Cryopreservation of Semen from Japanese White Rabbits for Use in Teratological Studies

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Abstract: Cryopreservation of semen from Japanese White rabbits was examined to reduce the number of their males for use in teratological studies. Semen was frozen with liquid nitrogen, preserved, thawed, and tested for motility according to the method of Chen et al. Even after cryopreservation an average of 52% of the thawed sperms were motile. In a previous study, frozen-thawed sperms with a motility of 40% resulted in a high conception rate (approximately 88%) on artificial insemination when New Zealand White rabbits were used [5]. These results indicate the possibility that cryopreservation of semen from Japanese White rabbits may be used in teratological studies to reduce the number of males.

Key words: cryopreservation, rabbit, semen

In teratological studies in rabbits, particularly in the safety evaluation of drugs, the use of a large number of males is unavoidable. Recently Kaneda et al. described the usefulness of an artificial insemination technique in teratological studies to reduce the number of males [6]. A combination of this technique with frozen semen would provide a number of advantages, including a reduction in the number of males, stabilization of semen supply, and labor-saving in artificial insemination. Weitze et al. have reported that the fertility rate of frozen spermatozoa was almost the same as that of fresh sperm when crossbred rabbits were used [8], but although sperm motility after freezing and thawing differed among strains [7], that of Japanese White rabbits, the strain generally used in teratological studies in Japan, has not yet been confirmed. In the present study sperm motility of cryopreserved semen from Japanese White rabbits was examined in an effort to reduce the number of males in a teratological study.

Male Japanese White rabbits (Kbl: JW, SPF) purchased from KITAYAMA LABES Co., Ltd., NAGANO were used at the age of 30 weeks. Semen was collected two times per week by means of an artificial vagina [2]. Gel was removed from the ejaculate and the percentage of motile spermatozoa was estimated at 37°C. If the motility of the collected semen exceeded 80% it was extended six times with an egg yolk-acetamide extender [1, 4, 5]. Sperms in extended semen were frozen in vapour of liquid nitrogen, preserved, thawed, and tested for motility according to the method of Chen et al. [3].

The motility of the frozen-thawed sperms is shown.

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in Table 1. Even after cryopreservation more than 52% of thawed sperms were motile.

Hanada et al. previously found a similar motility with sperms from New Zealand White rabbits under practically the same conditions except that dry-ice was used in place of the liquid nitrogen. In addition, they also found that New Zealand White rabbit sperms with a motility of 40% had a high conception rate (approximately 88%) on artificial insemination [5]. The present study and previous findings therefore indicate the possibility that frozen sperm of Japanese White rabbits is sufficiently fertile. Further studies are needed to elucidate the conception rate. The use of cryopreservation of semen from Japanese White rabbits in teratological studies would help to reduce the number of males, stabilize semen supply and save labor in artificial insemination.

Table 1. Motility of frozen-thawed sperms from Japanese White rabbits

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>No. of Animals</th>
<th>Motility (%) a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>50.0 ± 9</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>50.1 ± 5.66</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>51.9 ± 5.65</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>54.7 ± 1.38</td>
</tr>
<tr>
<td>Overall mean</td>
<td></td>
<td>52.2 ± 3.86</td>
</tr>
</tbody>
</table>

a Values are the mean ± SD. Number of ejaculates pooled in each experiment was 2, 2, 3 and 3, respectively.

References