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The effect of seminal plasma on post thaw motility and viability of ram spermatozoa

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Numerous studies have investigated the effect of supplementing ram spermatozoa with additional seminal plasma (SP) during cryopreservation in an effort to improve their post-thaw quality. However, the fundamental effect of the presence or absence of SP during cryopreservation has yet to be described. As such, this study investigated the role of SP in maintaining sperm function during cryopreservation. Semen collected from Merino rams (n = 3) via artificial vagina was centrifuged (200× ***g***, 10 min, 27°C) to remove SP, diluted to 100×10^6 spermatozoa/ml with tris-citrate-fructose + 0.03%BSA and frozen in straws in the presence or absence of 10% merino SP. Samples were assessed for motility (CASA), viability and acrosome integrity (PI/FITC-PNA) at 0, 2 and 4 h post-thaw. Spermatozoa frozen with SP consistently displayed higher motility ($p < 0.001$; 38.0 ± 2.23%, 22.3 ± 1.66% and 4.8 ± 0.61%, respectively) than spermatozoa frozen without SP (23.2 ± 1.80%, 8.4 ± 0.47% and 0.6 ± 0.00%, respectively) across all time points. At 0 and 4 h post-thaw, spermatozoa frozen with SP had a higher proportion of viable membranes and intact acrosomes ($p < 0.001$; 28.3 ± 1.58% and 12.1 ± 0.20%, respectively) than those frozen in its absence (15.6 ± 0.73% and 11.2 ± 0.58%, respectively). These results show that SP provides protection for ram spermatozoa during cryopreservation.

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Relation of sperm chromatin structure assay (SCSA™) parameters and morphological abnormalities in ovine semen

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Even morphologically normal mammalian sperm can suffer subtle chromatin damages. This study aimed to describe the relation between sperm morphology and chromatin integrity in the ram. Thus, weekly ejaculates (N = 387) were collected for 1 year from five Chios and four East-Friesian rams using an artificial vagina. Abnormalities of the head (pyriform, vacuolated, small, large, tapered, detached or misshapen head), the acrosome (knobbed acrosome or detached acrosome), the midpiece (distal midpiece reflex, Dag defect, segmental aplasia of the mitochondrial sheath, tail stump defect, bowed or bent midpiece, proximal or distal cytoplasmic droplet) and the principal tail piece (abaxial, coiled, bent or double/multiple tail) were microscopically (×1000) detected in eosin-nigrosin stained smears of fresh semen, as previously described by Barth and Oko (1989) for bull sperm. DNA-fragmentation index (DFI) and the percentage of cells with high DFI (%DFI) were determined using the SCSA™. Spearman correlation coefficients (rs) were calculated to describe the relation between morphological abnormalities and SCSA parameters. Results showed that 7.16%, 1.65%, 8.61% and 7.81% of sperm exhibited high DFI values, head, midpiece and principal piece abnormalities, respectively. SCSA parameters correlated significantly ($p < 0.05$) but weakly ($rs < 0.250$) with the percentage of sperm with vacuolated head, knobbed acrosome, distal midpiece reflex, Dag defect or bent tail. Only sperm with detached heads showed a $rs > 0.250$ with DFI and%DFI ($rs = 0.269$ and $rs = 0.324$, respectively, $p < 0.01$). In conclusion, chromatin integrity was weakly related to morphological abnormalities of ovine spermatozoa.

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Effect of a recombinant manganese superoxide dismutase in thawed bovine sperm

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Reactive oxidant species (ROS) are necessary for the normal cell functions, but high concentrations lead to the oxidative stress, causing damage to cell structures, mainly in the membranes. Sperm freezing and thawing processes are essential in veterinary and human practice of artificial insemination and lead to physical damage of membranes, resulting in loss of antioxidant enzymes such as superoxide dismutase (SOD). Adding external SOD to replace its loss into semen extenders has been repeatedly and successfully reported at a concentration that goes up to 100 UI. In the current study we investigated the effect of a novel recombinant isoform of human manganese SOD (rMnSOD) at a concentration of 0.56 UI and 1.12 UI/ml. Motility, viability, acrosome reaction and production of ROS were the analyzed parameters. A significant improvement was found for all the parameters evaluated: motility (41.73 ± 1.76 for the Control group and 53 ± 1.19 for the 1.12 UI/ml rMnSOD group), viability (53.77 ± 0.69 fo the Control group and 55.73 ± 0.64 for the 1.12 UI/ml rMnSOD group; $p < 0.01$). ROS production was lower when rMnSOD was added (13.21 ± 0.98 for the control group and 11.51 ± 0.83 for the 1.12 UI/ml rMnSOD group). This new rMnSOD has been shown to be effective at scavenging ROS after sperm thawing and at improving sperm parameters at a concentration much lower than the commercially available SOD. Future research will examin whether the site of activity lies intraspermatic or in the external medium.

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Bull sperm motility and molecular kinetic of Hoechst dye are effected by the buffer system of extenders

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Buffers are a key component of semen extenders. Their ability to control H+ -ion concentrations maintains the pH of a solution almost constant. which is a fundamental aspect for sperm storage. Furthermore, buffers also significantly affect the physiology of the exposed cells beyond what is expected from pH alone by changing molecular kinetics properties. In the here presented study two different buffers, 3-(N-morpholino)propanesulfonic acid (MOPS) and tris(hydroxymethyl)aminomethane (TRIS), were analysed concerning their influence on bovine sperm by observing fresh sperm standard parameters (motility, viability, morphology) as well as the sex sorting process. Compared to sperm stored in extender containing TRIS, sperm in a MOPS extender were found to have a significantly better progressive motility (73 ± 4.5 for MOPS and 53 ± 6.2 for TRIS; $p > 0.01$). Interestingly, the time required for the dye Hoechst to enter in spermatozoa enough to be detected by the flow cytometer during the sex sorting was also significantly shortened with the MOPS extender. No significant difference was seen in morphology and viability. In conclusion, since MOPS buffered extender positively effected sperm motility as well as reducing the incubation time necessary for staining sperm with Hoechst it is excellently suitable for the sex-sorting process in bovine sperm.