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Survival and Fertility of Boar Spermatozoa After Freeze-Thawing in Extender Supplemented With Butylated Hydroxytoluene

JORDI ROCA, MARIA A. GIL, MARTA HERNANDEZ, INMA PARRILLA,
JUAN M. VAZQUEZ AND EMILIO A. MARTINEZ

From the Department of Medicine and Animal Surgery, Faculty of Veterinary Medicine, University of Murcia, Murcia, Spain.

Correspondence to: Dr Jordi Roca, Department of Medicine and Animal Surgery, Faculty of Veterinary Medicine, Campus de Espinardo, University of Murcia, E-30071 Murcia, Spain (e-mail: roca[at]um.es).

This study evaluated the protective effect of butylated hydroxytoluene (BHT), a lipid-soluble antioxidant, against cryopreservation injuries to boar spermatozoa. In experiment 1, the lowest BHT concentrations able to reduce lipid peroxidation in boar spermatozoa were determined. Nine BHT concentrations (ranging from 0.025 to 3.2 mM) were evaluated, and the lowest ($P < .05$) production of malondialdehyde (MDA), as an indicator of lipid peroxidation, was obtained when BHT ranged from 0.2 to 1.6 mM. In experiment 2, sperm survivability was evaluated when BHT was added to a postthaw freezing extender by measuring the degree of sperm lipid peroxidation (using MDA production) and by measuring parameter such as motility, plasma membrane and acrosome integrity, and cell apoptosis. The ability of thawed spermatozoa to fertilize in vitro-matured oocytes and of embryos to develop to the blastocyst stage in vitro was also assessed. Pooled sperm-rich fractions collected from 3 mature Pietrain boars were frozen in 0.5-mL straws after dilution with lactose-egg yolk-glycerol-Orvus ES Paste extender supplemented with 0, 0.2, 0.4, 0.8, and 1.6 mM BHT. Postthaw sperm survival, evaluated 30 and 150 minutes after thawing, was higher in BHT-treated spermatozoa, being significant ($P < .05$) when the freezing extender was supplemented with 0.2, 0.4, and 0.8 mM BHT. The addition of BHT to the freezing extender resulted in a significant ($P < .05$) decrease in the MDA concentration in thawed spermatozoa, irrespective of the level of BHT used. BHT had no effect on oocyte cleavage rates, but the development to blastocyst was improved for embryos derived from spermatozoa frozen in extender supplemented with 0.4 mM BHT (16% vs 29% of blastocysts per total oocytes; $P < .05$). In conclusion, under the conditions tested in the present study, the addition of BHT to the freezing extender improved the overall efficiency of thawed boar spermatozoa.

Key words: Antioxidants, cryopreservation, sperm, porcine

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