Testicular microlithiasis negatively affects sperm quality in bovine sires

Microlitiase testicular afeta negativamente a qualidade do esperma em bovinos machos

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ABSTRACT
Testicular microlithiasis is an asymptomatic pathology of unknown origin, characterized by microcalcifications in the seminiferous tubules. In humans, it is correlated with subfertility, but it is unknown if the same occurs in animals. We evaluated testicular microlithiasis frequency and semen quality in 103 bovine sires. The evaluated animals were grouped by species (Bos taurus, Bos indicus and crosses thereof) and age (16 ≤20 months, >20 ≤30 months and >30 months). Animals positive for testicular microlithiasis were classified as grade 1 (5 ≤10 microlites), grade 2 (>10 ≤20 microlites) or grade 3 (>20 microlites). Semen was collected and individual motility, sperm concentration and morphology evaluated. Frequency of a positive testicular microlithiasis diagnosis was 41.74% overall. Positive animals had lower (p<0.05) semen quality values than negative animals: motility, 59.65% vs. 73.1%; concentration, 262.3 x 10^6 vs. 597.7 x 10^6 sp mL; and
normal sperm, 82.6% vs. 89.5%. Presence of testicular microlithiasis in bovine sires negatively affected sperm motility and concentration.

**Keywords:** mineralization, gonads, spermatozoids.

**RESUMO**
A microlitiase testicular é uma patologia assintomática de origem desconhecida, caracterizada por microcalcificações nos túbulos seminíferos. Em humanos, está correlacionado com a subfertilidade, mas não se sabe se o mesmo ocorre em animais. Avaliamos a frequência de microlitiase testicular e a qualidade do sêmen em 103 bovinos. Os animais avaliados foram agrupados por espécie (Bos taurus, Bos indicus e respectivos cruzamentos) e idade (16 ≤20 meses, >20 ≤30 meses e >30 meses). Os animais positivos para a microlitiase testicular foram classificados como grau 1 (5 ≤10 microlitas), grau 2 (>10 ≤20 microlitas) ou grau 3 (>20 microlitas). O sêmen foi coletado e a motilidade individual, a concentração de espermatozoíde e a morfologia foram avaliadas. A frequência de um diagnóstico positivo de microlitiase testicular foi de 41,74% no total. Os animais positivos apresentaram valores menores (p<0,05) de qualidade do sêmen do que os animais negativos: motilidade, 59,65% vs. 73,1%; concentração, 262,3 x 10⁶ vs. 597,7 x 10⁶sp mL; e espermatozóide normal, 82,6% vs. 89,5%. A presença de microlitiase testicular em bovinos afetou negativamente a motilidade e a concentração dos espermatozoides.

**Palavras-chave:** mineralização, gônadas, espermatozoides.

**1 INTRODUCTION**
Testicular microlithiasis (TM) is a known testicular pathology in humans. It is defined as the presence of microcalcifications in the testis as detected by hyperechoic image (BACKUS et al., 1994). Research links TM to testicular tumors (MILLER et al., 2007), subfertility, and testicular cancer (RASSAM et al., 2020; SAKAMOTO et al., 2006; YEE et al., 2011).

This pathology also occurs in bovids, although it has received limited attention. One case was reported in a non-breeding buffalo in Pakistan (CHANDOLIA et al., 2018), and it was reported in breeding bulls in Mexico (SAAVEDRA JIMÉNEZ et al., 2022). It has also been reported in antelopes (BORNMAN et al., 2010). Since TM is related to subfertility in humans, its presence in bovine sires is potentially worrisome. Sires are vital parts of cattle breeding systems, and any damage to the testicles can damage sperm quality, leading to subfertility (LOZANO, 2009). The present study objective was to analyze the effect of testicular microlithiasis on sperm quality in bovine sires.
2 MATERIALS AND METHODS

2.1 STUDY AREA

The study was conducted in the municipalities of Ometepec (16°40’ 57” N, 98°24’ 43” W) and Cuajinicuilapa (16°27’60” N, 98°24’60” W), in the state of Guerrero, Mexico. Predominant regional climate is warm sub-humid with summer rains, a temperature range of 22 to 28 °C, and average annual rainfall of around 1200 mm.

2.2 ANIMALS

Experimental animals were 103 bovine sires aged ≥16 months, without defects or pathologies in the reproductive organs and in good general health. All animals were grazing in semi-intensive systems with access to mineral salts and water. At night, they were housed in individual pens with free access to water. They were dewormed and vaccinated in compliance with regional health authority guidelines.

Bias during measurement of scrotal circumference (SC) was avoided by grouping the animal by species (Bos taurus, Bos indicus and B. taurus x B. indicus) and age (16≤20, >20≤30, >30 months) (Table 1). Body condition was estimated for each animal using a nine-point scale (1 = emaciated, 9 = obese (AYALA-BURGOS et al., 1998). For animal evaluation and data collection, the animals were placed in a handling chute, without sedation, and minimizing any stress (GRANDIN, 2019). Animal handling practices and study design were approved by the Institutional Bioethics Committee of the Autonomous University of Guerrero.

<table>
<thead>
<tr>
<th>Species</th>
<th>Age (months)</th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16 ≤20</td>
<td>&gt;20 ≤30</td>
<td>&gt;30</td>
<td></td>
</tr>
<tr>
<td>Bos taurus</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Bos indicus</td>
<td>21</td>
<td>31</td>
<td>22</td>
<td>74</td>
</tr>
<tr>
<td>Bos taurus x Bos indicus</td>
<td>0</td>
<td>17</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>All species</td>
<td>23</td>
<td>49</td>
<td>31</td>
<td>103</td>
</tr>
</tbody>
</table>

Source: Own data.

2.3 TESTICLE EVALUATION

Scrotal circumference (SC) was measured using a scrotal tape (Lane Manufacturing, Denver, CO, USA), as described by (KOZIOL; ARMSTRONG, 2018a). The testicular parenchyma was evaluated using an ultrasound machine (Chison Eco 5), with a 7.5 MHz transrectal linear transducer following an established technique (MOMONT; CHECURA, 2015). A compact, homogeneous
and echogenic testicular parenchyma was considered normal testicular appearance in the ultrasound image (BARTH, 2007), (Figure 1) The vesicular glands were evaluated to rule out the presence of other pathologies that could negatively affect sperm quality (PÁEZ-BARÓN; CORREDOR-CAMARGO, 2014).

Figure 1. Normal testicular parenchyma ultrasound image

![Image of normal testicular parenchyma](source: Own picture.)

Presence of TM was deemed positive upon identification of hyperechoic points within the testicular parenchyma (Figure 2). Four grades, or severities, of TM were assigned according to (CORNUD et al., 2001) and (AMARAWARDENA; SIYAMBALAPITIYA, 2016b): Grade 0 (G0, negative, no microliths), Grade 1 (G1, 5 -10 microliths), Grade 2 (G2, >10 ≤20 microliths) and Grade 3 (G3, >20 microliths).

Figure 2. Hyperechoic point in testicular parenchyma

![Image of hyperechoic point](source: Own picture.)
2.4 SEMEN EVALUATION

Semen was collected by electroejaculation (Standard Precision USA), and sperm observed under a microscope (i4 infinity, LW Scientific, USA). Sperm individual motility and morphology were evaluated using an established technique and parameters by (KOZIOL; ARMSTRONG, 2018). Sperm concentration was quantified by counting in a Neubauer Bright line chamber following the methodology of the World Health Organization (WHO, 2010).

2.5 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experimental design was completely random. The independent variable was presence or absence of microlithiasis, and the dependent variables were individual sperm motility (IM, %), sperm concentration (millions spermatozoa/mL) and normal or non-malformed spermatozoa (%). Results were analyzed with an analysis of variance (ANOVA), and any differences between the means identified with a Tukey's test (p<0.05). All statistical analyzes were performed with the SAS program (SAS, 2013).

3 RESULTS AND DISCUSSION

More than 40% of the evaluated bulls exhibited some degree of microlithiasis (Table 2). All the Bos taurus animals had TM, although the overall highest number of cases was observed in Bos indicus.

<table>
<thead>
<tr>
<th>Species</th>
<th>Animals (n)</th>
<th>TM+ (n)</th>
<th>TM+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bos taurus</strong></td>
<td>6</td>
<td>6</td>
<td>5.82</td>
</tr>
<tr>
<td><strong>Bos indicus</strong></td>
<td>74</td>
<td>26</td>
<td>25.24</td>
</tr>
<tr>
<td><strong>Bos taurus x Bos indicus</strong></td>
<td>23</td>
<td>11</td>
<td>10.67</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>103</td>
<td>43</td>
<td>41.74</td>
</tr>
</tbody>
</table>

Source: Own data.

Microlithiasis is usually an incidental finding in both humans (CATANZARITI et al., 2014; SAKAMOTO et al., 2006) and animals (BORNMAN et al., 2010; CHANDOLIA et al., 2018; SHIRAI; EVANS, 2018). Frequency in humans remains open to debate, with some studies finding higher incidence in African-Americans (COSTABILE, 2007), and others a higher incidence in Caucasians (PEDERSEN...
et al., 2019). Diagnosis in animals is almost fortuitous since ultrasound is rarely used to evaluate reproductive organs. It therefore remains quite difficult to state whether some non-human species are more likely than others to develop TM. One preliminary study done in cattle (SAAVEDRA JIMÉNEZ et al., 2022) found a higher TM percentage in Swiss Brown (*B. taurus*) sires. With such limited data in the literature, much more research will be needed before any conclusions can be reached on frequencies.

In the present results, presence of TM had a detrimental effect on the evaluated sperm characteristics (Table 3). Although TM frequencies remained below the acceptable limit (BRITO et al., 2007; KOZIOL; ARMSTRONG, 2018b), they were still higher than in TM-negative animals.

<table>
<thead>
<tr>
<th>Variable</th>
<th>TM-</th>
<th>TM+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual motility (%)</td>
<td>73.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Concentration (millions mL)</td>
<td>597.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>262.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal spermatozoids (%)</td>
<td>89.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Different letter superscripts in the same row indicate statistical difference (p<0.05).

Source: Own data.

Individual motility (BARTH, 2007) was good in TM-negative animals, but only fair in positive ones; it was 13% lower in TM-positive animals than in negative ones. Decreased sperm motility has been reported in humans (XU et al., 2014). In the evaluated animals, the presence of microliths may have affected motility. Dystrophic calcification, which occurs in damaged tissue, alkalinizes the surrounding environment due to its high calcium content, which in turn can cause decreased mitochondrial membrane potential (PUGA et al., 1987). This could prevent spermatid flagellum activation and thus impede or compromise movement (DASGUPTA; MILLS; FRASER, 1994).

Sperm concentration was 50% lower in the TM-positive animals than in negative ones, placing it at the lower acceptable limit for naturally-mated sires. Lower sperm concentration has been attributed to obstruction of the seminiferous tubules by microliths (AMARAWARDENA; SIYAMBALAPITIYA, 2016b), atrophy and decreased tubular diameter, and internal debridement of the seminiferous tubules (THOMAS et al., 2000) as well as arrest of spermatogenesis.
(AIZENSTEIN et al., 1998). However, other studies have found no correlation between presence of TM and sperm motility or concentration (MAZZILLI et al., 2005; SAKAMOTO et al., 2006).

No differences were observed in sperm morphology, with both TM-positive and -negative groups exhibiting greater than 70% of sperm with normal morphology (Table 3), which is the minimum accepted level (BARTH, 2007).

Semen quality was also found to be affected by degree of TM (Table 4). The G0 group had better values than all three TM-positive groups. In the latter, evaluated sperm parameters were lower as degree of TM increased. Sperm concentration was most obviously affected, with poor values (BARTH, 2007) in G2 and G3, which were 11.05% and 17.72% lower than G0, respectively. Increasing sample size and use of different sampling strategies may help to better identify differences in future research. The present results agree with studies done in humans (RASSAM et al., 2020; XU et al., 2014; YEE et al., 2011) which report that degree of TM is inversely proportional to sperm quality, with sperm concentration and motility being the most affected.

### Table 4. Mean values for evaluated sperm characteristics in bovine sires by degree of testicular microlithiasis (TM)

<table>
<thead>
<tr>
<th>Variable</th>
<th>G0</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual motility (%)</td>
<td>73.12</td>
<td>63.33</td>
<td>53.00</td>
<td>50.00</td>
</tr>
<tr>
<td>Concentration (millions/mL)</td>
<td>597.70</td>
<td>284.20</td>
<td>229.00</td>
<td>201.30</td>
</tr>
<tr>
<td>Normal spermatozoids (%)</td>
<td>89.48a</td>
<td>85.43ab</td>
<td>79.60ab</td>
<td>73.62b</td>
</tr>
</tbody>
</table>

G0: No microlithiasis; G1: 5-10 microliths; G2: >10 ≤20 microliths; G3: >20 microliths.
a,b Different letter superscripts in the same row indicate statistical difference (p<0.05)
Source: Own data.

4 CONCLUSION

Testicular microlithiasis was clearly present in the sampled bovine sires. Indeed, its presence negatively affects sperm motility and concentration, with higher degrees of microlithiasis having a more notable effect.
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