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Short communication

Efficiency of short-term storage of equine semen in a simple-design cooling system

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Abstract

Five experiments tested the efficiency of a simple, low-cost system (CP) for cooling and storing equine semen at 2.0 °C for 24 h and 48 h. Pantaneiro stallions of known fertility were used. Semen quality was evaluated for progressive motility (PM), plasma membrane integrity (PMI), and pregnancy rate. Experiment 1 showed that PM and PMI were similar between CP and the control (Equitainer^{TM}) in cooled semen. In Experiment 2, the influence was evaluated of combinations (four treatments) of two volumes (50/100 ml) and two sperm concentrations ($500/750 \times 10^6$) on sperm quality of semen cooled and preserved by CP (cooling system replaced at 24 h). While PM decreased gradually from before cooling to 24 h and 48 h, PMI decreased only at the least and greatest sperm volume and concentrations. Storage time did not affect PMI. Results from Experiment 3 showed that CP maintained semen PM $\geq$ 30% in all samples 24 h after cooling and decreased to about 70% 42 h after cooling. Results from Experiments 4 and 5 confirmed semen quality after cooling and storage (24 h and 48 h, respectively), achieving a 69% pregnancy rate in the first estrous cycle when insemination occurred. Thus, the CP system is satisfactory for cooling and preserving equine semen for up to 48 h.
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Keywords: Equine; Cooled semen; Sperm motility; Fertility; Plasma membrane integrity

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1. Introduction

Artificial insemination with equine semen destined for transport was expanded after the commercial launching of Equitainer™ (ideal cooling rate = −0.30 °C/min, Douglas-Hamilton et al., 1984). Despite being widely used, this high-cost method has stimulated interest in developing similar, low-cost products. Thus, in the present study a simple, low-cost system to cool, store and transport equine semen was tested. Such systems are useful for artificial insemination because they eliminate the transport of mares and their foals to stud farms and maximize the use of genetically valuable stallions. However, cooling systems should not impair semen quality by causing structural and functional damage to sperm by thermal shock during cooling (Varner et al., 1988). Extender quality, cooling rates, storage temperature and equipment used for transport are major factors to be considered. Ideal cooling conditions should maintain gamete integrity and reduce metabolic activity, thus increasing cellular longevity (Loomis, 1992).

2. Materials and methods

2.1. Animals and treatments

Fertile, 8–11-year-old Pantaneiro stallions were studied during two consecutive breeding seasons (November–January) in Terenos, Brazil (latitude 20°25′39, 72″ S; longitude 54°51′24,96″ W; altitude 389 m). In the first experiment, cooling system CP1 was compared to a widely accepted system (Equitainer™), but because the CP1 Styrofoam box was no longer available, a similar cooling system (CP2) was used in the following four experiments.

2.2. Cooling system

The isothermal container was a 25.5 cm × 19.5 cm Styrofoam box with 2.5 cm thick walls. The refrigeration unit had five recycling plastic bottles of (13.8 cm × 9.8 cm × 5.0 cm) loaded with freezable gel (Quimimax Indústria e Comércio de Produtos Químicos Ltda, Brazil) and placed inside the Styrofoam box. Experiment 1 showed the efficiency of this system for cooling and storing equine semen. CP2 had a larger Styrofoam box (20.4 cm × 17.1 cm × 25.0 cm), with 1.6 cm thick walls (Knauf Isopor, Brazil) with CP1 refrigeration units. CP1 and CP2 are patented under number 000037 of the Instituto Nacional da Propriedade Industrial – INPI/MS, Brazil. The semen was maintained in a cylindrical glass recipient, covered with 2.0 cm thick Styrofoam, and placed in a 0.5 cm thick isothermal cup (Knauf Isopor, Brazil).

2.3. Experimental strategies

In Experiment 1, efficiency of CP1 for cooling and storing equine semen for 24 h was evaluated. A total of 24 semen ejaculates were collected from six stallions (four ejaculates/stallion) and extended at a 3:1 (extender: semen) rate and fractioned into two equal aliquots for each sample. These aliquots were then processed by either CP1 or a control reference, Equitainer™ (Hamilton-Thorn Research, Danvers, MA, USA – a recognized system widely used for cooling and storing equine semen – Douglas-Hamilton et al., 1984). Cooling rates were measured by a digital thermometer (model Ioptherm 46®; IOPE – Instrumentos de Precisão Ltda, Brazil) with the probe inserted into the semen. After sealing the cooling system, semen temperature was recorded every 10 min for 6 h and then every 30 min for 18 h. Sperm progressive

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