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A009 Physiology of Reproduction in Male and Semen Technology

Different *in vitro* sperm challenge and its relationship with *in vivo* bull fertility

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The aim of this work was to challenge the laboratory quality of thawed semen and to compare the in vitro results with in vivo semen fertility. Frozen-thawed semen of 4 different batches from the same bull, which were previously used in a TAI program were used for insemination of 332 Brangus cows. For laboratory experiment, three repetitions of each batche was performed. For each semen dose, the following procedure was accomplished: initially, the semen sample was thawed at 37°C for 30 sec (control), sperm motility was assessed by CASA and plasma membrane integrity was evaluated by propidium iodide fluorescent probe. Then, an aliquot of 150 µL of the sample was incubated in a water bath at 45°C for 40 min (thermal challenge group; GDT) and another aliquot of 150 µL of the sample was centrifuged at 500 x g (Percoll gradient 45%/90%) for 15 min (centrifugation challenge group; GDC). The centrifuged semen was also subjected to another thermal challenge, being incubated (water bath) at 45°C for 40 min (centrifugation + thermal challenge group; GCDT). At the end of each challenge (GDT, GDC and GCDT), the same laboratory tests used for control group were repeated. The field data were analyzed by GLIMMIX of SAS and laboratory data by analysis of variance in GraphPad INSTAT. Significance level of 5% was established. No difference ($P>0.05$) between AI technitian, BCS or batches (B) was observed for conception rate (CR). The following CR were observed for each batch: B1 = 48.9% (44/90); B2 = 44.2% (23/52); B3 = 55.5% (40/72); B4 = 43.2% (51/118). Although no statistical difference was observed between batches, numerically higher CR was observed for B3 compared to B4. According to CASA results, it was interesting to note that B4 was the batch that presented lower ($P<0.05$) percentages of Progressive Motility (PM) both after thawing (control: 47.2 ± 8.5) and after all sperm challenges (GDT: 40.0 ± 4.6; GDC: 45.7 ± 7.3; GCDT: 4.7 ± 7.2) compared to B3 (control: 63.0 ± 5.3; GDT: 56.0 ± 1.7; GDC: 64.2 ± 12.5; GCDT: 7.7 ± 3.8). In addition, while B3 and B4 demonstrated similar percentage of plasma membrane integrity (MPI) in control (T3 = 66.7 ± 1.3 and T4 = 65.2 ± 3.3), the semen of B3 demonstrated higher ($P<0.05$) percentage of MPI (37.2 ± 2.5) than B4 (26.7 ± 3.3) after passing through the greatest challenge of this in vitro experiment (GCDT). According to the results, it was concluded that the semen of batch 3 was the most resistant to the proposed laboratory challenges, especially when compared to batch 4. Therefore, the present study suggests that to submit seminal samples to a laboratory challenge before to perform an in vivo semen quality assessment seems to be an interesting alternative for define semen batches that may present greater reproductive performance of field fertility.

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