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Review article

Comparative cryopreservation of avian spermatozoa: Effects of freezing and thawing rates on turkey and sandhill crane sperm cryosurvival☆

Juan M. Blanco[a,b], Julie A. Long[b,*], George Gee[c], David E. Wildt[d], Ann M. Donoghue[e]

[a] *Aquila Foundation and the Center for Studies on Iberian Raptors, CERI Toledo, Spain*
[b] *USDA, ARS, ANRI, ABBL, Beltsville, MD 20705, United States*
[c] *Patuxent Wildlife Research Center, Laurel, MD 20708, United States*
[d] *Center for Species Survival, Smithsonian's National Zoological Park, Conservation & Research Center, Front Royal, VA 22630, United States*
[e] *USDA, ARS, PPPSRU, Fayetteville, AR 71656, United States*

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ABSTRACT

A comparative approach was used to evaluate semen cooling rates, thawing rates and freezing volume on the cryosurvival of avian sperm. Turkey (*Meleagris gallopavo*) and sandhill crane (*Grus canadensis*) sperm were cryopreserved with dimethylacetamide (DMA) concentrations ranging from 6% to 26%. Experiments evaluated the efficacy of (1) rapid, moderate and slow cooling rates, (2) rapid and slow thawing rates, and (3) final volume of semen frozen (0.2 mL compared to 0.5 mL). For crane sperm only, additional experiments were conducted to evaluate the effect of sucrose on cryosurvival. The functionality of frozen/thawed crane sperm was evaluated by fertility trials. For all studies, sperm viability was assessed using the nigrosin–eosin stain. Higher percentages of crane and turkey sperm maintained intact membranes when frozen with moderate or slow cooling rates compared to rapid cooling rates ($P < 0.05$), regardless of DMA concentration. Turkey sperm viability was not affected by thawing rate at any DMA concentration ($P > 0.05$). Crane sperm viability was only affected by thawing rate for the 24% DMA treatment, where moderate thawing was better than slow thawing ($P < 0.05$). Sperm viability was not affected by the semen volume used for freezing for either species ($P > 0.05$). The percentage of membrane-intact crane sperm at lower DMA concentrations was improved by addition of 0.1 M sucrose ($P < 0.05$) but not 0.29 M NaCl. The mean fertility rate from frozen/thawed crane semen was 57.5%, and 71.4% of the fertile eggs hatched. The viability of crane sperm was always greater than turkey sperm, regardless of cooling rate, thawing rate or volume of semen frozen. These data verify avian-specific differences in sperm cryosurvival, further emphasize the need for species specific studies to optimize cryopreservation protocols.

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* Corresponding author at: Animal Biosciences and Biotechnology Laboratory, BARC-East, Building 200, Room 120, Powder Mill Road, Beltsville, MD 20705, United States. Tel.: +1 301 504 8580; fax: +1 302 504 8623.
E-mail address: julie.long@ars.usda.gov (J.A. Long).

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1. Introduction

A principal challenge for the survival of cells during cryopreservation is the lethality of an intermediate temperature zone (−15 to −60 °C) that is traversed twice during the cryogenic cycle as cells are cooled and re-warmed (Gao and Critser, 2000). Optimal freezing and thawing rates lessen the damage caused by intracellular ice formation, cell shrinkage and exposure to multiple osmotic gradients; these data are critical for developing successful semen cryopreservation protocols. Freezing rates have been evaluated for chicken sperm (Sexton, 1980; Williamson et al., 1981; Seigneurin and Blesbois, 1994; Blanco et al., 2000; Woelders et al., 2006) but have not been fully evaluated for the turkey, another important poultry species, or for sperm from non-domestic avian species, such as the sandhill crane.

Cells are subjected to "solution effects" during the cryogenic cycle (Mazur, 1977). During the cooling process, exposure to the non-frozen, hyperosmotic solute fraction causes water to withdraw from cells and results in cell shrinkage as well as an influx of ions. Upon re-warming, these dehydration effects are reversed and the subsequent water influx may disrupt the plasma membrane (Holt, 2000). Discordance between cooling and thawing rates poses additional problems. When rapidly cooled cells are re-warmed at a slow rate, small water crystals aggregate and form larger crystals that disrupt internal organelles and rupture membranes (Mazur, 1984). Conversely, cells that are frozen slowly and thawed rapidly will undergo swelling as a result of osmotic changes in the extracellular medium (Mazur, 1977). While these scenarios represent extreme examples in terms of cell damage during the cryogenic cycle, it is also important to note that optimal cooling and warming rates have been shown to be highly specific among various taxa (Barbas and Mascarenhas, 2009; Mansour et al., 2009; Medrano et al., 2009; Vuthiphandchai et al., 2009), as well as avian species (Blanco et al., 2000; Tai et al., 2001; Woelders et al., 2006; Kowalczyk, 2008).

A comparative approach has proven valuable where a species exhibiting good sperm cryosurvival, the sandhill crane, has been evaluated alongside a species with extremely poor sperm survival rates after cryopreservation, i.e., the domestic turkey (Blanco et al., 2008, 2011). In the first report, we showed that a vast difference in osmotic tolerance contributed to the superior cryosurvival of sandhill crane sperm. In the latter report, we demonstrated that the cryosurvival of turkey sperm was not influenced by the use of sucrose as a non-permeating osmoprotectant, whereas sandhill crane sperm responded favorably when sucrose was used in combination with lower dimethylacetamide (DMA) concentrations. The aim of this study was to evaluate the effects of freezing rates, thawing rates, and the volume of semen frozen on the cryosurvival of turkey and sandhill crane sperm. Also investigated was a possible mechanism for the improved cryosurvival of crane sperm frozen in a cryodiluent containing sucrose, as an extension of a recent study with sandhill crane sperm cryopreservation (Blanco et al., 2011). The final objective was to evaluate the fertility of frozen/thawed semen from the sandhill crane.

2. Materials and methods

2.1. Species/animals used and semen collection

Sexually mature turkey toms (*Meleagris gallopavo*; $n = 40$) were housed indoors in floor-pens (10 males/pen) and maintained under artificial photoperiod (14 h L: 10 h D) at the Beltsville Agricultural Research Center. Sandhill cranes (*Grus canadensis*; $n = 24$) were part of the captive stock at the Patuxent Wildlife Research Center, and all semen donors were paired with sexually active females with a history of reproductive success under natural photoperiod. Crane pairs were kept in breeding facilities consisting of 300 m^2 pens with barriers to avoid visual contact between pairs. Semen was collected from males of both species twice a week using the massage method (Quinn and Burrows, 1937), with specific modifications for cranes (Gee and Temple, 1978; Gee, 1983), and ejaculates on a given day were pooled before transport to the laboratory. Semen was maintained at room temperature during transport (15 min or less prior to dilution for cryopreservation).

2.2. Cryodiluent composition

Turkey and sandhill crane semen was diluted with different extenders based on known species-specific requirements for extender components and osmolality (Sexton, 1979; Gee and Mirande, 1996; Blanco et al., 2008).