect gamete or early embryo survival. Management of reproduction in camelids is complicated because the signs of estrus are not very reliable and the timing of breeding in relationship to growth and

size of the follicle are very critical for induction of ovulation. Diagnosis of the cause of repeat breeding can be challenging and a diagnosis can only be achieved with a complete and sound approach to the problem.[4]

Female reluctance to breed may be due to pregnancy. Behavioral problems are relatively common and may be associated to dominance or sexual differentiation problems. Reluctance to breed may be caused by pain during mating, due to either congenital or acquired defects of the reproductive tract.

Visible abnormalities of the vulva or vagina can be very easy for an owner to detect. Observed abnormalities include mainly vaginal discharge (bloody or mucopurulent) and abnormalities of development or perineal conformation.

Approach to diagnosis of reproductive failure

The objectives of a breeding soundness examination are to attempt to answer the broad categories of causes of reproductive failure which are: is the failure due to breeding management errors? Is the male a major factor? Does the female have normal genitalia? Does she have normal follicular dynamics and is she able to ovulate? Is fertilization occurring and followed by pregnancy loss? Judicious choice of and proper use of routine clinical examination techniques allows accurate diagnosis of the condition in the majority of cases(Tables 1 and 2).[4] Timing of the initial examination is very important in order to maximize the information obtained. We recommend to our clients to present the female camelid for an evaluation 14 days following a mating.[5]

History and physical examination

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

Female behavior provides a relatively accurate and inexpensive means for pregnancy diagnosis. Male rejection is very well correlated with high progestéronémie, while receptivity is not well correlated with ovarian status.

External genitalia

The vulva should be inspected for discharge or lesions and its size and conformation recorded. Females presenting increased size and edema of the vulva should be examined for recent abortion. Abnormal size and position of the vulva in maiden females may suggest presence of congenital disorders or intersexuality. Intersex 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 may have dysuria. Copious vaginal discharge beyond seven days postpartum is almost always pathological.[5]

Per rectum evaluation of the reproductive tract

Per rectum palpation of the genitalia is an essential part of the examination of the female llama and camel. 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. In the non-pregnant female, 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 relaxed and thin-walled during the luteal phase and pregnancy. Ovarian structures (corpus luteum, follicles) are palpable in the same manner as in cattle.[5]

Ultrasonography

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. Dominance is established when follicular diameter reaches 6 mm. The dominant follicle continues to grow steadily (0.6 mm per day in SAC and 1.5 to 2 mm/day in camels) until it reaches its maximal size (9 to 14 mm in llamas, 8 to 12 mm in alpacas, 12 to 25 mm in camels). The corpus luteum (CL) is easy to visualize by day 4 after mating of induction of ovulation. It is less echogenic than the ovarian stroma, protrudes from the ovarian surface, and measures 11 to 14 mm in SAC and 15 to 25 mm in camels. A central anechoic cavity may be visualized in a significant proportion of CL.[6-8]

During the follicular phase, the uterus is toned, the uterine horns are straight, and ultrasonography there is increased edema of endometrial folds. During the luteal phase, the uterus is homogeneous with a medium degree of echogenicity. Typical ultrasonographic features of the normal and abnormal reproductive tract are presented in Figures 1-4.[9]

Vaginal examination

Digital examination of the vestibulo-vaginal area should be performed on all maiden females to rule out persistent hymen and segmental aplasia, and all females suspected to have pyometra or hydrometra, due to vestibular or vaginal adhesions.[5,10]

Vaginoscopy is best performed using a tube speculum (2 to 3 cm in diameter in llamas and camels) or a human sigmoidoscope in alpacas. The vaginal mucosa should be moist and pink. The cervix is clearly visible and should be open during the follicular phase. A small quantity of mucus may be observed in some females with peak follicular development.

Endometrial cytology and bacteriology

Endometrial cytology and culture should be part of the basic infertility workup. Samples are taken from the uterine cavity using a mare double guarded swab. Swabs are examined routinely for aerobic and anaerobic bacteria, *Ureaplasma* spp., *Mycoplasma* spp., and for fungus. The bacteria responsible for endometritis in camelids are essentially those found in the equine and bovine species. The best endometrial specimens for cytology are obtained with a double-guarded cytobrush.[5]

Endometrial biopsy

Endometrial biopsy is essential in the diagnosis of inflammatory, degenerative, or neoplastic changes in the endometrium. Endometrial biopsy should be considered on any female falling into the following categories:

- barren female
- infertility or unsuccessful breedings
- embryo loss or abortion
- pre-purchase evaluation of breeding females
- screening of recipients for embryo transfer
- abnormal echotexture of the uterus

Samples are preferably obtained during the peak follicular phase when the cervix is easy to bypass and uterus has maximum tone. An equine endometrial biopsy punch is appropriate for llamas and camels. For alpacas, we recommend the use of a Turret® rectal biopsy punch (4 x 3 x 3 mm). The instrument is advanced into the uterine cavity with the aid of a vaginal speculum. Endometrial samples may also be obtained through a flexible fiberoptic endoscope during hysteroscopy. This technique offers the advantage of samples specific areas of the endometrium but the samples obtained are often very small (2 mm) and difficult to interpret. A biopsy classification method has been proposed but has not been fully investigated (Table 3).[11,12]

Endocrinological evaluation

Circulating progesterone concentration is the most widely used hormone assay in camelids. Progesterone levels above 1.5 ng/ml indicate the presence of a functional corpus luteum or luteinized anovulatory follicle. Progesterone assay may be used to confirm occurrence of ovulation seven to eight days after mating. Pregnancy is suspected if progesterone level remains high in a second sample taken two weeks after breeding. This method of pregnancy diagnosis is relatively precise if breeding history is accurate.

Plasma estrogen levels above 10 pg/ml in plasma indicate presence of follicular activity. However, this assay provides little information compared to ultrasonography. Follicular waves may be monitored by serial sampling (every 3 days for 10 days).

Testosterone and inhibin evaluation is critical for the diagnosis of granulosa-theca cell tumors. Evaluation of AMH has not been fully investigated in camelids.

Hysteroscopy

Hysteroscopy is performed under sedation using a 9 mm (outer diameter) flexible fiberoptic endoscope or videoendoscope (a human pediatric gastroscope is very appropriate for alpacas). 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. [13]

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. In our practice, this technique has been used to diagnose 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 retrograde oviductal flushing to verify the patency of the uterine tube.[4]

Cytogenetic evaluation

The normal karyotype in camelids has 74 chromosomes. Abnormal karyotype has been associated with different forms of reproductive problems in camelids. Cytogenetic studies should be considered when external sexual characteristics are ambiguous or when there is extreme aplasia of the ovary or genitalia. Cytogenetic abnormalities described in camelids include XO, XXX, XXY, XX/XY, and XX sex reversal. A minute chromosome has been described in camelids and may be implicated in infertility.[14]

Evaluation of specific complaints

Repeat breeding

Repeat breeding complaints can be further subdivided into several categories based on initial evaluation: repeat breeding with no ovarian follicular activity, repeat breeding with no ovulation, or repeat breeding after a normal ovulation and without a positive pregnancy diagnosis (failure of fertilization or very early embryo loss).

Absence of ovarian follicular activity. Absence of ovarian follicular activity can be confirmed by serial ultrasonographic evaluation or plasma estradiol levels (every two to three days for ten days) and may be congenital or acquired. Ovarian hypoplasia was found in 16.8% of 155 infertile alpaca females on postmortem examination.[15] The majority of affected females display continuous receptivity to the male. Ovarian hypoplasia has been associated with various chromosomal abnormalities in about 30% of the cases (XXX, XO, XX/XY and presence of the minute chromosome).[14] However, many females may have a normal karyotype. The uterus is often very small and flaccid.

Acquired ovarian inactivity is commonly observed in lactating camels and to a lesser degree in lactating llamas and alpacas. Negative energy balance during lactation or due to poor nutrition is also involved.[16,17] Ovarian inactivity is also suspected to occur due to trace mineral deficiencies and

hypothyroidism. Follicular development may be seen in some females but the follicles fail to develop to an ovulatory size. Poor ovarian activity is also observed with advanced age.

Ovulation failure. Camelids are induced ovulators. Ovulation is induced 30 to 36 hours following mating in response to the luteinizing hormone (LH) peak, which is elicited by a combination of uterine inflammation and the presence of □□nerve growth factor (□□NGF) in seminal plasma.[18,19] Failure of ovulation may occur following mating to a less potent male, when the follicle is immature, or when the follicle is past its ovulatory peak (anovulatory follicles).[20] Anovulatory hemorrhagic follicles are not uncommon and some females seem to more prone to them than others. Metabolic disorders (obese females) and endocrine disorders (hypothyroidism) seem to place a female at a higher risk of failure of ovulation and anovulatory hemorrhagic follicle development particularly in camels. Ovarian disorders such as oophoritis and ovario-bursal adhesions have been diagnosed by the authors in females with failure of ovulation.

Ovulation failure and repeat breeding can be observed in females with abnormalities of the vagina and cervix that preclude normal mechanism of induction of ovulation (which is dependent on in utero semen deposition). These situations include imperforate hymen, vaginal aplasia, and vaginal or cervical adhesions. In all these cases accumulation of various quantities of fluid may be seen on ultrasonography of the uterus and/or vagina. Some females may be extremely painful and may continue to strain after mating.[21]

Fertilization failure. In the presence of adequate management and normal male fertility, the most common cause of fertilization failure in the female camelid is endometritis. Uterine infection should be suspected in animals with a history of repeat breeding or early embryonic death. It is important to consider other primary reasons for reproductive failure as endometritis may be the result of repeat breeding in animals with ovarian or oviductal problems. Clinical evaluation may reveal thick mucopurulent vaginal discharge. On ultrasonography, the uterine wall is often thickened and intrauterine fluid may be visualized. Diagnosis is confirmed by uterine culture, uterine cytology, and eventually uterine biopsy.[22]

Samples for bacteriology should be kept at room temperature and shipped overnight to the laboratory in special transport media depending on the type of culture desired (Culturette®, Aimes transport medium). Samples should be examined routinely for aerobic and anaerobic bacteria, *Ureaplasma* spp., *Mycoplasma* spp., yeast and fungus. Refrigeration and transport in special media should be considered for *Mycoplasma* spp. and *Ureaplasma* spp. and for *Campylobacter* spp. (Cary-Blair transport medium). Samples from camels should also be examined for *Tritrichomonas* spp. and *Campylobacter* spp. which are suspected to be responsible for fertility failure in the dromedary. Bacteriological techniques should include sensitivity tests to the major antibiotics.

The most common bacteria isolated form camelids with endometritis are *Escherichia coli*, *Streptococcus equi zooepidemicus*, *β*-hemolytic *Streptococci*, *Enterococcus* spp., coagulase negative *Staphyloccocus* spp., *Proteus* spp, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Trueperella (*formerly *Arcanobacter) pyogenes*. *Pseudomonas aeruginosa*, *Campylobacter fetus fetus*, and *Tritrichomonas foetus* have been isolated from infertile camels and venereal transmission should be considered in cases of herd infertility or abortion outbreaks.[23] *Aspergillus* spp. and *Mucor* spp. have been isolated from female dromedaries with endometritis. *Chlamydophila* spp. and *Mycoplasma* spp. have been found in a few cases. It is important to realize that a negative culture does not negate the presence of infection. [22] The authors have seen outbreaks of *Pseudomonas aeruginosa* in some alpaca and camel herds due to venereal transmission by an infected male.

Treatment approaches are similar to those described for the equine and consist of uterine flushing followed by daily infusion of antibiotics for three to five days. Uterine lavage with antiseptic solutions may compromise the local defense mechanisms. In recent years we have been satisfied with the use of Equine Uterine Lavage solution® (Bioniche Animal Health, Pullman, WA) because it is buffered to pH 7.1 and contains a surfactant which helps clear mucus and cell debris. Oxytocin (5 IU for alpacas, 10 IU for llamas and 20 IU for camels, IM) is given to improve uterine clearance. If antibiotics are to be infused

in utero we prefer to infuse them a few hours after lavage. The antibiotic choice should be based on culture and sensitivity results. Alternately, infusion with a broad spectrum antimicrobial may be used while laboratory cultures are pending. The most common antibiotics used are potassium penicillin ($1.5x10^6$ units for llamas and alpaca, $5x10^6$ units for camels), gentamicin sulfate (200 to 300 mg for llamas and alpacas, 500 to 1000 mg for camels), ticarcillin and clavulonic acid (particularly for *Pseudomonas* infections), amikacin sulfate (for *Pseudomanas* spp. and *Klebsiella* spp. infections) and ceftiofur sodium (250 to 500 mg for alpacas and llamas, 1 g for camels). The third generation cephalosporin ceftiofur has a broad spectrum and is effective against gram negative and gram positive bacteria.

Agents such as DMSO or N-acetylcysteine may be used in chronic cases with thick mucopurulent discharge to help liquefy the mucus. Infusion with 30% DMSO solution prior to uterine lavage is used in our laboratory for chronic cases. Biofilm formation is a characteristic of many organisms found in camelid endometritis including *P. aeruginosa*, *S. epidermis*, *E. coli*, *Enterobacter* spp. and some fungi and yeast. Studies in mares have shown that addition of buffered chelating agents (tris-EDTA and Tricide®) help dissolve the biofilm and improve antimicrobial action.

In camels, the authors have used specially formulated intrauterine infusion products such as Metrijet® (1500 mg oxytetracycline hydrochloride solution) with good results. However, these products are not available in some countries. Intrauterine foam (Fatroximin®, Fatro, Bologna, Italy) provides a release of rifaximin, a synthetic derivative of rifamycin, over 72 hours and has been used in camels for post-breeding control of re-infection. A 4% metacresol-sulfonic acid and formaldehyde solution (Lotagen®) is commercially available in some countries and seems to provide good results for treatment of endometritis in camels.[24]

Most antibiotics used *in utero*, as well as orally administered trimetrhoprim/sulfamethoxazole and IV enrofloxacin achieve very good concentration in uterine tissue. Recently we have used long acting tetracyclines as well as ceftiofur (Excede®) in the management of camels with high susceptibility to uterine infection.

Treated animals should be sexually rested for two weeks. The duration of mating should be minimized (12 minutes) during the peak of the follicular to reduce chance of recurrent endometritis.

Failure of fertilization may be due to uterine tube and bursal abnormalities. The most common disorders of the uterine tube in camelids are inflammation, pyosalpinx, or hydrosalpinx. 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 is poor if bilateral and guarded if unilateral. In camels, ovario-bursal adhesions, ovariobursitis and hydrobursitis have been recently associated to *Chlamydophila* infections but these reports have not been thoroughly investigated.[25-27]

Recurrent pregnancy loss

Early embryonic death is commonly reported in camelids. Incidence of early embryonic death in these species can approach 60%. Most of the early embryonic loss occurs before day 45. Some of the possible etiologies of embryonic death in camelids include early breeding of maiden females (less than 45 kg in body weight), genetic causes (fiber production and quality), environmental factors (heat stress), systemic diseases (acute respiratory syndrome, trypanosomiasis and pox in camels), corpus luteum dysfunction, and uterine pathology including 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 three weeks). Reduced ovulation rate and embryonic viability may be seen in females in a negative energy balance due to heavy milk production.

Twinning may contribute to early pregnancy loss. Clinicians should always verify the location and number of CL during pregnancy diagnosis. Most twin pregnancies will either reduce to a singleton or be completely lost by 60 days of pregnancy.

Management of recurrent pregnancy loss is best addressed by the use of embryo collection and transfer. Females suspected of poor luteal function are often placed on progesterone supplementation in the form of hydroxyprogesterone caproate (250 mg every three weeks until 300 days). However this treatment should be used cautiously due to the risk of complications during pregnancy and at the time of parturition. Poor luteal function is often observed in very obese animals. Hypothyroidism is suspected to be a predisposing factor.

Other disorders of the reproductive tract that may be involved in failure of fertilization or recurrent pregnancy loss include segmental aplasia of the uterine tube or uterus, uterine cysts, uterine abscesses, peri-uterine adhesions, polyps, and uterine neoplasia. Peri-uterine 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.[11] Endometrial hemangioma has been diagnosed by the authors in three females presented for reproductive failure and persistent bleeding following mating.

Abnormal behavior

The main complaints regarding abnormal behavior in females are persistent rejection of the male or male-like behavior. Persistent rejection of the male may be due to persistent luteal activity or to adverse reaction to mating (painful mating due to vaginal obstruction). Persistence of CL may follow a sterile mating or a spontaneous ovulation. This problem is often encountered in the postpartum female or females with chronic degenerative uterine disorders. Luteolysis is easily induced by administration of cloprostenol (250µg, IM in SAC, 500 µg in camels). Persistent luteal function may also be due to luteinized hemorrhagic follicles. These structures do not readily respond to a single injection of cloprostenol.

Male-like behavior is normal to a certain degree and is observed in dominant females at the peak of follicular development. However, persistent aggressive male-like behavior may be due to testosterone-producing ovarian tumors such as granulosa-theca cell tumors and interstitial cell tumors.[21,28,29] Unilateral ovariectomy is the best course of action. Male-like behavior may also be observed in XX-sex reversed animals due to the presence of testicular tissue.[30]

Abnormal external genitalia

Abnormalities of the external genitalia are typically congenital in origin. Some of these abnormalities are obvious on inspection of the external genitalia but other may present as abnormal vaginal discharge (blood) or abnormal urination. Abnormalities of the external genitalia can arise from cytogenetic abnormalities. Ambiguous external genitalia with a long, prepuce-like structure is often encountered in XX sex reversal animals.[30] Intersex, freemartinism, and Sry-negative XX sex reversal have been reported with anomalies of the vulva and clitoris.[30-33]

Vulvar hypoplasia or aplasia is common. Total vulvar aplasia with accumulation of large quantities of urine in the uterus is seen in neonates and can be lethal if not addressed quickly. Vulvar hypoplasia is often observed in maiden females when mating is difficult due to the small vulvar opening.[4,34-36] Surgical correction is possible but the possible hereditary nature of the defect should be considered.

Animals with abnormal vaginal discharge may have congenital abnormalities or acquired vaginal or cervical adhesions that cause persistent inflammation during mating. Double cervix with some degree of didelphia (double vagina) has been described in llamas and alpacas.[37] The major complaints are infertility with a persistent vaginal discharge or repeat breeding.

Urinary incontinence as evidence by lack of good urine stream and/or urine scalding is often part of the clinical manifestation of congenital or acquired abnormalities of the caudal genitalia. These abnormalities may also include ectopic ureters. Females presenting for persistent straining and vaginal and/or rectal prolapse following mating should be examined for vaginal adhesions or persistent imperforate hymen.

Conclusion

Reproductive failure in the female camelid may be due to management as well as congenital or acquired disorders. The practitioner providing theriogenological services to camelid herds should be involved in client education. Areas of focus should includebreeding management as well as the importance of breeding soundness examination of maiden females, for a timely diagnosis of congenital defects and to avoid compromise of animal welfare. Timely and accurate diagnosis of acquired infertility is also important for prognosis for future fertility. Practitioners providing reproductive services should master the basic clinical techniques used for evaluation of reproductive function such as ultrasonography.

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Table 1: Reproductive disorders, by organ, diagnosed in female camelids presented for infertility, and methods used to suspect (SM = suspicion method) and confirm (CM = confirmation method) the diagnosis

Diagnosis	SAC	Camels	SM	CM
Vulva and Vagina				
Ambiguous gender	5	1	External examination	Cytogenetics, post-mortem
Enlarged clitoris	8	2	External examination	
Vulvar hypoplasia	8	2	External examination	
Vaginal aplasia	7	1	Vaginoscopy	Videoendoscopy
Vaginal adhesions	10	5	Vaginoscopy	Videoendoscopy
Vaginitis	1	2	Vaginoscopy	
Double vagina	2	1	Vaginoscopy	Videoendoscopy
Persistent Hymen	9	3	Digital palpation	Videoendoscopy
Recto-vaginal tears	8	6	External examination	
Cervix				
Cervicitis	5	3	Vaginoscopy	
Double cervix	2	1	Vaginoscopy	Videoendoscopy
Cervical adhesions	3	0	Manual examination	Videoendoscopy
Cervical rupture (incompetence)	11	3	Vaginoscopy	Videoendoscopy
Uterus				
Segmental aplasia uterus	5	1	Ultrasonography	Laparoscopy
Endometrial abscess	1	1	Ultrasonography	Videoendoscopy
Uterine cysts	8	4	Ultrasonography	Videoendoscopy
Endometrial fibrosis	32	15	Ultrasonography/palpation	Biopsy
Uterine luminal adhesions	12	7	Ultrasonography/palpation	Videoendoscopy
Endometritis	83	102	History, vaginoscopy	Culture, cytology, biopsy
Intestinal-uterine adhesions	2	2	Palpation	Laparoscopy
Mucometra - Pyometra	18	5	Palpation	Ultrasonography
Mummification	1	2	Palpation	Laparotomy
Uterine tube				
Segmental aplasia	1	1	All usual techniques failed	Laparoscopy
Hydrosalpinx/ Pyosalpinx	1	3	Ultrasonography	Laparoscopy
Salpingitis	6	8	All usual techniques failed	Laparoscopy / laparotomy
Ovary and Ovarian bursa				
Ovarian hypoplasia	37	4	Ultrasonography	Laparoscopy
Hemorrhagic follicles/cysts	41	86	Ultrasonography	Ultrasonography
Ovulation failure	8	3	History	Endocrinology
Persistent luteal structures	15	4	Ultrasonography	Endocrinology
Ovarian adhesions	3	11	Ultrasonography	Laparotomy
Ovarian tumor	3	4	Palpation, ultrasonography	Laparotomy
Ovario-bursal adhesions	3	117	Palpation, ultrasonography	Laparotomy

Table 2: Major complaints and value of specific examination procedures in the determination of the cause of infertility in camelids.

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
Behavioral problems	1	Physical, ultrasound	Physical examination and endocrinology
Intersex	1, 4	Physical	Laparoscopy, Cytogenetics
Multiparous females			
Anovulatory/hemorrhagic follicles	1	Ultrasound	Endocrinology
Luteal activity/persistent corpus luteum	1	Ultrasound	Endocrinology
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)

Table 3: Classification of endometrial biopsy and potential effect on fertility (according to Powers et al 1990)

Categories of biopsy	Histopathological characteristic	Effect on fertility
Grade 1A	Normal endometrium	Normal conception rates
Grade 1B	Few lymphocytes within the endometrium. Siderophages present.	Low-grade infection or remnants of previous inflammation. Mild surface irritations may indicate reaction to breeding. May be postpartum or post abortion (siderophages)
Grade 2A to 2B	Active and acute, chronic, or chronic active endometritis. Chronic inflammation tended to be more deeply located in the endometrium, compared with active and chronic active inflammation	Interferes with conception and may cause early embryonic death.
Grade 3A	Chronic endometritis with glandular fibrosis	Interferes with implantation and placentation. May cause early embryonic death.
Grade 3B	Uterine neoplasia	Pregnancy loss or abortion

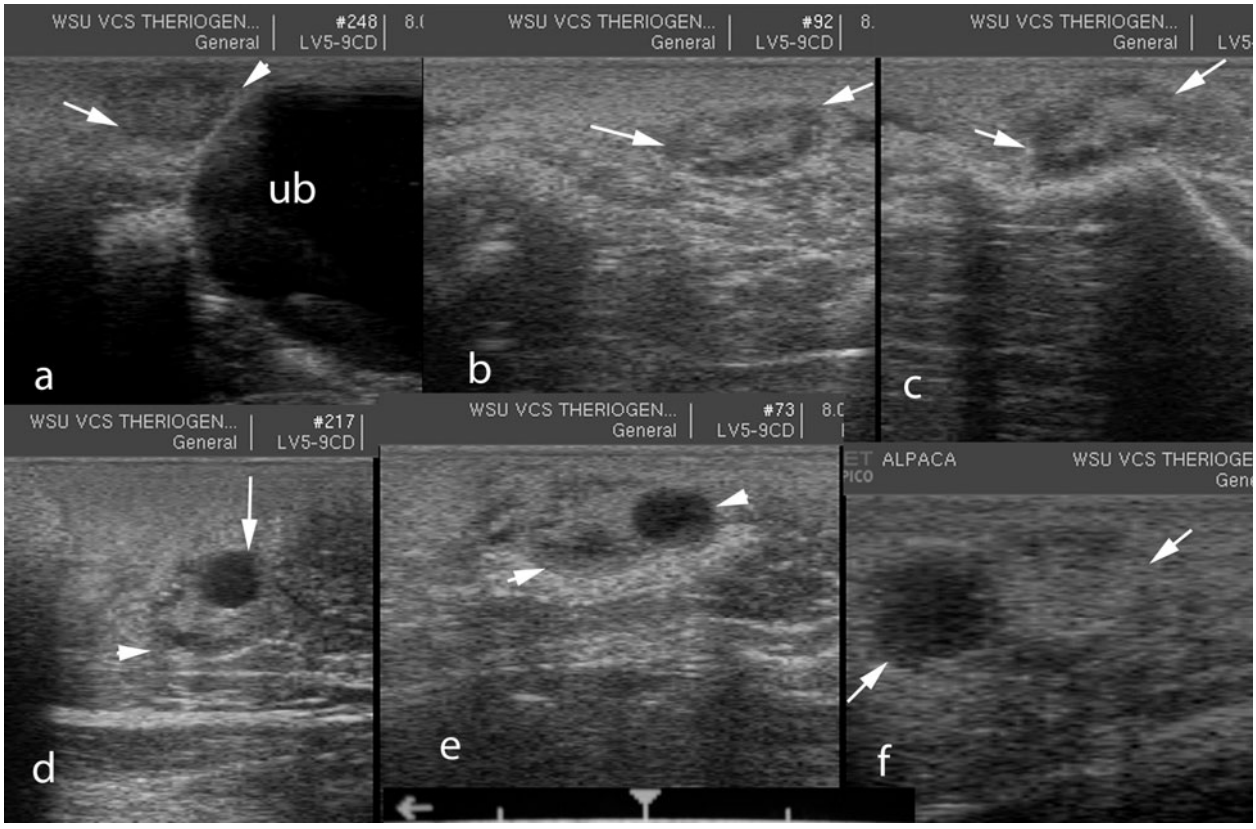

Figure 1: Ultrasonographic imaging of follicular dynamics in camelids. a) Quiescent ovary (arrows), ub= urinary bladder, b) and c) Follicular recruitment, d) Follicular dominance, e) Mature follicle, f) Regressing follicle. Note the hyperechoic ovarian stroma and the more hypoechogenic cortex.

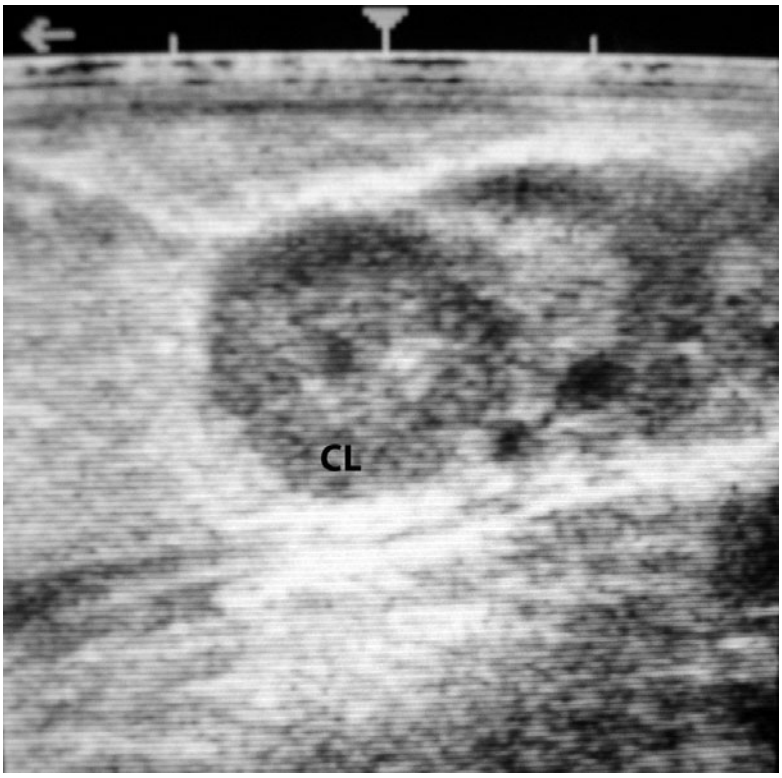

Figure 2: Ultrasonogram of a mature corpus luteum. Note the size and the small central cavity.

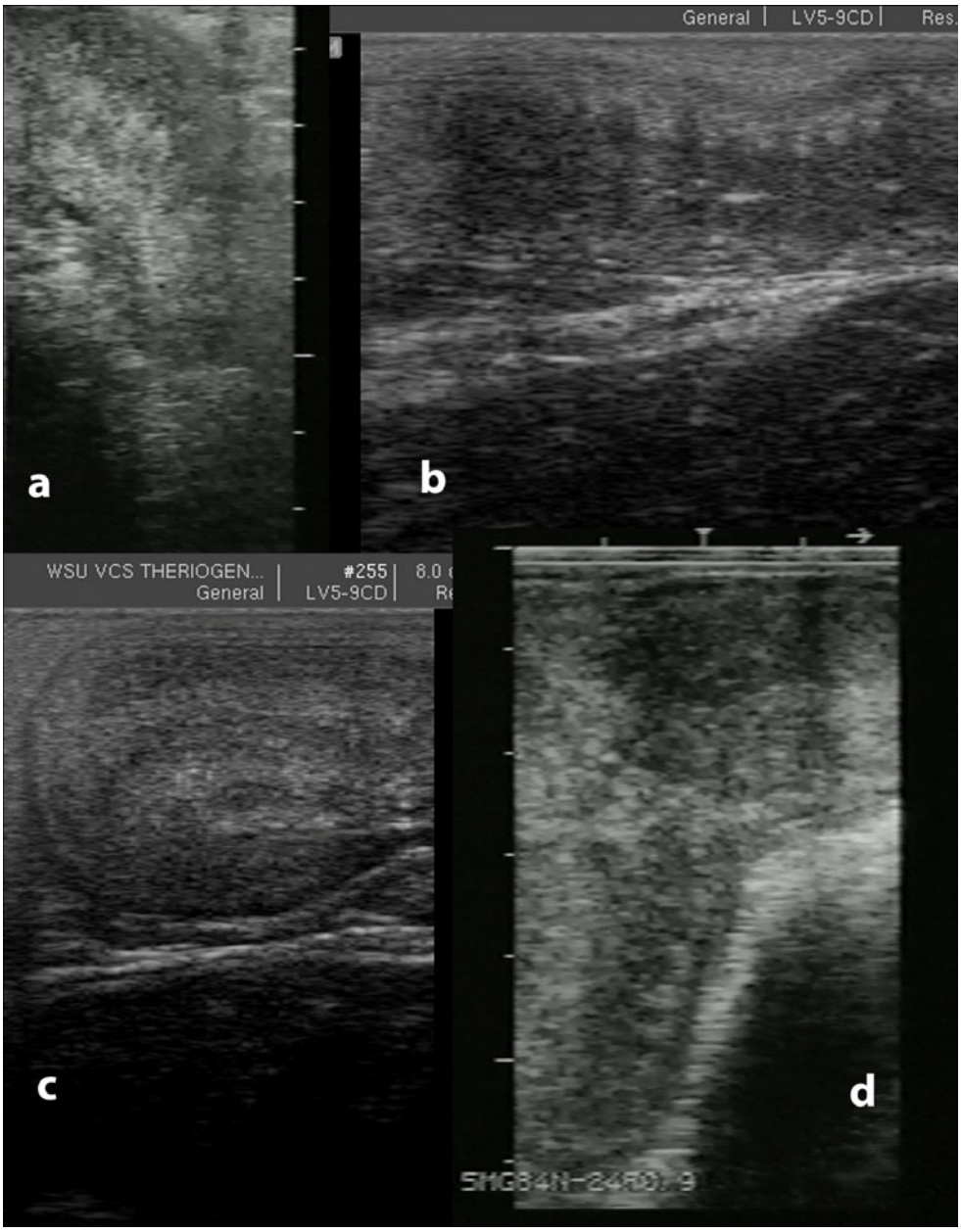

Figure 3: Ultrasonograms of the uterus during different stages of follicular development and after ovulation. a-b) Increased edema and tone (follicular phase), c-d) Relaxed and homogenous (luteal phase).

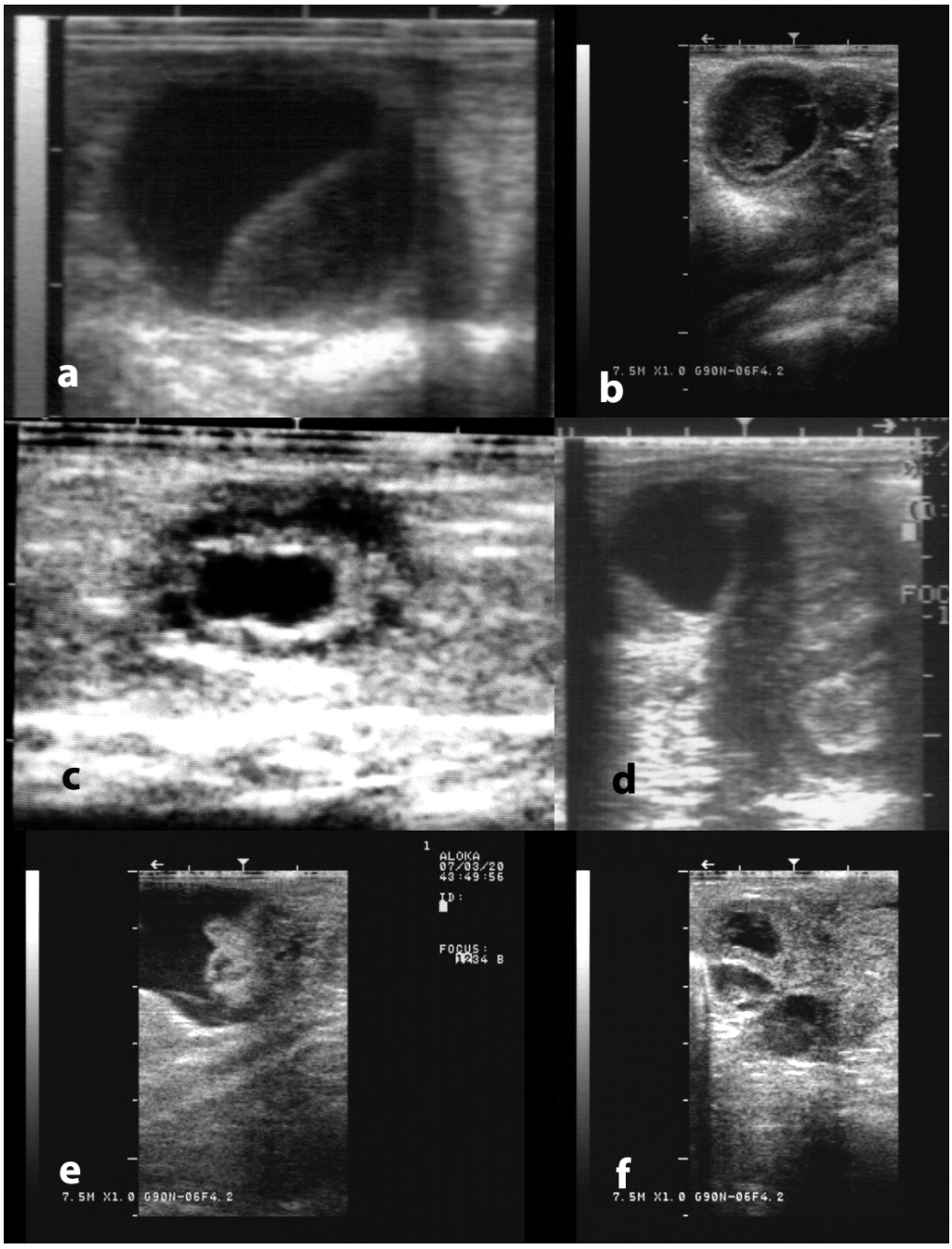

Figure 4: Ultrasonographic appearance of some ovarian and tubal disorders in camelids. a) Large anovulatory follicle, b) Luteinized/hemorrhagic anovulatory follicle, c) Cystic rete ovarii, d) Ovarian teratoma, e) Hydrobursitis, f) Hydrosalpinx.

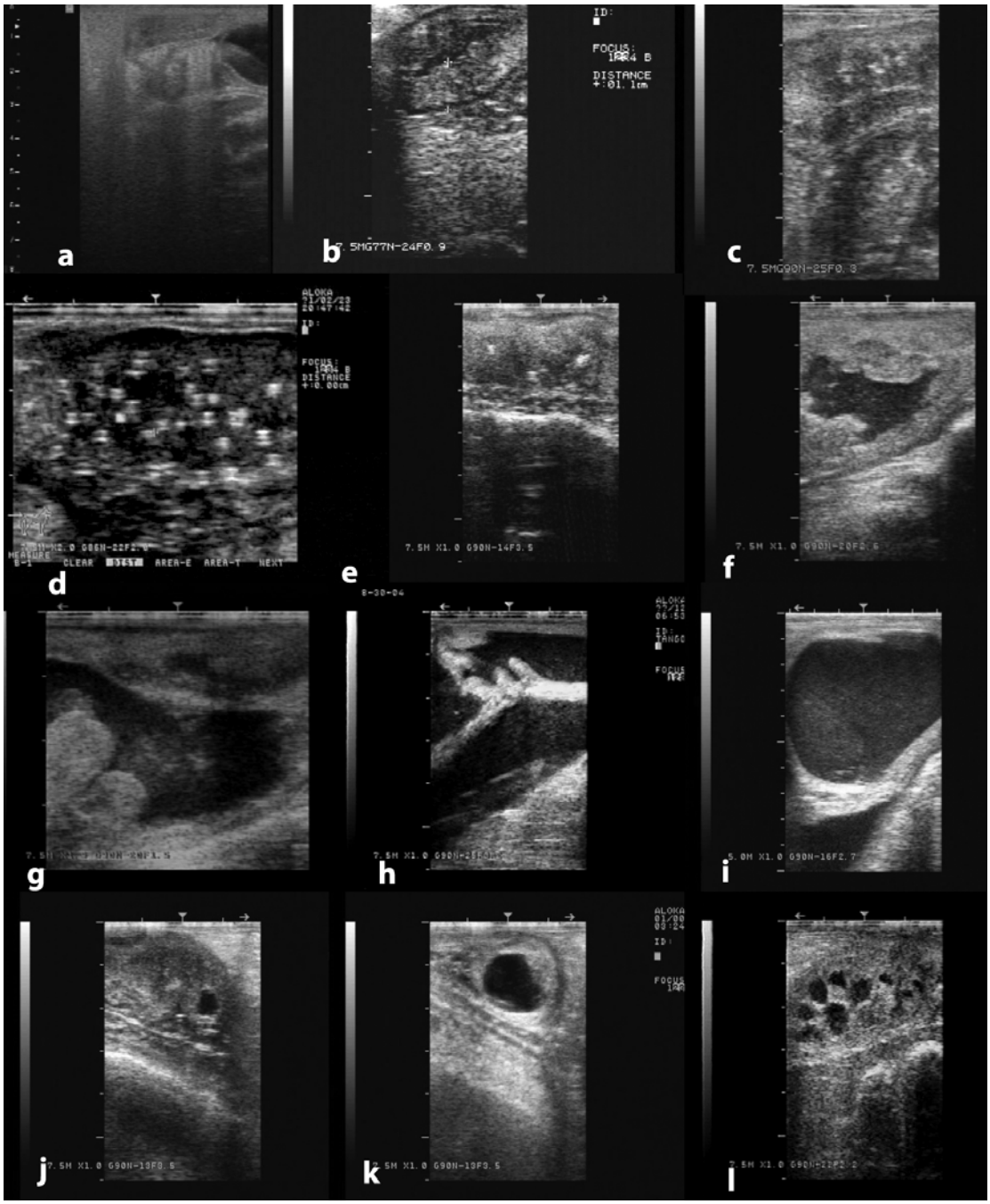

Figure 5: Ultrasonographic appearance of some disorders of the uterus. a) Infantile uterus, b) Endometritis, c) Chronic endometritis,d) Endometrial abscesses, e) Cervical adhesions, f) Pyometra; g) Post-fetotomy pyometra; g) mucometra due vaginal adhesions, note the open cervical rings, i) Mucometra due to vaginal aplasia, j-l) Uterine cysts.