Effects of nerve growth factor β added to extenders for cryopreservation of electro ejaculated and epididymal harvested bull semen

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Nerve growth factor β (NGF) is a protein in seminal plasma of mammalian species. Seminal plasma NGF concentrations and sperm bound NGF expression have both been positively associated with sire conception rates in bulls. Since bovine sperm express NGF in the head and tail and its receptor (TrkA) in the acrosomal cap, nucleus, and tail regions, it is likely that NGF influences sperm function. Seminal plasma NGF mRNA expression was positively associated with maintenance of post thaw functional membrane integrity in bull sperm, suggesting a role in sperm cryotolerance. Based on the aforementioned findings, we hypothesized that NGF supplementation enhances quality of frozen thawed bull sperm. Objectives were to compare postthaw semen quality from ejaculated and epididymal harvested bull sperm incubated with purified NGF prior to cryopreservation. Semen was obtained from Angus x Simmental crossbred bulls (n = 10) collected by electroejaculation, followed by castration and epididymal sperm harvest 3 days later. Semen samples from each bull were preextended with a commercial extender (Optixcell IMV, Maple Grove, MN) and divided into one of 4 treatments to achieve a final sperm concentration of 4 x 10^6 spermatozoa/ml and a final NGF concentration of 0, 0.5, 5 and 50 ng/ml (CONT, LOW, MED and HIGH, respectively). Immediately after final dilution, samples were manually loaded into 0.5 ml semen straws, sealed ultrasonically, and incubated in a cold room at 5°C for 3 - 5 hours per manufacturer's recommendation. Straws were then placed 4 cm above liquid nitrogen for 15 minutes and then submerged and stored in liquid nitrogen until post-thaw analysis. Prefreeze sperm motility was assessed in each sample prior to treatment using computer-aided sperm analysis. Frozen straws were thawed and incubated at 37°C for postthaw motility assessment in 30 minute intervals for 4 hours. Samples were stained with fluorescent probes for evaluation of sperm viability (SYBR 14/PI), acrosomal integrity (FITC PNA/PI), and chromatin stability (acridine orange) using flow cytometry. Kruskal Wallis rank sum test and ANOVA were used for statistical analyses, with significance set at $p < 0.05$. Postthaw sperm motility and velocity parameters were decreased, whereas linearity (LIN) was increased in HIGH versus CONT ejaculated samples ($p < 0.01$), but no differences were observed in epididymal samples ($p = 0.22$). HIGH ejaculated samples had a lower amplitude of lateral head displacement (ALH) at 2.5 and 3 hours postthaw ($p < 0.01$). Postthaw viability, acrosome integrity, and DNA fragmentation index were not affected by NGF treatment in either ejaculated or epididymal sperm ($p \geq 0.15$). Treatment with NGF did not significantly improve cryotolerance of sperm collected by electroejaculation or epididymal harvest in bulls. Supplementation of extender with high concentrations of NGF decreased postthaw curvilinear velocity and ALH and increased LIN, suggesting a potential role in preventing premature sperm hyperactivation and capacitation. Although the current study did not support a role of NGF in improving sperm cryotolerance, further studies should address potential effects on sperm fertility within the female reproductive tract.

Keywords: Bovine, motility, nerve growth factor β, spermatozoa