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Physiology of reproduction in male and semen technology

Evaluation of a new sperm purification device for preparing bovine frozen-thawed semen for in vitro fertilization

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Density gradient centrifugation is a common approach for preparing frozen-thawed semen for *in vitro* fertilization. This method is time and labor intensive, requires experience and sperm cell recovery is limited. Therefore, we tested a novel sperm purification device, the VetCount™ Harvester (MotilityCount ApS, Copenhagen, Denmark). The core elements of the VetCount™ Harvester are two chambers which are separated by a microporous membrane with a pore diameter of 10 µM. Spermatozoa are selected when actively swimming out of the semen-filled chamber, i.e., through the membrane, into the other chamber which contains a sperm collection medium. The handling of the VetCount™ Harvester is simple. Semen and collection medium, in this experimental approach a TALP (Tyrode's Albumin Lactate Pyruvate) based medium, are injected into the chambers and, after 30 minutes incubation at 38°C, the medium is aspirated and the spermatozoa are ready for further use. In a preliminary evaluation, we assessed sperm recovery and semen quality of frozen-thawed but otherwise untreated bull semen and frozen-thawed semen treated with the VetCount™ Harvester or BoviPure™ gradient centrifugation (Nidacon International AB, Mölndal, Sweden), a standard technique in our laboratory. Frozen semen samples from six different bulls (n = 6), ten straws of one ejaculate per bull, were analyzed. Sperm concentration was determined using a hemocytometer chamber and the total sperm count was calculated. Motility parameters were assessed using IVOS II, a computer assisted sperm analysis (CASA) system, and flow cytometry was used to simultaneously evaluate viability, acrosome integrity, membrane fluidity and intracellular Ca^{+2} concentration. Results were tested for significant differences using Wilcoxon's signed rank test with Bonferroni correction. A *p*-value of <0.05 was set as significance level. BoviPure™ and VetCount™ Harvester treatment increased the progressive sperm motility compared to frozen-thawed semen samples (82.4±18.3%, 78.8±8.4%, and 41.2±18.4%, respectively; $p<0.05$). The proportion of viable, acrosome intact sperm cells with low intracellular Ca^{+2} concentration and low membrane fluidity was increased after VetCount™ Harvester or BoviPure™ treatment (78.6±6.0%, 76.5±4.4%, and 37.1±13.2%, respectively; $p<0.05$). Following VetCount™ Harvester filtration, viable, acrosome intact sperm cells had a lower normalized intracellular Ca^{2+} concentration (67±10% of the concentration in untreated semen; $p<0.05$) compared to spermatozoa following gradient centrifugation (84±14%; $p<0.05$) or untreated sperm cells (normalized to 100%). There was no significant difference in recovery rate of sperm cells between the VetCount™ Harvester and BoviPure™ treatment (12.4±3.6% and 14.4±5.1%; $p>0.05$). The data demonstrate that the VetCount™ Harvester treatment selects a high-quality fraction of sperm from frozen-thawed bull semen with even lower free intracellular Ca^{2+} concentrations than a BoviPure™ gradient centrifugation. We are currently investigating whether sperm treated with a BoviPure™ gradient or the VetCount™ Harvester differ in cleavage rate, blastocyst rate and quality when they are used in bovine *in vitro* fertilization.

Keywords: cattle, sperm, purification