Age-related Changes in Semen Quality of Captive Bharal (Pseudois nayaur)

Hiroshi KUSUNOKI1, Tomoya NISHIKAKU2, Daisuke NAKAGAWA2, Toyoharu TAKIDA2, Daisuke KURITA2, Koji UEMICHI2, Kaoru UEDA2, Tomoko OOE2, Kazuo OKUDA2, Satoshi KUSUDA3 and Osamu DOI3,4

1) Faculty of Agriculture, Kobe University, Rokkodai-cho 1-1, Nada-ku, Kobe 657-8501, Japan
2) Himeji Central Park, Kamiy-a-aza-Ookura 1436-1, Toyotomi, Himeji 679-2214, Japan
3) United Graduate School of Agricultural Science, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan
4) Faculty of Applied Biological Sciences, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan

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飼育下パーサール (Pseudois nayaur) における加齢に伴う精液性状の変化

ABSTRACT. Age-related changes of semen parameters from 1.5 to 5.7 years were evaluated in captive bharal (Pseudois nayaur). In 28 trials, 25 semen samples were collected using an artificial vagina method from two males during four breeding seasons between December 2001 and February 2005. One male bharal was able to ejaculate semen at the age only of 1.5 years, but its quality was quite inferior (semen volume: 0.24 ml, semen pH: 8.3, sperm concentration: 13.8 million/ml, sperm motility index: 0.0, sperm viability: 0.0% and morphologically normal sperm: 54.1%). As age increased, semen quality improved and totally stabilized by the age of 4.5 years. The final quality (semen volume: >1 ml, semen pH: 6.8, sperm concentration: 3,500-4,000 million/ml, sperm motility index: <80, sperm viability: >70% and morphologically normal sperm: >95%) was virtually equal to that reported in domestic sheep and goat.

Key words : artificial vagina, bharal, Pseudois nayaur, semen quality, sexual maturity

INTRODUCTION

The bharal (Pseudois nayaur), which belongs to the family Bovidae, with the alias "blue sheep," is apparently more closely related to goat [1]. Bharals inhabit the alpine zone in Central Asia [2], and at present are not in a critical situation in their habitat. However, the dwarf bharal (P. schaeferi), the other species which belongs to the same genus and inhabits the lower zone in the same area, is classified as endangered [3]. Therefore, it is not possible to consider the future of the bharal optimistically.

It is known that the bharal is a seasonal breeder with a mating season from October to January, and gives birth from May to early July [4]. However, little research has been conducted on the details of their reproductive characteristics. The objective of the present investigation was to collect natural ejaculates by the artificial vagina method, to analyze the semen characteristics and to evaluate male reproductive potential in the captive bharal.

MATERIALS AND METHODS

Two bharals (A and B) were used for semen collection. They were captive-born (birth date: A, June 2000; B, June 1999) and maintained at the Himeji Central Park, Himeji, Japan. Semen was collected with a warmed artificial vagina for ram and goat (200 mm in length and 44 mm in inner diameter, NFA260, Fujihira Industry Co., Ltd., Tokyo, Japan) using a teaser female during four breeding seasons. The first season was from December 2001 to January 2002 when males A and B were 1.5-1.6 and 2.5-2.6 years old, respectively. The second season was January 2003, the third from December 2003 to January 2004, and the fourth from December 2004 to February 2005.
Immediately after collection, the volume and pH of ejaculates, sperm concentration, motility, viability and morphology were evaluated according to methods described previously [5]. Briefly, semen pH was measured using a compact pH meter (Type B-212, Twin pH, Horiba, Ltd., Kyoto, Japan). Sperm concentration was determined with aid of a haemocytometer, and sperm motility (percentage of motile cells and their progressive speed) was subjectively examined using a bright-field light microscope (×400 magnification) equipped with a heated (39 °C) stage (slide warmer, Type-III, FA222, Fujihira Industry Co., Ltd.). The percentage of motile spermatozoa was estimated in increments of the nearest 5% unit. Progressive speed was measured on a scale of 0 to 4 (0, no motility; 1 side-to-side movement with no forward progression; 2, slow forward progression; 3, steady forward progression; 4, rapid forward progression). The sperm motility index was calculated using the following formula:

Sperm motility index = percentage of motile spermatozoa × (scale of progressive speed/4).

Sperm viability and morphology were determined by modified eosin-nigrosin (EN) staining [6]. Approximately 200 spermatozoa in randomly-chosen fields were counted with the bright-field light microscope at ×400 magnification to determine the percentage of live (non-eosinophilic) spermatozoa and morphologically normal cells (Fig. 1). Sperm morphological abnormality was classified with the standard of Barth and Oko [6].

All data are presented as the mean ± standard error of the mean (SEM). Percentage data were transformed using arcsin before analysis. Data were first analyzed by the F-test for homogeneity of variance with significant levels of 5%. If the test revealed homogeneity of variance, a comparison was made between the means using the Student’s t-test. In the case of heterogeneity of variance, a comparison was performed using the Aspin-Welch’s t-test. A two-tailed P value of <0.05 was considered statistically significant.

**RESULTS AND DISCUSSION**

The two male bharals used in this study were able to be trained to ejaculate into the artificial vagina without difficulty. The optimum temperature in the artificial vagina to elicit a complete ejaculation was 40-50°C, and both lower and higher temperatures were unsuitable. The same response to temperature has been also observed in domestic ram [7]. During the four breeding seasons, a total of 28 collections were attempted in these males, and succeeded 25 times (89.3%). Two of the three failures happened in the second season when they were 2.5 or 3.5 years old, and the last failure was during the fourth season when they were 4.6 or 5.6 years old. The cause of the unsuccessful ejaculation seems to be not age but a temporary decline of libido at the time of collection.

Age-related changes of bharal semen characteristics are summarized in Table 1. The ejaculates collected from the male aged 1.5 years were slightly alkaline, of small volume, and contained only a small number of dead spermatozoa. With age, the ejaculates were acidified, sperm abnormality decreased.

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**Fig. 1** Bharal spermatozoa stained with eosin nigrosin (original magnification ×1,000). Three eosinophilic spermatozoa (arrows) were dead and the other non-eosinophilic types alive. All were morphologically normal.
Bharal semen characteristics

Table 1  Age-related changes of semen characteristics from two bharals using artificial vagina method

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Male No of semen</th>
<th>Semen volume (ml)</th>
<th>Semen pH</th>
<th>Sperm concentration (million/ml)</th>
<th>Sperm motility index (%)</th>
<th>Live sperm (%)</th>
<th>Morphologically normal sperm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5-1.6</td>
<td>A 4</td>
<td>0.24 ± 0.06</td>
<td>8.3 ± 0.2</td>
<td>138 ± 38</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>54.1 ± 10.9</td>
</tr>
<tr>
<td></td>
<td>A 3</td>
<td>0.54 ± 0.20</td>
<td>7.1 ± 0.6</td>
<td>1585.2 ± 496.7</td>
<td>38.3 ± 19.2</td>
<td>66.4 ± 3.3</td>
<td>91.4 ± 3.6</td>
</tr>
<tr>
<td>2.5-2.6</td>
<td>B 4</td>
<td>0.75 ± 0.10</td>
<td>7.5 ± 0.1</td>
<td>2077.5 ± 684.5</td>
<td>37.5 ± 14.4</td>
<td>39.3 ± 13.8</td>
<td>93.8 ± 19.0</td>
</tr>
<tr>
<td></td>
<td>Total 7</td>
<td>0.66 ± 0.10</td>
<td>7.3 ± 0.2</td>
<td>1866.5 ± 423.1</td>
<td>37.9 ± 10.6</td>
<td>50.9 ± 9.3</td>
<td>92.8 ± 2.3</td>
</tr>
<tr>
<td>3.5-3.6</td>
<td>B 1</td>
<td>0.81</td>
<td>6.9</td>
<td>2192.0</td>
<td>60.0</td>
<td>58.2</td>
<td>94.1</td>
</tr>
<tr>
<td></td>
<td>Total 4</td>
<td>0.73 ± 0.25</td>
<td>6.8 ± 0.1</td>
<td>1690.7 ± 622.6</td>
<td>50.8 ± 22.8</td>
<td>61.2 ± 12.5</td>
<td>94.8 ± 2.7</td>
</tr>
<tr>
<td>4.5-4.6</td>
<td>B 5</td>
<td>1.22 ± 0.13</td>
<td>6.8 ± 0.0</td>
<td>4025.4 ± 946.3</td>
<td>76.3 ± 24.9</td>
<td>76.2 ± 28.0</td>
<td>95.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Total 8</td>
<td>1.26 ± 0.10</td>
<td>6.8 ± 0.0</td>
<td>3845.6 ± 615.6</td>
<td>77.1 ± 17.5</td>
<td>73.6 ± 26.0</td>
<td>95.5 ± 1.2</td>
</tr>
<tr>
<td>5.6-5.7</td>
<td>B 2</td>
<td>1.00 ± 0.00</td>
<td>6.8 ± 0.0</td>
<td>3896.0 ± 1304.0</td>
<td>65.0 ± 5.0</td>
<td>60.9 ± 58.2</td>
<td>97.8 ± 18.0</td>
</tr>
</tbody>
</table>

Values (mean ± SEM) within the same column with different superscripts (a-d) differ significantly, P<0.05.

The volume and sperm number increased, and sperm motility and viability improved. Finally, sperm morphology stabilized at over 2.5 years old. Semen pH and sperm viability were over 3.5 years old, and semen volume, sperm concentration and motility were over 4.5 years old.

In the bharal, it has been reported that sexual maturity is attained at 18 months [4]. In this study, a captive bharal was able to ejaculate semen at this age, but the quality was quite inferior. Half of the spermatozoa were morphologically abnormal (Table 1), and the majority (90.1 ± 2%) were detached sperm heads. In bulls, it is reported that testicular hypoplasia has been associated with a high incidence of this defect [8]. This supports the immaturity of the bharals at 1.5-1.6 years old. Their testes and accessory genital glands, especially the seminal vesicles, may have been yet undevloped. Semen quality improved with aging, and all parameters stabilized at over 4.5 years of age. Wegge [9] indicated that male bharals do not reach trophy size until they are 5-7 years old, and it is unlikely that they are able to mate under natural conditions prior to those ages. Our result confirmed this observation. Finally, the quality of bharal semen reached to virtually the same level as those reported in domestic sheep and goat [10, 11]. This indicates that the male reproductive potential in the bharal is comparitively high.

In the artificial vagina method, the male requires training and there always is the danger of injury to the animal handler. However, compared with electroejaculation, this technique is a natural approach and can repeatedly obtain stable quality semen. This technique has been executed in various herbivores, including Grant’s zebra (Equus burchelli boehmi) [12], alpaca (Lama pacos) [13], llama (Lama glama) [13], bactrian camel (Camelus bactrianus) [14], Arabian camel (Camelus dromedarius) [15], red deer (Cervus elaphus) [16] and European mouflon (Ovis gmelini musimon) [17]. In the bharal, although there is an unofficial brief report of an attempt at semen collection using this method in the Kanazawa Zoological Garden, Yokohama, Japan [personal communication], this is the first paper examining this method in detail.

In Japan, bharals are now kept in only three facilities: Himeji Central Park, Kanazawa Zoological Garden and Kyoto Municipal Zoo. To maintain genetic diversity in these captive zoo populations, it is indispensable to adopt artificial reproductive technologies such as sperm cryopreservation and artificial insemination. The present result makes a promising contribution to the promotion of this practice.

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要 約

1.5歳から5.5歳までの4年間の加齢に伴うバーラル（Pseudois nayaur）の精液性状の変化を調べた。精液は、2001年12月から2005年2月までの間の4回の繁殖期に、2頭の雄から人工巣で採取した。28回中25回で精液が採取できた。得られた精液の性状は、1.5歳時ではかなり劣悪であったが（精液量：0.24ml、精液pH：8.3、精子濃度：138百万/ml、精子運動指数：0.0、生存精子率：0%、形態異常精子率：54.1%）、加齢に伴って向上し、4.5歳以上で概ね安定し、その時点での性状は（精液量：＞1ml、精液pH：6.8、精子濃度：3500～4000百万/ml、精子運動指数：＞80、生存精子率：＞70%、形態異常精子率：＞95%）、家畜のヤギやヒツジに匹敵する程良質であった。
REFERENCES

7. Rodin II. 1940. The influence of thermal and mechanical stimuli on ejaculation into the artificial vagina in rams. Trud Lab iskusst Osemen Zivoti 1: 223.