

## Original Article

# Use of a domestic Korean black goat (*Capra hircus coreanae*) with its chest crayon-harnessed in detecting estrus of Himalayan tahrs (*Hemitragus jemlahicus*)

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The reliability of a Korean black goat (*Capra hircus coreanae*) to detect estrus in Himalayan tahrs (*Hemitragus jemlahicus*) for an artificial breeding program was investigated. Estrus in six female Himalayan tahrs was synchronized using fluorogestone acetate (FGA) sponges. Thirteen days later, 200 IU of PMSG and 100 IU of hCG were injected before removing the sponges and simultaneously injecting 5 mg of PGF2 $\alpha$  the next day. Penetration of the cervical canal and the thickness and location of red crayon marks were examined 40~43 h later. Two females treated with sponges containing 60 or 45 mg of FGA had estrogen levels of 8.7 and 11.1 pg/mL, respectively. No red marks were found on the backs of these two tahrs. The remaining females had higher levels of estradiol, and the red crayon marks were clearly shown. The cervical folds of these tahrs were readily penetrated and the insemination gun was smoothly inserted into the uterine body. In conclusion, a Korean domestic goat with its chest crayon-harnessed was successfully used to detect estrus of Himalayan tahrs. This technique might be utilized as a part of breeding programs for wild goats and avoid the need for a vasectomy of conspecific males.

**Keywords:** estrus detection, goat, Himalayan tahr, teaser

## Introduction

The Himalayan tahr (*Hemitragus jemlahicus*) is a wild goat with a "near threatened" conservation status according to the International Union for Conservation of Nature (IUCN). The natural habitat of this animal covers a wide range of territory around the Himalayan Mountains. These goats are seasonal breeders with males rutting and fighting

for receptive females. The female Himalayan tahr is seasonally polyestrous with behavioral signs that are easily undetected compared to those of domestic goats. However, the tahr was found to be responsive to exogenous hormones during the process of estrus synchronization [21,22].

Detecting estrus is important for maintaining the economic value of domesticated livestock for which accurate and reliable methods have been developed to decrease any chance of missing the optimal time of fertilization [1,11,12,20]. Most zoo ungulates, like Himalayan tahrs, do not benefit because their annual breeding season is limited according to the length of sun exposure. In addition, the ability of males to find females in heat is possible only during the short annual breeding season [22]. Problems with artificial breeding programs for seasonal breeders do not involve females but rather males. Among Himalayan tahrs, this is due to the fact that estrus is induced in females by exogenous hormones at any time of the year while males show sexual interest only during the breeding season [21]. In preliminary studies performed during the non-breeding season, male Himalayan tahrs showed a low level of testosterone and only conducted non-breeding activities such as foraging, resting, and rumination even in the presence of standing-heat females in which estrus was induced with exogenous hormones. Testosterone functions so critically that even a cow treated with it was as available as vasectomized bull [14].

The Korean black goat (*Capra hircus coreanae*) is the only breed of goat indigenous to Korea and the most prolific of all domestic Korean ruminants. Additionally, this type of goat is able to breed throughout the year [19]. Phylogenetically, Himalayan tahrs are not very similar to Korean black goats. The Himalayan tahr has 48

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chromosomes ( $n = 24$ ), which is 12 fewer than the domestic Korean goat [3]. Notably, male Korean black goats are sexually interested in females throughout the year. Presumably, male Korean black goats would be ideal for detecting estrus of Himalayan tahrs that are scheduled to be used for artificial breeding programs during the non-breeding season. To date, the use of a domestic goat as a teaser has not yet been attempted as a method for determining the optimal insemination time of wild female goats in artificial breeding programs for wild caprinae species conservation [7-9]. In the present study, the response of a penile-deviated and vasectomized domestic Korean goat to estrus-synchronized Himalayan tahrs was examined during the non-breeding season. Receptivity of the females, hormonal changes, and ease of cervical canal penetration were evaluated.

## Materials and Methods

### Animals

Six female Himalayan tahrs (40~55 kg, 6~7 years old) and a male Korean black goat (60 kg, 5 years old) born in captivity at the Seoul Zoo (Korea; 37°25'N, 127°1'E) were included in the study. The animals were fed a diet of alfalfa hay, carrot, lettuce, cabbage, and sweet potato twice a day and water ad libitum. This study was performed in November 2009, and the experimental procedures were approved by the Institutional Animal Care and Use Committee of Kangwon National University (Korea) in accordance with the Guiding Principles for the Care and Use of Research Animals.

### Preparation of the Korean black teaser goat

The penis of a male Korean black goat was surgically

deviated 45 degrees. Nevertheless, intromission with conspecific female black goats was sometimes found to occur by moving the deviated penis obliquely, which is rare among bulls with a surgically translocated penis [13]. Therefore, the vasa deferentia were also severed before the teaser goat was allowed into an enclosure with female Himalayan tahrs. A crayon was attached to the chest of the male goat a month before estrus synchronization was induced in the tahrs (Fig. 1A).

### Anesthesia induction

The female tahrs were anesthetized with a blow dart in which 1.2 mL of xylazine (0.48 mg/kg; Kepro BV, the Netherlands) were loaded as previously described [5]. After anesthesia had been induced, blood was collected at the time of sponge insertion and removal as well as cervical penetration. The animals were revived with 1.2 mL of atipamezol hydrochloride (0.12 mg/kg, Antisedan; Orion Corporation, Finland).

### Experimental design

In this study, the vasectomized Korean black goat was tested to determine its suitability as a teaser for Himalayan tahrs. Marks left by the crayon strapped to the male goat, hormone levels, and penetration of the cervical canal were analyzed.

**Scoring the extent of standing heat:** Smudges left by the red crayon on the rump of the Himalayan tahrs were examined by caretakers. The distance between the crayon marks and trunk along with the thickness of the smudges were measured twice a day after sponge insertion. The marks were scored in the following manner: nothing on the back (–), slightly marked on the rump (+), heavily marked on the rump (++), slightly marked up to the back of trunk



**Fig. 1.** A vasectomized male Korean black goat harnessed with a crayon that had undergone penile translocation to serve as a teaser buck (A) and its ability to detect estrus (B). The crayon obviously marked the rump of a Himalayan tahr that displayed behavior associated with estrus including submission to mounting and intromission. The picture shown in panel B was incidentally taken by a zookeeper holding a cell phone at the moment of mounting.

(+++), and heavily marked up to the back of trunk. (++++). The thickness and location of the red smudges on the backs of the females were examined in detail on the day of cervical penetration while the animals were under anesthesia (Fig. 1B).

**Changes of estradiol and progesterone levels:** Blood was collected from the jugular vein during every step of the estrus synchronization procedure and centrifuged at  $1500 \times g$  for 20 min to obtain serum. The serum was stored in a freezer at  $-20^{\circ}\text{C}$ . The samples were transferred to Neodin Veterinary Laboratory (Korea) to measure estradiol and progesterone concentrations by electrochemiluminescence immunoassay (ECLIA) and radioimmunoassay (RIA).

**Estrus synchronization and cervical canal penetration:** Estrus was synchronized in six female Himalayan tahrs using 60-, 45-, or 30-mg fluorogestone acetate (FGA) sponges (Syncrite; Animal Health Supplies, Australia) implanted on November 3, 2009. Thirteen days later, 200 IU of PMSG and 100 IU of hCG (PG600; Intervet, the Netherlands) were intramuscularly injected before removing the sponges the next day on November 17 with an injection of PGF $2\alpha$  (5 mg of dinoprost tromethamin, Lutalyse; Pharmacia, Sweden). Penetration of the cervical canal was performed 40 to 43 h later using an insemination gun designed for goats and sheep (All-2-Mate Goat

Insemination Gun, 30.48-cm long; Agtech, USA) without semen. A vaginal speculum was used to clearly view the area from the vagina to the opening of the cervical canal.

### Evaluation of hormone changes

During the 14 days of FGA sponge placement, the natural release of estradiol was suppressed to maintain a low concentration from 13.5 to 25.4 pg/mL until the day of sponge removal. Among the tahrs that received 60 (A and C in Fig. 2) or 45 mg of FGA (E and G in Fig. 2), C and G had estradiol levels of 13.5 and 14.2 pg/mL, respectively. On the day of cervical penetration 3 days after PMSG and hCG were injected, the estradiol levels of C and G (Fig. 2) fell to 8.7 and 11.1 pg/mL, respectively, while the levels in A and E increased up to 21.7 and 23.0 pg/mL, respectively. In the group of that received 30 mg (I and K in Fig. 2) estradiol release was increased from 16.6 to 25.4 pg/mL and 10.1 to 19.4 pg/mL between sponge insertion and removal (Table 1).

### Thickness of red crayon marks smudged on the rump

On the day of cervical penetration, no red crayon marks were found on the entire backs of C and G tahrs that received 60 or 45 mg of FGA (Fig. 2). In contrast, A and E had well-defined red marks on the rump or back fur of the



**Fig. 2.** Appearances of the back and external genitalia of female Himalayan tahrs at the time of cervical penetration. Red crayon (square box) was smudged on the rump of animals shown in panels A, E, I and K. Cervical mucus of these females (panels B, F, J, and L) was also clearly observed on the external genital region at the time of cervical penetration 2 days after sponge removal. The Himalayan tahrs (panels A, C, E, G, I, and K) were headed in the right direction.



**Table 1.** Serum estradiol and progesterone concentrations during estrus induction and cervical penetration

Fluorogestone acetate dose	60 mg		45 mg		30 mg	
Tag number	A <sup>‡</sup>	C	E <sup>‡</sup>	G	I <sup>‡</sup>	K <sup>‡</sup>
Sponge insertion	11.0*/1.64 <sup>†</sup>	9.6/0.68	10.6/0.35	12.6/0.56	16.6/0.78	10.1/0.14
Sponge removal	19.4/0.61	13.5/0.31	18.3/0.16	14.2/0.21	25.4/0.47	19.4/0.21
Cervical penetration	21.7/0.11	8.7/0.25	23.0/0.11	11.1/0.57	17.0/0.07	23.2/0.32

\*,<sup>†</sup>The numbers shown are the estradiol (pg/mL) or progesterone (ng/mL) concentrations, respectively. <sup>‡</sup>These Himalayan tahrs had crayon smudged on their back. Cervical penetration into the uterine body was easily accomplished in these females.

thoracic region that were scored (+++) and (++) , respectively (Fig. 2). Thick smudges were also observed clearly on the backs of I and K tahrs (Fig. 2) that were scored as (++++).

### Ease of cervical canal penetration

Except for C and G tahrs (Fig. 2) that had estrogen levels of 8.7 and 11.1 pg/mL, respectively, and were unmarked (–), cervical folds of the females were readily penetrated and the penetrating gun was smoothly inserted into the uterine body. Transparent cervical mucus (B, F, J and L) was also clearly observed on the external genitals of A, E, I, and K, respectively (Fig. 2). As presented in Fig. 2D, penetration of the cervical canal was not actually accomplished in the C tahr. Also in the G tahr, the canal was barely penetrated even after repeated trials (Fig. 2H). No sign of cervical mucus discharge was found in the perineal and genital region of C or G tahrs (D and H in Fig. 2).

### Discussion

Estimating the optimal insemination timing and degree of estrous response is a primary concern for artificial breeding programs of zoo animals. If the exact time of standing heat can be determined, the time and cost required for estrous detection could be diminished or even eliminated [4,12,14]. Various types of methods have been used to detect the appropriate time of ovulation and insemination for reproductive programs of domesticated wild ungulates [2,9,18]. Detection of estrous behaviors can be directly accomplished with hormone and urine assays [8]. However, behavioral observation in person or via closed circuit television (CCTV) is time-consuming or labor-intensive. CCTV also has drawbacks such as the presence of several blind spots within large enclosures that make observation limited to several fixed spots and specific times during the day. If infrared cameras are used to make observations during the night, it is even more difficult to distinguish morphologically similar animals or select which ones are in heat. Although high-quality

devices developed to detect estrus have been used, an attempt by males to mount females is the most reliable external sign of estrus [2,7,8,10,17,23].

Among wild goats that breed seasonally, the use of conspecific males as estrus detectors during the non-breeding season is not possible due to low level of male sexual desire consistent with extremely low concentrations of testosterone [14,22]. Even during the breeding season or in artificial breeding programs, some male wild goats in captivity are too valuable to simply vasectomize and lose the potential contribution to genetic diversity. Considering the difficulty with determining the optimal insemination time for wild goats in artificial breeding programs, the use of domestic male goats might be an acceptable alternative for conspecific wild male goats as teasers. Because conspecific male wild goats are not functional until the rutting season has almost commenced, a main benefit of this strategy is that domestic teaser goats are functional during the entire year. In our preliminary study, Himalayan tahrs in which estrus had been induced were housed with a conspecific male during the non-breeding season. The male did not mount the females. Thus, a conspecific male is not an appropriate teaser for detecting estrus during the non-breeding season. Testosterone levels in seasonal breeders are greatly dependent on testicle size that is greatest during the mating season [6,14]. In contrast, the Korean black male goat maintains its fertility throughout the year.

The use of a domestic counterpart instead of conspecific males might be more desperate in artificial breeding programs conducted during the non-breeding season for wild goats in captivity. The history of using interspecific domesticated animals includes a dog trained to identify estrus-related odors in cows [12]. In the present study, the teaser goat did not need to be trained. The male goat only needed its own instinct to respond to female Himalayan tahrs in heat. A red crayon was attached to the chest of the Korean black goat. The goat was then observed chasing the female Himalayan tahrs, frequently looking up at the females standing on a far over-height fence, curling his lips, approaching the tahrs, kicking his front legs, making

noise, and finally mounting the females. The behavior of the male goat was similar to manner in which a dominant male tahr acts towards conspecific females. When the same goat was used to detect estrus in Barbary sheep, the difference of rump height seemed to cause an absence of smudging with the crayon (unpublished data).

In the current study, the crayon marks were thicker when estradiol levels were higher as measured on the day of penetration. Transparent vaginal mucus discharged from the external genitalia was found in four Himalayan tahrs that had high levels of estradiol. No vestige of red crayon smudges was found on the entire back of two Himalayan tahrs nor was any presumable trace of vaginal mucus observed. These findings were associated with difficulty of penetrating the cervical canal.

In a previous investigation, a vasectomized blackbuck (*Antilope cervicapra*) was used as a teaser for female blackbucks [8]. Using a male conspecific teaser may be a useful strategy for artificial breeding programs involving wild goats unless domestic male goats with similar behavior and characteristics are available and the wild female goats willingly permit themselves to be mounted. In the current study, a domestic Korean black goat was successfully used as an estrus detector and could replace conspecific males. In addition, the ability of the black goat is available year-round unlike seasonal breeders.

Ovarian responses to exogenous hormones in seasonal breeders vary with environmental factors such as nutrition, ambient temperature, season, and photoperiod [15,16]. Sponge-type devices including ones that deliver 30 mg of FGA might be effective for sustaining estradiol release and inducing estrus after injecting PMSG and hCG. In two tahrs treated with 60 or 45 mg FGA, the release of estradiol was too suppressed to induce estrus after injecting PMSG and hCG. However, 200 IU of PMSG and 100 IU of hCG might be insufficient for inducing estrus. In other words, the low doses of PMSG and hCG could be the reason why optimal estradiol levels required for standing heat were not reached. Effects of the FGA dose would be a very reasonable explanation for estrus induction failure in the C and G tahrs (Fig. 2).

The crayon used in this study was red and can leave conspicuous smudges on the white fur of sheep or goats. Crayons of various colors are commercially available for both goats and sheep. It is necessary to select a color suitable for making visible marks on the multicolored back fur of ungulates in artificial breeding programs. The fur of female Himalayan tahrs is reddish brown. As shown in Fig. 2E, the red crayon smudges on the rump were sometimes hard to find. Bright sunlight could also make it more difficult to observe the small red marks on the reddish brown fur.

Not many reports about detecting estrus in wild ungulates have been published. The detection of estrus is critical for

artificial breeding programs of wild goats such as Himalayan tahrs that have a harem pattern of reproduction. The loss of dominant males resulting from fighting during the mating season and the failure to sustain genetic diversity could be avoided if artificial breeding programs performed during the non-breeding season were conducted while knowing the optimal insemination time determined by domestic teaser goats. Similar to the domestic Korean black goat used in this study, other domestic species could be used to identify which females are in heat.

In conclusion, an indigenous domestic Korean black goat was found to have a reliable ability to find Himalayan tahrs in heat. This capability could make domestic goats suitable for use in artificial breeding programs designed for other zoo ungulates to increase the success rate. To the best of our knowledge, this is the first study in which a domestic goat served as an estrus detector in an artificial breeding program for Himalayan tahrs or other types of wild goats.

## Conflict of Interest

There is no conflict of interest.

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