INFERTILITY IN THE MALE LAMOID

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Male lamoid infertility is still not fully investigated due in part to the lack of routine evaluation and in part to the fact that male lamoids are generally not bred to a high number of females to determine their fertility limitations.

The male breeding soundness examination (BSE) is an important part in the evaluation of herd infertility and decision-making concerning selection or purchase of a herd sire. Unfortunately there is little data available on the incidence and types of infertility in the male camelidae. This is due in part to lack of routine examination of males under extensive management, and in part due to the fact that many clinical examination procedures, especially semen collection and evaluation, are not fully standardized and give variable results making interpretation very difficult.

Most often the evaluation of males for infertility is attempted only if a gross abnormality is seen or after a long period of unsuccessful matings which limits the ability of the clinician to reach a diagnostic in time to prevent economic loss.

Breeding soundness evaluation of the Male lamoid

Examination of the male should be conducted methodically to avoid oversight of any problem, which may affect reproductive performance. The evaluation should include the following aspects: Identification of the animal, History, Detailed description of the reason(s) for examination, General health examination and Special examination of the genital system[27, 28]

History and physical examination: The history of the animal is very important to establish examination criteria and identify potential problems. This history should include age of the animal, origin and type of management, breeding record, previous health problems and reason for examination. The type management relates mainly to the description of the herd (size, number of females and males) and housing (paddocks, individual stalls or pens). Breeding history should include the breeding management (free mating vs. in-hand mating, breeding frequency) and conception rate. Diseases of systems other than the genital tract can seriously affect reproductive performance of the male. Lesions of the musculo-skeletal system can impair the physical ability of the male to copulate. Poor conformation or weakness of the hind legs may compromise the mounting ability of the male lamoid. A prolonged febrile condition or debilitating diseases can affect spermatogenesis. In order to identify these underlying conditions the examiner should take a complete health history, including previous illnesses, vaccination and recent treatments.

Reasons for BSE can be generally divided into 3 categories: examination for purchase or selection of a stud, examination for infertility or obvious genital lesions and examination for insurance or expert opinion. A general physical examination should be performed on all animals regardless of the reason for examination. This examination includes general appearance and body condition and evaluation of each system. Special attention should be given to signs of

contagious diseases, neuromuscular, vision and locomotion problems. A sound musculo-articular system is critical for the breeding male because of the position and extreme strain during copulation in camelidae. A complete blood cytology and biochemistry should be done on all recently introduced males.

Evaluation of the external genitalia

Examination of the prepuce in the llama and alpaca may require restraint of the animal in lateral recumbency because the fleece usually hides it. Sedation may be required in order to exteriorize the penis or for detailed examination of an abnormally pendulous prepuce, presence of edema or laceration. Penile attachment to the prepuce is normal in young, prepuberal animals but can signal the presence of adhesions in the mature male. The penis should be completely free at 3 years of age. The most common lesions observed on the penis are lacerations, pustules or abrasions. The glans penis should be examined for signs of inflammation due to traumatic balanitis or presence of hair rings. Urolithiasis is a common problem and should be suspected in the case of dysuria.

Examination of the testicles and epididymis includes inspection, palpation, measurement and ultrasonography of the scrotum and its content. This examination can be completed on the male restrained in standing or sitting position. This facilitates manipulation of the organs especially in a suspected cryptorchism, hypoplasia or fluid accumulation. In some cases (aggressive male or very sensitive scrotum) sedation may be required.

Testes should be present within the scrotum at birth and visible in males by 2 years. In older males, the scrotum may sometimes be pendulous with a longer neck. One of the testicles may be slightly more ventral than the other but both are nearly equal in size. Absence of visible testicles in the scrotum may be due to severe testicular degeneration, ectopic testicles or cryptorchidism.

The scrotal skin is thin and smooth but can become thick and folded in case of severe testicular degeneration. It should be examined for bite wounds (by other males), insects bites and tick infestation.

On palpation, the normal testes should be smooth, firm and resilient. The testicles become hard and fibrotic or very soft in case of degenerative changes. The scrotal sac should be free from fluid. Normally only the tail of the epididymis is palpable as a small hard knob.

Testicular size is an important indicator of sperm production ability. Size of the testis can be evaluated by measuring its length and width. Average normal length x width of the testis at 2 years, 3 years and in the adults should be respectively 3.9 x 2.3 cm, 4.5 x 2.7 cm and 5.4 x 3.3 cm in llamas, and 3.3 x 2.2 cm, 3.6 x 2.4 and 3.7 x 2.5 in alpacas [2].

Pathological changes affecting testicular size include orchitis, hematoma or reduction in size due to hypoplasia or degenerative atrophy [1, 24, 26].

Ultrasonography (US) is an important diagnostic technique for evaluation of the testicular parenchyma as well as the surrounding tissues[25]. The animal is restrained, the scrotum is cleaned and US coupling gel is applied to facilitate close contact with the transducer. Alternatively to the gel, the US probe can be fitted with a pad in order to avoid artifacts[25].

Examination is preferably done using a linear 7.5 MHz transducer probe for the evaluation of scrotal content in llamas and alpacas. The image obtained shows a peripheral area on homogenous tissue corresponding to the testicular parenchyma and a central more echogenic area corresponding to the fibrous mediastinum testis. The epididymis is small and not easily visualized by ultrasonography. Accumulation of fluid in the testicular envelopes (hydrocele) is usually easy to identify because of the good contrast obtained between testicular parenchyma and the fluid. Hydrocele is observed frequently and with varying degree of intensity in heat stressed camelids.

Testicular ultrasonography is also used in llamas for precise measurement of the testicles. In one study on llamas, testicular measurements (length and width) taken by ultrasonography were found to be highly correlated with postmortem measurements and more accurate than those taken with calipers.

Evaluation of the accessory sex glands

Evaluation of the accessory sex gland is sometimes required and is limited to ultrasonography of the prostate and bulbourethral glands.

Evaluation of the mating ability

Mating ability of the male is best observed in the presence of a receptive female. During this evaluation the succession of the normal behavioral pattern is recorded as well as the times needed for each step: vocalization, chasing, forcing down, mounting, intromission and duration of copulation. Behavioral problems during mating can be due to shyness, inexperience or lack of libido.

Semen collection and evaluation

Several of the semen collection techniques described for other species have been modified and adapted to the camelidae with variable degrees of success. Semen collection in camelids presents many difficulties due to the nature of their copulatory behavior and the slow (dribbling) process of ejaculation. Although semen collection using a dummy mount fitted with an artificial vagina has been described in alpaca and llama [4, 9, 17-19], this technique is not commonly used. In practice, the main techniques used for semen collection in Lamoids are electroejaculation (EE) or post-coital vaginal aspiration.

EE is a relatively quick and simple method of semen collection but requires general anesthesia. A ram probe with the fixed or variable voltage EE can be used to ejaculate a llama [11]. To prevent gross contamination of the semen sample, it is recommended to grasp the penis and extend it out from the prepuce prior to ejaculation. Many llamas will ejaculate a small quantity (0.1 to 0. ml) of sperm-rich semen, which is adequate for semen evaluation. Some males may not respond to EE technique.

In practice, semen is collected directly from the vagina of a female after completion of mating. A plastic uterine pipette fitted with a syringe is introduced deep into the vagina and through the cervix and semen is collected by blind aspiration. Aspiration using a vaginoscope is preferred by some practitioners but does not necessarily yield better results.

Semen evaluation: In the alpaca, ejaculate volume ranges from 0.4 to 4.3 ml. Ejaculates obtained by EE are usually smaller in volume than the semen collected by AV. At least in alpacas, the volume of ejaculate does not seems to be significantly affected by repeated collection using an artificial vagina (for review see Tibary and Anouassi 2002 [27]). In llama, ejaculate volume varies from 0.3 to 2.5 ml using EE and from 0.2 to 7.9 using an AV [17, 29].

The color of alpaca and llama semen is opalescent to milky white whether it is collected by EE [6] or by AV [7, 9, 22] but can sometimes be creamy white [9]. In the alpaca, the percentage of seminal plasma is 88.5% and the volume of spermatozoa is 11.5%, with no significant differences amongst ages [9]. Ejaculates may be heterogeneous with some translucent material mixed with cloudy areas. Semen collected by aspiration from the vagina may be pink or even red due to contamination with blood.

Camelid semen is very viscous, which makes handling (pipeting, preparation of slide, dilution in extender) difficult until liquefaction is completed. The degree of viscosity depends on the individual male and on the proportion of seminal gelatinous fluid. Viscosity of the semen is usually attributed to the presence of mucopolysaccharides from secretions of the bulbourethral glands and/or the prostate[9]. Liquefaction of alpaca semen can be obtained by addition of trypsin or collagenase to the ejaculate [5]. The pH of semen is 7.5 and 8.1 for the alpaca [15] and the llama [17], respectively.

The concentration of spermatozoa may be determined by hemocytometer after proper dilution but this rarely done in practice. There is a great variability in the concentration of ejaculates reported in the literature. Sperm cell numbers per milliliter of ejaculate vary from 10 to 250 million in alpacas and 1 to 20 million in llamas (see Tibary and Anouassi 2002 [27]).

Mass motility in generally poor unless the semen is totally liquefied and constituted exclusively by the sperm rich fraction. Individual motility ranges from 20 to 95% in llamas and alpacas[4, 17].

Sperm morphology is assessed by examination of the stained slide of the semen using eosine-nigrosin stain or Wright's Giemsa stain. All sperm abnormalities found in other livestock species can be found in lamoid semen. However, the effect of these types of abnormalities on camelidae fertility is not yet determined. In the llama, the proportion of abnormal spermatozoa in the ejaculates collected by AV is highly variable and ranges from 20.9 to 96.1%[17, 29]. The most common abnormalities concern the head (20 ± 19%) and the acrosome (13 ± 12%). The incidence of cytoplasmic droplets in llama semen ranges from 0 to 45.4% (average 11.1 ± 12.4) [17]. In alpacas, most of the ejaculates collected from fertile males have less than 30% total abnormalities[4] although this percentage can be as high as 50% in sexually rested animals [7]. The incidence of abnormal heads, cytoplasmic droplets and abnormal tails being 9.6%, 3.6% and 14.5%, respectively [4]. Incidence of abnormalities is not affected by rank of ejaculate [3]. Incidence of cytoplasmic droplet in alpacas between 15 and 20 months of age can be as high as 90% (Tibary A. unpublished).

Other tests

Testicular biopsy: Testicular biopsy is not a routine procedure for the evaluation of the breeding soundness in the male. However, in some cases a diagnosis and/or prognosis of the male fertility cannot be reached based solely on physical examination, ultrasonography and semen evaluation. This is particularly true for camelids, in which semen collection is very difficult and gives inconsistent results. Testicular biopsy should be considered in males that have low fertility, testicular asymmetry, and abnormal testicular ultrasonography not consistent with hematoma or orchitis. This technique is useful for diagnosis of spermatogenic arrest, oligospermatogenesis, hypogonadism, inflammations and neoplasm.

Surgical wedge biopsy technique is not desirable because it is hard to perform and can cause budging of testicular tissue through the incision in the tunic and lead to complications such as hematoma, hemorrhage, adhesions, inflammation, autoimmune reactions and degeneration of germinal epithelium and tubules.

A 14 gauge self firing biopsy instrument (Biopty, Bard Radiology, Covington, GA, USA) has been tested in llamas and found to be safe and reliable. A skin stab with # 11 scalpel is made to introduce the instrument and perform the biopsy under aseptic condition [10].

A needle "core" biopsy can be performed using a 1 $^{1/2}$" 16 gauge needle after heavy sedation of the animal[12]. Fine needle aspiration technique is also possible and easy but samples are difficult to interpret [25].

Endocrinology: Testosterone level determination are indicated when a differential diagnosis between cryptorchismm and castration is need. Blood samples should be taken before, 2 hours and 24 hours after administration of hCG (3000 IU).

Causes of infertility in the male lamoid

Most of testicular abnormalities are detected during routine breeding soundness examination (Table 1 and 2)[27]. This prevents loss of time incurred by using a non-fertile male. The most common congenital abnormalities found on routine evaluation are testicular hypoplasia and testicular and epididymal cysts. Acquired conditions are brought to the attention of the practitioner as one of two complaints: 1) reduced fertility or infertility and 2) visible anomalies (swelling, reduced libido, reduced testicular size …) (Table 1 and 2)

There are limited reports on the pathology of the male reproductive tract in lamoids. The most comprehensive study on abnormalities of the reproductive tract in alpacas is that of Sumar (1983)[24]. This author examined 3015 males and 792 reproductive organs. The incidence of pathological conditions in breeding alpacas was 18.1% (testicular hypoplasia 10%, cryptorchism 5.7% and ectopic testis 2.5%). In the slaughterhouse material incidence of abnormalities was 30.5% (hypoplasia 10.8%, cryptorchism 3%, ectopic testis 1.9%, cysts 14.5%).

Affections of the prepuce and the penis are relatively rare because these organs are protected due to their anatomic position. Preputial swelling is due to local inflammation caused by contact with chemical or physical irritants, parasitic infestation, or by rupture of the urethra. Preputial

swelling can also be part of a large ventral edema in some animals suffering from heat stress. If the preputial swelling is due to urethral rupture, the urine accumulated in the subcutaneous space should be drained and a urethrostomy performed. Preputial prolapse was reported in at least one alpaca [16].

Paraphymosis can become complicated by the presence of dirt in the preputial opening and lead to a balanoposthitis with sometimes necrosis of the tip of the penis. Early detection of paraphymosis and treatment will avoid these complications. In the llama, paraphymosis and balanoposthitis can be due to the presence of "hair rings" if the females are not clipped before breeding[8].

Urolithiasis has been reported in the male camelidae [14, 20]. Most of these calculi occur at the level of the distal part of the urethra or at the level of the sigmoid flexure. The affected animals initially show signs of colic, which become more and more frequent. At later stages of progression of the affection, the animal becomes lethargic and anorexic. Deterioration of the animal's health usually signals rupture of the bladder and peritonitis. Relief of the condition can be attempted via urethrostomy or tube cystotomy. Recurrence of obstruction is common even after surgery.

Scrotal trauma due to bites from other males is common. Prognosis for the reproductive life of the individual male depends on the extent of the injury and the time elapsed until detection. The affection should be differentiated from orchitis or hydrocele. Deep lacerations are frequently complicated by testicular hemorrhage, infection and development of schirrous cord and require urgent surgical intervention (castration).

Hydrocele can be due to inflammatory or non-inflammatory causes. The scrotal sacs become pendulous and increased in size. Initial diagnosis is based on palpation of the scrotum and its content. The scrotum is not painful and the testes are usually free within the scrotal sac and fluid can be isolated in one area. Confirmation is easily done by visualization of the fluid by ultrasonography of the scrotal sac. The nature of the fluid varies from anechoic (clear serous fluid) to slightly echoic. Moderate hydrocele is often seen in older males during the peak of the summer. The condition resolves progressively with decreasing ambient temperature. Hydrocele can develop following obstruction of the normal blood flow in the spermatic cord. A case of hydrocele in a llama was attributed to the presence of an abscess at the level of the external inguinal ring. Long standing hydrocele affects the thermoregulation of the testes and decreases the quality and quantity of semen.

There are very few reports on orchitis in the male camelidae. Systemic antibiotics may be considered for treatment of infectious orchitis but in most cases this is not efficacious and castration is recommended[1].

Testicular degeneration is probably the most common cause of infertility due to testicular pathology. The degenerated testicles are smaller than normal and either soft or hard and fibrous.

Partial or total testicular hypoplasia or atrophy has also been reported. The incidence of testicular hypoplasia is estimated at 10%. In the adult alpaca, the testicles are considered hypoplastic when their length and width are smaller than 3.7 cm and 2.5 cm respectively. Most hypoplasia cases

are bilateral. The left side seems to be more affected than the right in bilateral cases [24]. Histology of the testicular parenchyma shows small seminiferous tubules with absence of spermatogenesis. Testicular biopsy has been suggested for the diagnosis of these cases[10, 12, 13, 25].

Cryptorchism or failure of testicular descent into the scrotum is a relatively rare [23]. Cryptorchism can be unilateral or bilateral and is suspected when inspection of the perineal region shows a flat or absent scrotum. In Sumar's study[24], cryptorchidism was found in approximately 3% of the population examined. The undescended testicles are usually found close to the internal inguinal opening but could be also be found intra-abdominally, caudal to the kidney or within the inguinal canal. Cryptorchidism was also reported in related vicuñas (3 cases in a population of 60 individuals), which suggests that the affection may be hereditary [23]. Differentiation between cryptorchidism and castration may be achieved using hormonal diagnosis[21].

There are very few reports of affection of the epididymis in camelidae. Epididymal cysts have been reported in the llama and alpaca. In alpacas, cystic structures were found in 14.5% of slaughtered animals mainly on the anterior aspect of the head of the epididymis and near the ventral border of the testis. The majority of these cysts were 1 to 5 mm in diameter although in one case the cyst was 50 mm [24]. Cystic formations within the testicular tissue or epididymis have been diagnosed in several males with various degrees of infertility (Table 2).

Other reproductive problems are reported by owners but their exact etiology is not known. Amongst these are lack of or reduced libido, erection or ejaculation failure and unexplained sub-fertility or infertility. Lack of libido may be associated with hormonal imbalance, high temperature, stress and presence of debilitating diseases. Decreased libido can be one of the first signs of illness. Substandard semen quality can be involved in male infertility especially in overused or old animals.

Despite using all the available tests some cases of male infertility remain a mystery. In one case, all seminal parameters were normal and yet no pregnancies could be obtained after natural cover. This suggests that other factors (immunological or genetic) may be involved in male infertility in these species. Other suspected conditions that need further investigations include incapacity or low capacity to achieve ovulation.

Table 1: Documented diseases of the reproductive organs in the male camelidae

Prepuce	Penis	Testis and epididymis	Accessory sex glands
Preputial edema (heat stress) Preputial edema (trypanosomiasis) Preputial obstruction Preputial laceration Preputial prolapse Preputial necrosis Posthitis Preputial warts Phymosis	Prolapse penis Paraphymosis Balanitis Ulcerations/Abrasions Hair ring Penile warts Urethral rupture Urethritis Urolithiasis	Cryptorchidism Ectopic testicles Hydrocele Testicular degeneration Testicular hypoplasia Testicular cyst Orchitis Epididymitis Epididymal segmental aplasia	Prostate hypertrophy Prostate abscess

Table 2: Complaints and conditions diagnosed in Male camelids

Diagnosis	Complaint	# cases	Methods
Cryptorchidism	Routine BSE	4 (3 unilateral)	Clinical, endocrinological
Testicular hypoplasia	Routine BSE	14	Clinical, semen evaluation
	Infertility	7	Clinical, semen evaluation, biopsy
Testicular degeneration	Infertility	32	Clinical, semen evaluation, biopsy
Testicular hematoma	Scrotal swelling	1	Ultrasound
Orchitis/epididymitis	Scrotal swelling	3	Clinical, semen evaluation, ultrasound
Testicular cysts	Infertility	3	Ultrasound, oligozoospermia,
	Sterility	1	Ultrasound, azoospermia
	Routine BSE	6	Ultrasound
Seminoma	Testicular enlargement	1	Ultrasound, histology
Prostatic enlargement (?)	Infertility, hemospermia	1	Semen evaluation, ultrasound
Preputial swelling	Preputial swelling	2 (llamas)	Clinical
Not determined	Sterility	1	
Not determined	Poor libido	1	
Not determined	Erection failure	1	

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