

## Treatment of vesiculitis in bulls with mesenchymal stem cells increases seminal quality and the production of semen doses

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The aim of this study was to evaluate the effects of applying allogeneic mesenchymal stem cells (MSCs) directly to inflamed vesicular glands in the treatment of vesiculitis. Twelve bulls with acute and chronic vesiculitis with two or more recurrences were selected at Semen Collection and Processing Centers (SCPC). The proposed method of intraglandular injection of MSCs was to be applied through the ischiorectal fossa with a 30-35 cm long needle and a 25-30 cm long guide directly into the affected vesicular gland. The MSCs were cultured and frozen in the BioCell Cell Therapy® laboratory. Two applications were carried out (intervals of 40 to 60 d) with  $3 \times 10^6$  MSCs per vesicular gland. Comparisons were made based on the average of 5 semen collections before the application and five semen collections 20 d after the last application of the treatment. The variables analyzed were ejaculate volume (mL), initial motility (%), concentration (million sperm/mL), sperm morphology (% - phase contrast), post-thaw motility (CASA system), presence of leukocytes per field, number of semen doses produced, and number of doses per ejaculate. All variables were measured before and after treatment. The Student's *t*-test was used to identify statistical differences between variables before and after treatment. A probability of  $P \leq 0.05$  was considered a significant difference. Data is presented as mean  $\pm$  standard error of the mean (SEM). Improvements were observed in initial motility from  $60.09 \pm 4.8$  to  $69.89 \pm 4.6$  ( $P < 0.05$ ), as well as in post-thaw motility from  $26.26 \pm 6.77$  to  $42.5 \pm 5.99$  ( $P < 0.05$ ). The number of doses produced increased after treatment with MSCs, from  $95.61 \pm 23.31$  units to  $337.84 \pm 67.75$  units ( $P < 0.05$ ) per ejaculate. The number of pyocytes observed per field decreased from  $5.83 \pm 0.48$  to zero, demonstrating recovery of the inflamed vesicular glands. The other variables analyzed had no statistical differences. It was concluded that the application of  $3 \times 10^6$  MSCs in the vesicular glands of bulls with vesiculitis was safe and efficient, as it improved several parameters evaluated in this study, especially the production of semen doses per ejaculate.

## Fertility prediction in fixed-time artificial insemination according to semen analysis methodology

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Analyses of cryopreserved semen batches are carried out using various technologies, including microscopy with subjective analysis and CASA (Computer-assisted semen analysis), focusing on different abilities of the sperm. The choice of semen for AI largely affects the final result. Thus, it was proposed to evaluate the applicability of fertility prediction models and the efficiency of two CASA (Hamilton Thorn IvosII, hCASA, and iSperm) and subjective analyses. Doses of cryopreserved semen from 15 bulls (Nelore and Angus) used in fixed time artificial insemination (FTAI) were analyzed. After thawing for 30 s in a water bath at 38°C, the semen was diluted using Easy Buffer (IMV) with a standard of 1/8 for subjective motility (%), vigor (1 to 5) and sperm defects (%) evaluation as recommended by the CBRA and analyzed with the settings for bovine semen according to the manufacturer's instructions, using hCASA and iSperm. Motility and vigor were evaluated again after incubation at 46°C for 30 min for a thermoresistance test (rapid TRT). Fertility prediction models were used (Silva et al. *Theriogenology* 212, 148-156, 2023): Conventional (P (%) =  $49.781 + (0.243 \times \text{MotilityTRT}) - (0.483 \times \text{Major Defects})$ ); hCASA/iSperm (P (%) =  $9.788 + (0.200 \times \text{PROG}) + (0.289 \times \text{VAP}) + (0.179 \times \text{VSL})$ ). Statistical analyses were carried out by SAS (Pearson correlation). Pregnancy/AI (P/AI; %) of field pregnancies was  $50.49 \pm 12.39$ , whereas the predictions made by the conventional model were  $59.80 \pm 3.17$ , with the model using hCASA presenting  $64.83 \pm 8.15$  and the model using iSperm  $48.48 \pm 9.81$ . The fertility prediction made with subjective assessments (conventional model) did not correlate with that observed in the field ( $-0.36$ ;  $P = 0.11$ ); however, when evaluated using only bulls with a field conception rate  $> 55\%$  (7 of 15) indicated a strong correlation ( $0.78$ ,  $P = 0.035$ ). P/AI and iSperm fertility prediction had a correlation ( $0.44$ ;  $P = 0.05$ ), but hCASA prediction did not ( $0.24$ ,  $P = 0.29$ ), although iSperm had a correlation between the fertility prediction made with hCASA ( $0.822$ ,  $P < 0.001$ ). The correlation between predictions and actual results varied depending on the model used. Further research is required to validate and improve fertility prediction models that reflect field results.

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