Cryosurvival of epididymal and ejaculated bovine spermatozoa frozen in liposome and egg yolk based extenders

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Egg yolk based semen extenders are commonly used to freeze bull semen. However, there are growing concerns regarding use of animal based products, given potential for contamination with pathogens. Liposome based extenders are becoming a popular alternative to animal-based products. Egg yolk lipoprotein prevents cryoinjuries by binding to seminal plasma proteins, whereas liposomes incorporate into the sperm plasma membrane. It is unknown if these extenders are comparable to freeze epididymal sperm (EP). We hypothesized that there are no differences in response to cryopreservation between electroejaculated (EE) and EP sperm, but egg yolk-based extender is superior to a liposome-based extender for EP sperm, due to differences in cryoprotective mechanisms. This study evaluated functional sperm parameters associated with cryotolerance in EP and EE sperm frozen with 2 semen extenders. One ejaculate was collected from each bull (n = 10) by electroejaculation. Each ejaculate was divided into 2 aliquots and cryopreserved with a liposome-based extender (Optixcell, IMV Technologies, Maple Grove, MS; EEO) or an egg yolk-based extender (BotuBov,® Botupharma, Scottsdale, AZ; EEB). Bulls were castrated 3 days later. Sperm were recovered from 1 cauda epididymis from each bull and cryopreserved with Optixcell (EPO). Sperm from the other epididymis were processed with Botubov® (EPB). Concentration, motility, and morphology were evaluated before freezing. After thawing, total (TM) and progressive (PM) sperm motility, capacitation (Merocyanin 540/Yo Pro1, flow cytometry), acrosomal and plasma membrane integrity (FITC PNA/PI, flow cytometry) and mitochondrial potential (JC-1/PI, fluorescence microscopy) were evaluated and compared among treatments using ANOVA. Results are expressed as mean ± SEM. Percentage of normal sperm did not differ (p = 0.001) between EE (76 ± 4.7%) and EP samples (75.1 ± 6.1%). There were fewer distal droplets in EE (11.6 ± 3.6%) than EP (41.4 ± 6.7%) samples. Pre freezing PM but not TM, was higher in EE than EP sperm extended in BotuBov® but not Optixcell (p = 0.049). Post thaw TM but not PM, was higher (p = 0.015) in EPB (58.6 ± 5%) than EPO (41.8 ± 4%). Percentages of sperm with intact plasma and acrosomal membranes were higher (p = 0.001) with BotuBov® (EEB 60 ± 3%, EPB 59.7 ± 4%) than Optixcell (EEO 37.8 ± 6%, EPO 25.8 ± 6%). Motility and acrosomal integrity did not differ between EE and EP sperm. Percentage of capacitated sperm differed (p = 0.009) with treatment (EEB 2 ± 0.4%, EEO 6.9 ± 2.4%, EPB 0.9 ± 0.3%, EPO 4.2 ± 1.4%. Sperm in EP samples had fewer sperm with high mitochondrial potential (EPB 6.6 ± 4.4%, EPO 14.6 ± 6.2%) than EE sperm (EEB 46.7 ± 9.8%, EEO 41 ± 14.6%), with no difference (p = 0.026) between extenders. In conclusion, samples cryopreserved in egg yolk-based extender had higher TM, less cryocapacitated sperm and more intact acrosomes. Egg yolk-based extender was more effective at mitigating effects of cryocapacitation on membrane fluidity and acrosomal integrity. Ejaculated sperm had fewer distal droplets and had less mitochondrial damage than epididymal sperm.

Keywords: Bull, electroejaculation, cryopreservation, semen, diluents