CASE REPORT

BMC Veterinary Research

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A premier case report of a congenital pseudocyst of the testis (*cystis spuria testiculi congenita*) in a three-week-old calf

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Abstract

Background Cysts in the testicular parenchyma are extremely rare in bulls and are typically detected during ultrasound examinations. In contrast, pseudocysts are exceedingly uncommon. This paper describes a case involving a large pseudocyst in a few-day-old calf.

Case presentation The circumference of the identified structure was 25 cm, with a length of 14 cm and a width of 8 cm. The skin of the scrotum was movable, and the consistency of the formation was elastic. The structure was positioned obliquely, with its apex reaching the proximal part of the ankle. Ultrasonography revealed the presence of a large normoechoic fluid space, with small, irregularly shaped fragments of tissue exhibiting significant hyperechogenicity. The structure was surgically excised. Histopathological analysis indicated changes consistent with a pseudocyst. The bull's karyotype was normal (60XY), and the biochemical and haematological parameters were within physiological ranges. Testosterone concentrations on the day of surgery and at 10 months of age were measured at 0.02 ng/ml and 0.07 ng/ml, respectively.

Conclusions This case report represents the first documented instance of an abnormally large testicular pseudocyst in a bull. The surgical removal of the pseudocyst was performed safely and successfully, with no adverse effects observed on the calf's subsequent growth and development.

Keywords Pseudocyst, Congenital testicular pseudocyst, Bovine reproductive disorders, Testicular abnormalities, Bovine reproductive health

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Background

Testicular tumours and inflammation have been reported in numerous animal species [1]. They often exhibit malignant characteristics or arise from injuries or infections. Similar conditions have been observed in the epididymis of boars, rams, and bucks [1]. Additionally, epididymal cysts have been described in wisents of various ages, ranging from 3 months to 20 years [2]. These cysts affected 61% of the males studied and were primarily located in the head of the epididymis, with fewer occurrences in the body and tail. Small cysts have also been identified in the parenchyma of bull testicles

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during ultrasound examinations. Notably, all affected sires exhibited varying degrees of marked infertility [3]. Cysts and pseudocysts commonly develop as a result of circulatory disorders. Typically, transudate accumulates in the free spaces of tissue structures, such as glands or ducts, leading to the formation of true cysts - closed structures lined with epithelial cells that separates the cyst cavity from the surrounding tissue. Less frequently, fluid accumulates in the connective tissue space, causing systematic enlargement of the cyst cavity, which has a wall composed of connective tissue and is referred to as a pseudocyst. Such pseudocysts can appear in various animal species, and usually result from angiogenesis disorders that may occur during the prenatal period or chronic inflammation. Testicular pseudocysts, in particular, are extremely uncommon.

Case presentation

In May, 2022, a male Holstein-Friesian calf was born. The sire was a high-genetic-value American bull, and the dam was a multiparous domestic cow. A notable characteristic observed by the staff was a large umbilical hernia.

Visit of the herd veterinarian

Five days later, during a visit by the veterinarian the hernia was ruled out, as the veterinarian identified the structure as an enlarged scrotum, comparable in size to that of a several-month-old bull with a severely enlarged testicle (Fig. 1). The veterinarian responsible for the herd decided to surgically remove the detected formation as a preventive measure. The case was also reported to the Institute



Fig. 1 View of the enlarged scrotum (red arrow) of a three-week-old calf

of Veterinary Medicine of the Nicolaus Copernicus University in Toruń.

Surgical procedure and samples collection

Approximately 2 weeks later, surgery was performed under farm conditions to remove the abnormalities. Prior to the operation, the circumference of the scrotum was precisely measured, and an ultrasound examination was conducted using the Esaote MyLab One (Esaote, Italy) ultrasound scanner with a 13 MHz linear transducer and full frequency adjustment. The circumference of the identified structure measured 25 cm, with a length and width of 14 cm and 8 cm, respectively. The skin of the scrotum was movable, and had an elastic consistency. A single structure was identified within the scrotum, oriented obliquely with its apex reaching the proximal part of the hock joint (Fig. 1). The ultrasound examination revealed a large normoechogenic fluid space with small fragments of highly echogenic and irregularly shaped tissues visible in some areas (Fig. 2).

The calf was sedated with xylazine (Sedazin, Biowet Puławy, Poland), administered intravenously at a dose of 10 mg/100 kg b.w. The calf was estimated based on body weight records from the zootechnical documentation. Local anesthesia was administered along the greater curvature of the testis and intracavitarily at the level of the superficial (external) inguinal ring using 5 ml of 2% lidocaine (Lidor, Richter Pharma AG, Austria). Intraoperatively, the presence of the second testicle in the scrotum and inguinal canal was excluded. Castration was performed using the bloody technique, involving removal of the testis enclosed within the common vaginal tunic, following the Muir method [4]. The fluid-filled structure and its sheaths were excised from the scrotal skin. After dissection and incision of the scrotal sac, the wound was carefully closed in two layers.

Subsequently, a detailed examination of the testis and its associated sheaths was performed. Upon incision of the tunica vaginalis communis, a small volume of clear fluid was observed between this space and the tunica vaginalis testis. Following the incision of the tunica vaginalis testis, a considerable amount (126.7 g) of clear, straw-yellow fluid was evacuated. The total weight of the testis, including the tunica vaginalis communis and the accumulated fluid, was 206 g. After fluid removal, the interior of the testis was examined, revealing a small tissue fragment corresponding to a hyperechoic lesion previously identified within the lumen of the fluid-filled space (Fig. 2). All excised materials (testis and associated tunics) (Fig. 3) were fixed in formalin for subsequent histopathological analysis. Following the orchiectomy, the bull was transferred for continued fattening.

On the day of the surgical procedure, blood was collected from the jugular vein and sent for haematological,



Fig. 2 Sagittal sonogram of a testicular pseudocyst showing a large normoechogenic fluid-filled space (white arrow) with small fragments of highly echogenic tissue (red arrow)

biochemical, hormonal and cytological analysis. The testosterone level in the blood on the day of surgery was reported to be below 0.02 ng/ml. Upon subsequent measurement when the bull was 10 months old, the testosterone level was found to be 0.07 ng/ml.

The results of the remaining hematological and biochemical parametres were within the reference range established for cattle.

Genetic and histological examinations

A cytogenetic examination was conducted at the Department of Molecular Biology of Animals of the National Research Institute of Animal Production, confirming a normal karyotype 60, XY (Fig. 4).

Tissue samples were collected following the resection of the structure and fixed in 10% buffered formalin. Paraffin-embedded sections were stained with haematoxylin and eosin. The tissue specimens were evaluated using slide preparations with a Leica DM2000 LED light microscope (Leica, Germany), equipped with a LEIKA MC190 HD digital camera, at magnifications ranging from 12.5x to 30x. Photographs were taken using LEIKA LAS V4.12 microscope imaging software. Multiple tissue fragments were collected from the vicinity of, and wall of, the testicular cyst post-resection:

- Cranial region of the structure (Fig. 5): The presence of the serous membrane (*lamina parietalis tunicae vaginalis testis*- *periorchium*), underlying loose connective tissue with blood vessels, and a layer of skeletal muscles was observed.
- Intermediate region of the cyst (Figs. 6 and 7): The appearance of the cyst wall varied segmentally. Some areas exhibited residual structures morphologically corresponding to elements of the normal testis histological structure (Fig. 6A). A well-developed connective tissue capsule was present along the entire wall section, characterized by an compact external layer transitioning into a looser arrangement in the deeper layers, with perpendicular extensions of loose connective tissue bands. These bands demarcated regions containing vestigial testicular lobes. The lobes were delineated by dense and loose connective tissue bands with numerous blood vessels and straight tubules (Fig. 6B). Deeper connective tissue bands were interspersed with



Fig. 3 Image of the pseudocyst (black arrow) after resection with the sheath of the testis (red arrows)



Fig. 4 Karyotype of the examined bull with marked sex chromosomes

narrow fissures lined by two-layered columnar epithelium, resembling the *rete testis* (Fig. 6C). Some lobes contained residual, dilated convoluted tubules lined with a single layer of flattened cells exhibiting hyperchromatic nuclei. Sectionally, the tubule walls consisted of densely packed cells with loose chromatin (Fig. 6D). Solitary clusters of cells with eosinophilic cytoplasm and hyperchromatic nuclei, consistent with Leydig cells, were observed within the intralobular connective tissue (Fig. 7A). The innermost cyst wall layer comprised dense connective tissue. In other cyst wall sections beneath the *tunica albuginea*, irregularly shaped cavities surrounded by single peripheral cells were noted (Fig. 7B). These cavities lacked vestigial convoluted tubules but contained numerous clusters of cells with hypochromatin and hyperchromatic nuclei within the deeper connective tissue (Fig. 7C and D).

 Caudal region of the cyst (Fig. 8): The cyst wall comprised a thick layer of connective tissue.
Irregular clusters of loose connective tissue extended towards the cyst lumen, containing small blood vessels, isolated cells, or small cell clusters with hypochromatin or hyperchromatic nuclei.

Discussion

The testicular circumference, regardless of breed, in one-month-old bull calves is approximately 10 cm and increases linearly with body weight until around 7 months of age [5, 6]. A scrotal circumference of approximately 13.68 cm has been reported in mithun bulls (Bos frontalis) aged between 0.1 and 1 year [7, 8], increasing to 28.5 cm in dairy bulls aged 7–12 months [9]. Our previous studies demonstrated that at 8 months of age, the testicular circumference of Holstein-Friesian bulls averaged 25.1 mm [6], a value comparable to the specimen observed in the present case. Moreover, in a 10-monthold swamp buffalo bull, the testicular diameter was 15.1 cm, increasing to 25 cm by 48 months of age. In young bulls, the testes are typically small, often weighing only a few grams, with relatively stable size until approximately six months of age, followed by a period of rapid growth [7, 10]. The testicular weight of Holstein-Friesian calves at 12 months of age has been reported to be approximately 9 g [10]. Although lower testosterone levels are characteristic of calves [8, 11], in our case, the measured level was below the typical range (2.28 ± 0.09) ng/mL) for calves of a similar age [12].

The values of selected hematological and biochemical blood parameters were within the broad physiological range for Holstein-Friesian calves, and, therefore, did not contribute significantly to the diagnostic process [13, 14].

The ultrasound examination revealed a substantial fluid-filled cavity, indicating the presence of a cyst. Cysts in various parts of the bovine reproductive system are uncommon; however, one study reported the presence of fluid-filled cavities inside the testes and epididymis of



Fig. 5 Tissues adjacent to the cephalad pole of the pseudocyst (inguinal canal area). Periorchium (black arrows) with deeper loose connective tissue, a layer of skeletal muscles, and blood vessels. H/E staining. (x12.5)

one out of ten infertile breeding bulls [3]. In that case, the affected bull exhibited markedly enlarged testicles with a soft, pulpy consistency, measuring 63 cm in circumference, and a high proportion (43.4%) of dead sperm in the semen [3]. The lesion described was notably large, approximately the size of a melon. In contrast, Curtis et al. [15] reported that bull testicles typically weigh slightly over 80 g at 32 weeks of age.

The histological findings clearly indicate a congenital pseudocyst, likely resulting from abnormal organogenesis during embryonic or fetal development. To our knowledge, congenital testicular pseudocysts in bulls have not been previously documented in the literature. The presence of residual histological structures of normal testicular tissue within the cyst wall suggests that the cyst cavity initially developed within the testicular parenchyma. Considering the volume of fluid present, it can be inferred that the pressure exerted on the surrounding testicular tissue likely impeded proper testicular development. In bulls, testicular descent typically occurs towards the end of the third month of fetal life [16]. Given the narrow inguinal canal, it would have been impossible for a significantly enlarged gonad to pass through due to mechanical resistance. Therefore, it can be assumed that the cyst was either absent or relatively small during this period, with its primary development occurring after the gonad descended into the scrotum. The resected lesion appeared as a single mass within the scrotum, and the fate of the contralateral testicle remains unclear. Its absence from the scrotum raises the possibility of abdominal cryptorchidism, supported by the lack of findings in both the inguinal canal and ectopic locations. To the best of our knowledge, this case represents the first report of such a condition in a neonate. The large size of the lesion was particularly remarkable, as it was several times larger than the typical testicular size of neonatal calves. The likely cause of the pseudocyst formation may involve circulatory disorders, leading to excessive secretion of serous fluid or impaired absorption by the testicle sheath. The observed changes were definitively not indicative of hydrocele, which involves transudative fluid accumulation between the testicles and does not cause such advanced atrophic changes. In hydrocele, only vestigial functional remnants of the testicular parenchyma are typically observed. Hydrocele is rare in cattle [17], with prevalence rates in bulls ranging from 1.6 to 2.4% according to various studies [18, 19].

A testicular hydrocele is a type of cyst– an oval structure filled with fluid and surrounded by a sheath (referred to as a capsule). It is considered one of the conditions leading to cyst formation in the scrotal area. Testicular hydroceles develop as a result of improper descent of the testicles into the scrotum, with the direct cause being the failure of the vaginal process to fuse. In normal fetal development, the vaginal process closes and forms an empty sac located on the anterior surface of the testicle.



Fig. 6 The middle section of the pseudocyst wall (in the middle between the inguinal canal and the distal part of the scrotum). **A** Residual testicular lobes (red arrows) with convoluted tubules (black arrows) and interstitium are visible. H/E staining. (x12.5). **B** Loose connective tissue bands with numerous blood vessels and straight tubules with secretions (black arrows). H/E staining. (x30). **C** Narrow gaps in the interstitium lined with a two-layer columnar epithelium corresponding to the structure of the rete testis (black arrows). H/E staining. (x30). **D** The dilated lumen of the convoluted tubules is visible, lined with a single layer of flat cells with hyperchromatic nuclei (black arrows) and/or segmentally layered cells with hypochromatic nuclei (red arrows). H/E staining. (x30)

Peritoneal fluid may accumulate in an unclosed vaginal process, and over time, this fluid accumulation leads to the development of a hydrocele. The direct cause of a leak in the adhesion of the vaginal sac and the formation of a congenital testicular hydrocele may involve disturbances in the testicular descent process or trauma occurring in the postnatal period. Cysts that develop peripherally outside the tunica albuginea of the testicle lead to the fluid accumulation which increases the volume of the scrotal sac. However, this does not disturb the flow of body fluids to an extent that could lead to the atrophy of the testicular histological structures. This applies to epidermoid cyst formation within the scrotal sac. Additionally, the accumulation of fluid in the testicular structures is not a result of cysts in the testicular ducts, as their histological structure indicates that they are not true cysts, i.e., those with an epithelial lining [20].

Conclusion

This case represents the first documented instance of a testicular pseudocyst in a newborn calf. The lesion's substantial size, attributed to the accumulation of a significant volume of fluid, suggests that the cyst cavity likely originated within the testicular parenchyma, gradually displacing spermatogenic tissue. However, given the current stage of knowledge, it remains challenging to definitively determine the etiology of this condition. Jaśkowski et al. BMC Veterinary Research (2025) 21:306



Fig. 7 The middle section of the pseudocyst wall (in the middle between the inguinal canal and the distal part of the scrotum). **A** In the intralobular tissue, single aggregates of cells with eosinophilic cytoplasm and a large, hypochromatic nucleus are visible, consistent with Leydig cells (black braces). H/E staining. (x30). **B** Visible under the tunica albuginea, variously shaped free spaces (black arrows) with single cells on the periphery, without vestigial structures of the convoluted tubules. H/E staining. (x12.5). **C** Numerous clusters of cells with hypochromatic nucleus (red arrows) and/or cells with a hyperchromatic nucleus (black arrows) are visible in the area of loose connective located in the deeper layer of the cyst wall. H/E staining. (x30). **D** Numerous cells with hypochromatic (red arrows) and hyperchromatic nucleus (black arrows). H/E staining. (x40)



Fig. 8 Caudal fragment of the pseudocyst wall (in the distal part of the scrotum). Visible irregular clusters of loose connective tissue directed to the lumen of the cyst accompanied by small blood vessels. Additionally, there are single cells or small clusters of cells with loose chromatin or a hyperchromatic nucleus. H/E staining. (x12.5)

Acknowledgements

Not applicable.

Author contributions

Conceptualization: JMJ; Material collection: JMJ, MK and MG; Material analysis: MG and BMJ; Writing- original draft: JMJ, BMJ, SK and MG; Writing- editing: JMJ, BMJ and MG; Final revision: JMJ, MG and BMJ. All authors read and approved the final manuscript.

Funding

This research received no external funding.

Data availability

The data analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable. The described case originates from routine veterinary practice. In accordance with applicable regulations, approval from the local ethics committee was not required.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 11 February 2025 / Accepted: 21 April 2025 Published online: 02 May 2025

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