ABSTRACTS

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CANINE CHILLED SEMEN: INFLUENCE OF LATEX AND AIR

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Introduction - Preservation of liquid semen at 5°C is an important technique in the breeding management of dogs. The aim of this kind of semen storage is to preserve gametes at low temperatures without reaching the freezing point. In fact, the longer the fertility potential of cooled semen can be extended, the easier it should be for breeders to time the insemination and transport the AI doses. But, oxidative damage to spermatozoa during storage is a potential cause of the decline in motility and fertility during hypothermic storage of liquid semen. The aim of this experiment was to evaluate the storage effect of canine chilled semen on syringes with and without latex and with and without air column.

Materials and methods - Ten healthy and sexually mature dogs between 2.5 and 8 years old and different breeds were used in this study, all of them have proven fertility. Two ejaculates were obtained with a 45min interval (4), from each dog by digital manipulation. Ejaculates were analyzed to determine its semen concentration, total number of spermatoza, sperm motility and morphology, so that adequate semen quality was secured. Only ejaculates with total number of spermatoza >400 x 10^7 spetz, sperm motility >70% and < 30% of total pathology, were included in this study. Sperm concentration was determined using a Neubauer haemocytometer. Skim milk extender described by Kenney (8) was used to dilute the samples of semen to be chilled. This extender was prepared once and then it was frozen on -70°C and thawed before every trial.

After initial analyses, semen was extended up to 16mL, and than it was divided into four groups, 4mL each. They were storage in: syringes without latex, with (G1) and without (G2) air column; syringe with latex, with (G3) and without (G4) air column. Each group was divided into 4 aliquots, 1mL each. One aliquot of each group was warmed at 32°C for 10 minutes after 6, 24, 48 and 72 hours of cooling, and then it was analyzed for sperm motility and vigor, as described by (5), plasma membrane integrity, as described by (2), acrosomal status as described by (8). Results were analyzed by ANOVA and tukey test and orthogonal contrast using Genes program.

Results

Table 1: mean results found to sperm motility and vigor at all moments that were evaluate.

<table>
<thead>
<tr>
<th>Sperm motility (%)1,2</th>
<th>Sperm vigor (0-5)1,2</th>
</tr>
</thead>
<tbody>
<tr>
<td>6h</td>
<td>24h</td>
</tr>
<tr>
<td>6h</td>
<td>24h</td>
</tr>
<tr>
<td>G1 86aA</td>
<td>74aA</td>
</tr>
<tr>
<td>G2 86aA</td>
<td>76abA</td>
</tr>
<tr>
<td>G3 68aB</td>
<td>34bB</td>
</tr>
<tr>
<td>G4 68aB</td>
<td>39.5bB</td>
</tr>
</tbody>
</table>

1 Different small letters on the same line means statistic difference (p= 0.05)
2 Different capital letters on the same column means statistic difference (p= 0.05)
Table 2: mean values to acrossome status and sperm morphology

<table>
<thead>
<tr>
<th></th>
<th>Acrossome intact (%)</th>
<th>Total sperm defect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6h 24h 48h 72h</td>
<td>6h 24h 48h 72h</td>
</tr>
<tr>
<td>G1</td>
<td>78.6abA 63.2bA 74.5abA 84.3aA</td>
<td>3.9aA 5.3aA 6.1aA 8aA</td>
</tr>
<tr>
<td>G2</td>
<td>43.5bB 50.3aAB 61.3AB 43.5bB</td>
<td>8.6aA 15.3aB 9.5aA 14.9aB</td>
</tr>
<tr>
<td>G3</td>
<td>41.7abB 41abB 49.1aBC 42.1abB</td>
<td>7.5aA 11.7aAB 9.3aA 10.6aAB</td>
</tr>
<tr>
<td>G4</td>
<td>23.3abC 14.6aC 39.2cC 31bcB</td>
<td>9.3aA 17.4bB 9.3aA 23bC</td>
</tr>
</tbody>
</table>

1 Different small letters on the same line means statistic difference (p= 0.05)
2 Different capital letters on the same column means statistic difference (p= 0.05)

No difference between groups was found on membrane integrity.
On orthogonal contrast it was found a difference between groups with and without latex (p>0.001) but no difference was found between groups with and without air column.

Discussion - It has been described that if the semen is going to be transported on syringes the piston shouldn’t have latex, because it can be lethal to spermatozoa (1). On experiment using canine semen, it was observed that with only 2 minutes, more than a half of spermatozoa lost their motility (4). But on the experiment that we made, it was observed that that if the semen is properly extended and cooled, sperm on latex syringe can last for at least 6 hours. It has been described that when human spermatozoa is exposed to an air concentration of only 5%, fertility characteristics is better preserved than with 20% (6). They related that with a bigger formation of reactive oxygen species (ROS) that can kill sperm. Our results shows that latex is more toxic to spermatozoa than ROS, since it wasn’t found difference between groups with and without air column. More than that, it was observed that G1 had better results than the others, showing that sperm need some extra oxygen during storage. Based on that, it can be concluded that for canine chilled transportation up to 72 hours the best way to do it is on syringe without latex and with 1cm of air column.

References