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Evaluation of Percoll PLUS as a cushion solution during single layer centrifugation of fresh bull semen: Effects on frozen/thawed spermatozoa motility

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Traditional semen centrifugation can pack spermatozoa on bottom of conical centrifuge tube. Thus, this study was carryed out to verifify the influence of Percoll PLUS (PP) as a cushion solution during single layer centrifugation (SLC) of fresh bull semen on sperm motility parameters. Eighteen ejaculates from 3 Nellore bulls (1 of each bull/trial) were polled and divided into 3 groups, as follows: no centrifuged sperm (NC), cushioned centrifugation (CS; with PP as commercially available) and non-cushioned centrifugation (NCS) both during SLC. SLC was performed by layered 1 billion of spermatozoa on top of 9-ml column of PP (70%) followed by centrifugation ($839 \times g$) for 13 min at room temperature. Then, the supernatant was discarded and the sperm pellet diluted in freezing extender to a final concentration of 24×10^6 spermatozoa/straw. After cooling and freezing sperm samples were thawed in a water bath (37 °C/30 s) and assessed for total motility (TM, %), progressive motility (PM, %), curvilinear velocity (VCL, μm/s), straight line velocity (VSL, μm/s), average path velocity (VAP, μm/s), amplitude of lateral head displacement (ALH, μm), beat cross frequency (BCF, Hz), linearity (LIN, %), straightness (STR, %), and wobble (WOB, %) using computer assisted sperm analyzer (CASA). For statistical analysis ANOVA and Tukey test were used (values expressed as mean ± SD) with $P < 0.05$ taken as significant. Higher percentage for PM (51 ± 8.6 and 49.8 ± 7.1), STR (78 ± 0.02 and 79 ± 0.04), and WOB (72 ± 0.03 and 72 ± 0.04) were found, respectively for CS and NCS than for NC (26.5 ± 1.7, 65 ± 0.04, and 64 ± 0.03, respectively). No difference was observed among groups for the other sperm motility parameters, except for ALH which was better for CS and NCS. In conclusion, cushioned centrifugation with PP during SLC yielded similar results as compared to non-cushioned centrifugation, but these findings suggest that PP could be a substitute for other cushion solutions commonly used. [Acknowledgements: FAPESP (grant 2015/20986-3), Tairana AI Station, Botupharma Ltda, Master Fertility Animal Reproduction, Brazil.]

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Addition of iodixanol in bull freezing extender improves the sperm membranes integrity

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This study was carried out to verify the effects of different concentrations of iodixanol added to the freezing extender on plasma and acrossomal membrane integrity of post-thawed sperm bull. Therefore, ejaculates from three Nellore bulls ($n = 18$) were pooled and extended in a commercial freezing medium (egg yolk-sugar-glycerol) supplemented with iodixanol at concentrations of 0% (control group), 2.5% (I-2.5), 5% (I-5), and 10% (I-10). Subsequently, semen samples were cooled for 5 h at 4 °C, filled in 0.50 ml straws and frozen in a programmable freezer machine. After that, the straws were stored in liquid nitrogen until sperm evaluation, which were performed by thawing the samples in a water bath at 37 °C/30 s. Plasma and acrossomal membrane integrity (PAMI) were simultaneously assessed using Propidium Iodide and FITC-PSA probes, respectively, while translocation of phosphatidylserine (TPS) was identified by Annexin V and plasma membrane destabilization (PMD) by YO-PRO-1. All sperm samples were analyzed by flow cytometry. ANOVA and Tukey's test were used for statistical analysis (data of six replicates), with $P < 0.05$ taken as significant. Higher percentage ($P < 0.05$) of MPAI was observed in I-10 (65 ± 1.4) than for other groups, with control group showing a lower value (56.7 ± 2.1), while I-2.5 (59.8 ± 2) did not differ from control and I-5 (62 ± 1.6) groups. Regarding to TPS, higher percentage of spermatozoa without TPS (considering intact membrane cells) was observed in I-10 (61.2 ± 1.4) with lower percentage found in control group (45.6 ± 2.4). However, I-2.5 (56.3 ± 1.9) and I-5 (57.7 ± 1.5) exhibited similar results but differed significantly from the other groups. For PMD the values of I-10 and I-5 were no different (67.8 ± 1.9 and 67 ± 1.9, respectively), but they were higher than in control (62.4 ± 2.7) and I-2.5 (61 ± 1.9) groups, both with similar results. In conclusion, the addition of iodixanol to the bovine freezing extender significantly improves the sperm membrane integrity in a dose-dependent manner. [Acknowledgements: CAPES,

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A controlled comparison study of two insemination lubricants for spermatotoxic potential

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Sperm may be exposed to catheter lubricant as part of the artificial insemination (AI) procedure. If the lubricant is toxic to the sperm, there is a risk of reduced reproductive performance. The aim of this study was to evaluate the effect of two different catheter lubricants on boar sperm motility, acrosome integrity and membrane viability (permeability), using CASA (computer-assisted semen analysis) and flow cytometry. Extended semen doses from three different extender classifications (short-term, long-term non-BSA, and long-term with BSA) were tested across three lubricant concentrations (1%, 2.5% and 5% v/v) and three exposure times (3, 30 and 60 min). Kuster Research and Consulting (KRC) performed blinded replicate testing using two insemination lubricants: water soluble Clarity, supplied by Aurora Pharmaceutical and a frequently used commercial propylene glycol-based lubricant, Priority Care® manufactured by First Priority Inc. Pooled semen doses were obtained from commercial boar studs during routine collection. Only doses that met standard production criteria (75% gross motility; 70% normal morphology) were included in this study. Results indicated that spermatotoxic potential differed significantly between the two lubricants included in this study. Exposure to Priority at all concentration × time combinations in all extenders reduced gross and progressive motility parameters to the extent that reproductive performance could be compromised. For example, following 3 min exposure to Priority (5%, v/v) gross motility was $33 \pm 11\%$ in long-term extender without BSA, while sperm viability measured at 60 minutes was reduced to $57 \pm 8\%$ and $51 \pm 10\%$ in short-term and BSA+ extenders respectively, compared to controls of $75 \pm 3\%$ and $81 \pm 3\%$. In contrast, Clarity had no significant effect (Dunnet's one-way multiple comparison test) on motility parameters in short-term or long-term non-BSA extenders. Motility reductions in the BSA+ extender were limited to the highest Clarity concentrations with smaller magnitude than Priority. Exposure to Clarity (5%, v/v) for the longest duration (60 min) had no detrimental effect on sperm viability or acrosome status in any extender. Although insemination lubricants may claim they are "non-spermicidal" we present evidence that a popular commercial lubricant has a negative effect on sperm motility and damages sperm plasma membranes [Research supported by Aurora Pharmaceutical.]

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