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JOURNAL ARTICLE

Premature capacitation of bovine spermatozoa is initiated by cryopreservation

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Poor motility and abnormal acrosomal morphology only partially explain the reduced fertility of cryopreserved bovine spermatozoa. To test the hypothesis that cryopreservation procedures (dilution, cooling, freeze-thaw) induce capacitation in bovine spermatozoa, two experiments were conducted using semen diluted in egg yolk-Tris-glycerol extender (EYTG) (Tris, tris(hydroxymethyl)aminomethane). Capacitation was determined prior to and following incubation with various concentrations of heparin using the chlortetracycline (CTC) fluorescence assay or after preexposure to EYTG using in vitro fertilization (IVF) of bovine cumulus-oocyte complexes (COC) in the absence of heparin. Fresh ejaculates were divided into four treatments and the first was diluted with noncapacitating medium, NCM (+0.3% polyvinyl alcohol (PVA); control), then maintained at 23 degrees C for 4 hours. The remaining semen was diluted with EYTG; the second treatment was held at 4 degrees C (EYTG-4), and the third treatment was held at 23 degrees C (EYTG-23) for 4 hours. The fourth treatment was cooled to 4 degrees C over 4 hours, as per the normal industry protocol, cryopreserved, and thawed (frozen-thawed). After the 4-hour maintenance periods or thawing, all treatments were resuspended either in capacitating medium (CM; +0.6% BSA) for the CTC experiment (n = 3) or in NCM for the IVF experiment (n = 9-11). Prior to incubation in conditions that support capacitation, the percentage of cells exhibiting pattern B (capacitated according to the CTC assay) was similar for all treatments with fresh-extended spermatozoa. Immediately following the addition of heparin (0, 2, or 10 micrograms/ml), three times more frozen-thawed than fresh-extended spermatozoa exhibited pattern B ($P < 0.05$). After 3 or 6 hours of incubation, however, the percentages of cells displaying pattern B did not differ among treatments. In the absence of heparin, spermatozoa preexposed to EYTG-4 fertilized 2.6x more COC than did control cells ($P < 0.001$) and 9.2x more than spermatozoa preexposed to EYTG but held at 23 degrees C (EYTG-23; $P < 0.0001$). No differences were observed among fertilization rates for fresh-extended (EYTG-4) and frozen-thawed spermatozoa. This study provides evidence that premature capacitation occurs in partially (extended and cooled) and fully cryopreserved bovine spermatozoa.

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