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The post-thaw quality of ram sperm held for 0 to 48 h at 5 °C prior to cryopreservation☆

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Abstract

The effects of holding diluted ram semen at 5 °C for up to 48 h prior to cryopreservation were investigated. Semen from six rams was collected by electro-ejaculation in the autumn and again from six different rams in the spring. The sperm concentration and motility were determined using spectrophotometry and computerized automated semen analysis, respectively. Samples were diluted at 23 °C to 400×10^6 cells/ml in a one-step Tris–egg yolk–glycerol (5%, v/v) media, cooled to 5 °C over 2 h and maintained at 5 °C for the duration of the experiments. Aliquots were loaded into 0.5 ml French straws at 0, 24 or 48 h after cooling, frozen in liquid nitrogen vapor for 12–13 min, 4.5 cm above the liquid nitrogen, and plunged into liquid nitrogen for storage. After thawing, autumn samples frozen after 0, 24, or 48 h of storage exhibited similar percentages of motility (29, 31, 36%, respectively), progressively motility (16, 15, 17%, respectively), plasma membrane integrity (28, 35, 29%, respectively) and live acrosome-reacted cells (0.4, 0.6, 0.8%, respectively; $P > 0.05$). In addition, the quantity of sperm that bound to hen's egg perivitelline membranes after being held at 5 °C for 0, 24, or 48 h was not significantly different when the values were expressed as means of the quantity of sperm (155, 177, 106 sperm, respectively) or as the proportion of sperm inseminated (0.39, 0.49, 0.34, respectively; $P > 0.05$). Likewise, ram sperm collected in the spring and frozen at 0, 24 and 48 h after cooling had similar ($P > 0.05$) total motility (21, 25, 20%, respectively), progressive motility (14, 15, 11%, respectively), plasma membrane integrity (26, 33, 31%, respectively) and live acrosome-reacted cells (3.7, 3.5, 3.2%, respectively; $P > 0.05$). The 0 h holding time had significantly

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less sperm bound to a hen's egg perivitelline membrane compared to the 48 h holding time (250 and 470 sperm, respectively) although the 24 h holding time was not different from the 0 or 48 h holding time (281 sperm; $P < 0.05$) but analysis of the proportion of the total sperm inseminated resulted in no significant differences observed ($P > 0.05$). These results indicate that ram sperm can be held at 5 °C for up to 48 h prior to freezing with no injurious effects on motility, membrane integrity, or fertilizing potential as indicated by membrane binding ability.

Keywords: Spermatozoa; Cryopreservation; Holding time; Ram

1. Introduction

The goal of the National Animal Germplasm Program is to bank viable germplasm from agricultural species; however, most animals of interest are not within reasonable proximity of our facility. Consequently, it is necessary to collect and transport semen samples from the place of collection to our laboratory while maintaining the semen quality before and after cryopreservation. Ideally, a 48 h time span from collection to cryopreservation would be most opportunistic for our needs. These same barriers exist for sheep producers, as little infrastructure exists to efficiently collect, process, and cryopreserve ram semen for artificial insemination.

Semen from many species such as bulls (Graham et al., 1957; Batra et al., 1981; Foote and Kaproth, 2002), boars (Kikuchi et al., 1998; Guthrie and Welch, 2005), stallions (Crockett et al., 2001; Backman et al., 2004) and rams (Jones and Martin, 1964; Lightfoot and Salamon, 1969; Fiser and Batra, 1984) can be cooled to and maintained at 5 °C for time periods up to 24 h, depending on species, prior to cryopreservation and still have acceptable post-thaw sperm quality and fertility. Ram sperm held at 6 °C for 24 h, or up to 12 days maintain acceptable motility (53%, Tiwari and Sahni, 1976; and 50%, Lopez-Saez et al., 2000), but documentation concerning cryopreserving ram sperm following incubations of up to 48 h have not been reported. Therefore, the objectives of this study were to determine the effects of holding time prior to cryopreservation on post-thaw ram sperm quality and fertilizing potential.

2. Materials and methods

2.1. Semen collection, pre-freeze evaluation, and cryopreservation

All chemicals were reagent grade and were purchased from Sigma-Aldrich, St. Louis, MO (see footnote to the title, unless otherwise noted).

Semen was collected from rams in the autumn ($n = 6$) and in the spring ($n = 6$) using an electro-ejaculator as described by Evans and Maxwell (1987). The sperm concentration was determined using spectrophotometry (Hammerstedt, 1975) and the sperm motility estimated using phase contrast microscopy (400×). All samples had an initial total motility of at least 70%.