Animal Reproduction

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Male reproductive physiology and sperm technology

# Effects of thawing and storage temperature on sperm viability of Nelore and Holstein bull's

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## Resumo

Artificial insemination is the one of the most used biotechnologies in animal production. Although well established and used worldwide, some critical steps such as thawing still need improvement. Therefore, we aimed to investigate the effect of thawing temperature on cryopreserved sperm and to evaluate sperm viability after storage for 8 hours. Frozen semen from Holstein (Hol, n=5) and Nelore (Nel, n=5) bulls were thawed at 37°C or at 4°C. After thawing, the samples were kept in the same temperature up to 8 hours. Samples were evaluated at 0, 2, 4, 6 and 8h for total (TM) and progressive motility (PM) by CASA (Hamilton Thorne Biosciences, Beverly, Massachussetts – USA) and membrane (MI) and Acrosome Integrity (AI) by flow cytometry (AMNIS Flow Sight, Amnis Corp., Setattle, WA). Data were analyzed by ANOVA using a 2X2 factorial experimental design, based on breed (Hol or Nel) and thawing temperature (4°C or 37°C), and their interactions. Data were compared among groups at the same time point and within group as repeated measure. TM was affected by breed and time ($P<0.05$). It decreased over time ($P<0.05$) for Nel (0h= 78.4% vs 8h =50.6%) and Hol (0h= 63.4% vs. 8h = 30.2%) semen, when thawed at 37°C. However, time did not affect TM ($P>0.05$) when Nel (0h= 62.2% vs 8h =65.5%) and Hol (0h= 43.8% vs. 39.6%) semen was thawed at 4°C. Regarding PM, except for Hol semen thawed at 37°C that had a decrease ($P<0.05$). After 4h of incubation, in all other treatments, PM was not affected by time and at 8h it was similar among all three groups (Nel 4=24.8%; Nel 37=26.6%; Hol 4= 26.6%). The AI and MI were affected by treatment ($P<0.05$) and time ($P<0.05$). The percentage of sperm with AI and MI was similar among all groups at 0h ($P>0.05$) and were preserved up to 2h. After 4 h there was a decrease in AI and MI on Hol and Nel semen thawed at 37oC ($P<0.05$) but had no impact on those at 4oC ($P>0.05$). No interactions were found between breed and treatment ($P>0.005$) for any of the assessments. In conclusion, Hol and Nel semen are susceptible to be thawed and stored at 4oC or 37oC until 8h post thawing. However, 4°C thawing/storage, is able to maintain the sperm quality for longer storage time than 37oC. Further studies in vitro and in vivo are needed to confirm such fertilization potential.

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