A quantifiable biochemical marker for cold shock damage to porcine sperm

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Porcine sperm are extremely sensitive to the damaging effects of cold shock. Accidental cooling below 15–17 °C, the temperature used to ship boar semen for AI, results in cold shock damage and decreased fertility. A method to evaluate the degree of cold shock damage in shipped boar semen would be of great benefit to swine producers. We previously reported a quantifiable increase in protein tyrosine phosphorylation (PY) following cold shock that correlated with temperature and the percentages of spontaneous acrosome reacted (AR) and dead sperm [1]. Therefore, we hypothesized that protein PY is a quantifiable biochemical marker of cold shock damage to porcine sperm. The purpose of this study was to localize cold shock- and capacitation-induced increases in protein PY, and to determine whether or not the same protein undergoes PY in both situations. The sperm-rich fraction was collected from three Yorkshire cross boars, filtered, extended, and washed by centrifugation. The pellet was re-suspended in capacitating medium containing 0.8 mM 2-hydroxypropyl-*b*-cyclodextrin (CD). Sperm in CD medium were washed in PBS, allowed to settle onto coverslips, fixed with 4% paraformaldehyde, permeabilized with methanol (−20 °C), stained with 1:200 antiphosphotyrosine antibody (4G10) and 1:200 Texas Red anti-mouse secondary antibody, and assessed via color fluorescence microscopy (300 cells/slide, 630 × magnification). Data are presented as means ± S.E.M. ($n = 5$, 3 boars used) and were evaluated by ANOVA, followed by Tukey's test (significance set at $P < 0.05$). Indirect immunofluorescence and differential interference contrast microscopy revealed that PY localized to the equatorial region (intact pattern) in intact sperm, to the equatorial and acrosomal regions (AR pattern) in sperm that have recently undergone the AR, but staining of the equatorial region was lost and acrosomal staining decreased (dead pattern) under conditions that promoted cell death (additional incubation following AR induction). The predominant pattern of staining shifted significantly from intact (90.0 ± 1.5% intact, 7.3 ± 1.2% AR, 2.7 ± 0.3% dead) at 0 h before cold shock or incubation to the AR pattern following cold shock (1.3 ± 0.3% intact, 88.0 ± 1.5% AR, 10.7 ± 1.8% dead) or AR induction in capacitated sperm (4.3 ± 3.8% intact, 80.3 ± 3.5% AR, 15.3 ± 5.2% dead). This acrosomal staining could represent proacrosin binding protein PY, as identified previously in capacitated porcine sperm. Sperm proteins extracted before and after cold shock or incubation to promote capacitation were evaluated by two-dimensional gel electrophoresis using the ZOOM™ system and reagents (Invitrogen, Carlsbad, CA, USA). Antiphosphotyrosine immunoblots revealed PY proteins migrating at identical relative molecular mass and isoelectric point after cold shock or capacitation. We inferred that protein tyrosine phosphorylation of an acrosomal protein was a sensitive and quantifiable marker of porcine sperm cold shock damage.

Acknowledgement

Supported by grants from PA Dept. Ag. (ME 446735) and NIH (5-K08-HD041430).

Keywords: Boar sperm; Acrosome reaction; Tyrosine phosphorylation; Cold shock; Cryopreservation

Reference

[1] J Androl 2007;(Suppl.):52

DOI: 10.1016/j.theriogenology.2008.05.013

Effect of cryopreservation medium on post-thaw viability of equine testicular cells

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Although spermatogonial stem cell (SSC) cryopreservation has been proposed for preserving genetics from valuable stallions, a freezing protocol has not been developed for horses. Our objective was to evaluate the effect of three freezing media on post-thaw viability of equine testicular cells. Testes were obtained from 6- to 12-month old colts presented for routine castration ($n = 12$), and testicular cells were isolated by enzymatic digestion. The cell suspension was incubated overnight in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% Fetal Bovine Serum (FBS) at 38 °C in 5% CO_2 in air. The following day, non-adherent cells were recovered and divided into three aliquots. Each aliquot was frozen at a final volume and concentration of 1 mL and 3×10^6 cells/mL, respectively, in one of three freezing media: (A) DMEM with