Estrous Synchronization and Artificial Inseminationin in European Mouflon (*Ovis gmelini musimon*)

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ABSTRACT

To establish a protocol of estrus induction and synchronization in European mouflon, we performed artificial insemination using frozen-thawed semen and exogenous hormones. CIDR was inserted into vaginas of four mouflons for 16 days. A day before removal of CIDR, PG 600 was injected intramuscularly. $PGF_2\alpha$ was injected when removing CIDR. Artificial insemination was cervically conducted with injecting LHRH 48 hours after CIDR withdrawal. Even though no pregnancy was confirmed, estrous signs were notified like open cervix, congestion of vaginal wall and discharge of cervical mucus. Further research in the wild sheep would be needed for development of artificial breeding methods and advancing sustainability of domestic zoos.

(Key words : estrus, insemination, mouflon, semen, synchronization)

INTRODUCTION

The mouflon (*Ovis gmelini musimon*) is a subspecies group of the wild sheep *Ovis aries* (Wilson *et al*., 2005). This species is now classified as "Near Threatened" by IUCN (International Union for the Conservation of Nature). In domestic sheep industry, a lot of research has been working on to find more efficient methods of artificial insemination in breeding, non-breeding and transition period (Evans *et al*., 2004; Lida *et al*., 2004; Fukui *et al*., 2008; Contreras-Solis *et al*., 2009). The cloned mouflon was born in 2001 and survived 7 months after birth (Loi *et al*., 2001). A domestic sheep was used as a surrogate, which means domestic animals could be used to create clones of wild animals. Since mouflons were introduced to North America for the purpose of hunting, they had been interbred with domestic sheep like Barbado sheep, Dall sheep and Jacob's sheep in game branches. Therefore, a lot of purebred mouflons could be found mostly at zoos. It is one of the reasons why we should develop more efficient methods of artificial breeding by which the genetic diversity of this species can be guaranteed in Korea.

The purpose of this retrospective study is to understand reproductive system of European mouflon and hormonal changes in the procedure of artificial insemination.

MATERIALS AND METHODS

1. Animals

The present research was conducted at Seoul Zoo in Gwacheon, Korea, during the breeding season. Six European mouflons aged 3 to 5 years old were used for estrus induction and semen collection.

2. Semen Collection and Cryopreservation

Two male mouflons were not fed for two days before collection of semen. For anesthesia, medetomidine (0.11 mg/kg) and ketamine (2.5 mg/kg) were intramuscularly injected by a blow gun. After collecting semen by electrical shock on Nov 5, two males were recovered soon after injection of atipamezol (0.55 mg/kg). The collected semen was diluted and frozen by Triladyl (Minitube, Germany)-based method (Mejia *et al*., 2008). One of two male mouflons showed no motility of spermatozoa, and the semen was discarded. The collected semen was 1.5 ml, 8.4×10^8 /ml, and was diluted in 1:2 (v/v semen:extender). The extender was composed of Triladyl, egg yolk and tri-distilled water at a ratio of 1:1:3 (v/v). The final concentration of semen after dilution was 3.15×10^8/ml. After cooling the mixture of semen and extender slowly down to 5℃ for 2 hours, the cooled semen was loaded into 0.5 ml straws and exposed to liquid nitrogen vapor for 5 min before plunging them into

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liquid nitrogen.

3. Artificial Insemination after Induced Estrus

Estrus synchronization was conducted by a modified method of domestic sheep (Fukui *et al*., 1993). Intravaginal progesterone-releasing devices (EAZI-BREED CIDR®; 300 mg progesterone per device, Pfizer Animal Health, New Zealand) were inserted into the vaginas of four mouflons on October 20, 2009 (Fig. 2B). On the 15th day of CIDR insertion, PMSG 200 IU and hCG 100 IU (PG600®, Intervet, Netherlands) was intramuscularly injected, and CIDR was withdrawn next day with injection of $PGF_2\alpha$ (78.9 ug of cloprostenol sodium, PGF Veyx forte®, Veyx-Pharma GmbH, Germany). Forty six to forty eight hours later, cervical insemination was performed with injection of gonadorelin acetate (25 ug, Fertiline®, Vétoquinol Inc., Canada) (Fig. 2C and 2D). All blood samples at every step were venipunctured from jugular veins, and estradiol and progesterone were analyzed by ECLIA (electrochemiluminescence immunoassay) and RIA (radioimmunoassay), respectively at Neodin Vet. Lab. (Seoul, Korea).

RESULTS

1. Levels of Estradiol and Progesterone

The levels of estradiol and progesterone were 26.7/3.26 (No.1), 19.1/1.83 (No.2), 30.3/3.97 (No.3), and 26.5/1.68 (No.4) (pg/ml and ng/ml) on the day of CIDR insertion. The levels changed to 23.0/0.4 (No.1), 53.8/0.83 (No.2), 50.7/0.73 (No.3), and 37.8/0.22 (No.4) (pg/ml and ng/ml) on the day of artificial insemination.

2. Vaginal Appearances

Except for No. 1, the other three mouflons showed cervical mucus discharge (Fig. 2A). Artificial insemination gun was readily penetrated into uterine horns.

3. Motility of Frozen-thawed Sperm

At the moment when diluted semen was cooled down to 5℃, the motility was excellent (> 90%) but it decreased to less than 10% at the time of artificial insemination.

DISCUSSION

Body weights of female mouflons used in this study were around 18 kg. The length extended from the entrance of vagina to cervix os is about 10 cm. Male reproductive organ showing sigmoid flexure of the penis typically observed in ungulates has big testes and spermatic cords compared to their petit body size (Fig. 1A and 1B). There are no follicles in the ovaries of a mouflon died in non-breeding season (Fig. 1C). Two uterine horns are separated by a septum, and many caruncles were shown on the uterine walls connected with a uterine body beneath which more than 7 cervical folds present in the cervix (Fig. 1D). The number of cervical folds is the reason that laparoscopic artificial insemination is more popular in the sheep than in goats.

Ovulatory activity is related with latitude, ram exposure, prolactin and melatonin concentrations in wild and domestic sheep (Gonzalez *et al*., 2001; Evans *et al*., 2004). Methods used for estrus synchronization of sheep have been successfully applied to mouflon in breeding, non-breeding and transition period (Gonzalez *et al*., 2001; Berlinguer *et al*., 2003; Berlinguer *et al*., 2005; Berlinguer *et al*., 2007).

Various types of devices with progesterone released into body system have been used to achieve estrus synchronization by extending the luteal phase (Pendleton *et al*., 1992; Evans *et al*., 2004; Lida *et al*., 2004; Awel *et al*., 2009). CIDR devices inserted for 16 days were effective to induce estrus and synchronization in this study. Not yet clear is the identification of which gonadotrophin is more effective, duration of CIDR or FGA (fluorogestone acetate) insertion, usefulness of $PGF_2\alpha$ and LHRH, exposure of teaser ram (Donovan *et al*., 2004). But we think the quality of frozen-thawed semen and the optimal time of artificial insemination are most important because we confirmed ovaries responded well to exogenous hormones in this study. The ewes do not breed until they are 2～3 years of age and the males don't breed until approximately 7 years of age due to social factors. The male mouflon showing no motility of semen could be sexually suppressed by the other higher-ranked males. The estradiol level might be indicative of becoming estrus period and showing behavioral estrus when it is more than 50 pg/ml. Korea is much behind than advanced countries in assisted reproductive technology of zoo and wild animals (Loi *et al*., 2001; O'Brien *et al*., 2006; Sontakke *et al*., 2009). Through zoo's cooperation with veterinary schools, zoology should be first advanced to the scientific level of Southeast Asian countries.

To our knowledge, this article is the first about European mouflon's reproductive system, estrous synchronization using exogenous hormones and artificial insemination in Korea.

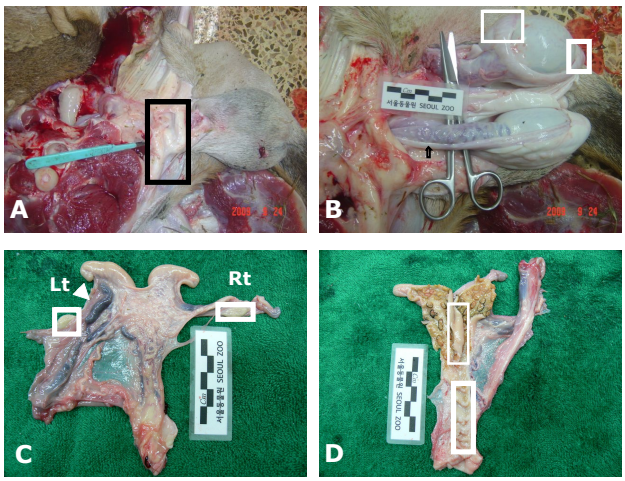

Fig. 1. Reproductive organs of European mouflon. (A): Sigmoid flexure of the penis was shown in the box. (B): A big box and a small box indicate the head and tail of epididymis, respectively. An arrow shows a vas deference. (C): Boxes show ovaries and an arrow indicates an oviduct. (D): The thin-lined box indicates a septum that separates left and right uterine horns. Seven cervical folds are shown in the thick-lined box.

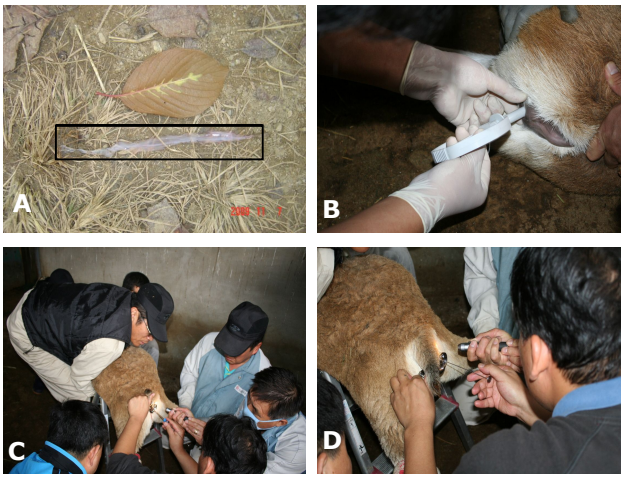

Fig. 2. Procedures of artificial insemination in European mouflon. (A): At the day of artificial insemination, a lot of cervical mucus (box) discharged from the cervix. (B): A CIDR device was inserted into the vagina. (C,D): Artificial insemination was performed using a pen light, vaginal speculum and artificial insemination gun fitted to sheep or goats.

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