REGULATION OF MOTILITY IN FRESH AND COOLED, STORED BOAR SPERM

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Motility of extended-cooled, stored boar semen declines over time and, concurrently, fecundity decreases (Althouse, 1997; Cheminade, 2002; Anil, 2004). Interestingly, most of the immotile cells remain viable long after motility is lost (Conejo-Nava, 2003; Dube, 2004; Vyt, 2004). Our first objective was to examine the cell signaling pathways that regulate boar sperm motility. Our second objective was to attempt to improve motility in extended-cooled, stored boar semen samples using agonists of specific signaling pathways. Calcium/calmodulin (Ca/CaM) and Protein Kinase-A (PK-A) signaling pathways are involved in the regulation of murine sperm motility. Our first hypothesis was that Ca/CaM and PK-A pathways also are involved in the regulation of porcine sperm motility. We used *N*-(6-aminohexyl)-5-chloro-1-naphthalenesufonamide hydrochloride (W-7, Calbiochem), a cell permeable CaM inhibitor. Motile, ejaculated porcine sperm were treated with varying concentrations of W-7 (50–250 μM) and motility was determined using a computer assisted sperm analysis (CASA) system (IVOS Analyzer, Hamilton Thorne Research). Treated sperm had a concentration-dependant decline in total and progressive motility compared to controls ($P < 0.05$). Sperm motility was restored to control levels when W-7 treated sperm were exposed to agonists of the PK-A pathway (1 mM db-cAMP (a cell-permeable cAMP analogue) and 100 μM IBMX (a phosphodiesterase inhibitor)). We inferred that Ca/CaM and PK-A pathways are involved in the regulation of boar sperm motility. Additionally, stimulation of the PKA pathway can compensate for the reduction in sperm motility after inhibition of CaM.

Our second hypothesis was that stimulation of the PK-A pathway would improve total and progressive motility in extended-cooled, stored boar sperm. Ejaculated boar semen was extended (following the manufacturers' recommendations) short-term Beltsville Thaw Solution (BTS, IMV International) or long-term Androhep® product (Minitube of America)). Extended semen was stored for 72 h at 17 °C and motility was determined daily using CASA. Once daily, an aliquot of the stored semen was removed and treated with 100 μM IBMX/1 mM db-cAMP. The percentages of total and progressive sperm motility decreased over time in untreated semen, regardless of extender. However, at all time points examined and for both extenders, the addition of IBMX/db-cAMP increased total and progressive motility ($P < 0.05$) that was sustained for over 2 h. Therefore, stimulation of the PK-A pathway significantly improved motility of extended-cooled, stored boar sperm. We inferred that Ca/CaM and PK-A pathways are involved porcine sperm motility. Additionally, the motility of extended-cooled, stored boar sperm was substantially improved by manipulating the PK-A pathway.

Keywords: Boar; Sperm; Motility; Signaling; Cooled semen