USE OF 2-HYDROXYPROPYL-β-CYCLODEXTRIN AND CHOLESTEROL TO IMPROVE PORCINE SPERM VIABILITY FOLLOWING CRYOPRESERVATION AND COLD SHOCK

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Porcine sperm are extremely sensitive to the damaging effects of cold shock and cryopreservation. Cholesterol-binding molecules, such as 2-hydroxypropyl-β-cyclodextrin (HBCD), improve post-thaw and post-cooling porcine sperm viability when added to an egg yolk-based extender, but also enhance sperm capacitation in other species. HBCD can act either as a cholesterol shuttle or sink to increase or decrease, respectively, sperm plasma membrane cholesterol content depending upon the environmental cholesterol content. Increasing the sperm cholesterol to phospholipid ratio reduces cold shock sensitivity while decreasing the ratio initiates the process of sperm capacitation. The objectives of this study were two-fold: to determine an optimal concentration of HBCD for porcine sperm cryopreservation using an extender containing 20% egg yolk (BF5) and to determine the effects of HBCD and cholesterol 3-sulfate (ChS), in a defined medium lacking egg yolk, on porcine sperm viability and capacitation following cold shock (10 °C for 10 min). For the cryopreservation study, porcine sperm were frozen into pellets on solid CO_2 in BF5 extender containing 0, 20, 40, 60, or 80 mM HBCD. Viability was assessed using CFDA/Propidium Iodide staining. Sperm capacitation following cold shock was assessed by three assays: quantification of protein tyrosine phosphorylation via antiphosphotyrosine (clone 4G10) immunoblots, the percentage of acrosome reactions induced by addition of calcium ionophore, and chlortetracycline (CTC) staining. We report here that post-thaw viability was significantly improved in the presence of 40 mM HBCD (43.1 ± 3.5%, mean ± S.E.M., $n = 68$) and 60 mM HBCD (45.9 ± 2.3%) when compared to 0 mM or 20 mM HBCD (25.9 ± 3.6% and 29.8 ± 3.1%, respectively). At a concentration of 80 mM HBCD, post-thaw sperm viability is adversely affected (24.0 ± 6.6%) presumably due to a shift in the function of HBCD from cholesterol shuttle to cholesterol sink. Following cold shock, porcine sperm incubated in defined medium containing both 0.8 mM HBCD and 0.5 mM or 1 mM ChS have significantly improved viability (77.2 ± 5.7% and 76.6 ± 2.9%, $n = 65$) when compared to cold shocked sperm incubated without HBCD or ChS (46.2 ± 7.6%), or with either component alone. Treatment with 0.8 mM HBCD plus 0.5 mM or 1 mM ChS completely inhibited the increase in protein tyrosine phosphorylation of the 28 kDa band induced by the cold shock treatment. It is possible that the appearance of this band indicates a cytotoxic event rather than capacitation. Sperm capacitation, as assessed by the rate of calcium ionophore-induced acrosome reactions and chlortetracycline staining, was not significantly altered by HBCD and ChS following cold shock. These results indicate that the manipulation of sperm plasma membrane cholesterol content affects sperm viability and could therefore be useful to protect sperm from cold shock during cryopreservation by improving viability without promoting premature capacitation.

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