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Fertility disturbances of dimethylacetamide and glycerol in rooster sperm diluents: Discrimination among effects produced pre and post freezing-thawing process

F.M.K. Abouelezz[a,*], M.A.M. Sayed[a], J. Santiago-Moreno[b]

[a] Department of Poultry Production, Faculty of Agriculture, Assiut University, 71526 Assiut, Egypt
[b] Departamento de Reproducción Animal, Instituto Nacional de Investigación y Tecnologia Agraria y Alimentaria (INIA), 28040 Madrid, Spain

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ABSTRACT

With avian sperm cryopreservation protocols, the most widely used cryoprotectants (CPAs) are the glycerol (GLY; in gradual freezing: in-straw freezing method), and the dimethylacetamide (DMA; in pellets by plunging into liquid nitrogen: in-pellet rapid freezing method). Use of both methods results in a small portion of thawed live sperm with lesser fertilizing ability compared with the semen samples immediately after collection. This study was conducted to assess the pre-freezing damage occurring to the sperm due to the interaction with the cryoprotectants (CPAs) GLY (8%) and DMA (5%), as well as the post-freezing damage resulting from both freezing methods Data for each treatment, in fresh and frozen-thawed samples, were compared for sperm motility, fertilizing capacity and sperm-egg penetration holes/germinal disc (SP holes/GD). Hens (n = 50) were artificially inseminated (10 hens/treatment) six times with 3 day intervals between inseminations. The treatment of fresh sperm with DMA led to a reduction ($P < 0.05$) in the count of SP holes/GD (21.4) and the fertility rate (66.7%). The addition and elimination of GLY in fresh samples resulted in a lesser ($P < 0.05$) number of SP holes/GD (11.8) and the fertility rate (i.e., 50.0%). The number of SP-holes/GD was least in frozen-thawed samples using both DMA and GLY (14.2 and 9.2, respectively). The fertility rate when using semen frozen with DMA in- pellets was greater ($P < 0.05$) than with use of semen that had been frozen using GLY in straws (46.4% compared with 31.3%). The reduction in fertility compared with the control when semen was cryopreserved using GLY was 64.1%; the GLY addition and elimination was responsible for two thirds of this reduction. The reduction in fertility when using semen cryopreserved with DMA was 46.7%; half of the reduction was attributed to the treatment with DMA. In conclusion, the mechanical damage attributed to the process for reducing GLY concentrations was more harmful to sperm fertilizing capacity than the toxicity of DMA and freeze/thaw process. For both freezing methods, the amount of sperm cryo-damage was similar, when the damage attributed to the CPA addition and elimination process was excluded.

1. Introduction

Even with the great advances in sperm cryopreservation technology, there is a significant inconsistency in results from previous research regarding the optimal cryoprotectant and cryopreservation protocols. There are many factors responsible for these inconsistencies, such as poultry breeds used, different composition of diluents, concentrations of the cryoprotectant agent (CPAs), methods

* Corresponding author at: Department of Poultry Production, Faculty of Agriculture, Assiut University, CP. 71526, Assiut, Egypt.
E-mail address: abollez@aun.edu.eg (F.M.K. Abouelezz).

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of freezing/thawing, the cooling rate, the method of sperm packaging, and number of sperm cells used for insemination (Blesbois 2006; Long et al., 2010). Studying the factors involved in causing sperm damage would improve the understanding of the problem and contribute to optimizing cryopreservation protocols to improve sperm viability and fertilizing capacity.

Glycerol (GLY) and dimethylacetamide (DMA) are considered the most adequate CPAs to freeze avian sperm (Woelders et al., 2006; Blesbois et al., 2007). The CPA, GLY, is most commonly used to freeze rooster sperm. Unlike mammals, however, GLY has a contraceptive effect when hens are intravaginally inseminated (Tajima et al., 1989; Hammerstedt and Graham, 1992). When GLY is used as a CPA, semen must undergo further processing to reduce GLY. Abouelezz et al. (2015a) and Hammerstedt and Graham (1992) among others recommended reducing GLY concentration to < 1% prior to insemination. The step-wise dilution with use of a centrifugation method is commonly used to reduce GLY concentrations (Tajima et al., 1989; Tajima et al., 1990). This processing of semen is detrimental to the sperm due to osmotic and physical damage that results in a reduction in fertility (Hammerstedt and Graham, 1992).

The use of DMA is a primary alternative to the use of GLY as a CPA, which does not require the processing of sperm to reduce the concentrations of CPA as is the case with GLY before insemination. One major disadvantage of use of DMA, however, is its toxic effect when used at the greater concentration (Blanco et al., 2000). Zaniboni et al. (2014) reported reductions in sperm viability after 30 min of equilibration with DMA using concentrations as low as 0.69 M (6%).

The effectiveness of sperm cryopreservation may also depend on the interaction between the type of CPA used and sperm freezing and packaging methods (Tselutin et al., 1999). It has been suggested, therefore, that the most desirable results are obtained when GLY is the CPA used in combination with the in-straw method for sperm storage, and when DMA is used as the CPA in combination with the pellet method of sperm storage (Blesbois et al., 2007).

The success of sperm cryopreservation is often assessed by *in vitro* measurements such as sperm motility and plasma membrane integrity (Chalah and Brillard, 1998; Donoghue and Wishart, 2000; Blesbois et al., 2008). These methods, however, were reported to be inadequate and when used result in an overestimation of the actual fertilizing capacity of sperm (Donoghue and Wishart, 2000). A fertility test is, therefore, considered invaluable because it reflects the ultimate success of the cryopreservation procedure (Graham and Mocé, 2005). The sperm-egg penetration assay was used in the present study because it demonstrates the number of sperm that pass through the oviduct and initiate the first stage of the fertilization process by hydrolyzing the ova perivitelline layer (Mocé et al., 2010; Lemoine et al., 2011; Abouelezz et al., 2015b). Other researchers have used the acrosome reaction (AR) technique (Lemoine et al., 2011 and Mocé et al., 2010), which is an *in vitro* technique that is labor and time consuming as compared with conducting the *in vivo* sperm-egg penetration assay. The current study was conducted to delineate the detrimental pre-freezing effects occurring due to the use of the CPAs, GLY or DMA, from the detrimental effects of the freezing/thawing procedure when these two CPAs were used for freezing rooster semen.

2. Materials and methods

2.1. Birds

Male (n = 10) and female (n = 50) 1-year-old Dandarawy chickens, an indigenous Egyptian chicken breed, were used in this study. The birds were developed on the experimental farm of the Poultry Production Department, Faculty of Agriculture, Assiut University, Assiut Governorate, Egypt. All birds were housed in individual cages of 30 × 40 × 40 cm with the photoperiod that was imposed being 16 h light:8 h dark between January and March 2016. Birds were fed a commercial diet containing 16% crude protein, 2800 kcal ME/kg, 3.5% calcium and 0.5% available phosphorus during the entire experimental period.

2.2. Sperm collection and pre-freezing preparation

Semen was collected six times with 3-day intervals between collections in a 15-mL graduated Falcon tube using the massage technique (Burrows and Quinn, 1937). After each collection, a semen pool was made from all roosters and was immediately diluted 1:1 (v/v) using the medium (Lake and Ravie, 1984), which comprised glucose (0.8 g), sodium glutamate (1.92 g), magnesium acetate 4HO (0.08 g), polyvinylpyrrolidone [relative molecular mass (Mr) = 10 000] (0.3 g), potassium acetate (0.5 g), and H2O (100 mL). The final pH was 7.08 and final osmolality 343 mOsm/kg. This diluted, pooled semen was then cooled to 5 °C and transported to the laboratory where sperm concentration and motility values were evaluated within 20 min of collection.

The motility percentage and quality were subjectively assessed in 1:20 (v/v) diluted samples under 400 × magnification (Olympus IX53; Olympus America INC., 3500 Corporate Parkway, Center Valley, Pennsylvania 18034-0610, U.S.A.). Sperm motility was expressed as the percentage of total motile sperm. The quality of motility was scored on a scale of 0–5; 0 = no movement, 1 = tail movements but no sperm progression, 2 = only circular sperm movements, 3 = large percentage of sperm showing progressive but no rectilinear movement, 4 = large percentage of sperm showing rectilinear but not very vigorous movement, and 5 = large percentage of sperm showing vigorous, rectilinear progressive movement (Santiago-Moreno et al., 2011). Sperm concentration was estimated using a hemocytometer (Neubauer chamber). The semen pH was measured using a digital pH meter (ADWA, AD11 Waterproof pH-TEMP Pocket Tester, 6726 Szeged − Hungary). Data for the characteristics of the 10 Dandarawy semen donors and evaluation of the fresh semen samples are presented in Table 1.