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A comparison between freezing methods for the cryopreservation of stallion spermatozoa

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Abstract

The effects of sperm freezing concentration (40×10^6 mL^{-1} vs. 400×10^6 mL^{-1}), straw size (0.25 mL vs. 0.5 mL) and freezing method (liquid nitrogen vapour in a Styrofoam® box vs. programmable freezing machine) were evaluated in a $2 \times 2 \times 2$ factorial experimental design using 3 split ejaculates from each of 4 stallions. Immediately after thawing, the total motility and forward progressive motility of spermatozoa frozen at a concentration of 40×10^6 mL^{-1} was higher than for spermatozoa frozen at 400×10^6 mL^{-1}. No significant differences were observed in the semen parameters assessed after cryopreservation in either 0.25 or 0.5 mL straws. However, the programmable freezer provided a more consistent and reliable freezing rate than liquid nitrogen vapour. We conclude that an effective protocol for the cryopreservation of stallion spermatozoa at low concentrations would include concentrations of 40×10^6 mL^{-1} in 0.25 mL straws using a programmable freezer. This freezing protocol would be suitable for emerging sperm technologies such as sex-preselection of stallion spermatozoa as the sorting process yields only low numbers of spermatozoa in a small volume available for either immediate insemination or cryopreservation.

Keywords: Stallion; Equine; Spermatozoa; Sperm concentration; Cryopreservation

1. Introduction

Current protocols for the commercial use of frozen-thawed stallion semen for artificial insemination (AI) include the insemination of 250×10^6 to 500×10^6 motile spermatozoa (Householder

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et al., 1981) frozen at concentrations ranging from 100×10^6 to 500×10^6 mL^{-1} in 0.25 to 5 mL straws (Cochran et al., 1983; Wockener and Schuberth, 1993; Heitland et al., 1996). However, for emerging sperm technologies such as sex-preselection, these freezing protocols require refinement to cope with the reduced number of spermatozoa available for each insemination dose and the detrimental effects of prolonged processing.

Foals of a pre-determined sex have been born after insemination of mares with flow cytometrically sorted, fresh or stored stallion spermatozoa (Lindsey et al., 2001, 2002a). Flow cytometric sorting of stallion spermatozoa is time consuming as cells are routinely sorted at rates of 3000–5000 cells/s (Maxwell et al., 2004). This results in a processing time of up to 1.5 h to sort 16×10^6 cells, providing only a small number of spermatozoa for insemination or cryopreservation. Current protocols for the cryopreservation of sex-sorted stallion spermatozoa use 0.25 mL straws and sperm concentrations ranging from 20×10^6 to 90×10^6 mL^{-1} (Lindsey et al., 2002b, 2003).

The reported fertility of sex-sorted frozen-thawed stallion spermatozoa has been disappointingly low (Lindsey et al., 2000). In order to cryopreserve sex-sorted spermatozoa, it is necessary to freeze spermatozoa at regular intervals throughout the process in order to minimise any loss of sperm viability over time. In addition, it may be beneficial to modify these conventional protocols to optimise the fertility of frozen spermatozoa after freezing at a low concentration (40×10^6 mL^{-1}) in smaller straw volumes (0.25 mL) suitable for low dose insemination as reviewed by Ball (2004) and Morris (2004).

The aim of the present study was to compare the post-thaw function of stallion spermatozoa frozen at two different sperm concentrations in 0.5 or 0.25 mL straws in either liquid nitrogen vapour or a programmable freezer.

2. Materials and methods

2.1. Animals and experimental design

Three ejaculates from four stallions ($n = 12$) of various breeds and ages were collected and processed for cryopreservation. Stallions and ejaculates were selected based on acceptable (>60%) total motility of spermatozoa before freezing and good recovery of sperm motility after thawing.

The ejaculates were split and each fraction allocated to groups for examination of three treatment factors (Fig. 1): freezing concentration of spermatozoa (40×10^6 mL^{-1} vs. 400×10^6 mL^{-1}), freezing package (0.25 mL vs. 0.5 mL straw) and freezing method (liquid nitrogen vapour in a Styrofoam® box vs. programmable freezing machine). This split-ejaculate approach resulted in a $2 \times 2 \times 2$ factorial experimental design.

2.2. Preparation of spermatozoa

Semen was collected using a Missouri artificial vagina (Nasco, Fort Atkinson, WI) with an inline filter (non-woven semen filter, Minitube Australia, Smythes Creek, Australia) to exclude the gel fraction of the ejaculate. Filtered semen was immediately extended (1:1, semen:diluent, v/v) with Kenney's extender (Kenney et al., 1975) and transported (60 min) to the laboratory in an Equitainer® (Hamilton Research, South Hamilton, MA, USA) maintained at 15 °C using one frozen ice brick and one room temperature ice brick.