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Influence of various cryoprotectants on the sperm mobility of Muscovy semen before and after cryopreservation

V. Gerzilov

Department of Animal Science, Agricultural University, 12 Mendeleev, 4000 Plovdiv, Bulgaria

Abstract. A study for influence of five cryoprotectants - glycerol, dimethyl sulfoxide (DMSO), ethylene glycol, 1,3 propandiol and polyethylene glycol in different concentrations: 3%, 5% and 7% with use of HIA-1 and AU extenders on the mobility of Muscovy spermatozoa after freeze-thaw was carried out. The semen was collected with artificial vagina from 9 one-year-old Muscovy drakes by using a female as a teaser twice a week. The sperm was diluted with HIA-1 and AU extenders with 15% egg yolk (v/v) at the ratio of 1:3 (semen:extender), respectively, and divided equally. Cryoprotectant was added in one of following concentrations: 3-5-7% into each semen sample, respectively, a process of equilibration in a refrigerator followed at 4 °C for 60 min. Afterwards they were directly dropped in concave cavities of dry ice at -79 °C for 10 min. The semen pellets were placed in an atmosphere of liquid nitrogen (LN2) vapors for 5 - 10 min and finally they were put in cryotubes and plunged into liquid nitrogen. The pellets were kept frozen in the LN2 container for at least 2 months before being thawed for evaluation. The sperm samples were thawed at 42 °C with HIA-1 and AU extenders, respectively. Comparatively the highest sperm mobility was established at using 5% and 7% glycerol and 7% DMSO. Both HIA-1 and AU extenders are suitable to semen dilution. Cryopreservation of Muscovy semen caused damages in the morphological integrity of sperm cells.

Keywords: Muscovy duck, semen, sperm mobility, cryopreservation, cryoprotectant

Abbreviations: LN - liquid nitrogen, spz – spermatozoa, DMSO - dimethyl sulfoxide, ME - metabolizable energy, CP - crude protein

Introduction

The preservation of poultry semen in frozen state has been the subject of intense scientific interest beginning approximately 60 years ago by Polge (1949, 1951) with the discovery of glycerol as a good cryoprotective medium. Despite the fact that this scientific breakthrough was accomplished with rooster semen (Polge, 1951), the overall fertility rates with frozen-thawed poultry semen are highly variable and not reliable enough for use in commercial production or preservation of genetic stocks. According to Long (2006), the greatest progress in commercializing semen preservation has been achieved by the dairy and beef cattle industries, where semen cryopreservation has been optimized, standardized, and automated. This high level of success with bull semen has not been achieved with other livestock species, such as pigs or sheep (Holt, 2000), and the fertility rates of cryopreserved poultry sperm are dramatically lower than any of the domestic mammalian species.

Despite extensive research on poultry semen cryopreservation, there is limited success in applying these procedures either to “on farm” use or to the enhanced management or conservation of rare wild avian species (Blanco et al., 2000). The sperm cryopreservation for ex situ management of genetic resources in poultry is still a big problem. According to Blesbois and Brillard (2007), cryobanking for species other than chicken remains extremely limited. The lack of knowledge required to develop the appropriate technology for cryopreservation of sperm in poultry species is another challenge for the years to come. Cryopreservation of Muscovy duck semen is more difficult than Pekin ducks (Tseltin et al., 1999). However, the experiments indicate that spermatozoa of commercial lines of these two species of ducks are able to be frozen with reasonable success of fertility of frozen-thawed semen (Blesbois, 2007).

One of the most critical steps in the cryopreservation of avian semen is the choice of the cryoprotectant and its use during the process (Tseltin et al., 1999). Many compounds have been tested for their efficiency as sperm cryoprotectants (Holt, 2000), but most extensively – glycerol, dimethyl sulfoxide, dimethyl acetamide, dimethyl formamid, diethyl formamide, ethylene glicol, propylene glicol (Lake and Ravie, 1982; Hammerstedt and Graham, 1992; Surai and Wishart, 1996; Tseltin et al., 1999; Tai et al., 2001; Łukaszewicz, 2001).

The aim of this study is to compare the effect of two HIA-1 and AU extenders, and five cryoprotectants - glycerol, dimethyl sulfoxide, ethylene glicol, 1,3 propandiol and propylene glicol in 3%, 5% and 7% as a final concentration, respectively, on the mobility of Muscovy spermatozoa before and after cryopreservation.

Material and methods

Birds

The experiment was carried out on the Poultry farm of the Agricultural University, Plovdiv with 9 one-year-old Muscovy drakes. During the entire natural breeding season the males were kept individually in cages (0.6/0.8/0.6 m in size) under natural light. The birds were fed with diet consisting of ME - 11.5 MJ/kg and CP – 16.0 %, and daily ration from 200 to 250 g/bird.
Semen collection

The semen was collected individually by placing a female (teaser method) in the cage of the Muscovy drake using an artificial vagina, two times per week (Tan, 1980; Gerzilov, 2000). The artificial vagina consisted of a rubber muff and a graduated test-tube.

Semen evaluation

Only good quality ejaculates (color – pearly-white; cleanliness - free of any contamination with cloacal products; volume – above 0.3 ml; sperm mobility – above 65%, sperm concentration – above 1 x 10^9 sperm cells/ml) were used for cryopreservation. The pooled semen was divided in two parts and diluted with HIA-1 and AU-extenders by Gerzilov (2003) with added 15% egg yolk (v/v) at a ratio 1:3 (semen:extender), respectively (Figure 1).

The HIA-1 extender consists of 0.25 g D-glucose, 0.25 g D-fructose, 0.07 g saccharose, 0.50 g sodium citrate, 9.00 g sodium chloride, and 100 mL double distilled water. The osmolarity was 290 mOsmol/kg and pH - 7.00. The AU extender consists of 0.40 g D-glucose, 0.80 g D-fructose, 0.80 g saccharose, 0.90 g sodium citrate, 0.84 g sodium glutamate, 0.40 ml glycolcol, 0.04 g ethylenediamine tetra acetic acid disodium salt dihydrate, and 100

![Diagram of cryopreservation process]

**Figure 1.** Design of cryopreservation of Muscovy semen

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The diluted semen was distributed equally in 15 sterile glass tubes again. A cryoprotectant - glycerol, dimethyl sulfoxide, ethylene glycol, 1,3 propanediol and polyethylene glycol in 3%, 5% and 7% as a final concentration, respectively, was supplementary to each tube as a final concentration. The semen samples were equilibrated in a refrigerator at 4°C for 60 min, and then they were directly dropped in concave cavities of dry ice at -79°C for 10 min. Semen pellets were placed in an atmosphere of LN vapor for 5-10 min and finally they were put in cryotubes and plunged into liquid nitrogen. The pellets were kept frozen in the LN container for at least 2 months before being thawed for evaluation. The sperm samples were thawed at 42°C with HIA-1 and AU extenders (1:3 v/v), respectively.

The sperm mobility (%) of the pooled semen, diluted, cryoprotectants in different concentration to the diluted semen (Table 1). After the use of HIA-1 extender, sperm mobility was in the range of 66.50±4.95% to 71.67±0.71% and 71.00±1.08% in the use of AU extender. In both cases the lowest values in sperm mobility were in 7% concentration of polyethylene glycol. The highest values of sperm mobility were obtained in 5% concentration of glycerol. Sperm mobility after the equilibration was decreased but the differences were not significant.

The Table 1. Sperm mobility in semen before freezing and after thawing

<table>
<thead>
<tr>
<th>Cryoprotectant</th>
<th>%</th>
<th>Diluted semen x ± S,</th>
<th>Equilibrated semen x ± S,</th>
<th>Frozen/thawed semen x ± S,</th>
<th>Diluted semen x ± S</th>
<th>Equilibrated semen x ± S</th>
<th>Frozen/thawed semen x ± S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>3</td>
<td>74.50±2.43</td>
<td>71.25±2.01</td>
<td>6.67±7.36</td>
<td>76.00±1.41</td>
<td>68.50±2.12</td>
<td>17.00±2.12</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>76.67±0.65</td>
<td>72.67±4.08</td>
<td>35.33±5.31</td>
<td>78.00±1.22</td>
<td>73.33±2.48</td>
<td>31.33±3.63</td>
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<tr>
<td></td>
<td>7</td>
<td>75.00±2.43</td>
<td>70.75±1.06</td>
<td>29.25±4.17</td>
<td>73.33±2.04</td>
<td>70.67±1.47</td>
<td>25.33±6.72</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>3</td>
<td>70.50±5.51</td>
<td>71.67±4.08</td>
<td>11.67±4.08</td>
<td>72.50±3.53</td>
<td>70.50±3.54</td>
<td>8.00±2.82</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>74.75±1.91</td>
<td>70.75±0.87</td>
<td>19.00±8.16</td>
<td>76.00±1.41</td>
<td>70.00±2.12</td>
<td>9.00±1.41</td>
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<tr>
<td></td>
<td>7</td>
<td>76.25±2.76</td>
<td>72.00±1.41</td>
<td>18.25±8.00</td>
<td>78.00±2.83</td>
<td>71.67±5.40</td>
<td>17.33±1.78</td>
</tr>
<tr>
<td>1.3</td>
<td>3</td>
<td>70.50±5.51</td>
<td>61.00±8.49</td>
<td>6.50±4.95</td>
<td>76.00±1.41</td>
<td>73.50±4.95</td>
<td>6.33±1.08</td>
</tr>
<tr>
<td>Propandiol</td>
<td>5</td>
<td>74.75±1.91</td>
<td>62.50±10.61</td>
<td>15.00±7.07</td>
<td>73.00±1.54</td>
<td>72.50±3.54</td>
<td>6.50±2.12</td>
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<tr>
<td></td>
<td>7</td>
<td>76.25±2.76</td>
<td>65.00±6.12</td>
<td>10.00±7.01</td>
<td>76.25±2.76</td>
<td>68.50±2.12</td>
<td>8.00±2.83</td>
</tr>
<tr>
<td>DMSO</td>
<td>3</td>
<td>72.67±1.78</td>
<td>71.67±2.04</td>
<td>isolated spz</td>
<td>73.67±1.00</td>
<td>66.00±1.41</td>
<td>11.50±7.78</td>
</tr>
<tr>
<td>Polyethylene glycol</td>
<td>5</td>
<td>71.67±5.40</td>
<td>68.33±2.04</td>
<td>22.00±13.91</td>
<td>75.00±7.01</td>
<td>67.50±3.54</td>
<td>21.00±5.66</td>
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<tr>
<td></td>
<td>7</td>
<td>71.75±5.13</td>
<td>68.25±3.28</td>
<td>25.00±7.71</td>
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<td>3</td>
<td>71.50±2.12</td>
<td>70.50±0.71</td>
<td>isolated spz</td>
<td>73.67±0.82</td>
<td>69.67±1.78</td>
<td>isolated spz</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>71.00±1.23</td>
<td>68.50±2.12</td>
<td>isolated spz</td>
<td>71.50±2.12</td>
<td>71.50±2.12</td>
<td>isolated spz</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>66.50±4.95</td>
<td>64.00±1.41</td>
<td>7.00±4.24</td>
<td>71.00±1.23</td>
<td>69.00±4.14</td>
<td>4.50±0.71</td>
</tr>
</tbody>
</table>

The mobility in the thawed semen ranged from several isolated sperm cells using polyethylene glycol to 35.33 ± 5.31% using glycerol, respectively. The thawed semen with the highest mobility using 5% and 7% glycerol and 7% DMSO in comparison with all other cryoprotectants. The cryoprotectants - 1.3 propandiol and polyethylene glycol were extremely unsuitable and toxic. The cryopreservation process induced a significant decrease of sperm mobility vs. diluted and equilibrated semen (P<0.001).

In our previous works we established high percentage of dead and abnormal spermatozoa in unfrozen semen (Gerzilov et al. 2009; Kazachka et al., 2009). The morphological damages of sperm cells affected the membrane integrity, midpiece and mitochondrial helix mainly. Xia et al. (1988) established similar changes in rooster sperm. According to Maeda et al. (1984), the most radical change in rooster spermatozoa was the complete separation of acrosome from the apical part of the nucleus. In general, avian spermatozoa are more sensitive to the freezing/thawing process and fertility rates of cryopreserved poultry sperm are dramatically lower than any of the domestic mammalian species (Donoghue and Wishart, 2000; Long, 2006).
Conclusion

Comparatively highest sperm mobility was established at using 5% and 7% concentration of glycerol and 7% concentration of DMSO. Both HIA-1 and AU extenders are suitable as semen diluents. Cryopreservation of Muscovy semen caused damages in the morphological integrity of sperm cells.

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