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Hormone Analysis during Artificial Estrus Induction in Korean Black Goats

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흑염소의 인공수정 발정동기화 처리방법에 따른 호르몬 변화분석

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Abstract The purpose of the study is to investigate the change in the blood progesterone (P4) and estrogen (E2) levels when applying different estrus induction protocols to Korean black goats, and this was done to gain understanding about their reproduction physiology. For the experiment, we performed three estrus induction protocols that are commonly used in bovine: controlled internal drug release (CIDR) + prostaglandin F2α (PGF2α), PGF2α + gonadotropin-releasing hormone (GnRH) + PGF2α, and CIDR + PGF2α + PMSG. The P4 and E2 concentrations showed different patterns until the last treatment of the three protocols. However, similar concentration patterns were shown after the last treatment in all the protocols. In conclusion, we monitored the blood P4 and E2 levels in Korean black goats following three different estrus induction protocols. Our findings may be used in other breeding programs of Korean black goats, such as artificial insemination and embryo transfer.

요 약 본 연구의 목적은 한국 재래흑염소 번식생리의 이해를 돕고자 서로 다른 발정동기화법을 적용하였을 때 혈액내 프로게스테론과 에스트로겐의 농도변화를 알아본 것이다. 발정동기화법은 소의 방법을 기본으로 하여 품종의 크기에 따라 호르몬의 용량을 조절하는 것을 가장 많이 사용되고 있으나, 흑염소에 적용하였을 때 발정동기화 후 인공수정 적기의 판단이 정확하지 않아 효율이 매우 낮게 조사된다. 연구에 조사된 발정동기화 방법으로는 소에서 많이 이용되어왔던 CIDR + PGF2α, PGF2α + GnRH + PGF2α, 그리고 CIDR + PGF2α + PMSG 세가지를 적용하였다. 혈액내 프로게스테론과 에스트로겐은 발정동기화 각 처치 후와 최종 처치 후 수일간의 변화를 조사하였다. 혈액내 프로게스테론과 에스트로겐 농도를 조사한 결과, 세가지 발정동기화 방법 마지막 처치 전까지는 모두 다른 변화를 보였으나, 그 후로는 서로 유사한 패턴으로 호르몬이 변화하는 것을 관찰할 수 있었다. 결론적으로 발정동기화를 위한 호르몬 주사가 개체에 서로 다르게 작용하여 발정을 유도하고 최종 처치 후로는 유사한 발정이 유도되어 유사한 호르몬 상태를 보인 것으로 생각된다. 본 연구의 결과를 한국 재래흑염소 번식에서 인공수정이나 수정란 이식과 같은 기술에 접목한다면 효율적인 개량유도에 기여할 것으로 판단된다.

Keywords : Estrogen, Estrus Induction, Hormone Analysis, Korean Black Goat, Progesterone

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1. Introduction

Recently, Korean black goat (*Capra hircus coreanae*) meat has gained popularity among the general public owing to its promotion as a healthier alternative to beef, pork, and chicken. Many studies have investigated the reproduction, feed management, genetics, and meat science[1-8]. Assisted reproductive technologies, such as semen cryopreservation, artificial insemination, and oestrus synchronization, have been researched to overcoming the natural reproductive inefficiencies of the goat population[9-11]. The reproduction efficiency of Korean black goat was found to below owing to the limited number of studies on the relationship between the basic physiology and reproduction of Korean black goat.

Oestrus induction protocols using hormones have been widely used in the management of animal reproduction. Oestrus induction is a key factor for artificial insemination and reproductive failure in Korean black goat. Thus, goat oestrus induction may be an important technique in the breed management of this species[12,13]. In ruminants such as goats, artificial oestrus is induced by various methods such as hormone feeding, injection, and devices[9,14,15]. However, most methods are based on initialization of oestrus cycle through regression of corpus luteum by $PGF_2\alpha$ and control of oestrus time by prolonged administration of P4. In many cases, goat oestrus induction was performed using modified cattle oestrus induction methods[16,17].

In cattle, intravaginal progestogen device has been commonly used either alone or in combination with other hormones[9,18-20]. Therefore, with modified methods of estrus induction, which are commonly applied, the breeding efficiency in goats remained low in the field[21]. Although goats and cattle are both ruminant animals, differences in their physiological systems are thought to affect the success of goat breeding programs, including estrus induction. Therefore, it is necessary to investigate the physiological features of Korean black goats.

In this study, to better understand estrus induction in Korean black goats, we aimed to monitor changes in the blood levels of progesterone (P4) and Estrogen (E2) of Korean black goats after estrus induction protocols. Our findings would serve as a foundation for other breeding programs, such as artificial insemination and embryo transfer.

2. Materials and Methods

2.1 Animals

Twenty-one female Korean black goats (average body weight: 36 kg) with 2-3 maternity experience were used in this experiment (Table 1). All animal experiments were conducted according to the Guidelines for the Care and Use of Laboratory Animals of the National Institute of Animal Science, Korea.

Table 1. Experimental design

Group	Estrus induction protocol	No. of animals
Group 1	CIDR +PGF_2a	7
Group 2	PGF_2a+GnRH +PGF_2a	7
Group 3	CIDR+PGF_2a +PMSG	7

CIDR, controlled internal drug release; GnRH, gonadotropin-releasing hormone; PGF2α, prostaglandin F2α; PMSG, pregnant mare serum gonadotropin.

2.2 Estrus induction

Before estrus induction, the pudenda were cleaned with sterile saline and 70% alcohol solutions using cotton swab. Three protocols of estrus induction were performed (Fig. 1). Briefly, Group 1 was treated with controlled internal

drug release (CIDR) and prostaglandin F2α (PGF2α), in which Eazi-Breed™ CIDR® for sheep and goat (New Zealand Zoetis Australia, Pty, Ltd.) was inserted into the vagina of the goats, and removed five days after insertion. Next, simultaneous with CIDR removal, 15 mg PGF2a (Lutalyse, Zoetis, Belgium) was injected intramuscularly to the goats. Group 2 was first injected intramuscularly with 15 mg PGF2a, with 50 μg gonadotropin-releasing hormone (GnRH; Fertagyl) at 8 days after the first injection, and once again with 15 mg PGF2a at 7 days after the second injection. Group 3 was first subjected to CIDR insertion. Next, at 7 days after CIDR insertion, the goats in this group were injected intramuscularly with 15 mg PGF2a. Finally, at 2 days after injection, the CIDR was removed and 200 IU PMSG was injected to the goats.

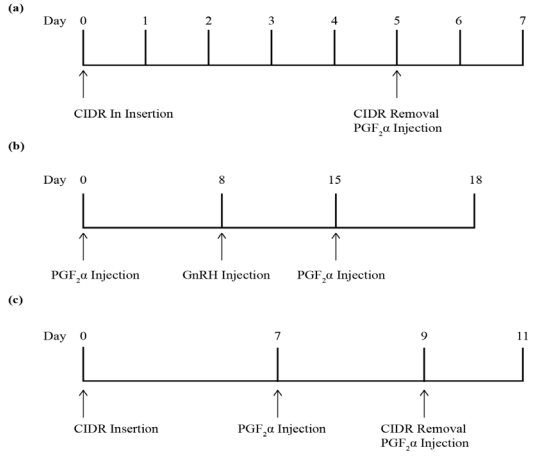

Fig. 1. Systematic diagram of estrus synchronization procedure

2.3 Measurement of P4, E2 in blood

To measure changes in the internal concentrations of P4 and E2, blood samples were collected via the jugular vein at approximately 9 a.m. daily at the time of hunger before 3 days of hormone treatment, and refrigerated for 12 h. Serum samples were obtained by centrifugation at 3,000 rpm for 10 min and then stored in a cold freezer at -70°C until experiments. Serum P4 and E2 concentrations were measured using electric chemiluminescence immunoassay.

2.4 Statistical analysis

Statistical analysis was conducted using SAS 9.2 version 2014. Duncan's multiple range test was conducted to determine significant differences.

3. Results

3.1 Blood P4 and E2 change in group 1

Through CIDR insertion, changes in the blood concentrations of P4 and E2 in Korean black goats were synchronized with estrus cycle (Table 2 and Fig. 2). As shown in Fig. 2, P4 level rapidly decreased to below 1 ng/mL after CIDR removal; however, it increased after remaining at a low level for 4 days. On the contrary, E2 level rapidly increased (20-30 pg/mL) after CIDR removal, and remained stable for 2 days.

Table 2. Changes in P4 and E2 concentrations in Group 1

Time of measurement		Progesterone (ng/mL)	Estrogen (ng/mL)
CIDR insertion		9.19±2.45	19.26±2.47
CIDR removal		12.48±1.73	12.25±1.66
After CIDR removal	D+1	0.57±0.06	25.19±2.28
	D+2	0.31±0.04	24.24±2.10
	D+3	0.25±0.04	17.22±2.82
	D+4	0.46±0.08	15.95±2.68
	D+5	1.37±0.25	24.45±2.99
	D+6	2.42±0.33	21.86±2.50
	D+7	3.21±0.48	15.40±1.67
	D+8	3.60±0.53	9.07±1.53

Data represented as mean±SE.

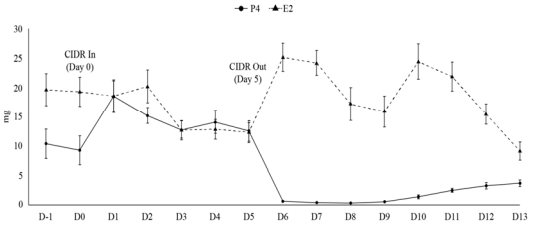

Fig. 2. Changes in P4 and E2 concentrations following estrus synchronization by controlled internal drug release (CIDR)

3.2 Blood P4 and E2 change in group 2

Changes in the blood concentrations of P4 and E2 in Korean black goats were synchronized with estrus by hormone injection (Table 3 and Fig. 3). As shown in Fig. 3, P4 level decreased to below 1 ng/mL following the second PGF2a injection and slowly increased after remaining at a low level for 4 days. In contrast, E2 level increased (20-30 ng/mL) after the second PGF2a injection and remained stable for 2 days.

Table 3. Changes in P4 and E2 concentrations in Group 2

Time of measurement		Progesterone (ng/mL)	Estrogen (ng/mL)
PGF2α injection		8.17±2.41	16.95±2.43
GnRH injection		10.90±1.85	15.90±2.50
PGF2α injection		17.65±2.81	14.02±1.96
After CIDR removal	D+1	1.08±0.12	19.95±2.19
	D+2	0.99±0.30	25.03±1.67
	D+3	0.95±0.38	28.85±2.97
	D+4	1.03±0.53	19.13±3.37
	D+5	1.55±0.75	16.36±2.83
	D+6	2.31±0.70	14.68±1.92

Data represented as mean±SE.

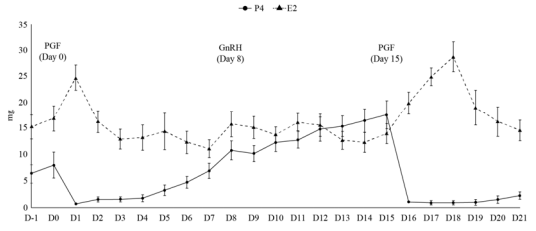

Fig. 3. Changes in P4 and E2 concentrations following estrus synchronization by prostaglandin F2α (PGF2α) and gonadotropin-releasing hormone (GnRH) injections

3.3 Blood P4 and E2 change in group 3

Changes in the blood concentrations of P4 and E2 in Korean black goats were synchronized with estrus through CIDR insertion combined with hormone injection (Table 4 and Fig. 4). As shown in Fig. 4, P4 level decreased after injection of 1 mg PGF2a and remained at low levels. On the contrary, E2 level rapidly increased (40 ng/mL) after PGF2a injection, but decreased after CIDR removal and PMSG injection. After reaching a peak, E2 concentration decreased within 2 days and remained at a stable level.

Table 4. Changes in P4 and E2 concentrations in Group 3

Time of measurement		Progesterone (ng/mL)	Estrogen (ng/mL)
CIDR insertion		28.43±3.88	16.44±2.09
PGF2α injection		6.30±0.474	14.19±1.87
CIDR removal and PMSG injection		0.73±0.08	41.25±3.24
After PMSG injection	D+1	0.48±0.04	30.99±3.32
	D+2	0.45±0.07	24.11±2.02
	D+3	1.45±0.36	24.79±2.25
	D+4	2.63±0.42	27.88±2.58
	D+5	3.62±0.89	25.66±2.84

Data represented as mean±SE.

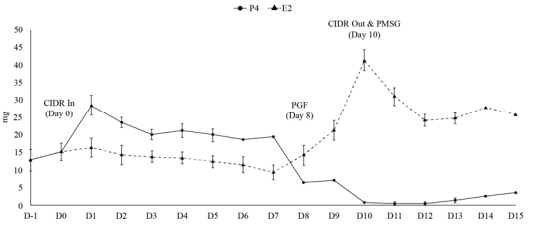

Fig. 4. Changes in P4 and E2 concentrations after estrus synchronization by controlled internal drug release (CIDR) and hormone injection prostaglandin F2α (PGF2α) and PMSG, pregnant mare serum gonadotropin

4. Discussion

Goat is a ruminant animal like cattle, but it is thought that different such as body weight, estrus cycle, gestation period and others. For that reason, it is necessary to investigate the physiological feature in Korean black goat. This study aimed to determine changes in blood P4 and E2 concentrations in Korean black goats after three different protocols of estrus induction to attain a better understanding of hormonal changes in this species during the estrus induction period.

The overall results of present study, different patterns were shown about P4 and E2 concentration until the last treatment in the three other protocols for estrus induction. Hormone treatment could affect to ovaries and induced a follicle growth and ovulation. However, it is estimated that hormone administration was not affect to blood hormone change. But it was assumed that first and second group showed different hormone patterns during same CIDR insertion period because of different estrus status before hormone administration to Korean black goat.

Although this study only two hormones results were shown, but luteinizing hormone as well as P4 and E2 were also examined for predict of ovulation time. The luteinizing hormone is a reliable marker for predicting ovulation in mammals. However, luteinizing hormone was not detected in blood of all groups during experiment period. It is presumed that luteinizing hormone was maintained very low concentration that difficult for measurable during pro and/or pre ovulation. Furthermore, oocyte ovulation was occurs after luteinizing hormone surging but the time is very short for measurable. For that reason, we seemed failing of luteinizing hormone measurement during experiment period in Korean black goat.

5. Conclusions

In conclusion, P4 and E2 concentrations showed different patterns until the last treatment in all three protocols of estrus induction. However, the results of the present study are not adequate determine the optimal time of artificial insemination in Korean black goats based on hormonal levels during estrus cycle. Although the optimal artificial insemination time can be roughly estimated, future studies involving a larger number of test animals and behavioural analysis are warranted.

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