Fertility of Ewes Inseminated Intrauterinally with Frozen Semen Using Extender Containing Bovine Serum Albumin

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Abstract. The present study was conducted to examine the fertility of ewes inseminated intrauterinally with frozen semen using semen extender containing either egg yolk or bovine serum albumin (BSA). Sixty Suffolk and cross-bred ewes were treated with controlled internal drug release (CIDR) devices during the non-breeding season (July 2006). A CIDR was inserted into the vagina for 12 days and an intramuscular injection of 500 IU equine chorionic gonadotropin was administered one day before its removal. Ejaculates from a suffolk ram were diluted with a Tris-based extender containing either 15% (v/v) egg yolk or 10% (w/v) BSA, and the diluted semen was frozen in 0.25 ml straws. A fixed-time intrauterine artificial insemination (AI) was performed 43–47 h after CIDR removal, regardless of incidence of estrus. There was no significant difference in pregnancy rates at 60 days after AI between the extenders containing egg yolk (66.7%, 20/30 animals) or BSA (65.5%, 19/29 animals). Furthermore, there were no significant differences in the lambing rates (66.7% and 62.1%) and prolificacy (1.25 and 1.56) between the two semen extenders. The present study indicates that a semi-defined semen extender containing 10% BSA produces fertility after intrauterine AI that is similar to that achieved with semen extender containing egg yolk.

Key words: Artificial insemination, Bovine serum albumin, Frozen semen, Semen extender, Sheep

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Most semen extenders for freezing of spermatozoa, including ram semen contain egg yolk, milk, or a combination of the two as a basic ingredient. Although egg yolk is beneficial for sperm cryopreservation because it protects against cold shock [1], there are several disadvantages of addition of egg yolk to semen extender. Preparation of uniform semen extenders containing egg yolk is difficult because individual egg yolk quality may vary depending on the number of days after laying and the storage period. Furthermore, addition of egg yolk reduces the acrosome integrity of goat spermatozoa [2], and high egg yolk concentrations reduce the post-thawing viability of ejaculated spermatozoa in several species, such as goats [3], rams [4] and water buffaloes [5]. Finally, development of a chemically defined semen extender would be required in order to eliminate possible infectious origins from egg yolk added to the semen extender. Several investigations have been conducted for development of semen extenders that do not contain egg yolk, such as a defined extender containing soybean lecithin for bovine and wild gazelle semen [6–10], and bovine serum albumin (BSA) has also been used as a substitute of egg yolk for rainbow trout and turkey spermatozoa [11, 12].
Our previous study [13] showed that 10% or 15% BSA could be substituted for egg yolk in a semen extender for freezing of ram spermatozoa. Therefore, in the present study, we examined the fertility of ewes inseminated intrauterinally with frozen semen extender containing 10% BSA in comparison with an extender containing 15% egg yolk.

Materials and Methods

Animals
The present study was conducted at the Shibecya Sheep Farm in Hokkaido, Japan during the non-breeding season (July 2006). A total of 60 mature (2 to 9 years old) Suffolk and Suffolk × Southdown ewes were used. The ewes were fed 3 kg/day of hay (mainly orchards), supplemented with 300 g/day of concentrates (13% crude protein and 76% total digestible nutrients) and were provided with free access to fresh water and mineral blocks throughout the study.

Treatment
All 60 ewes 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. A CIDR was inserted into the vagina for 12 days, and an intramuscular injection of 500 IU equine chorionic gonadotropin (eCG, Serotropin; Teikoku Zoki, Tokyo, Japan) was administered one day before its removal.

Chemical reagents
All chemical reagents for preparation of semen extenders were of the highest purity commercially available. Tris-hydroxymethyl aminomethane (Tris) was purchased from Merck (Darmstadt, Germany). Fructose and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). Citric acid and glycerol were purchased from Wako (Osaka, Japan).

Artificial insemination (AI)
Semen was collected from a Suffolk ram (3 years old) using an artificial vagina and was diluted (semen : extender=1:4) in a water bath (30 C) with one of the two Tris-based extenders containing 297.58 mM Tris, 96.32 mM citric acid, 82.66 mM fructose, 5% (v/v) glycerol, and either 15% (v/v) egg yolk [14] or 10% (w/v) BSA [13]. The diluted semen was gradually cooled to 4 C for 2–3 h. The diluted semen was frozen in 0.25 ml straws according to the methods described previously [13]. In brief, the semen samples were packed in straws and kept at 4 C before freezing. They were then exposed to liquid nitrogen (LN2) vapor (−125 to −130 C) for 3–4 min, plunged into LN2 (−196 C), and stored in LN2 until AI.

Before AI, the frozen straws was thawed at 37 C and the motility of the spermatozoa in each straw was evaluated. The straws with a percentage of motile spermatozoa of approximately 50% or more were used for AI. The CIDR was expelled from one of the 60 ewes during the insertion period, and the ewe was excluded from the present study. A fixed-time intrauterine insemination was performed for the remaining 59 ewes (30 and 29 ewes for the semen extender containing egg yolk and BSA, respectively) 43–47 h after removal of CIDR [15–19], regardless of the incidence of estrus. In regard to the insemination dosage (0.2 to 0.3 ml per ewe), the numbers of spermatozoa per ewe ranged from 50 to 100 × 10^6 and the number of motile spermatozoa per ewe was approximately half that (25 to 50 × 10^6 per ewe). Half the volume of each insemination dose was deposited into each uterine horn using an insemination pipette (No. 20887: I.M.V., Rue Clémenceau, France) with the aid of a laparoscope.

Pregnancy was diagnosed 60 days after AI by real-time ultrasonic scan. Lambing rate (number of ewes lambed/number of ewes inseminated) and prolificacy (number of lambs born/number of ewes lambed) were recorded.

Statistical analysis
The pregnancy (number of pregnant ewes/number of ewes inseminated) and lambing (number of lambed ewes/number of ewes inseminated) rates of the inseminated ewes were analyzed using the chi-square test. Prolificacy (number of lambs born/number of ewes lambed × 100) was compared using Student’s t-test. A value of P<0.05 was chosen as an indication of significance.

Results
As shown in Table 1, there was no significant
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difference ($X^2=0.03$) in pregnancy rates 60 days after AI between the extenders containing egg yolk (66.7%, 20/30 animals) or BSA (65.5%, 19/29 animals). Furthermore, there were no significant difference in lambing rates (66.7% and 62.1%, $X^2=0.01$, for semen extenders containing egg yolk or BSA, respectively) and prolificacy (1.25 ± 0.10 and 1.56 ± 0.12, $t=1.97$) were between the two semen extenders.

Discussion

The present field study demonstrated that a semen extender containing BSA without egg yolk results in acceptable fertility that is similar to that achieved with extender containing egg yolk. To the best of our knowledge, this is the first study of sheep AI using a semi-defined semen extender containing BSA.

Egg yolk is generally accepted to be an effective agent in semen extenders for protection of spermatozoa against cold shock and the lipid-phase transition effect [1]. However, it is difficult to produce semen extenders consistent with quality standards because of the individual quality differences inherent in egg yolk. Therefore, it seems that removal of chicken egg yolk from semen extenders would produce several advantages, such as improved consistency in the components of semen extenders and elimination of hygienic risks. For these reasons, it is necessary to develop a synthetic semen extender free of egg yolk [6–9]. Bovine serum albumin (BSA) is one of proteins available for replacement of egg yolk [11, 12]. Matsuoka et al. [13] reported that the rates of post-thaw sperm progressive motility were significantly higher in 10% and 15% BSA than in control extender (Tris-fructose-egg yolk) and concluded that 10 or 15% BSA could be substituted in place of egg yolk in a semi-defined semen extender. The present field trial confirmed these laboratory results [13]. However, complete elimination of animal ingredients, including BSA, is desirable for production of a defined egg yolk-free semen extender. A soybean lecithin based extender (AndroMed, Minitube, Tiefenbach, Germany) has been developed and utilized for bovine and the Mountain gazelle semen [8–10]; its results in a higher non-return rate after AI compared with extender containing egg yolk [9]. As reported above, elimination of egg yolk from semen extender would make the whole process for semen cryopreservation and AI significantly better and safer.

The lambing rate achieved using semen extender containing BSA in the present study was comparable to those achieved in our previous studies (55–70%) over the last decades. In those studies, we used fixed-time intrauterine AI with frozen-thawed semen containing egg yolk in ewes treated with different progestogens, including CIDRs, during the non-breeding and breeding seasons [15–20]. Conventional cervical insemination with frozen semen has not been fully accepted by the sheep industry due to low fertility (10–30%), although some studies in Norway have shown higher fertility (a lambing rate of over 70%) [21, 22]. Taking the present results into consideration, the effect of addition of BSA to frozen semen extender on fertility after cervical insemination should be examined in the future trials.

The present study indicates that a semi-defined semen extender containing 10% BSA produces fertility after intrauterine AI that is similar to that achieved with semen extender containing egg yolk.

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<th>Extenders</th>
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a Percentages of the ewes inseminated.

b The CIDR was expelled from one ewe during the insertion period.

Table 1. Fertility of ewes inseminated with frozen-thawed semen using extender containing egg yolk or BSA
Shibecya Sheep Farm for allowing us to use facilities and sheep for the present study.

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