Japanese Journal of Veterinary Research 72(2): 51–55, 2024

JJVR

SHORT COMMUNICATION Experimental Research

The attempts to improve the conception rate of transvaginal artificial insemination using frozen-thawed semen by using a Foley catheter to retain semen adjacent to the cervical os in ewes

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Received for publication, February 6, 2024; accepted, June 19, 2024

Abstract

A new transvaginal artificial insemination (AI) procedure by frozen-thawed semen using a Foley catheter in ewes was performed in the present study. A 30-ml ballon was fixed in the vagina and frozen-thawed semen expelled into the vagina was retained near the cervical os for approximately 30-60 min. In the first trial, a single AI was performed on eight ewes after estrus synchronization and one ewe became pregnant (12.5%). In the second trial, AI was performed for two consecutive days on the seven ewes that did not become pregnant in the first trial. Three of the seven (42.9%) ewes become pregnant. These results suggest the usefulness of transvaginal AI by frozen-thawed semen using a Foley catheter for two consecutive days.

Key Words: Foley catheter; Sheep; Transvaginal artificial insemination

Artificial insemination (AI) is a basic technique in livestock breeding programs and has been used as a method to breed genetically superior animals. Many farms now perform AI by frozen-thawed semen to breed cattle[1,13)]. Pig breeding is also mainly conducted through AI by liquid-stored semen[9,15)]. However, sheep AI has received substantially less attention than cattle and swine AI[19)]. The cervix of ewes is a small, narrow, and tortuous structure with several folds; therefore, an AI catheter cannot be inserted into the uterus through the cervical canal[1,2)], and we cannot deposit semen into uterus directly as in cattle, horses, and pigs. It resulted in low conception rates by transvaginal AI in sheep[6,8)]. Accordingly, frozen-thawed semen has been injected into the uterine lumen through the uterine wall using laparoscope (laparoscopic AI) in sheep[2,11)]. Laparoscopic AI achieves higher fertilization rates than cervical AI, which may be attributed to the significantly larger number of sperm per oocyte/embryo[5)]. Therefore, laparoscopic AI is the most commonly used method for sheep breeding due to its acceptable fertility rates[12)]. However, this procedure is complex, highly invasive for animals, and expensive because it requires veterinarian expertise[14)]. In addition, the timing of insemination is important for successful conception. In cows, the most reliable indicator of when to inseminate is standing estrus and

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doi: 10.57494/jjvr.72.2_51

high fertilization rates are generally achieved when cows are inseminated once 12–14 hr after when they first stand to be mounted followed by a second insemination 17–24 hr after the first mount[16]. Sumiyoshi et al.[17] reported that if ovulation had not occurred by 30 hr after AI, additional AI may be required to increase the conception rate. Ovulation typically occurs 14 to 26 hr after the LH surge in sheep[4]. This timing coincides with approximately 21 to 45 hr after the beginning of estrus[4]. The length of estrus may vary depending on the breeds[4]. Cavalcanti et al.[3] showed that when ewes were treated with vaginal sponges impregnated with medroxyprogesterone acetate for 6 days and 300 IU equine chorionic gonadotropin (eCG) and 30 μg d-cloprostenol were then administered 24 hr prior to sponge removal, the interval to estrus ranged between 13.5 and 63.1 hr. Based on these findings, performing AI several times after the onset of estrus may increase the conception rate. However, since laparoscopic AI requires a surgical procedure, it is not applicable to performing AI several times in one estrous cycle. Therefore, the development of a non-surgical transvaginal AI technology that achieves a high conception rate in sheep is needed.

Dogs have a bulbus glandis at the base of their penis, which swells up inside the vagina during copulation and helps keep sperm securely in the vagina, thereby increasing the chance of pregnancy. Therefore, in dog AI, some catheters with an inflatable cuff, *e.g.*, Foley catheters, are used for transvaginal AI[10]. We speculated that the conception rate in sheep by transvaginal AI may be increased with a catheter comprising an inflatable cuff to plug the vagina and keep semen near the cervical os. However, the use of a catheter with an inflatable cuff for sheep AI has not yet been reported. Therefore, we herein performed transvaginal AI by frozen-thawed semen in sheep using a Foley catheter.

All trials were conducted on eight Manx Loghtan ewes (1-7 years old, body weight 27.8-39.0 kg, three multiparous and five nulliparous) at the Experimental farm in the School of Veterinary Medicine, Kitasato University (longitude of 141° 13'E and latitude of 40° 37'N, Towada, Aomori, Japan). The ewes were allowed to graze at pasture (leguminous grass, poaceae grass, and forbs) throughout the day and they were fed 100 g of concentrate mixture (Super Beef A, Michinoku Feed Co., Ltd., Hachinohe, Japan) twice a day (am 9:00 and pm 3:30). Hay, fresh water, and mineral block were offered *ad libitum*. The animal experimentation protocol was approved by the President of Kitasato University through the judgment of the Institutional Animal Care and Use Committee of Kitasato University (Approval ID: 19-138).

The first trial was performed in August 2020. Eight ewes were used and estrus was synchronized by the insertion of handmade progesterone sponges as previously reported[7] with a slight modification. Progesterone cream consisted of 500 mg progesterone mixed with 1.5 g ointment (Oronain-H-Nankou; Otsuka Seiyaku Co., Ltd, Tokyo, Japan), the sponge was inserted into the vagina for 9 days, and ewes received an intramuscular injection of 300 IU eCG (Serotropin; ASKA Animal Health Co., Ltd., Tokyo, Japan) 24 hr before and 10 mg/ml dinoprost (Pronalgon F, Zoetis Japan, Tokyo, Japan) at the time of sponge removal. Fixed-time AI was performed to each ewe using a Foley catheter (14 Fr with a 30-ml balloon, Norta®, Terumo Corporation, Tokyo, Japan) 44-45 hr after sponge removal.

AI using a Foley catheter was performed as follows (Fig. 1). A stylet was introduced into a Foley catheter attached to a three-way stopcock (Terumo) and the catheter was inserted into the vagina until resistance was felt. Then the catheter was fixed by filling the balloon with 30 ml physiological saline warmed to 37°C (Fig. 1A, B) and the stylet was removed. After that, semen filled in 0.5-ml straw was immersed into a 37°C water bath for one minute and thawed. Semen used in the present study was donated by Rare sheep research association (Hinohara, Tokyo). Thawed semen was aspirated using a 1-ml syringe and the syringe was attached to the three-way stopcock. After introducing semen into the Foley catheter, 2.0 ml of a semen extender was introduced from the other injection port of the three-way stopcock and then completely expelled by a 3-ml air injection.

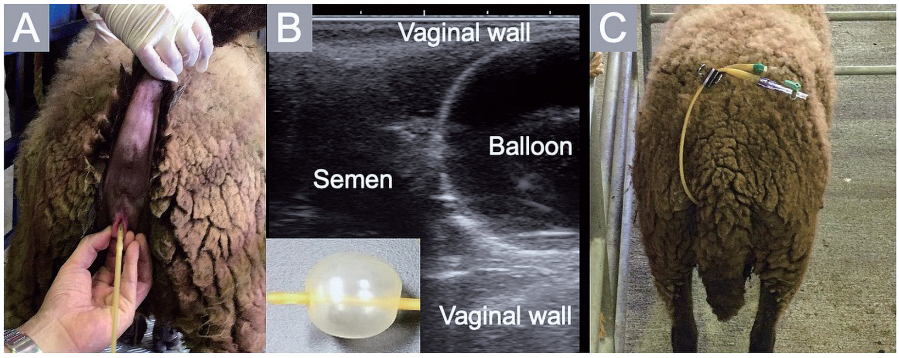

Fig. 1. Intravaginal artificial insemination using a Foley catheter.
A: A catheter with a stainless stylet is introduced into the vagina.
B: The ultrasound image of the ballon fixed in vagina with 30 ml physiological saline (HS-1600V, Honda Electronics Co., Ltd, Toyohashi, Japan) and the tip of catheter with filled balloon.
C: The three-way stopcock is closed to prevent the leakage of semen and the Foley catheter is kept on the ewe's back using a clip for 30-60 min.

The three-way stopcock was closed to prevent the leakage of semen and the Foley catheter was clipped on the back of ewes to keep it in position for 30-60 min to retain semen near the external cervical os (Fig. 1C). In AI in the first trial, two 0.5-ml straws were used for each ewe and the number of inseminated spermatozoa was 4.3-9.7 $\times 10^7$ cells. The percentages of motile sperm were evaluated as 30 to 50% under the light-microscopic observation. The semen extender used in the first trial was Ham's F-10 medium (N2147, Merck, Darmstadt, Germany) supplemented with 1 mM L-glutamine (Merck). Pregnancy was diagnosed by ultrasonography 40 days after AI. One ewe (4 years old) became pregnant (12.5%, 1/8) and delivered a male lamb the following January.

The seven ewes that did not become pregnant in the first trial were used in the second trial in December 2020. Estrus synchronization was performed using the same protocol as that in the first trial; however, dinoprost was intramuscularly injected at the same time as the administration of eCG. Fixed-time AI with a Foley catheter was performed using the same method as that in the first trial; however, each ewe was inseminated twice 44-45 hr and 63-64 hr after sponge removal. We used one straw that included 2.2-2.9 $\times 10^7$ spermatozoa for each AI. The semen extender was Ham's F-10 medium supplemented with 1 mM L-glutamine (Merck) and 1 mM creatine (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), which was reported to maintain the motility of mouse sperm *in vitro*[18]. Pregnancy was diagnosed by ultrasonography 40 days after the first AI. Three ewes (1, 2, and 7 years old) became pregnant (42.9%, 3/7) and delivered one lamb each. In the second trial, we expected the maintenance of sperm motility in the vagina and that sperm could pass through the cervix by adding creatine. No detrimental effects of creatine addition to the semen extender on sheep sperm motility was confirmed by our preliminary experiment. In further study, we should perform more AI trials and confirm the efficacy of creatine addition to the semen extender.

The present study demonstrated the usefulness of a Foley catheter for sheep AI. Namely, three of the seven ewes became pregnant (42.9%) when AI was performed twice using the Foley catheter. A previous study that attempted transcervical AI using frozen-thawed sheep semen[8] achieved a moderately high pregnancy rate (31.5%, n=89) when AI was performed twice and 5×10^8 spermatozoa (total 10×10^8 spermatozoa) were deposited in the uterus. Similar results were obtained herein, although we only performed AI on seven ewes. In addition, the previous study reported a low pregnancy rate (17.9%) when AI was performed twice and semen was deposited between the cervical os and second

cervical fold. We also noted a low pregnancy rate (11.1%, 1/5 Suffolk and 0/4 Manx Loghtan ewes) by conventional transvaginal AI using an AI gun for cattle (data not shown). These results suggest the effectiveness of semen retention near the cervical os by the Foley catheter.

In conclusion, transvaginal AI by frozen-thawed semen using a Foley catheter resulted in three pregnancies out of seven ewes (42.9%), which may be attributed to the retention of semen adjacent to the cervical os. Furthermore, since this method is less invasive, AI was performed on two consecutive days, which improved outcomes. Although the number of offspring is a very important factor in sheep AI, we were able to obtain only one lamb in each AI in the present study. In addition, first and second trials were carried out in August and December, respectively. There is a possibility that the high ambient temperature resulted in the low pregnancy rate of the first trial. In further study, we should investigate about the reason of the present outcome and improve the procedure to increase the fecundity of ewes.

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