Abstract. The present study was conducted to investigate fertility of estrus-induced ewes during the non-breeding season, artificially inseminated with frozen semen imported from New Zealand. A total of 122 Suffolk and Suffolk-crossed ewes in three different sheep farms (T, S, M) were treated with an intravaginal progesterone release device (CIDR-G) for 12 days, and an intramuscular injection of 500 IU equine chorionic gonadotropin one day before CIDR removal. Effects of intrauterine insemination doses (0.2 and 0.4 ml per head) and two rams on fertility were compared at Farm T using 75 ewes. At the other two farms (S and M), 0.1 to 0.2 ml doses were inseminated into the uteri on the fixed-time basis. The lambing rates of ewes inseminated with 0.2 and 0.4 ml doses were 68.4 and 59.5%, and the lambing rates of ewes inseminated with the two rams were 61.3 and 65.9%. No significant differences were found between any of the factors. The lambing rates on Farms S and M were 54.2% and 47.8%, respectively. Overall, there was no significant difference in the lambing rates (58.3% and 59.5%) and prolificacy (2.03 and 1.61) between the two rams. These results indicate that the fertility of ewes inseminated during the non-breeding season with frozen-thawed semen imported from New Zealand is acceptable and that new blood lines of Suffolk sires and dams can be produced on Japanese sheep farms.

Key words: Frozen semen, Insemination, Insemination dose, CIDR, Sheep

Cryopreservation technology for ram spermatozoa has made considerable progress toward practical and economic application of artificial insemination (AI) under field conditions. Especially, the techniques for intrauterine AI using frozen-thawed spermatozoa has been developed during the last two decades [1–3]. Fertility after intrauterine AI has varied depending on many factors such as types of progestogen treatment, timing of AI, insemination doses, semen sources from different rams, and different sheep flocks. For intrauterine AI, the optimal insemination dose has been decreasing to 20–50 × 10^6 motile spermatozoa per inseminated ewe [4]. A low number of spermatozoa per insemination dose could utilize genetically high value ram semen at the maximum.

For this study, the authors imported frozen ram semen from New Zealand. It was the first importation of frozen ram semen to Japan from another country, and the fertility of those imported semen needs to be investigated before field application. Two hundred 0.25 ml straws from two rams were used for the present study on three sheep farms. At one of the farms, effects of...
insemination doses (0.2 and 0.4 ml) and the two rams were compared on fertility (numbers of pregnant and lambed ewes per inseminated ewes) and prolificacy. The difference at the other two sheep farms using the two rams was also investigated.

**Materials and Methods**

**Animals**

The present study was conducted at three sheep farms (T, S, M) located in Hokkaido, Japan during June to July, 2001. A total of 122 mature (3- to 6-year old) Suffolk and Suffolk-crossed ewes were used.

**Treatment**

Seventy-five, 24, and 23 ewes at Farms T, S, and M, respectively were treated with an intravaginal progesterone release device (CIDR-G: Inter Ag, Te Rapa Road, Hamilton, New Zealand) containing 0.3 g progesterone per device. CIDRs were inserted into vagina for 12 days and an intramuscular injection of 500 IU equine chorionic gonadotropin (eCG: Serotropin: Teikoku-zoki Co., Tokyo, Japan) was administered to the ewes one day before the removal of CIDR.

**Insemination**

The frozen ram semen used in this study was imported on February 6th, 2001 from New Zealand (AgTech, Hamilton, New Zealand). The freezing procedures are described in the report of Vivanco and Alarcon [5]. Semen collected by artificial vagina from 2 two-year old Suffolk rams were diluted with a TRIS (hydroxymethyl aminomethane)-based diluent containing 20% egg-yolk, and 6.4% glycerol. The diluted semen was frozen in 0.25 ml straws. Before insemination on each farm, the frozen straws were thawed at 37 C, and the percentage of motile spermatozoa was determined as acceptable. The numbers of motile spermatozoa in each straw from the two rams (A and B) were 18 × 10^6 and 19 × 10^6, respectively. The thawed semen from each ram was separately expelled into a 10 ml glass test tube in a 37 C water bath. At Farm T, the effects of insemination dosage (0.2 and 0.4 ml) and the two rams on fertility after AI were investigated. The numbers of motile spermatozoa in the two insemination doses (0.2 and 0.4 ml) were 14.4–15.2 × 10^6 and 28.8–30.4 × 10^6, respectively. At the other two farms (S and M), 0.1–0.2 ml of the thawed semen from rams A and B were used for AI. The ewes on Farms S and M were examined before AI and given a body condition score (1=emaciated to 5=fat).

AI at each sheep farm was performed by the fixed-time intruterine insemination method for all ewes 44–52 h after CIDR removal, regardless of estrus incidence. Half the volume of each insemination dose was deposited into each uterine horn using an insemination pipette (No. 20887: I.M.V., France) with the aid of a laparoscope.

Sixty days after AI, pregnancy was diagnosed by a real-time ultrasonic scan on Farm T. The lambing rate (number of ewes lambed/number of ewes inseminated) and prolificacy (number of lambs born/number of ewes lambed) were recorded at all farms.

**Statistical analysis**

Data of pregnancy (number of pregnant ewes/number of ewes inseminated) and lambing rate, and prolificacy in ewes inseminated at Farm T were analyzed by analysis of variance using the general liner model procedure of SAS (Statistical Analysis System). Differences were also analyzed by Tukey’s ω-procedure [6]. For the data from Farms S and M, the lambing rates and prolificacy were compared between rams A and B, and the body condition scores (mean ± SEM: units) were also compared for the ewes between Farms S and M.

**Results**

As shown in Table 1, the frozen semen from the two rams made no significant difference to the rates of pregnancy and lambing (61.3 and 65.9%) and prolificacy (2.11 and 1.72). The lower insemination dose (0.2 ml per head) resulted in higher rates of pregnancy and lambing (68.4%: 26/38) than the higher insemination dose (0.4 ml per head: 59.5%, 22/37), but there was no significant difference in fertility and prolificacy between the two different insemination doses.

The fertility and prolificacy of ewes inseminated with the frozen semen from the two rams were not significantly different between Farms S and M (Table 2). In the comparison of the overall fertility
and prolificacy of the inseminated ewes among Farms T, S and M, Farm T had a higher lambing rate (64.0%) and prolificacy (1.88) than those in Farm S (54.2% and 1.38) and M (47.8% and 1.82). However, no significant difference was found in either the lambing rate or the prolificacy among the three farms. The mean body condition scores in the ewes at Farms S and M were 3.5 ± 0.8 (n=24) and 2.4 ± 0.3 (n=23), respectively, and the scores were not significantly different between the two farms.

**Discussion**

The present study shows that the frozen ram semen imported from New Zealand resulted in acceptable fertility (overall 59% in lambing rate) among estrus-induced ewes artificially inseminated by the fixed-time basis during the non-breeding season. The lambing rate was similar to our previous studies [2, 7]. Although the fertility of inseminated ewes on the 3 farms were not directly comparable due to the difference in the insemination doses between Farm T (0.2 or 0.4 ml) and the other two (S and M) farms (0.1–0.2 ml), the lambing rates on the 3 farms (48 to 64%) were not significantly different. The fertility of inseminated or naturally mated ewes may be influenced by the body condition and physiological status of each ewe, and the different management feeding systems at each farm [8, 9]. The mean body condition score in ewes at Farm S (3.5 units) was higher than that at Farm M (2.4 units) probably due to the short period from the previous lambing, but the score difference was not significantly different and did not affect the lambing rates of the ewes between Farms S and M.

In the trial at Farm T, the effects of two insemination doses and two rams on the fertility were investigated, and no effects were found. A higher insemination dose (0.4 ml per head) had no advantage for pregnancy and lambing rates compared with a lower insemination dose (0.2 ml per head), which tended to show a high lambing rate (59.5 and 68.4%). For intrauterine insemination in ewes, a semen dose of 20 to 50×10⁶ spermatozoa

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**Table 1.** Fertility of ewes inseminated with different insemination doses and rams on Farm T

<table>
<thead>
<tr>
<th>Ram</th>
<th>Insemination doses/head (ml)</th>
<th>No. of ewes</th>
<th>Prolificacy</th>
<th>Inseminated</th>
<th>Pregnant</th>
<th>Lambed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.2</td>
<td>16</td>
<td>10 (62.5)</td>
<td>10 (62.5)</td>
<td>2.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>15</td>
<td>9 (60.0)</td>
<td>9 (60.0)</td>
<td>2.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sub-total</td>
<td>31</td>
<td>19 (61.3)</td>
<td>19 (61.3)</td>
<td>2.11</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Fertility of ewes inseminated with frozen-thawed semen on two different farms

<table>
<thead>
<tr>
<th>Farm</th>
<th>Ram</th>
<th>No. of ewes</th>
<th>Prolificacy</th>
<th>Inseminated</th>
<th>Lambed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>6</td>
<td>4 (66.7)</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>B</td>
<td>18</td>
<td>9 (50.0)</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sub-total</td>
<td>24</td>
<td>13 (54.2)</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td>11</td>
<td>5 (45.5)</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>B</td>
<td>12</td>
<td>6 (50.0)</td>
<td>1.67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sub-total</td>
<td>23</td>
<td>11 (47.8)</td>
<td>1.82</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>47</td>
<td>24 (51.1)</td>
<td>1.58</td>
<td></td>
</tr>
</tbody>
</table>

*1 Diagnosed by a real-time ultrasonic scan 60 days after insemination. *2 Number of lambs born/number of ewes lambed.
per ewe has provided satisfactory lambing rates (55–85%) [2–4, 10]. But, it should be noted that the number of spermatozoa per insemination dose does not indicate the number of motile spermatozoa. Ham and Brogliatti [11] reported that there was no significant difference in pregnancy rates among ewes inseminated with 10 to 45 × 10⁶ motile spermatozoa per insemination dose (58 to 66%). Our previous study [2] also showed no significant differences among the lambing rates of ewes inseminated with 0.4, 0.2 or 0.1 ml of insemination dose containing 100, 50 and 30 × 10⁶ motile spermatozoa, respectively. The present frozen ram semen imported from New Zealand had 18 to 19 × 10⁶ motile spermatozoa/0.25 ml straw and the post-thaw motility ranged from 40 to 50%. Therefore, the actual number of motile spermatozoa per insemination dose were 14 to 15 × 10⁶ and 29 to 30 × 10⁶ for 0.2 and 0.4 ml, respectively. The present study shows that a lower insemination dose containing 14 to 15 × 10⁶ motile spermatozoa per ewe resulted in an acceptable fertility (68% of lambing rate) in 38 ewes inseminated during the non-breeding season. Recently, a low-dose of insemination number of spermatozoa has been investigated to utilize the flow sorted, sexed mammalian spermatozoa for AI [11, 12].

In conclusion, the present results show for the first time the acceptable fertility of ewes inseminated with frozen semen imported from New Zealand during the non-breeding season. The newborn lambs could contribute to new blood lines of Suffolk flock in Japanese fields.

Acknowledgments

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References