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Research Article

Breeding Soundness Evaluation in Alpaca *(Vicugna pacos)* Males: A Long Retrospective Study of the Effects of Cystic Masses and Environmental Temperatures on Scrotal Measures [1]

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Abstract: Male alpacas have reproductive peculiarities that can influence the breeding soundness evaluation (BSE). This study was aimed to analyse the frequency of epididymal and testicular cystic lesions in alpacas *(Vicugna pacos)* retrospectively during different environmental temperatures. A total of 45 subject from 120 males were evaluated with linear probe (10 MHz; MyLab VetONE) between at 5 different alpaca farms in Italy. Semen was collected via artificial vagina and semen parameters were evaluated. Twenty % (9/45) of the males demonstrated the presence of abnormal testicular and epididymal cyst. Those cystic lesions could represent a simple result of drainage defect of the fluid produced (ectasia of the rete testis), congenitally derived from a probable hereditary pattern (2/9) or affecting epididymal structures (3/9). Cystic lesions can increase the scrotal volumetric asymmetry due to the most common mono-lateral lesions (P<0.05). Some diagnostic biochemical parameters such as the seminal plasma alkaline phosphatase was higher in affected males (P<0.05). Besides, environmental temperature can influence scrotal swelling independently by the presence of cystic lesions thus alter the scrotal volume. The ultrasonography can provide more specific information about the presence of epididymal and testicular anomalies influencing the BSE results.

Keywords: *Alpaca, Breeding soundness evaluation, Ectasia, Cystic degeneration, Epididymal cyst, Ultrasonography*

Alpaka *(Vicugna pacos)* Erkeklerinde Üreme Sağlamlığı Değerlendirmesi: Kistik Kitlelerin ve Çevresel Sıcaklıkların Skrotal Ölçümler Üzerindeki Etkilerinin Uzun Bir Retrospektif Çalışması

Öz: Bu çalışmanın amacı epididimal ve testiküler kistik lezyon frekanslarının, farklı ortam sıcaklıklarında retrospektif olarak incelenerek ortaya koyulmasıdır. İtalya'da bulunan 5 farklı işletmeden toplam 120 erkekten seçilen 45 birey USG lineer prob (10 MHz; MyLab VetONE) muayene edildi. Sperma örnekleri suni vajinayla alındı ve sperma (Visközite, renk, konsantrasyon ve motilite) parametreleri değerlendirildi. Erkeklerin %20'si (9/45) anormal testis ve epididimal kisti varlığı gösterdi. Bu kistik lezyonlar, üretilen sıvının drenaj defekti (rete testis ektazisi), konjenital bir kalıtsal patern (2/9) veya epididimal yapıları etkileyen (3/9) bir problem olarak değerlendirildi. Kistik yapılar, en sık görülen monolateral lezyonlar nedeniyle skrotal hacimi ile asimetriyi artırdığı belirlendi (P<0.05). Seminal plazmada bulunan bazı biyokimyasal tanı parametrelerden, alkalin fosfataz patolojik erkeklerde daha yüksek bulundu (P<0.05). Ayrıca, çevresel sıcaklık, kistik lezyonların varlığından bağımsız olarak skrotal ödemi etkileyebilmekte ve böylece skrotal hacmi değiştirebilmektedir. Sonuç olarak, ultrason, ÜSM sonuçlarını etkileyen epididimal ve testis anomalilerinin varlığı hakkında daha spesifik bilgi sağlayabilmektedir.

Anahtar sözcükler: *Alpaka, Üreme sağlamlığı değerlendirmesi, Ektazi, Kistik dejenerasyon, Epididimal kist, Ultrason*

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Introduction

Breeding soundness evaluation (BSE) is a standard protocol used to classify domestic animals breeders into five categories: exceptional, satisfactory, questionable, unsatisfactory and deferred. Despite a fair amount of research on alpaca male reproductive characteristics [1,2], there is a limited amount of information available on reference ranges of semen quality and productivity [3-5]. Today, ultrasonography (US) is routinely used as a collateral diagnostic approach in addition to the traditional visual assessment and trans- scrotal palpation to evaluate the male reproductive content. Among the abnormalities that can be detected by US, scrotal swelling (SS) and cystic masses (CM) are commonly observed. Scrotal swelling can have various causes, including transudative or exudative origins, and can represent a simple reduction of blood flow when CM are present at the testicular or epididymal level. This pathology has been reported in multiple species, including canines, bovines, equines, and humans [6-10].

Scrotal CM can be differentiated into three types: tubular ectasia of the rete testis (TERT), cystic degeneration of the rete testis (CD) and epididymal cysts (EC). Rete testis is an intra-testicular area that originates from the testicular hilum and contains numerous anechoic areas. The typical US image represents the enlargement of the numerous canaliculi of the rete testis. The exact cause of TERT is not yet fully understood, however, possible causes include mechanical obstruction, primary congenital deformity, ischaemic degeneration of the efferent ducts (from the rete testis to the epididymis) and hormonal mechanisms, particularly androgen deficiency. Pathogenesis wise, during embryogenesis, the mesonephric duct forms the efferent ducts, epididymis and vas deferens, whereas the germinal epithelium separately forms the rete testis and the testicular cord. The connection between the mesonephric duct and germinal epithelium occurs at the rete testis level and CD could be related to a disorder of the connection between the mesonephric duct and germinal epithelium. Cystic degeneration has ultrasonographic similarities with TERT, but it is a congenital deformity [11]. Interestingly, different studies reported scrotal cystic lesions and specific anatomical features indicating the unknown origins in South American Camelids [2,5,12,14]. Bott et al.[5] reported an incidence of cystic lesions of 18.5 % considering a total of 173 male alpacas scheduled for castration. Sumar [14], considered the importance of culling these males with CM lesions from the breeding plan, since it may have a hereditable predisposition. Hence, the integration of US as a collateral tool for breeding soundness evaluation is becoming essential, as it can give useful information about the quality in terms of breeder utility [14,15].

Therefore, our aim was to retrospectively analyse collected data from BSE in male alpacas, in addition to characterizing scrotal cystic lesions during scrotal ultrasonography under different environmental temperatures and assessing impact on scrotal measurements.

Material and Methods

Ethical Statement

This study was conducted during routine clinical visits to Alpaca di Marano in Piacenza, Antico Feudo in Prato, Poggio Piero in Grosseto, and Piani degli Alpaca in Viterbo, which are private farms. As such, the study did not require ethical permission, as it involved no additional interventions or procedures beyond what was necessary for the normal veterinary care provided to the animals. Moreover, as a European country, Italy has certain exemptions for animal experiments under the EU Directive 2010/63/EU, which includes procedures that are part of veterinary practice or routine monitoring of animal health. As a result, the study did not meet the criteria for a research study that requires ethical permission. The study was conducted in an ethical and responsible manner, with the protection of the welfare and rights of the animals as the top priority.

Animals

In the initial phase of our study, we conducted a retrospective analysis of ultrasonography (US) images collected from South American Camelids raised in Italy between 2010-2018. We carefully selected and evaluated individual data and US images of males to examine and classify the intra-scrotal anomalies observed. A total of 45 out of 120 males were selected for analysis in our study. These individuals were chosen due to their classification as breeders and the fact that they underwent a complete physical examination, including the use of scrotal ultrasonography as a collateral diagnostic tool.

In the second phase of the study, we analyzed data collected from a total of 9 males (6 normal, without lesions, and 3 abnormal, with lesions). These males were chosen due to their origin from a single farm and their completion of a full breeding soundness evaluation (BSE), including semen collection and evaluation, as well as collateral diagnostic exams (scrotal and accessory gland ultrasonography, and evaluation of seminal plasma biochemical composition). In our study, semen collection and ultrasonography (US) evaluation were conducted twice, each time under different environmental-seasonal temperatures. This was done as the first BSE was classified as deferred. The study was conducted on 5 farms, with 3 located in northern Italy and 2 in central Italy. All the farms presented a balanced diet consisting in *ad libitum* hay (Dry substance - DS - 63-91.71%, Fat 1.12-2.95% DS, Protein 6.61-14.91% DS, Ash 8.21-11.43% DS, NDF 57.26-59.71% DS, ADF 32.98-38.32% DS), small amount (around 250 g) of concentrate during the breeding times (Protein 16.3%, Crude fiber 10%, Fat 2.7%, Ash 10.3, Vit. A 100000 UI, Vit. D_3 1000 UI, Vit. E 174 mg, Niacine 900 mg, Vit. B_1 43.6 mg, Vit. B_2 9.5 mg, Vit. B_6 32.4 mg, Vit. B_{12} 0.72 mg, Pantothenic acid 72 mg, Vit K 6 mg, Choline chloride 2.25 mg, Fe 73.6 mg, I 6 mg, Co 3.2 mg, Mn 120 mg, Zn 148 mg, Se 1.04 mg) and mixed pasture.

Scrotal Measurements

The length, width, and thickness of both testes were measured using calipers, testicular volume was calculated using the following formula [16]: Testicular volume: length x width x height x 0.5236.

Scrotal and Accessory Glands Ultrasonography

Ultrasonography images were collected using MyLab VetONE (Esaote®, Italy) with linear array probe (10 MHz; Gain max, Deepness 6 cm). The scrotal content was examined with sagittal and transversal sections of the testicular parenchyma and the appearance of the epididymal structures of 45 males. To collect the images of accessory glands, feces were removed manually, and the US probe was inserted and localized above the upper portion of the prostate and bulbourethral glands of 9 males. In our study, we used 10 MHz probe, changing only the deepness from 6 cm to 4 cm for testicle and accessory glands respectively. Each US image was classified depending on the normality or abnormality of testicular, epididymal and accessory glands structures. Abnormal findings were classified as SS, TERT and CD and EC.

Semen Collection

Nine adult males were studied to compare normal semen (n=6) to that of males with cystic lesions in the testicles (n=3). The males were abstinent from mating for at least two months prior to semen collection. Semen was collected twice from each male, under both high and low environmental temperatures, using a modified ovine artificial vagina in the presence of a teaser female [17]. The artificial vagina was equipped with a foam structure resembling a cervix and a glass tube for semen collection. The artificial vagina was heated to 38°C during semen collection and immediately afterward, the semen was placed in a water bath at 37°C. Standard semen parameters such as volume, consistency, concentration, and percentage of motile sperm were evaluated. The percentage of motile sperm was determined by using a microscope (Olympus CX-31) with a magnification of 400x. The samples were observed under a 18mm x 18mm coverslip on a warm glass slide maintained at 37°C. There is very little to no progressive motility in normal alpaca semen due to the high viscosity. Therefore, sperm motility, detected as an oscillatory motion of the flagellum, is given as the percentage of motile spermatozoa. Sperm concentration was measured using a Cell VU Sperm counting chamber, and motility was determined by the percentage of sperm displaying oscillatory motion. After analysis, the semen samples were centrifuged, and the seminal plasma was separated and stored at -20°C for further biochemical analysis. Environmental temperatures were recorded using a thermos-hygrometer. (Oregon Scientific THGR122NX).

Seminal Plasma Biochemistry

Biochemical analysis of the seminal plasma was conducted using an automatic analyzer and specific kits (912 Automatic Analyzer; Hitachi Boehringer Mannheim, Mannheim, Germany). The concentrations of alanine aminotransferase (ALT), g-glutamyl transferase (GGT), and alkaline phosphatase (ALP) were measured, as well as levels of glucose, total protein, triglycerides, cholesterol, phosphorus, and calcium.

Data Analysis

Reproductive ultrasound images were used to classify the males in the study. The first phase involved determining the frequency of pathological conditions affecting the male reproductive tract. In the second phase, data collected was analyzed using the GLM function in SIGMASTAT 2.03 software. A two-way ANOVA was performed, with testicular volume and semen quality parameters as dependent variables and the presence/absence of testicular dysplasia and seasonal changes in environmental temperature as independent variables. A P-value of less than 0.05 was considered statistically significant.

Results

A total of 9 out of the 45 males (20%) had cystic lesions in their scrotums. *Fig. 1*, *Fig. 2*, *Fig. 3* and *Fig. 4* show the ultrasound appearance of these cystic lesions. *Table 1* lists the age of the subjects and the type of lesions they had. Epididymal cysts, accounting for 6.67% of scrotal lesions, were found in males of different ages (2.5, 3.5, and 4 years) and could occur as either a single (1/3) or bilateral (2/3) lesion.

Cystic degeneration and TERT together accounted for 13.33% of the frequency of cystic masses at the scrotal level. The classification of cystic degeneration instead of TERT was considered in two juvenile cases (case 1 and 2) because their conditions were like that of their father (case 8). Testicular cystic masses were classified as cystic degeneration/TERT when the congenital origins were not known. *Table 2* shows the variability of the parameters during the two collection times during semen collection.

All adult males used as breeders (3 out of 9) had a

***Table 1.** List of scrotal pathological conditions in Alpaca males*

Male ID	Alpaca	Age	Type	Figure
1	Huacaya	1.5	Cystic degeneration RT	1-A
2	Huacaya	1.5	Cystic degeneration RT	1-B
3	Huacaya	2.5	Tubular Ectasia/Cystic degeneration RT	2-A
4	Huacaya	3	Tubular Ectasia/Cystic degeneration RT	2-B
5	Huacaya	2.5	Epididymal cyst	3-A
6	Huacaya	3	Epididymal cyst	3 B
7	Huacaya	4	Epididymal cyst	3 C
8	Huacaya	6	Tubular Ectasia/Cystic degeneration RT	4 A
9	Huacaya	10	Tubular Ectasia/Cystic degeneration RT	4 B

***Table 2.** Value of the parameters evaluated (normal vs pathological) during low and high environmental temperature*

Parameter	Normal (12)		Abnormal (6)	
	Low Temp.	High Temp.	Low Temp.	High Temp.
Age (year)	7.5 (5.56-10.58)	7.91 (4.84-13.6)	9.68 (6.40-12.08)	8.92 (5.61-11.37)
BCS	3.2±0.13	3.13±0.17	3±0.25	2.83±0.08
Right Testicular Volume	7.23±1.08[a]	16.69±2.28[b]	10.36±2.08[a]	25.48±4.53[a]
Left Testicular Volume	9.45±1.77[a]	18.38±2.39[b]	11.68±1.73[a]	25.45±0.47[b]
Volume (ml)	1.78±0.52	1.10±0.28	1.77±0.14	1±0.5
Viscosity	2	1.67±0.21	2.67±0.33	2.33±0.66
Colour	1.75±0.2	2.33±0.33	2.67±0.66	2.00
Concentration ($1x10^6$/ml)	46.17±2.08[a]	7.67±2.39[b]	120.83±15.02[a]	43.33±21.86[b]
Motility (%)	20	16.67±9.19	50.00±5.77	46.33±21.85

Different number of asterisks indicates a significant difference between low and high temperature within normal and pathological conditions [a,b]= $P<0.05$

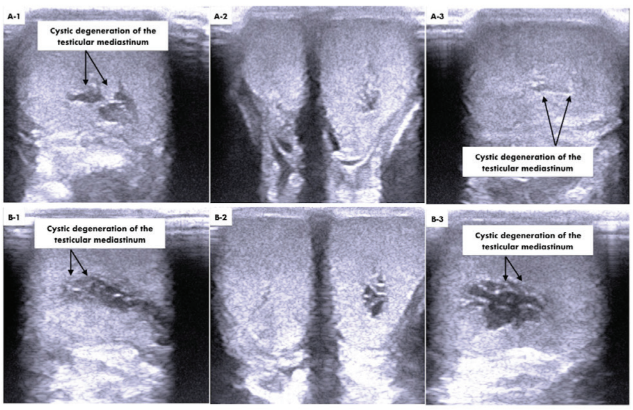

Fig 1. Juvenile mono-lateral cystic degeneration of the rete testis. Case 1 and 2 are classified as juvenile origin due to brotherhood, also being male offspring of case 8. Case no 1. A-1 Longitudinal left section; A-2 Transversal section; A-3 Longitudinal right section. Case no 2. B-1 Longitudinal left section; B-2 Transversal section; B-3 Longitudinal right section

unilateral, intra-testicular lesion. The presence of cystic masses affected the testicular volume, reducing it by around 40% (*Table 3*). Most of the lesions causing this decrease in volume were located on the left testicle. There was a significant difference in environmental temperature between seasons, ranging from 22°C (15-30°C) in the summer to 12°C (7-16°C) in the spring. The testicular volume was more affected by the hot environment, with a 130% and 145% increase for normal and pathological cases, respectively (*Table 3*).

Sperm concentration was found to be affected by temperature, regardless of the presence of cystic masses. High temperatures caused a decrease of around 80% in sperm concentration (*Table 2*). Some seminal plasma parameters also varied between seasons, with an increase observed during high temperatures.

***Table 3.** Variability of the parameters considered during BSE of alpaca males with normal and pathological intra-scrotal contents*

Parameter	Normal (12)	Abnormal (6)	Average Value
Age (year)	7.73 (4.84-13.60)	9.30 (5.61-12.08)	8.28 (4.84-13.60)
BCS	3.16±0.36	2.92±0.3	3.07±0.35
Right Testicular Volume	12.39±6.55	17.92±9.92	14.34±8.06
Left Testicular Volume	14.32±6.82	18.56±7.79	15.82±7.24
Testosteronemia (ng/mL)	205.50±116.67	178.50±7.78	192.0±69.29
Volume (mL)	1.37±0.97	1.38±0.71	1.38±0.86
Viscosity	1.80±0.42	2.50±0.84	2.06±0.68
Colour	2.1±0.74	2.33±0.82	2.19±0.75
Concentration (1x10^6/mL)	26.92±40.36	64.50±63.87	45.71±54.59
Motility (%)	17.14±20.59	46.67±25.03	30.77±26.6

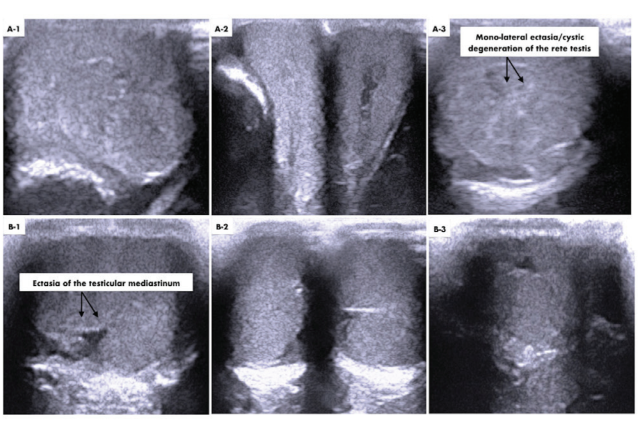

Fig 2. Mono-lateral ectasia-cystic degeneration of the rete testis. Case no 3. A-1 Longitudinal left section; A-2 Transversal section; A-3 Longitudinal right section. Case no 4. B-1 Longitudinal left section; B-2 Transversal section; B-3 Longitudinal right section

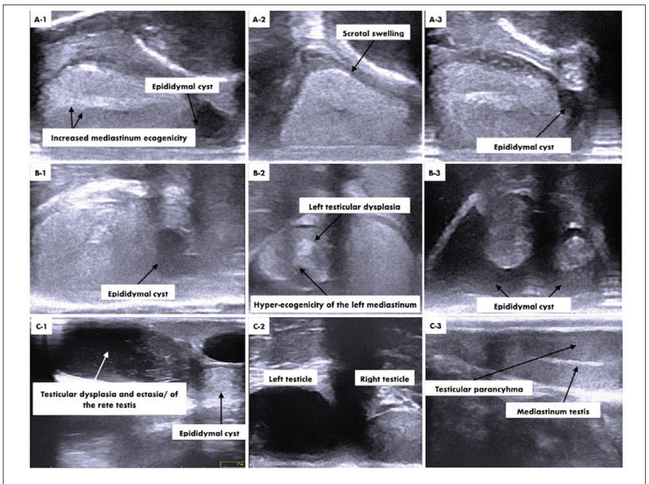

Fig 3. Bilateral epididymal cyst, scrotal swelling and increased echogenicity of left testicular mediastinum. Case no 5. A-1 Longitudinal left testicular and epididymal section; A-2 Transversal section; A-3 Longitudinal right testicular and epididymal section. Case no 6. B-1 Longitudinal left testicular and epididymal section; B-2; Transversal section; B-3 Transversal section of emi-scrotal areas. Case no 7. Mono-lateral epididymal cyst and extreme testicular dysplasia and ectasia of rete testis. C-1 Longitudinal left testicular and epididymal section; C-2 Transversal scrotal section; C-3 Longitudinal right testicular and epididymal section

Fig. 5 reports the US appearance of the accessory glands (prostate and bulbourethral glands) which is not influenced by the seasons. *Fig. 6* is clearly showing the presence of scrotal swelling (hydrocele) during the hot season.

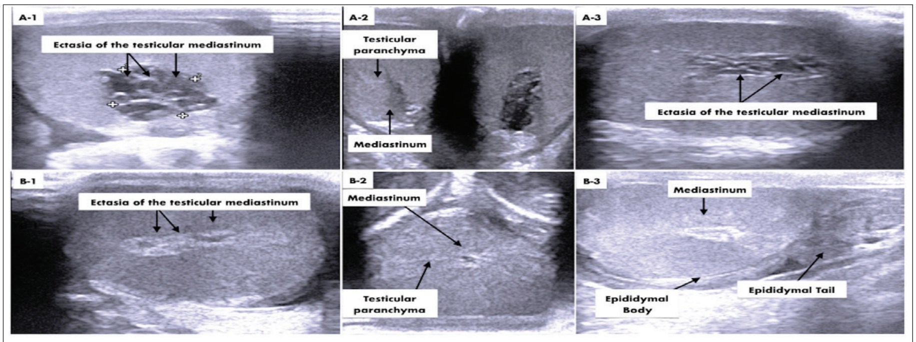

Fig 4. Mono-lateral epididymal cyst and extreme testicular dysplasia and ectasia of the rete testis. Case no 8. Adult male father of the cases 1 and 2. A-1 Transversal scrotal section; A-2 Transversal left testis advanced; A-3 Longitudinal left testicular section. Case no 9 Bilateral ectasia/cystic degeneration of the rete testis; B-1 Longitudinal right testicular section; B-2 Transversal right testicular section; B-3 Longitudinal testicular and epididymal left section

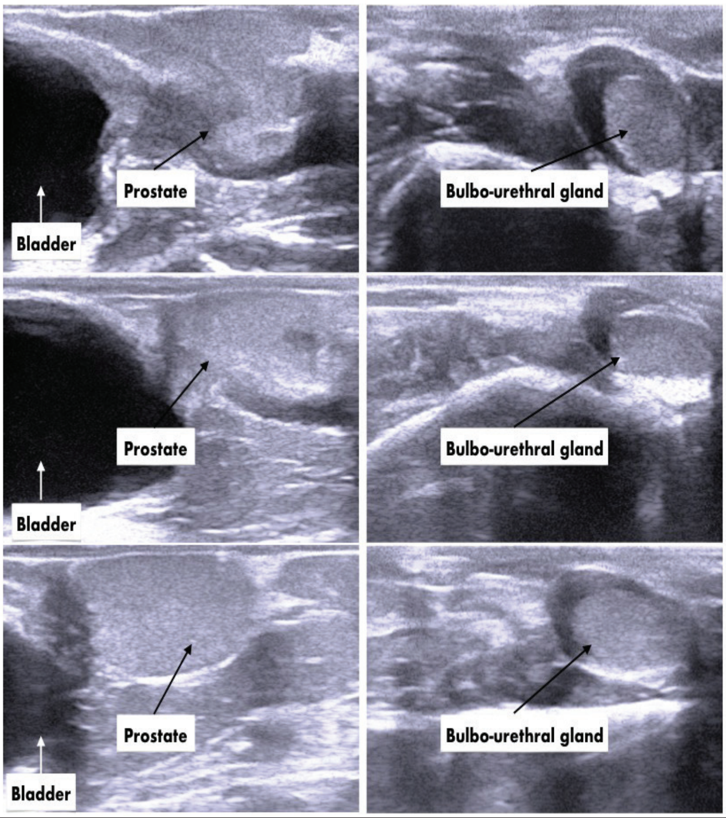

Fig 5. Accessory glands of male alpacas

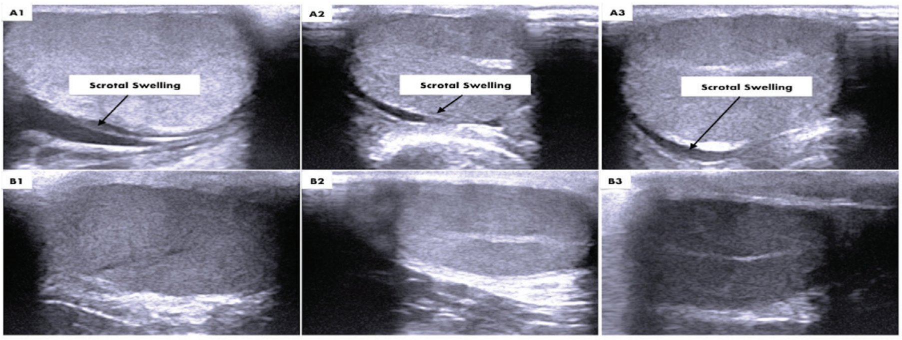

Fig 6. Scrotal swelling in alpacas

Seminal alkaline phosphatase (ALP) was found to be the most indicative parameter, showing a 140% increase in level between normal and pathological cases and a 244% (normal) to 520% (pathological) increase between seasons *(Table 4)*. The levels of other seminal plasma components were also reported in *Table 4*.

Discussion

Breeding soundness evaluation is an essential protocol to classify breeder males. It is based on physical measurements such as scrotal circumference or testicular diameters. However, in alpaca males, obtaining these measurements can be difficult due to their species-specific position of the scrotum and a high percentage of cystic masses. In the past, there was a general agreement on the relationship between testicular size and productivity. However, in males affected by cystic masses, this relationship may not hold as the testicular volume may be higher due to the cystic masses, even if the productive tissue is partially absent. Therefore, ultrasonography measurements are considered the most appropriate approach to determining testicular volume in these cases.

The most significant finding of our study is the higher sperm concentration and motility in individuals diagnosed with reproductive anomalies such as tubular ectasia of the rete testis (TERT), cystic degeneration of the rete testis (CD), and epididymal cysts, compared to

Table 4. *Seminal plasma biochemical parameters evaluated (normal vs pathological) during low and high environmental temperature*

Parameter	Normal (12)		Abnormal (6)		Average
	Low Temp.	High Temp.	Low Temp.	High Temp.	
Glucose (mg/dL)	0.85± 0.37	0.85±0.17	1.74± 0.71[a]	0.26±0.14[b]	1.02±0.28
Cholesterol (mg/dL)	2.24± 0.96	1.11±0.43	2.75± 1.68	2.97±1.41	1.96±0.63
Triglycerides (mg/dL)	14.8± 3.99	29.72±13.39	14.78± 4.95	41.38±16.68	23.23±7.53
Total Protein (mg/dL)	1.94± 0.6	4.3±1.98	3.91± 1.09	6.91±1.02	3.83±1.13
GGT (UI/L)	32.6±20.68	122.2±53.7	141.6±52.25	247.1±90.6	115.11±36.38
ALP (UI/L)	67.9±43.6[a]	234.2±128.7[a]	184.1±101.8[a]	1142.2±336.1[b]	255.83±118.60
Ca (mg/dL)	11.55± 0.91	10.26±2.77	16.3±2.36	13.39±1.54	12.38±1.74
P (mg/dL)	0.28± 0.05	0.71±0.31	0.33±0.04	0.63±0.06	0.50±0.17
Mg (mg/dL)	4.45± 0.52	2.99±0.44	4.15±0.56	2.54±0.58	3.61±0.4
Creatinine	0.72± 0.30	0.59±0.17	0.49±0.03	0.36±0.04	0.58±0.14
Urea	39.8±12.2	40.59±4.70	47.67±6.92	45.97±5.41	42.62±5.27

Different number of asterisks indicates a significant difference between low and high temperature within normal and pathological conditions [a,b] = $P<0.05$

those classified as normal. This increase is believed to be caused by an enlarged scrotal volume and obstructed sperm ducts. However, these anomalies also result in higher sperm viscosity. Although these conditions are not detectable by macroscopic or manual examination, they can be observed through ultrasound. We therefore suggest that alpaca males undergo scrotal and testicular examination with ultrasound as part of their reproductive health assessment. It should be noted that the standard deviation among sperm findings in this study was substantial, due to the unique biology of alpaca males during sperm retrieval, handling, viscosity, semen analysis, and evaluation method. The consirable variations in the results are consistent with those from other studies [18,19], with an average ejaculate volume of 1.8±0.8 mL and sperm concentration of 17.6±26.1x10^6 sperm/mL reported by Flores et al.[20] without evaluating sperm motility. It has been reported that repeated sperm retrieval reduces these standard deviations [21].

In veterinary practice, B-mode ultrasonography is the most used method, and it is easily performed with minimal restraint or discomfort for the animal. It provides highly sensitive information using a 5-7.5 MHz probe, with up to 100% accuracy in diagnosing hydroceles, haematoceles, and para-testicular masses, but less informative for testicular abscesses or epididymo-orchitis. Higher MHz probes (7-10 MHz) with or without color flow Doppler are the preferred probes for evaluating the testicles, epididymal structures, and spermatic cords. The study found an increased testicular volume during the summer collection, which was independent of the presence of cystic masses at different levels. This increase in volume during the summer months could be due to local circulatory impairment because of a general thermoregulatory response during the hot season. The cystic masses were reported as unilateral in most cases, which contributed to the testicular asymmetry. The seminal plasma biochemical composition indicated a testicular/epididymal response to elevated environmental temperatures.

In addition to the absence of a relationship between testicular volume and cystic masses, the study found that the effect of environmental temperature on the thermoregulatory response was evident with a decrease in semen quality and biochemical composition of the seminal plasma. Spontaneous recovery of simple testicular cysts has been reported only in humans [22]. In the study population, almost all the cases were unilateral and had a probable degree of heritability based on congenital cystic degeneration in two half-brothers. Epididymal cysts in alpacas have been reported by other authors [3,13], and little is known about their persistence or natural regression, as reported for humans [22-24]. These lesions are usually visible unilaterally. The cystic transformation of the rete testis is often associated with ipsilateral renal agenesis or other renal dysplasia. Congenital epididymal cysts (EC) are the most frequently observed anomaly of the male internal genitalia of Wolffian origin. However, traumatic, and inflammatory causes are also possible. The efferent ducts are part of the head of the epididymis because of their embryological origin from mesonephric/Wolffian structures. In the study population of alpaca males, one case had an EC lesion that increased the influence on the rete testis and a derived TERT for 18 months without showing any distress due to the enlarged testicle (case 8). To the best of our knowledge, this study reported the highest incidence of cystic masses compared to previously published percentages [5], and even though the study did not include histological investigations as the animals were not scheduled for castration, it aimed to identify the effects of cystic masses on testicular/scrotal functionality and breeding soundness evaluation results. However, there is an interesting report by Barrios et al.[25], and reproductive anomalies such as small testicles, uni- and bilateral epididymal cysts were found in 102 out of 177 alpaca males in a slaughterhouse evaluation in 2011, which has higher rates than our study.

During the second phase of the study, data regarding sperm motility was found to range between 5% and 80%, with high levels of variation among the males, and mean motility was found to be quite low (35%). The mean sperm motility in this study was within the range reported for alpaca semen collected using an artificial vagina (15.3% - 63.7%) in previous studies [21, 27]. Additionally, only oscillatory motility was observed in the collected samples, which agrees with previous studies that also described the oscillatory motion [26-30]. The study found that semen concentration was negatively influenced by high environmental temperature, with a decrease in values between seasonal changes. The increased testicular volume and decreased semen concentration seem to be linked by a pathophysiological cause, such as an impairment of the thermoregulatory vascularization of the scrotal content. Semen volume and concentration have shown clear tendencies, identifying, independently of pathological conditions, the negative effect of seasonal high temperature.

The most interesting biochemical parameter in the study was seminal plasma alkaline phosphatase (ALP). The value of this enzyme in seminal plasma is an important indicator of membrane damage and changes in membrane function resulting from increased sperm production [17, 26]. ALP is also an important parameter for testing the health and functionality of accessory glands. In azoospermic canine and equine ejaculates, high ALP levels can indicate a testicular origin of azoospermia, while low ALP levels may correspond to bilateral outflow obstruction [31,32]. In a study conducted on bulls, it was observed that ALP enzyme levels decreased in chronic efferent duct obstruction that causes ectasia in the epididymal, ampullary canal, and ductus deferens. In the study, the ALP value measured in the seminal plasma of animals with cystic lesions was 140% higher than in healthy animals [15]. This value was also affected by high temperature, with an increase of 244% in healthy animals and 520% in animals with cystic lesions.

Cystic masses in Alpaca are very common compared to other species. The presence of these lesions may influence testicular measurement and breeding soundness evaluation. Differential diagnosis between cystic dysplasia of the rete testis and simple ectasia should be further investigated to exclude carriers from breeding programs. More research is needed to identify the hereditary origin and any potential ipsilateral renal problems, making this species a potential animal model for human pathology. Breeding soundness evaluation in alpaca should be carried out during periods when environmental temperature is not high, as high temperatures can influence scrotal swelling and give false information on testicular dimensions. Furthermore, the use of ultrasound is essential to rule out the presence of cystic masses and to calculate the correct parenchymal testicular volume.

Availability of Data and Materials

The data presented in this study are available on request from the corresponding author (K. Tekin). The data are not publicly available due to the agreement with funding bodies.

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Conflict of Interest

The authors declare no conflict of interest.

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