Animal Reproduction

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Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

Assessment of seminal cell-free DNA as a potential marker for bovine sperm cryoresistance

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Resumo

Sperm cryopreservation is a great impact technique used in the fertility preservation and animal production. Although it is well established in some species, the response to cryopreservation is varies depending on individual. Thus, the identification of a marker that can indicate whether a semen sample is of high or low freezeability, prior to freezing, would be of great value to the semen industry. Them, searching for a new assessment that can predict the post-thawed sperm quality, this study evaluated the quantity of cell-free DNA (cfDNA) and mtDNA copy number in seminal plasma of Nelore bulls. Semen from nine bulls (1/bull) was collected by electroejaculation, half of the ejaculate was used for fresh semen evaluation and molecular analyses, the other half was cryopreserved. Evaluation of movement parameters by CASA (IVOS 12.3/Hamilton-Thorne, EUA) and of plasm membrane integrity (PMI), acrosome integrity (AI), plasm membrane stability (PMS) and apoptosis (Apo) by flow cytometry (AMNIS FlowSight - Amnis Corp., EUA) were performed in fresh semen and frozen/thawed semen at 0, 3, 6 and 12h post thawing. Seminal plasma was used for cfDNA isolation and quantification. The mtDNA copy number was quantified by qPCR. The data were analyzed by ANOVA and Tukey test. The concentration of cfDNA present in seminal plasma ranged from 15.23 ng/µL to 519.71 ng/µL. The median was calculated (58.95), and two groups were defined according to the cfDNA concentration: low-cfDNA (<58.95 ng/µL (n=5) and high-cfDNA (>58.95 ng/µL (n=4). All parameteres were compare between the two groups. The cfDNA average was 38.09±53.49 for low-cfDNA and 273.70±59.80 for high-cfDNA group (P=0.02). The mtDNA copy number was similar (P>0.05) between groups (low=9.14±3.42; high=7.91±3.82). When fresh semen was evaluated, the percentage of cells with membrane stability was higher (P<0.05) on low (84.24%) compared to high (52.72%) group. The effect of group according to cfDNA concentration was evaluated over time on thawed semen. Analysis was performed as repeated measure whitin individual, using the SAS PROC GLIMMIX. Model tested group fixed effect (lowcfDNA vs high cfDNA), time after thawing (0, 3, 6 and 12 h) and the group and time interaction. The results showed no differences for all parameters evaluated over 12 hours between the low-cfDNA and high-cfDNA groups. In conclusion, cfDNA in seminal plasm may have some implication on sperm quality, but after freezing process this effect disappears, therefore cfDNA in plasm cannot predict sperm freezability.