ABSTRACTS

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Freezing of sperm from chilled epididymides of dogs using two extenders

Martins MIM1*, Justino RC1, Sant’Anna, MC1, Trautwein LG1, Souza FF2

1Departamento de Clínicas Veterinárias, UEL, Campus Universitário – Caixa Postal 6001 – Postal Code 86051-990 – Londrina, PR, Brasil. 2Hospital Veterinário, Universidade de Franca – UNIFRAN, Av. Dr. Armando Sales de Oliveira, 201, Postal Code 14404-600 - Franca, SP, Brasil.

*imartins@uel.br

OBJECTIVES AND METHODS. During the cryopreservation process, sperm cell is exposed to many stressors, which may be linked to thermal shock during cooling of the semen, the formation of intracellular ice crystals, stress and action on the addition of cryoprotectant or osmotic shock during freezing and thawing (1). The aim of this study was to evaluate the effect on sperm viability using two different extenders, a commercial bovine extender (Bovimix®, Nutricell Ltda, Campinas, São Paulo, Brazil) and the Tris extender cited by Peña and Linde-Forsberg (2) and modified by Martins (3) to freeze the epididymal sperm.

Epididymides were obtained from 13 adult dogs by elective orchiectomy. After surgery, the testicles/epididymides were kept at 5°C for 24 hours in saline solution. The tail of epididymis was dissected and squeezed toward the vas deferens with a clamp into a Petri dish containing ringer without lactate. Immediately after the collection, the semen from all dogs was pooled and evaluated to spermatic motility and sperm concentration. The semen pooled were divided in 12 samples and centrifuged at 800xg for 10 minutes. Supernatant was removed and the pellet was diluted in one step, with two extenders (Bovimix® and Tris). The samples were packed in 0.5 mL French straws containing 80x10⁶ sperm/straw, at room temperature. Then, straws were equilibrated at 5°C, during 1 h, and frozen in nitrogen vapor for 20 min; thus stored at –196°C. Thus, they were stored in cryogenic container. The straws were thawed at 56°C for 10 sec. Thawed semen samples were evaluated for motility by CASA (Computer Assisted Sperm Analyzer, Hamilton-Thorne IVOS, Beverly, MA, USA). The fresh sperm samples that showed mean of 80% motility and 85% of membrane integrity was frozen. The sperm parameters from two treatments were tested to normality by Shapiro-Wilk test. The parametric variables were analyzed by the one way analysis of variance and non-parametric were by Kruskal-Wallis one way analysis of variance on ranks. The tests were followed by Student-Newman-Keul’s method. The level of significance was set at $p \leq 0.05$.

RESULTS. After thawing, the results ± SD obtained in samples frozen with Bovimix® versus Tris extenders are described in the Table 1 and 2.

Table 1. Mean ± SD of velocity parameters sperm canine epididymides evaluated with CASA system, after dilution with two extenders, freezing and thawing.

<table>
<thead>
<tr>
<th>Extenders</th>
<th>VAP (μm/s)</th>
<th>VSL (μm/s)</th>
<th>VCL (μm/s)</th>
<th>ALH (μm/s)</th>
<th>BCF (μm/s)</th>
<th>STR (μm/s)</th>
<th>LIN (μm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovimix®</td>
<td>88.1± 2.2</td>
<td>73.6 ± 2.9</td>
<td>123.8±2.6</td>
<td>5.6 ± 0.3</td>
<td>12.8 ± 1.1</td>
<td>83.2 ± 1.0</td>
<td>62.2 ± 3.0</td>
</tr>
<tr>
<td>Tris</td>
<td>80.1± 8.9</td>
<td>65.6 ± 7.4</td>
<td>121.6±12.4</td>
<td>6.5 ± 0.3</td>
<td>15.4 ± 1.8</td>
<td>81.5 ± 1.3</td>
<td>56.0 ±2.9</td>
</tr>
</tbody>
</table>

The results ± SD obtained in samples frozen with Bovimix® versus Tris were: sperm velocity index 248.2 ± 3.1 versus 233.8 ± 23.9 and sperm movement index 268.4 ± 8.6 versus 248.8 ± 11.5.

Table 2. Mean ± standard deviation of morphology parameters from canine epididymal sperm, fresh and frozen/thawed with Bovimix® and Tris.

<table>
<thead>
<tr>
<th>Extenders</th>
<th>Minor defects</th>
<th>Major defects</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>27.0 ± 8.5</td>
<td>38.0 ± 8.5</td>
<td>35.0 ± 0.0</td>
</tr>
<tr>
<td>Bovimix®</td>
<td>9.0 ± 4.2</td>
<td>81.2 ± 5.9</td>
<td>9.8 ± 2.6</td>
</tr>
</tbody>
</table>

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The sperm movement index (268.4 vs 248.8, \( P = 0.035 \)), LIN (62.3 vs 56.0, \( P = 0.025 \)), total motility (69.3 vs 54.5, \( P < 0.001 \)), progressive motility (40.5 vs 24.0, \( P = 0.029 \)) and rapid sperm (50.0 vs 30.8, \( P = 0.029 \)) were increased to Bovimix® than Tris. In contrast, ALH (5.7 vs 6.5, \( P = 0.006 \)), slow sperm (50.0 vs 30.8, \( P = 0.010 \)) and static sperm (2.5 vs 8.5, \( P = 0.029 \)) were decreased to Bovimix® than Tris.

CONCLUSION. We concluded Bovimix®, an extender development to freezing bovine semen, can be used to epididymal sperm of dog.


(2) Peña A, Linde-Forsberg C. Effects of equex, one or two step dilution, and two freezing and thawing rates on post-thaw survival of dog spermatozoa. Theriogenology, 2000; 54:859-75.
