

## Case Report

# Testicular degeneration, fibrosis, and mineralization in Limangus bulls

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## Abstract

A group of Limangus heifers had a high pregnancy rate after timed artificial insemination but low final pregnancy rate after 75 days of natural service with 2 Limangus bulls. Bulls (A and B) suspected of subfertility and 2 other bulls (C and D) with the same genetic background were examined. Bull A was clinically normal but had no sperm in the ejaculate. Bulls B and C had abnormal testicular consistency resembling fibrosis on palpation and ultrasonography; however, semen was normal. Bull D was clinically normal. Bulls were negative for brucellosis and venereal diseases. Bulls A, B, and C were culled and slaughtered. Bull A had a normal genital tract; however, nonstructured 5B gene was detected by reverse transcription polymerase chain reaction, suggesting an infection with bovine viral diarrhea virus-1 (pestivirus A). Bulls B and C had macroscopic and microscopic signs of testicular degeneration, fibrosis, and mineralization. Although the specific cause for testicular lesions in bulls B and C was not identified, these lesions were probably responsible for the low pregnancy rate.

**Keywords:** Bulls, infertility, testes, degeneration, fibrosis, mineralization

## Background

A common reproductive strategy in beef herds is to use timed artificial insemination (TAI) at the beginning of the breeding season and 15 days after TAI, use natural service (exposing cows or heifers to bulls for 60 days).<sup>1</sup> Various factors affect bull fertility; breeding soundness examination (BSE) is of great value to recognize the fertility of bulls and to maximize the reproductive efficiency of cow-calf operations.<sup>2</sup>

A standard BSE identifies bulls with substantial deficits in fertility but does not consistently identify subfertile bulls.<sup>3</sup> Routine BSE includes physical examination, estimation of sperm production based on scrotal circumference (SC), evaluation of semen quality, and occasionally, libido or copulation ability is assessed.<sup>4</sup> Ultrasonography is a complementary tool to identify testicular lesions that are not detected by palpation.<sup>5</sup> In addition, laboratory investigation of venereal diseases (*Tritrichomonas foetus* and *Campylobacter fetus venerealis*) should be included, since bulls maybe asymptomatic but infected cows will have signs of infertility. Other infectious

diseases may affect fertility of beef herds; therefore, core vaccination is recommended for bovine viral diarrhea (BVD), infectious bovine rhinotracheitis, leptospirosis, and clostridial diseases.<sup>4</sup>

We evaluated 4 Limangus bulls during the investigation of infertility in a cow-calf operation located in the south of Cordoba province, Argentina. In the fall of 2018, Limangus heifers (n = 62) were subjected to TAI and then exposed to natural service 15 days later with 2 Limangus bulls. During natural service, more heifers (n = 37) were added to the group. Pregnancy per TAI was 74% (46/62); however, pregnancy for 75 days of natural service was only 69.8% (37/53). In the winter of 2019, another group of Limangus heifers (n = 92) were bred and then exposed to natural service 15 days later with the same 2 bulls. Pregnancy per TAI was 50% (46/92); however, pregnancy for 75 days of natural service was only 63% (29/46). The referring veterinarian suspected infertility after detecting low SC and abnormal testicular consistency, since heifers were in good body condition and cycling. Two months later, a complete BSE (including ultrasonography of testis)

was conducted and preputial swabs for venereal diseases and blood samples were collected in these 2 bulls (A and B) and 2 other bulls (C and D) that were recently included. Bulls A and B were 3 years and bulls C, and D were 2 years (confirmed by second and first pair of incisors, respectively).

### Breeding soundness examination and complementary studies

Bulls underwent BSE according to criteria;<sup>4</sup> accessory sex glands were assessed by transrectal palpation. External reproductive organs, scrotal temperature, and painful responses were evaluated by palpation. SC was measured by pushing the testis to scrotal bottom by placing the thumb and fingers laterally on the side of scrotal neck and then pushing ventrally. A metal tape was applied over the scrotum and its contents; SC (in cm) was taken at the greatest diameter of the scrotal contents. Genital tract ultrasonography was conducted with a B-mode ultrasound scanner (Honda HS 1600) with a 7.5 MHz linear array transducer using described methodology.<sup>6</sup> High-quality ultrasonographic gel was used to generate clear ultrasonograms. One testis was pushed upwards (out of the transducer's ultrasound wave) while the other testis was pushed downwards for examination, thus scrotal wall was stretched improving the contact between transducer and scrotum. Transducer was applied longitudinally on the scrotal skin to evaluate the testis halfway between the head and epididymis tail.<sup>6</sup>

Semen samples were collected by electroejaculation and evaluated for sperm motility and morphology. Clean, prewarmed microscope slides, and coverslips were prepared. Sperm motility was evaluated subjectively under brightfield microscopy (400 x); morphology was evaluated using semen smears stained with eosin and nigrosin (E/N) and observed under oil immersion with brightfield microscopy (1,000 x). Sperm (n = 200) were evaluated for head abnormalities in each smear.<sup>4</sup> In addition, semen was diluted in 10% neutral buffered formalin (wet mount) and sperm (n = 100) were evaluated for tail defects, abnormal acrosomes, detached sperm heads, and proximal droplets using differential interface contrast microscopy (1,000 x).<sup>7</sup> Serologic testing for *Brucella abortus* was performed using buffered acidified plate antigen assay and preputial swab was obtained for testing sexually transmitted diseases (*Tritrichomonas foetus* and *Campylobacter fetus*) using a polymerase chain reaction (PCR) test.

A month later, bulls that failed BSE (A, B, and C) were culled and slaughtered; genital tracts were examined. Samples of testis (dorsal, mid, and ventral regions) were frozen at -20°C in sterile vials until processed for BVD virus.<sup>8</sup> In addition, 2 mm<sup>2</sup>

samples were taken for histopathological evaluation: 1. dorsal, middle, and ventral regions of testis; 2. head, body, and tail epididymis; 3. vas deferens; 4. vesicular glands; and 5. lesions. Samples were fixed in Bouin's solution for 6 hours, then placed in 10% formaldehyde solution (with 3 previous washes) and submitted for histology. Fixed tissues were trimmed and subjected to routine paraffin processing. Tissue sections were stained with E/N and examined by brightfield microscopy.

### BSE and necropsy findings

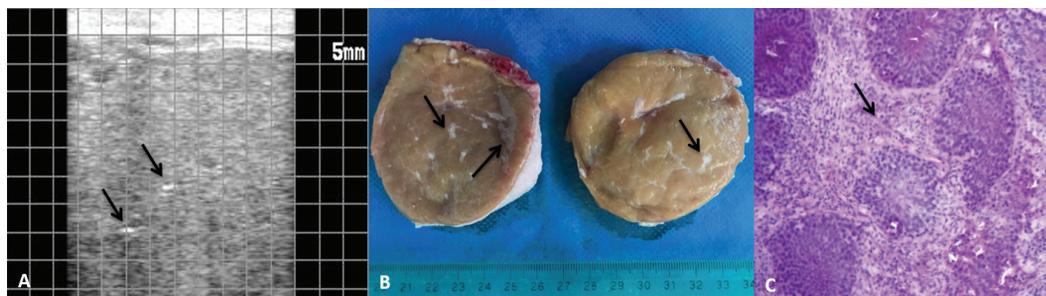
#### Bull A

BSE: Scrotum had a distinct neck; testes were symmetrical with normal tone and were freely moveable. Epididymis and spermatic cords were within normal limits. SC was 35.5 cm, and the scrotal temperature was normal without painful responses. Internal reproductive organs, prepuce, and penis were within normal limits. Ultrasonography revealed small focal hyperechoic areas (Figure 1A) affecting < 1 % of the area examined. Semen (5 ml) collected via electroejaculation was without sperm.

Necropsy: Samples were collected from the left and right epididymal tails. Sperm from the left had fair gross motility; individual motility was 70% and in E/N-stained slides 98.5% sperm were morphologically normal with ~ 3% head defects. Morphologically normal sperm on the wet mount was 93% (1% proximal droplet, 2% detached heads, 2% midpiece defects, and 2% principal piece defects). Sperm from the right had good gross motility; individual motility was 70% and in E/N-stained slides 99% sperm were morphologically normal with ~ 2% head defects). Morphologically normal sperm on the wet mount was 96% (4% had principal piece defects). Small focal white streaks of < 3 mm<sup>2</sup> (Figure 1B) were observed in both testicular histology. Epididymis, vas deferens, ampullae, and vesicular glands were within normal limits. Stromal fibrosis (Figure 1C) was evident in left testis. Ampullae and vesicular glands had mild fibrosis. In addition, nested multiplex reverse transcription polymerase chain reaction (RT-PCR) identified nonstructured 5B (NS5B) gene of BVD virus (BVDV-1 [pestivirus A]).

#### Bull B

BSE: Scrotum had a distinct neck; testes were symmetrical and were freely moveable. Right testis consistency was abnormal and had palpable intratesticular firm nodules. Epididymis and spermatic cords were within normal limits. SC was 35.5 cm, and the scrotal temperature was normal without painful



**Figure 1.** Bull A: A. Ultrasonogram of left testis; note small hyperechoic areas (black arrows). B. Cut section of testes; note small < 3 mm<sup>2</sup> in both testes (black arrows). C. Light microscopic image (1000 x) of left testis; note stromal fibrosis (black arrow)

responses. Internal reproductive organs, prepuce, and penis were within normal limits. Ultrasonography revealed diffuse hyperechoic areas and were more pronounced in the right testis (Figure 2A) affecting 20% of the examined area. Collection of semen by electroejaculation resulted in 5 ml of ejaculate that had poor gross motility and 65% individual motility. E/N-stained slides had 98.5% morphologically normal sperm (2% with head defects and 1% with midpiece defects). Wet mount had 93% morphologically normal sperm (4% with sperm head defects, 1% with midpiece defects, and 2% with principal piece defects).

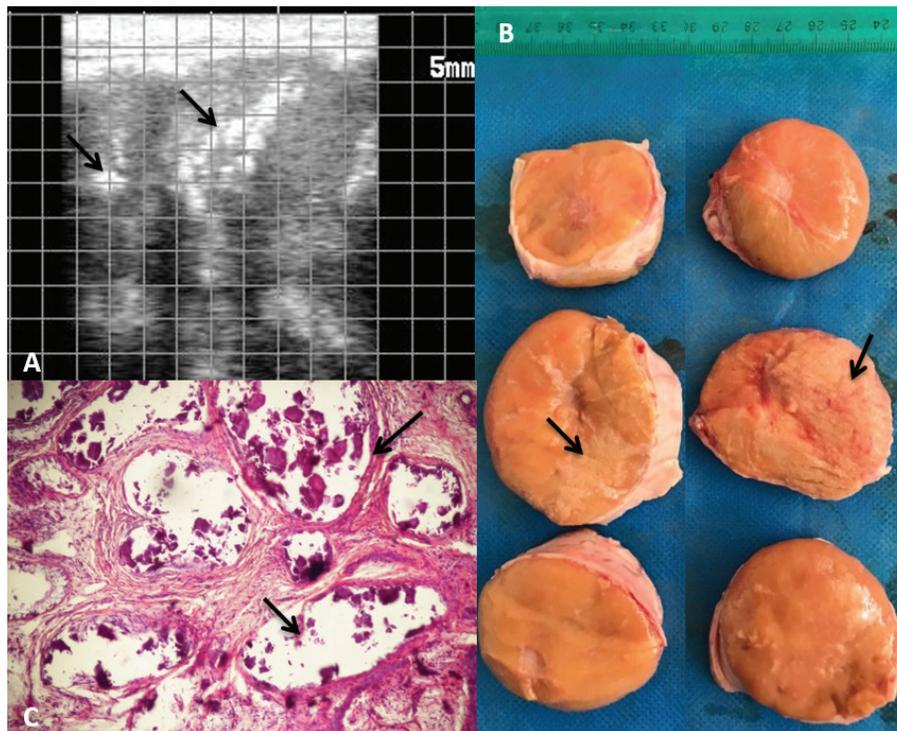
**Necropsy:** Left testis had focal white streaks of 5 mm<sup>2</sup> in the parenchyma and small areas of hard consistency and granulomatous appearance. Right testis had a large lesion of hard consistency and granulomatous appearance from the mediastinum toward the periphery involving 80% of testis (Figure 2B). Testicular parenchyma had abnormal appearance and atypical color. Epididymis, vas deferens, ampullae, and vesicular glands were within normal limits. In histology, intraluminal dystrophic calcification and peritubular fibrosis of the epididymis, fibrous tissue and calcification with mononuclear and polymorphonuclear cells (Figure 2C) were observed. The diagnosis was severe stromal and intratubular fibrosis. Nested multiplex RT-PCR was negative for NS5B gene of BVD virus identified as BVDV-1 (pestivirus A).

#### Bull C

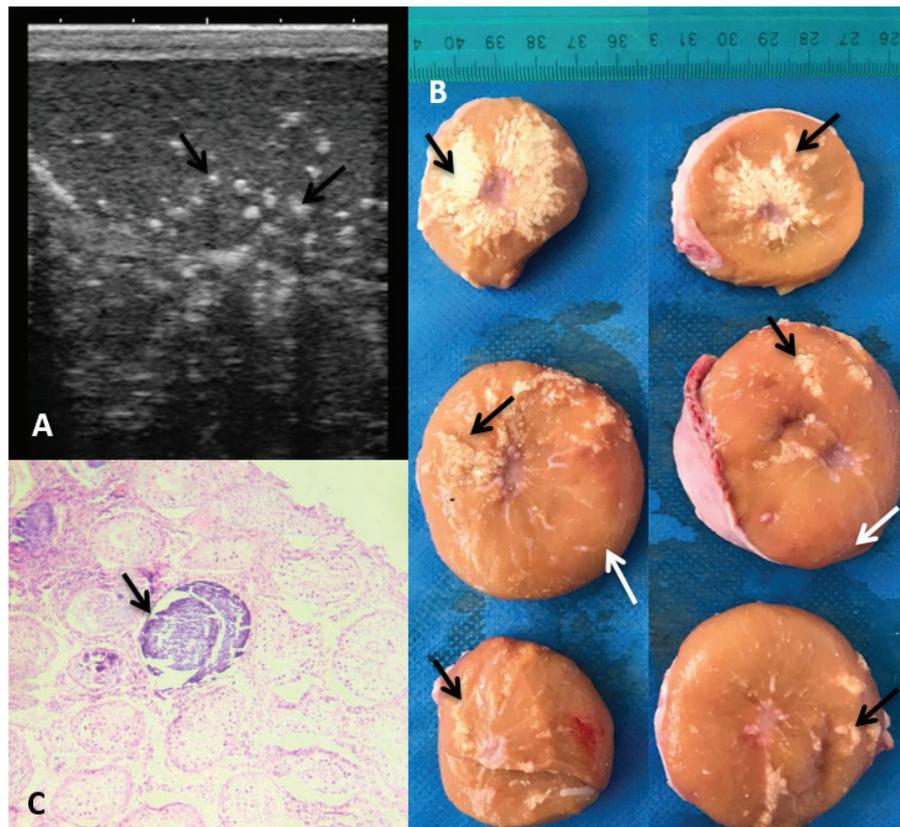
**BSE:** Scrotum had a distinct neck; testes were symmetrical and were not freely moveable. The tone was abnormal with palpable intratesticular firm swelling nodules in right testis.

Left epididymis tail was adhered to the testis. Right epididymis and spermatic cords were within normal limits. SC was 33.5 cm, and the scrotal temperature was normal without painful responses. Internal reproductive organs, prepuce, and penis were within normal limits. Ultrasonography revealed large diffuse and focal hyperechoic areas (Figure 3A) in testes affecting ~ 30% of examined areas. Collection of semen by electroejaculation resulted in 5 ml of ejaculate that had very good sperm gross motility and 65% individual motility. E/N-stained slides had 93.5% morphologically normal sperm (13% sperm head defects). The percentage of morphologically normal sperm in wet mount was 92% (3% defects of sperm head, 1% with proximal droplets, and 4% with principal piece defects).

**Necropsy:** There were adhesions between the parietal and visceral vaginal tunics. Testes had large white focal and diffuse streaks from the mediastinum toward the periphery of the parenchyma with lack of bulging (Figure 3B). Epididymides had adhesions to the vaginal tunic; vas deferens, ampullae, and vesicular glands were within normal limits. Testes histopathology revealed 70% mineralization of the seminiferous tubules with active fibroblasts and inflammatory cells (lymphocytes and macrophages) in the interstitial compartment. Seminiferous tubules had abnormal epithelium and intratubular concentric laminated calcification. There was a reduction in germinal cells; germinal cells were missing entirely in some tubules (Figure 3C). The diagnosis was severe testicular degeneration with moderate interstitial fibroplasia including areas with necrosis. Nested multiplex RT-PCR was negative for NS5B gene of BVD virus identified as BVDV-1 (pestivirus A).



**Figure 2.** Bull B: A. Ultrasonogram of right testis; note large hyperechoic areas (black arrows). B. Cut section of testes; note the hard consistency and granulomatous appearance of parenchyma (compatible with fibrosis) with severe lesions in right testis (black arrows). C. Light microscopic image (400 x) of testis; note mineralized seminiferous tubules with reduction on germinal cells (black arrows)



**Figure 3.** Bull C: A. Ultrasonogram of testis; note large hyperechoic areas (black arrows). B. Cut section of testes; note severe fibrosis from the rete toward the peripheral parenchyma (black arrows) with lack of bulging (white arrows). C. Light microscopic image (400 x) of testis; note seminiferous tubules with abnormal epithelium and intratubular concentric laminated calcification (black arrows)

#### Bull D

BSE: Scrotum had a distinct neck; testes were symmetrical with normal tone, and they were freely moveable. Epididymis and spermatic cords were within normal limits. SC was 38.0 cm, and the scrotal temperature was normal without painful responses. Internal reproductive organs, prepuce, and penis were within normal limits. Ultrasonography of testes revealed small focal hyperechoic areas affecting < 1% of the examined area. Collection of semen by electroejaculation resulted in 9 ml of ejaculate with very good gross motility and 90% individual motility. E/N-stained slides had 99% morphologically normal sperm (1% defects of sperm head). The percentage of morphologically normal sperm cells in wet mount was 97% (2% of detached normal sperm heads, 1% simple bent tail).

#### Discussion

There were severe testicular lesions in these 3 bulls. Bull A had stromal fibrosis in the left testis and NS5B gene was detected by RT-PCR suggesting an infection with BVDV-1 (pestivirus A). Bulls B and C were suspected of testicular fibrosis on palpation and ultrasonography revealed abnormal hyperechoic areas. They also had low SC; macroscopic signs of degeneration, fibrosis, and mineralization that were confirmed by histopathology.

Testicular degeneration, fibrosis, and mineralization have been described in bulls with reduced fertility; causes suggested

were biopsy, trauma, heat, cold, radiation, nutritional disorders, toxic agents, blind efferent ductules, vascular lesions, and aging.<sup>9</sup> Testicular biopsy could result in hemorrhage, infarction, and degeneration<sup>9</sup>; however, in this case, no signs of previous biopsy were observed, and the lesions were in the mediastinum and toward the periphery. Testicular trauma is rare in bulls,<sup>9</sup> and in these cases, classical hematomas and necrotic lesions were not observed. Blind efferent ductules or obstruction at the level of epididymis were suspected, especially in bull A that did not respond to electroejaculation. Lesions on the hemato-testicular barrier with spermiostasis often cause testicular degeneration, fibrosis, and mineralization.<sup>9</sup> However, duct permeability was normal in that bull and no macroscopic and microscopic signs of obstructive lesions were observed in any bulls. Infectious agents could cause inflammation of the arterioles and capillaries in the testes inducing necrosis and fibrosis.<sup>10,11</sup> In fact, there is evidence that bovine respiratory syncytial virus could be involved in the etiology of testicular fibrosis.<sup>11</sup> BVD virus can be detected in the testes for a long duration after acute infection.<sup>12</sup> Mass motility, sperm concentration, and herd fertility were significantly lower in serologically BVD-positive bulls.<sup>13</sup> However, although BVD virus was detected in a testicular sample of bull A, the role of the virus on infertility cannot be determined.

Testicular degeneration is an acquired condition that results in testes softer than normal on palpation, low concentration of sperm, with a high percentage of morphological defects.<sup>4</sup> In the present case, the specific cause of testicular degeneration,

fibrosis, and mineralization could not be confirmed; however, it could have been the cause of low pregnancy rate in this group of heifers. Testicular degeneration can decrease SC and it may be temporary if the underlying cause is removed.<sup>11</sup> The SC provides a reliable estimate of testicular mass and is useful in estimating daily sperm production and semen quality.<sup>4</sup> Severe testicular degeneration was diagnosed in bull C and the SC was below the normal range. However, semen parameters were within normal range for all 3 bulls. Probably, there was enough healthy testicular tissue to produce a fair amount of normal semen. The lack of sperm at electroejaculation in bull A was probably due to lack of response to the technique. Occasionally, it is difficult to collect from bulls that have been active with cows in the last 24 hours or nervous, irritable, or hard to manage.<sup>14</sup>

Although macroscopic lesions in the testes detected by ultrasonography have been associated with low semen quality and an increase in sperm abnormalities,<sup>15</sup> others reported that the degree of testis fibrosis was not associated with semen quality,<sup>11</sup> and mild lesions would not affect testicular function or sperm quality.<sup>16</sup> In the present case, the extension and severity of testicular degeneration, fibrosis, and mineralization probably reduced the amount of testicular tissue producing sperm and consequently reduced fertility.

### Learning points

- Breeding soundness examination of bulls before breeding season is reaffirmed
- Ultrasonography of testes is of great value
- Limangus breeders should be alerted to detect early testicular problems

### Conflict of interest

None to declare.

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