Reproduction in Domestic Animals

Reprod Dom Anim **45**, 368–372 (2010); doi: 10.1111/j.1439-0531.2009.01495.x
ISSN 0936-6768

Short Communication

Clinical and Pathological Findings of a Sac-like Formation in the Tunica Vaginalis of a Nelore (*Bos indicus*) Bull

J Chacón[1] and A Berrocal[2,*]

[1]*Section of Andrology, Research Program on Applied Animal Andrology;* [2]*Department of Pathology, School of Veterinary Medicine, Universidad, Nacional (UNA), Heredia, Costa Rica*

Contents

A seven-month-old purebred Nelore calf was diagnosed with a bilateral finger-shaped swelling although more prominent at the left side of the scrotum, located longitudinal and parallel to epididymis corpus. The finding was present from 7 months of age up to castration (performed at 25 months of age). Scrotal circumference, testicular and epididymis consistency and symmetry as well as seminal quality were normal during the follow-up period. The ultrasonographic appearance of the scrotal wall, pampiniform plexus, gonad and epididymis was normal. However, an anechoic region surrounded by a wall forming a sac-like structure with a blind end at its dorsal pole was seen on the swelling area. The examination of fluid aspirated from the saccular contents revealed the presence of mononuclear cells mainly from lymphocytic and histiocytic type as well as some loosing degenerated mesothelial cells. Gross examination at castration revealed a blind sac-like appendix derived from an evagination in the parietal layer of the tunica vaginalis. The structure of approximately 5.0 cm in length extended from the dorsal edge of the epididymis cauda. No adherences with surrounded tissues were observed. Histopathology of the sac wall showed a smooth inner surface composed by scarce mesothelial cells forming in some areas papillary-like projections protruding to the vaginal cavity. Underneath, a thick layer of fibrous tissue mixed with collagen fibres and mononuclear inflammatory cells were seen. The potential consequences of this saccular formation at an older age in a bull are unknown.

Introduction

The clinical assessment of the scrotum and its contents is an important step during the breeding soundness examination (BSE) in the bull. A careful evaluation must include palpation of the spermatic cord, the aspect and symmetry of the scrotum, testicular mobility and consistency by palpation, besides measurement of scrotal circumference. Furthermore, a throughout valuation of the epididymis, including symmetry and palpation of its caput, corpus and cauda is of outmost importance to diagnose abnormalities at this level. Some of the pathologies that can be diagnosed during the scrotal examination are testicular asymmetry (i.e. hypoplasia and atrophy), low scrotal circumference, orchitis, epididymitis, hydrocele and adherences between tunica vaginalis parietalis and testicular capsule (Chacón 2000). Besides, there are some findings that may be indicators of disrupted testicular function. For example, a decreased testicular consistency has been associated with pathological changes in the seminiferous epithelia and abnormalities in the ejaculate compared with normal bulls (Chacón et al. 1999; Chacón 2001).

Regarding the tunica vaginalis in the bull, abnormalities reported in this serosa are mainly limited to conditions such as hydrocele and adherences within tunica layers secondary to orchitis. Blom and Christensen (1958) described also the presence of cyst like formations (paradidymis), in the funiculus spermaticus (mesorchium), presumable derived from remnants of the mesonephric duct. It is unknown that sac-like formations in the tunica vaginalis of the bull or any other domestic animal have been previously published in the literature. Therefore, this paper aimed at describing the clinical and pathological features concerning the first report of a sac-like formation in the tunica vaginalis parietalis of a Nelore (*Bos indicus*) bull.

Materials and Methods

The animal

The affected male was diagnosed out from a group of 157 purebred Nelore (*Bos indicus*) calves located in four different farms in the dry North Pacific of Costa Rica (94°3′–11°13′N, 84°46′–85°57′W). Clinical and spermiogramme assessments were carried out on those males as part of a follow-up study on testicular growth. The animals were kept grazed in padlocks of Brachiaria sp and supplemented with minerals *ad libitum*. At 24 months of age, a serological diagnosis by ELISA of Brucellosis, Bovine Viral Diarrhea Virus, Leptospirosis, Bovine Leukemia Virus and Herpes Virus-1 was performed.

Clinical and spermiogramme evaluation

The same operator carried out the examinations of the sire at the farm at monthly intervals initiating at weaning (7 months of age) up to 24 months of age. This included a visual inspection, recording of body weight (BW) and a careful examination of the scrotal contents, including the scrotal circumference (SC) and a thorough palpation of the epididymis.

Ejaculate collections using a three-electrode electro ejaculator (Standard Precision Electronics®, Boulder, CO, USA) were initiated at 16 months of age and repeated every 60 days up to 24 months of age. Ejaculated volume (ml), overall motility (%), sperm output ($\times 10^6$), and morphological abnormalities (%) were the

variables studied at the spermiogramme. Immediately after collection, sperm motility was estimated subjectively using a phase contrast microscope (×400, Zeiss®; Hanover, Germany). Sperm morphology was evaluated under phase contrast microscopy (×1000) on 400 spermatozoa per sample, fixed in buffered formol-saline (Hancock 1952). Additionally, a semen smear was prepared and stained with carbol-fuchsin (Williams and Utica 1920) to assess the morphology of the sperm head under light microscopy (×1000). Sperm output was determined using a Neubauer chamber haemocytometer (Brand®, Wertheim, Germany).

Ultrasound assessment

The ultrasonographic appearance of the scrotum and its contents in the affected bull was performed at 24 months of age. After a thick layer of coupling gel was applied on the smoothed scrotal skin, a 5 MHz linear array transducer (Aloka®, Tokyo, Japan; model SSD 500) was placed longitudinally for assessment of testicular parenchyma. Likewise, the ultrasonography of the saccular formation was determined by placing the transducer at the medial face of each testicle.

Tissue sampling

At 25 months of age, the sire was anaesthetized using Xilazine clorhidrate 2%. Immediately after, the scrotum was carefully disinfected and with a fine needle (#22) an aspiration biopsy was taken from the sac contents using a disposable syringe. The fluid obtained was immediately centrifuged for 5 min at 6400 ***g*** and smears were prepared from its sediment for staining with May–Grünwald–Giemsa. Surgically excised biopsies were taken from different sections of the testicular parenchyma, epididymis (caput, corpus and cauda), vaginal parietal tunic and from the saccular formation and then fixed in buffered formalin solution (10%). Additionally, control biopsies were taken from the tunica vaginalis of three normal 2-year-old Nelore bulls raised at the same farm. Once trimmed, the samples were processed and stained with haematoxylin–eosin and Masson's trichrome.

Results

All the serological tests were negative. Body weight (kg) and SC (cm) at 7, 12, 18, 22 and 24 months of age were 181 and 13.0, 290 and 17.2, 345 and 22.3, 421 and 27.3 and 502 and 29.9 respectively. Testicular and epididymis symmetry and consistency were normal throughout the follow-up assessment. During the first clinical examination at 7 months of age, a bilateral well-delimited finger-shaped swelling area was detected (although more evident at the left side), running longitudinal and parallel to the epididymis corpus (Fig. 1). The approximate length and width of the structure at the left and right sides of the scrotum were 5.0 and 1.5 cm vs 2.5 and 1.5 cm respectively. The affected area was painless and soft at palpation, seeming to contain fluid and was persistently present during the monthly examinations. At 12 months of age, the swelling embraced approximately two thirds of left testicle length (Fig. 1).

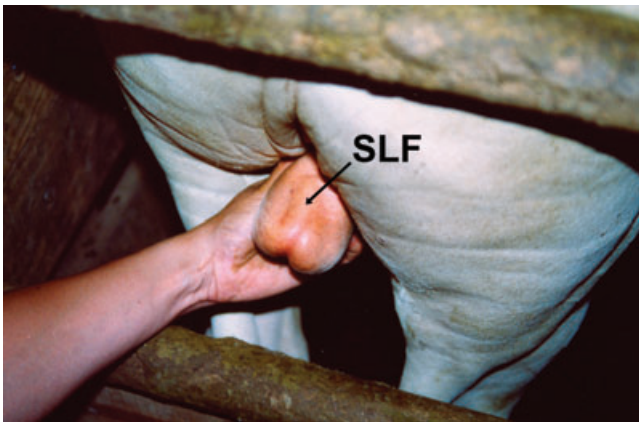

Fig. 1. Clinical appearance of the sac-like formation (SLF) in the tunica vaginalis during the scrotum examination of the Nelore bull at 12 months of age. Notice the finger-shaped swelling caused by fluid accumulation in the sac located parallel to the medial surface of both testicles, and embracing approximately 2/3 of the left gonad length

The first ejaculate containing spermatozoa was obtained at 18 months of age (5.2 ml, 65% overall motility and 130.0×10^6 spermatozoa). In addition, the % of morphological sperm abnormalities for acrosome, head (including nucleus), middle piece, tail and proximal droplets was 1.0, 12.0, 2.0, 6.5 and 86.0 respectively. At 22 months of age (BW 421 kg and SC 27.3 cm), a 12.6 ml ejaculate with 90% motility, 1543.5×10^6 spermatozoa and 94% normal morphology was obtained. The ultrasonographic appearance of the scrotal wall, testicular parenchyma and cauda epididymis was normal (Fig. 2). However, an oval-elongated shaped anechoic area was noticed delimited by a wall and extended from the dorsal edge of the epididymis cauda up to approximately half the testis length (Fig. 2) ending up in a blind sac structure (Fig. 3).

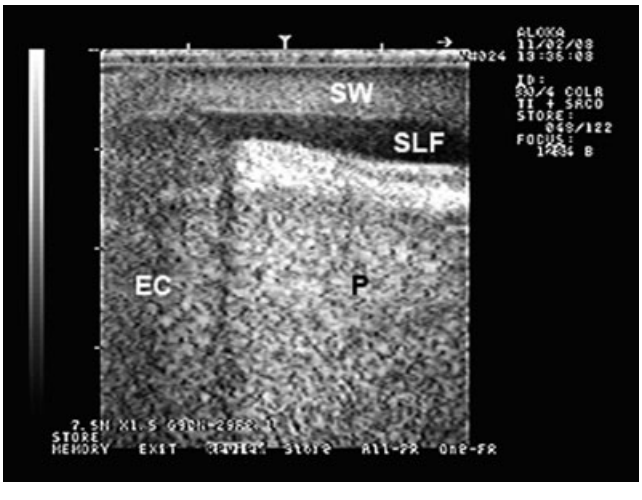

Fig. 2. Ultrasonic appearance of the sac-like formation (SLF). The transducer (5.0 MHz) was placed longitudinally at the medial face and ventral pole of left testicle. The anechoic area reflects fluid accumulation in the structure and extends dorsally on the epididymis corpus. (SW, scrotal wall; P, testicular parenchyma; EC, epididymis cauda; SLF, sac-like formation). The echogenic area between SLF and P may be caused by 'enhancement or through-transmission artifacts' present when a fluid filled structure increases the amplitude of the sound pulses

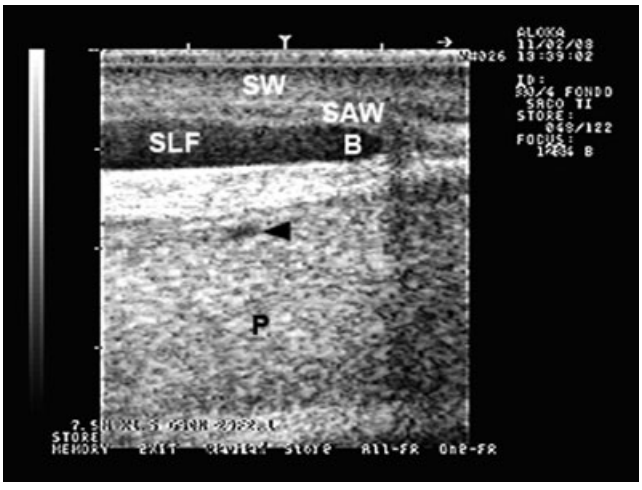

Fig. 3. Ultrasonic appearance of the sac-like formation (SLF) as seen placing the transducer (5.0 MHz) on the upper surface of the sac in the left testicle. The sac wall (SAW) can be differentiated at this level from the scrotal wall (SW). The anechoic spot (arrowhead) correspond to a superficial testicular vein (B, blind end of the sac formation; P, testicular parenchyma)

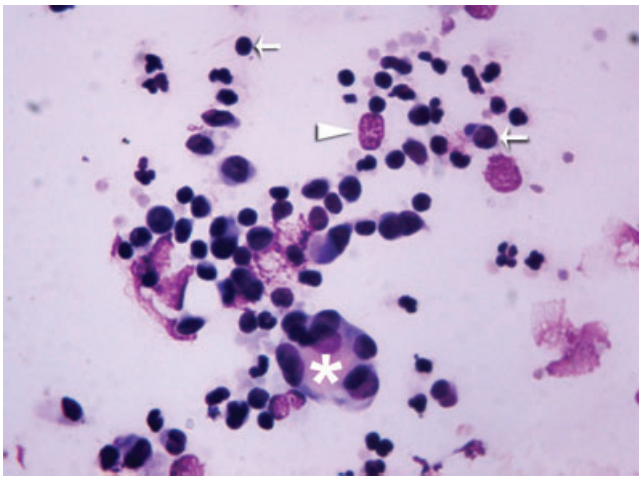

Fig. 4. Cytology of the accumulated fluid aspirated from the sac formation. Mononuclear cells (lymphocytes and histiocytes) predominates (arrows). Some of the cells likely from mesothelial type either joined into aggregates forming multinuclear reactive clusters (asterisk) or degenerates showing a granulated cytoplasm and lysed nuclei (arrowhead) (May–Grünwald–Giemsa 600×)

The cytological examination of the clear yellowish background transuded (5.0 cc) showed a slightly eosinophilic aspect and it was composed mainly by lymphocytes and histiocytes. Besides, there were spindle cells, few neutrophils and some mesothelial cells either degenerated or forming multinuclear clusters (Fig. 4). At the time of castration (25 months of age), the swelling in the left testicle had the same length as seen at 7 months of age and comprised about half the testis length. The macroscopic aspect of testicles, epididymis caput and corpus and vas deferens was normal. However, the epididymis cauda was partially detached from the left testicle at the level of its sigmoid flexure, showing a tongued-like appearance (Fig. 5a). A sac-like structure was found in the tunica vaginalis parietalis of both gonads although more pronounced in the left compared with the right testicle. These were located at the medial surface of each testicle, running parallel and longitudinal to epididymis corpus. In addition, the sac was closed at its dorsal pole and showed a smooth internal surface (Fig. 5b). No adherences were found between the sac and surrounding tissues. Microscopically, the parietal vaginal tunic from the control bulls showed an undulated inner surface formed by a monolayer of mesothelial cells with connective tissue underneath (Fig. 6a). On the contrary, a smoother inner surface with few mesothelial cells and papillary-like projections protruding to the lumen was seen in different areas of the saccular formation with infiltration of mononuclear inflammatory cells (Fig. 6b). Beneath, a thick layer of connective fibrous tissue and collagen fibres were observed with increase of hyperemic new vessels and presence of adipose tissue at the basal layer (Fig. 6b). Epidydimal and seminiferous epithelia showed a normal histology.

Discussion

Abnormalities in the tunica vaginalis of mammalians, particularly in the bull, are seldom reported in scientific literature and limited mainly either to inflammatory conditions associated in most cases to fluid accumulation in the vaginal cavity (hydrocele), or mesotheliomas (Wolfe et al. 1991). At our current state of knowledge, no previous reports of saccular formations in the tunica vaginalis parietalis have been published in the bull. In the male under study, the sac-like formation was a blind appendix originated from an evagination of the tunica vaginalis parietalis. The young age at which the sac was clinically found, besides no background of trauma, adherences or infectious diseases in the reproductive organs support the hypothesis of a congenital origin for this structure. The sac seemed not to increase its size with ageing and appeared to be permanent. It is unknown if the partial detachment of the epididymis cauda is related with the genesis of the sac. In the normal male, the cauda epididymis is attached to the posterior pole of the gonad through the proper ligament of the testis (Martin 1995).

During the final stage of testicular migration, a peritoneum evagination originates the processus vaginalis, which surrounds the posterior attachment of the gubernaculum testis forming the tunica vaginalis (Setchell 1978; Langman 2004). This tunica adheres to the gonad, epididymis and spermatic cord through its visceral layer and then reflexes forming the tunica vaginalis parietalis which outlines a uniform vaginal sac (Setchell 1978; Martin 1995; Langman 2004). Therefore, it is probable that the saccular formation found in the present case was originated during the inguinal phase of testicular descent, although the cause for the formation of this blind appendix is unknown.

Although no information is available in the literature about the approximate quantity of fluid contained in the vaginal cavity of a normal bull, we consider the amount of effusion drained out from the saccular formation as excessive. The accumulation of this fluid into the vaginal

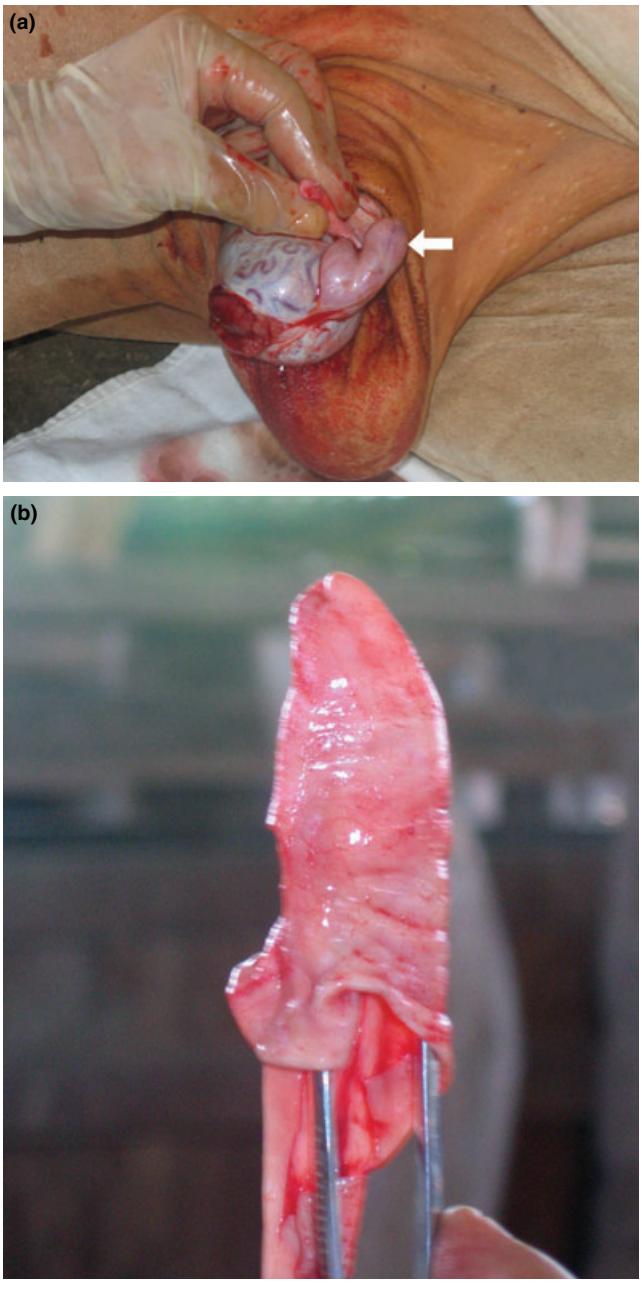

Fig. 5. (a–b) The inner surface of the sac-like formation (reverted) during castration of the left testicle is held by the operator's hand (a). Notice the partial detachment of epididymis cauda (arrow). In (b), the inner surface of the removed blind sac is shown

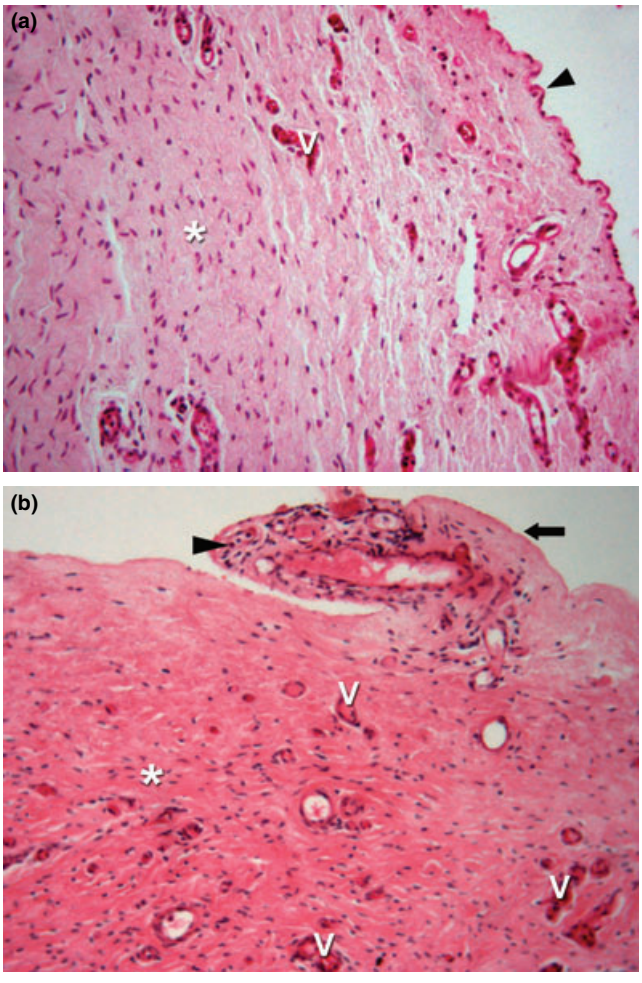

Fig. 6. (a–b) The normal histological aspect of the tunica vaginalis from a control Nelore bull is shown in (a). An undulated monolayer of mesothelial cells is seen in the inner surface (arrowhead) followed by fibrous tissue and collagen fibres (asterisk) with small sized vessels (V) underneath. In (b), the tunica vaginalis from the saccular formation shows papillary-like projections (arrow) protruding to the vaginal cavity with infiltration of mononuclear inflammatory cells (arrowhead). Notice the smoother inner surface and the scarce presence of mesothelial cells as compared with the control (haematoxylin–eosin 200×)

cavity is normally due to excessive production by the tunica vaginalis or decreased drainage through lymph vessels beneath the parietal tunic (Ladds 1993). It is possible that the sac favours the collection of fluid in its blind cavity by disturbing in some way the drainage of liquid at this level. Similarly as it occurs in cases of typical hydrocele effusions, the pressure caused by the fluid accumulation may induced a consequent inflammatory response in the tunica vaginalis thus contributing for degeneration and losing of mesothelial cells. However, it is important to point out that in the present case the collection of liquid was restricted only to the saccular formation which makes a difference with the classical hydrocele where it is distributed in the whole vaginal cavity.

There is very little information in the literature about the cell content in hydrocele effusions in domestic animals. Even though, the cytology found in the fluid contained in the sac formation is identical to that reported in hydrocele cases in humans, where nearly all the cells are macrophages and lymphocytes mixed with mesothelial degenerated cells (Spriggs and Boddington 1989).

Although the sac formation had no impairment on testicular or epididymal function during the study period, the potential consequences in an adult bull are unknown. A careful and throughout clinical examination of the scrotum should be performed by the practitioner during a BSE under field conditions to diagnose similar cases as the present report. Differential diagnosis should include scrotal hernia, hydrocele, serosal and epididymal cysts and neoplasm.

Acknowledgements

The authors express their deep gratitude to Mr José Rodríguez and Mrs Laura Alvarado for their valuable technical assistance. The authors also extend their special thanks to Las Pavas farm for allowing the follow up of the case. This study received financial support from the Research Program on Applied Animal Andrology (PIAAA) through the CRIOSEMEN project, Andrology Section, Veterinary School-UNA.

Author contribution

J. Chacón supported in follow up and diagnosis of the case, writting, design, analysed data. A. Berrocal involved in histological processing of samples and diagnosis. revision and discussion of manuscript.

References

Blom E, Christensen O, 1958: Cyst and cyst like formations (Inter Alia Spermiostasis) in the genitals of the bull. Studies on pathological conditions in the testis, epididymis and accessory sex glands in the bull. Bulletin from the ambulatory clinic and the state veterinary serum laboratory. Separatum Royal Veterinary and Agricultural College, Copenhagen, pp. 101–133.

Chacón J, 2000: Breeding Soundness Evaluation of Zebu Bulls. PhD Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden, pp. 11–46.

Chacón J, 2001: Assessment of sperm morphology in zebu bulls under field conditions in the tropics. Reprod Domest Anim **36,** 91–99.

Hancock L, 1952: The morphology of bull spermatozoa. Br J Exp Biol **29,** 445–453.

Chacón J, Müller E, Rodríguez-Martínez H, 1999: Morphological features of the seminiferous and cauda epididymides epithelia of breeding Zebu bulls with normal and decreased testicular consistency. J Reprod Dev **45,** 119–128.

Ladds P, 1993: The male genital system. In: Jubb K, Kennedy P, Palmer N (ed.), Pathology of Domestic Animals, **Vol. 3**. 4th edn, Academic Press Inc, CA, USA, pp. 476–478.

Langman J, 2004: The urogenital system. In: Sadler T (ed.), Medical Embryology. 9th edn, Williams and Wilkins, Baltimore, USA, pp. 377–379.

Martin E, 1995: The development of the genital peritoneum in domestic mammals. An analysis of the literature and nomenclature. Anat Histol Embryol **24,** 285–287.

Setchell B, 1978: Testicular migration. In: Finn C (ed.), The Mammalian Testis. Cornell University Press, Ithaca, NY, pp. 33–39.

Spriggs A, Boddington M, 1989: Hydrocele of the tunica vaginalis testis. In: Gresham A (ed.), Atlas of Serous Fluid Cytopathology: A Guide to the Cells of Pleural, Pericardial, Peritoneal and Hydrocele Fluids. Springer, Cambridge-UK, pp. 107–114.

Williams W, Utica N, 1920: Technique of collecting semen for laboratory examination with a review of several diseased bulls. Cornell Vet **10,** 87–94.

Wolfe D, Carson R, Hudson R, Boosinger T, Mysinger P, Powe T, Claxton M, Angel K, 1991: Mesothelioma in cattle: eight cases (1970–1988). J Am Vet Med Assoc **4,** 486–491.

Submitted: 20 Mar 2009

Author's address (for correspondence): Dr Jorge Chacón, Andrology section, Veterinary Medicine School, Universidad Nacional, PO Box 2556-3000, Heredia, Costa Rica. E-mail: jchacon@medvet.una.ac.cr
*Present address: Histopatovet, PO Box 904-3000, Heredia, Costa Rica