Changes of Sex Hormones and Cervical Mucus in Estrus-synchronized Himalayan Tahrs (*Hemitragus jemlahicus*) in Non-breeding Season

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ABSTRACT

Eight female Himalayan tahrs (*Hemitragus jemlahicus*) were estrus-synchronized, and transcervically inseminated with frozen-thawed semen in September, 2009, about 2 to 3 months earlier than their natural breeding season. Intravaginal progesterone-releasing devices were inserted into vaginas of six Himalayan tahrs on September 7, and the other two on September 8 to suppress luteal function of ovaries. The devices had been placed deep inside the vagina prior to withdrawal on September 23. A day before CIDR removal, a combination of PMSG 400 IU and hCG 200 IU was intramuscularly injected. Forty eight hours later, frozen-thawed semen was transcervically inseminated. Pregnancy diagnosis was performed 39 days later by analyzing progesterone level of serum. Every treatment was done under anesthesia inducted by xylazine injection. In conclusion, vaginal discharge of cervical mucus, hormonal changes induced by implant-typed or muscularly injectable hormones and widening of cervix enough to insert an insemination gun into uterine body were achieved in non-breeding season. Moreover, the first inseminated Himalayan tahr, 36 hours after CIDR removal was assumed to be pregnant but the fetus may have been lost due to the use of anesthetic drug.

(Key words : Himalayan tahr, estrus synchronization, semen, cryopreservation, laparoscopic, insemination)

INTRODUCTION

Himalayan tahr (*Hemitragus jemlahicus*) is one of three tahr species. This species belongs to conservation status of "Near Threatened" (NT).

Developing techniques of artificial breeding is much more important than proliferation of captive wild species only through natural mating especially in seasonal breeders. These techniques would include induction of fertile estrus, estrus synchronization, cryopreservation of genetic material, artificial fertilization and embryo transfer throughout a year (Asher et al., 1993; Keskintepe et al., 1998; Johnston et al., 2000; Mejia et al., 2009).

Using many species of wild ungulates, hormonal changes in breeding or non-breeding season had been shown (Holtz et al., 1988; Asher et al., 1993). However, the appearances of vaginal region, like swelling of vulva, congestion of vaginal mucus, and discharge of cervical mucus were not much introduced to people who are directly working on the animals at *in-situ* or *ex-situ* habitats.

In this study, hormonal changes shown after estrus synchronization, swollen vagina, congestion of vaginal mucus, and discharge of cervical mucus were investigated in non-breeding season of Himalayan tahrs.

MATERIALS AND METHODS

1. Animals

Eight female Himalayan tahrs were used for estrus synchronization, and two male tahrs were electrically ejaculated before the semen had been frozen for further use. All of them were born in captivity. Females are 6~7 years old, multiparous except for one, and ranged 40 to 45 kg while about 55 kg for the males. Feeding was served twice with alfalfa hay, carrot, lettuce, cabbage, and sweet potato with access to water *ad libitum*.

2. Semen Collection and Cryopreservation

Semen was collected and cryopreserved in September 2009 by the method performed with bighorn sheep, European mouflon, barbary sheep, and follow deer (Mejia et al., 2009; Yong et al., 2010). Briefly explaining, under anesthesia of xylazine hydrochloride (0.58 mg/kg; Rompun®, Bayer, Germany), semen was collected using electrical stimulation (ElectroJac IV, Neogen; USA) followed by diluting it with Triladyl (Minitüb, Ger-

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many)-based solution, cooling to 4℃ for 2 hours, and finally plunging into LN_2.

3. Induction of Estrus Synchronization

To synchronize estrus of female Himalayan tahrs, intravaginal progesterone-releasing devices (Eazi-Breed CIDR®; Progesterone 300 mg per implant, Pfizer Animal Health, New Zealand) were inserted into the vagina of six Himalayan tahrs on September 7, and the other two on September 8. The devices had been placed for 15 or 16 days prior to withdrawal on September 23. Twenty- four hours later, a combination of PMSG 400 IU and hCG 200 IU (PG 600®; Intervet, Netherlands) was intramuscularly injected. Frozen-thawed semen, stored about two weeks after collecting from two male tahrs, was transcervically inseminated once thirty six or forty eight hours later.

4. Anesthesia

Every treatment such as CIDR insertion, PG 600 injection, CIDR removal, cervical AI, and pregnancy diagnosis was conducted under anesthesia of xylazine hydrochloride (Rompun®, 0.58 mg/kg; Bayer, Germany) with no use of antidote.

5. Transcervical Insemination

Frozen-thawed semen was transcervically inseminated once into uterine body of each of the four female Himalayan tahrs 36 or 48 hours after CIDR removal following the method mentioned in our previous study (Yong et al., 2010). Briefly explaining, after anesthesia, the female Himalayan tahr was put on the table in a positioned of left or right lateral recumbency (Fig. 1A). Using a pin light and vaginal speculum, the entrance of cervix was visually identified and an artificial insemination gun loaded with 0.5 ml of frozen-thawed semen was guided into the cervical entrance remaining vagina widened by vaginal speculum (Fig. 1A). The AI gun was smoothly penetrated into cervical folds and frozen-thawed semen was deposited into uterine body right after passing the final cervical fold (Fig. 1B).

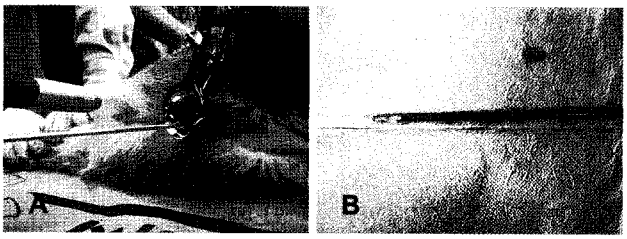

Fig. 1. Transcervical insemination of frozen-thawed semen in Himalayan tahrs. (A): With help of a pen light, vaginal speculum, artificial insemination was performed by AI gun that has been used in sheep or goat industry. (B): Cervical mucus smudged with a little bit of blood was shown on the sheath of AI gun after insemination.

6. Diagnosis of Pregnancy

On November 3, 39 days after AI, blood sera collected from eight female Himalayan tahrs were transferred to local vet lab (Neodin Co., Ltd, Seoul, Korea) to analyze progesterone level.

RESULTS

1. Hormone Levels

Judging by the hormone levels at the day of AI, it is difficult to say that 15 or 16 days of CIDR insertion is optimal to induce estrus synchronization. Except for 2, 6, and 7 of ear-tagged individuals, the others showed the low levels of estradiol commonly known in non-breeding season (in our preliminary study; not published) (Table 1). As comparing progesterone levels between the days of CIDR insertion and removal, the effect of CIDR, suppressing the release of endogenous progesterone, was not compromised.

2. Cervical Mucus at the Time of AI

At the moment of AI, most female Himalayan tahrs showed transparent, sticky discharge of cervical mucus (Fig. 2). Ear-tagged 6 and 7 individuals did not show cervical mucus discharged out of vagina but AI gun was smoothly penetrated into cervical folds. In addition, the appearances of vagina were distinctive to female Himalayan tahrs in heat (Yong et al., 2010). The motility of frozen-thawed semen was 50～60%.

3. Pregnancy Diagnosis

The presence of fetus was not confirmed by ultrasonography or X-ray but the high level of progesterone observed in Ear-tagged No. 1 can be the proof of being pregnant. Twenty days later, the progesterone level decreased to 2.53 ng/ml. The pregnant female was the one that was first inseminated at the day of AI, 36 hours after CIDR removal.

DISCUSSION

The cause of failure in maintaining pregnancy, we believe, was the early use of an anesthetic drug like xylazine hydro-

Table. 1. Changes of hormonal levels analyzed from CIDR insertion to pregnancy diagnosis

Treatments	Date	Hormone*	1	2	3	4	5	6	7	8**
CIDR insertion	Sep 7	E	17.2	12.2	18.9	13.8	14.7	11.3	26.2	26.4
		P	1.91	0.57	0.62	0.64	0.24	1.14	0.49	0.36
PG 600 injection	Sep 22	E	21.2	17.8	15.8	17.1	11.2	11.9	18.8	14.2
		P	1.32	1.36	2.06	1.78	1.82	3.63	2.35	1.44
CIDR removal	Sep 23	E	23.6	21.2	23.4	20.8	18.4	16.6	21.0	18.1
		P	0.97	1.14	1.41	1.09	1.37	1.11	1.02	1.46
Cervical AI	Sep 25	E	17.0	27.6	13.6	15.4	15.6	20.6	26.8	12.7
		P	0.58	0.16	0.77	0.17	0.24	0.39	0.14	0.67
Pregnancy diagnosis	Nov 3	E	10.9	11.8	11.0	9.6	10.6	12.6	16.6	10.1
		P	6.28	1.01	1.64	0.68	0.35	0.56	0.78	0.14

*The units of E (estradiol) and P (progesterone) are pg/ml and ng/ml, respectively.
**No. 7 and 8 were inserted with CIDR on Sep 8.

Fig. 2. Discharge of cervical mucus observed on the day of transcervical insemination in Himalayan tahrs. (A~H): At the day of artificial insemination, a lot of cervical mucus, discharged from the cervix, was shown in most of Himalayan tahrs. * The figures of external genital region from A to H are from No. 1 to No. 8 of ear tags.

chloride (Sakamoto et al., 1996). At the day of CIDR insertion, a female Himalayan tahr was sedated with more dose of xylazine hydrochloride than the normal dose of the other tahrs because it was not easily anesthetized by a blow gun. After it was down, a lot of ticks were found feeding on blood, and attached to the skin of neck, eyelids, trunk, and limbs hiding under the fur. As an antidote against xylazine hydrochloride, yohimbine hydrochloride was not proper to Himalayan tahrs (Dematteis et al., 2006). This female tahr was excluded in this study.

The duration of CIDR insertion would not be critical for estrus synchronization in the non-breeding season of seasonal breeders like Himalayan tahrs. The injection of $PGF_{2\alpha}$ routinely used in goat's estrus synchronization may be necessary at the time of PG 600 injection or CIDR removal in September, non-breeding season (Sohnrey and Holtz, 2005). The time of AI after CIDR removal is also important (Asher et al., 1993; Yong et al., 2010). Making the optimal time of AI limited to less than 36 hours would be beneficial no matter how many times AI are performed. As considering the fair circumstances of zoos, like the lack of surveillance cameras covering whole outdoor enclosures, the use of teaser buck can be a sure

thing to guarantee the optimal time of AI (Holtz et al., 1988). Especially in zoo ungulates, every time researchers treat on, anesthesia is not avoidable (Asher et al., 1993; Mejia et al., 2009; Yong et al., 2010). Therefore, proper restraint devices were developed to decrease the stress or unexpected injury that animals could get in the time of CIDR or sponge insertion and removal under anesthesia (Johnston et al., 2000).

The semen of wild ungulates was widely collected by electrical stimulation, and frozen to the time of further use (Watson, 1976; Asher et al., 1993; Keskintepe et al., 1998; Garde et al., 2003; Purdy, 2006; Mejia et al., 2009). Collecting in the natural breeding season may be more contributive to the success of AI. The semen collected in September, non-breeding season, could be a partial cause leading to the low pregnancy. In addition, the use of vasectomized teaser male commonly used in goat and sheep industry must be considered to do AI at the optimal time, resulting in offspring production through artificial breeding technique anytime in a year. To enhance fertilization rate, sperm cryopreservation technique should also be far more developed for species conservation.

In conclusion, we showed the method of estrus synchronization of Himalayan tahrs in non-breeding season. A further study of using vasectomized teaser is needed to range the time of AI leading to virtual production of kids. Even though this trial ended up with failure of kid production, the protocols of inducing estrus, sperm cryopreservation, artificial insemination, and hormone fluctuation during treatments will be fundamental and essential data for conservation of wild ungulates of which importation become gradually prohibited.

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