ABSTRACTS

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Cryopreservation of canine semen contaminated with blood

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OBJECTIVES AND METHODS: Haematospermia in dogs usually occurs secondary to benign prostatic hyperplasia (BPH) or trauma of the penis during semen collection. It is already known that the presence of blood is detrimental to cryopreservation of spermatozoa (1). However, cryopreservation cannot always be postponed to the time after treatment of a dog affected with BPH. Moreover, a number of conservative procedures to treat BPH are harmful to sperm quality with a consequent negative impact on suitability of semen for freezing. Selection methods have been already used in dogs with acceptable results to separate motile, viable and morphologically normal spermatozoa and to remove blood, bacteria, debris and pus (3). To our knowledge, studies on the effect of selection methods on dog sperm cryosurvivability have not yet been reported.

The aim of our study was to ascertain whether separation of erythrocytes can improve the outcome of semen cryopreservation from a dog with haematospermia and to find a convenient method of separation.

An ejaculate from a 10-year-old American Pit Bull Terrier with no clinical signs of BPH was collected and analyzed. Sperm and red blood cell (RBC) counts were determined using a Bürker chamber. Sperm motility was evaluated under a light microscope at 200x magnification, sperm morphology in phase contrast by evaluation of sperm fixed with formalin, at 1000x magnification, using immersion oil. Acrosomal integrity was determined by Pisum sativum agglutinin (PSA) conjugated with FITC. Even the spermatic fraction was brownish-pink and the concentration of RBC was 100 x 10^6/ml which corresponds approximately to 2% of RBC concentration in the blood. The ejaculate was divided into 4 aliquots. One was immediately frozen acc. to the Uppsala Equex-2 System (2) and three remaining aliquots were subjected to the following separation methods: swim-up and gradient centrifugation with two separation media [40/80 PureSperm® and 40/80 CaniPure® (both Nidacon International AB, Sweden)]. Separated semen samples were analyzed and cryopreserved. All semen samples were frozen at a concentration of 100 x 10^6 spermatozoa/ml. Total and motile sperm and erythrocyte yields after separation were calculated as the number of cells after separation/number of cells before separation x 100%.

RESULTS: Total and motile sperm and RBC yields were 54%, 47.2% and 51.4% in semen samples after swim-up; 46.5%, 45.9% and 1.7% in PureSperm® pellet; 42.5%, 42.5% and 4.9% in CaniPure® pellet. The quality of fresh semen, samples after separation and frozen/thawed sperm is shown in Table 1. Better survival ability during 24 h after thawing was shown in sperm treated with gradient separation methods than untreated or sperm after swim-up (Fig. 1).

Table 1: The effect of different separation methods upon sperm motility, morphology and acrosomal integrity of fresh and frozen/thawed canine spermatozoa.

<table>
<thead>
<tr>
<th></th>
<th>Untreated semen</th>
<th>Swim-up</th>
<th>PureSperm</th>
<th>CaniPure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility (progressive) %</td>
<td>80 (77)</td>
<td>50 (40)</td>
<td>70 (65)</td>
<td>60 (50)</td>
</tr>
<tr>
<td>Morphologically normal sperm %</td>
<td>62.5</td>
<td>44</td>
<td>69</td>
<td>40.5</td>
</tr>
<tr>
<td>AI %</td>
<td>89.5</td>
<td>72.5</td>
<td>89</td>
<td>62</td>
</tr>
</tbody>
</table>

FT: frozen/thawed; AS: after separation; AI: acrosome intact sperm.
CONCLUSION: In comparison with the swim-up technique, gradient centrifugation provides significantly more efficient separation of RBC from ejaculates. Moreover, it allows the elimination of bacterial contamination which is often connected to prostate disorders. According to this preliminary study, the use of gradient separation seems to be a suitable solution for freezing of blood contaminated semen. Sperm frozen after removal of RBC by gradient centrifugation showed a higher quality than spermatozoa from untreated ejaculates or after swim-up. On the other hand, in the case of a lower amount of blood in semen, what should be considered is whether or not separation methods should be used, due to a high loss of spermatozoa during the procedure.


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