ELSEVIER

Animal Reproduction Science 90 (2005) 347–354

ANIMAL REPRODUCTION SCIENCE

www.elsevier.com/locate/anireprosci

Short communication

Effects of extender, incubation temperature, and added seminal plasma on capacitation of cryopreserved, thawed boar sperm as determined by chlortetracycline staining

Melissa L. Vadnais [a], Roy N. Kirkwood [a,*], David J. Specher [a], Karen Chou [b,c]

[a] *Department of Large Animal Clinical Sciences, Michigan State University, College Vet Med, East Lansing, MI 48824-1314, USA*

[b] *Department of Animal Science, Michigan State University, East Lansing, MI, USA*

[c] *Institute for Environmental Toxicology, Michigan State University, East Lansing, MI, USA*

Received 19 January 2005; accepted 23 February 2005
Available online 11 April 2005

Abstract

The effect on apparent capacitation status of frozen-thawed (FT), washed boar sperm was examined in capacitation-supporting medium at 39 °C without added seminal plasma (SP) or supplemented with either 10 or 20% (v/v) boar SP. The thawed sperm from three boars were washed to remove the egg yolk-based freezing medium (EY) and then incubated for 1–8 h after addition of SP. Capacitation status of the sperm was determined microscopically using chlortetracycline staining patterns. At 1 h after the addition of 10 or 20% (v/v) SP, capacitated sperm decreased from 59.7 to 30.3% and from 59.5 to 26.8%, respectively ($P < 0.001$). Subsequent studies examined the effect of 10% SP on capacitation status of FT sperm extended in either phosphate buffered saline or commercial thawing extender with or without prior washing of sperm to remove EY and incubated at 17 or 39 °C. No effect of SP resulted from addition to sperm when EY remained or when the temperature was maintained at 17 °C ($P > 0.1$). These results indicate that SP appears able to reverse capacitation of

* Corresponding author. Tel.: +1 517 432 5198; fax: +1 517 432 1042.
E-mail address: kirkwood@cvm.msu.edu (R.N. Kirkwood).

0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2005.02.007

FT boar sperm, but that this effect is dependent on both temperature and composition of the thawing extender.

Keywords: Boar; Frozen; Spermatozoa; Seminal plasma; Capacitation

1. Introduction

Frozen-thawed (FT) boar semen has not received widespread acceptance for commercial breeding in the swine industry due to higher semen costs and reduced sow fertility (lower farrowing rates and smaller litter sizes) associated with the use of FT sperm (e.g. Almlid and Hofmo, 1996). The decrease in fertility is believed to be a result of the FT sperm being effectively capacitated upon thawing, a reaction termed cryocapacitation (Bailey et al., 2000). The ability of capacitated sperm to bind to the oviductal epithelial cells is less than that of uncapacitated sperm both in vitro and in vivo (Medeiros et al., 2002; Tienthai et al., 2004). Thus, fewer functional FT sperm would be available in the oviductal sperm reservoir to fertilize ova.

Capacitation is a sequential process including the removal of protein decapacitation factors from the sperm membrane that were acquired in the epididymus or at ejaculation (Watson and Green, 2000). It has been shown that the cold shock-induced membrane damage in ram sperm was reversed following incubation with a solution containing proteins extracted from ram SP (Barrios et al., 2000). In the boar, the epididymal protein anti-agglutinin that is bound to sperm acrosomes during maturation is released at an early stage of capacitation, but this loss was attenuated in sperm incubated in 5 or 10% (v/v) SP (Harayama et al., 1999).

Using chlortetracycline staining, we previously demonstrated that the addition of 20% SP appeared to reverse capacitation in freshly ejaculated, washed sperm maintained in a capacitation-supporting medium and capacitation induced by cooling sperm to 5 °C (Vadnais et al., in press). The objectives of the present study were to determine the effect of SP on capacitation status of FT boar sperm and how this effect is influenced by prior washing of sperm to remove the egg yolk-based freezing extender and the incubation temperature.

2. Materials and methods

2.1. Media, semen, and seminal plasma

The capacitation-supporting medium (mM199) was prepared as described previously (Vadnais et al., in press). The phosphate buffered saline (PBS) medium contained 160 mM sodium chloride, 8 mM $NaHPO_4$ (anhydrous), and 2 mM $NaHPO_4 \cdot H_2O$ with pH adjusted to 7.4. Prior to use, the mM199 and PBS were equilibrated overnight in a humidified incubator at 39 °C and 5% CO_2 in air. The commercial semen extender employed was X-Cell® (IMV, Maple Grove, MN).