Corpora Lutea Growth and Development in Thai-native Goats Throughout the Estrous Cycle

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ABSTRACT

This study was conducted to determine the growth and development of corpora lutea (CL) in Thai-native goats throughout the estrous cycle. Non pregnant Thai-native goats (n=12), 8 months of age were randomly assigned for estrous synchronization and superovulation protocols (progesterone 14 days+PMSG vs. progesterone 14 days+FSH). After progesterone removal, goats underwent laparotomy to determine number of CL post ovulation at 24, 48, 72 and 96 h. Blood sample were taken for progesterone concentration throughout the estrous cycle. Number of CL in goats receiving the protocols were not significantly different at times after ovulation (P>0.05) except for at 24 h (P=0.04). Concentration of plasma progesterone (P4) were not significantly different among goats difference in CL numbers (1, 2 and 3), with the concentration of 6.36, 5.45 and 5.72 ng/ml respectively. Plasma P4 concentrations of goats were low (<1 ng/ml) during the first 5 days of the estrous cycle, but significantly increased by day 7 (2.05 ng/ml), due to the presence of the developing CL. By day 19, the P4 concentration was less than 1 ng/ml due to the regression of CL. Goats underwent laparotomy to collect the ovary and CL using bilateral ovariectomy. Fresh weight of ovaries and CL were 841.8±164.47 and 311.75±63.59 mg, respectively. Percentages of ovarian weight represented by luteal and non-luteal tissues were 43.93 and 56.07%, respectively. The study was also indicated that estrous synchronization and superovulation protocols were not affected the number of CL. In addition, growth and development of CL gradually increased in early, but rapidly increased in mature or growing CL until day 13 or 14 due to the peak of P4 concentration. The regression of CL or luteolysis occurred between day 16 or 17 due to the secretion of $PGF_{2\alpha}$.

Key words: Corpora lutea, Growth and development, Thai-native goat

INTRODUCTION

The corpus luteum (CL) is a transient endocrine gland formed from the wall of Graffian follicle after the release of the egg, by a complex mechanism involving morphological and biochemical changes (Sangha et al., 2002). It is a dynamic endocrine gland showing variations in size, structure and steroidogenic activities in different stages of the estrous cycle and pregnancy (Fields and Fields, 1996). The CL, primary product progesterone (P4), is required for the establishment and maintenance of pregnancy. Inadequate P4 production is a major cause of infertility and embryonic loss since P4 is a necessary requirement for both endometrium development and embryo survival (Webb et al., 2002).

In previous studies found a strong relationship between CL size and plasma P4 on the luteal phase (Green et al., 2005). Low peripheral concentrations of P4 during the early luteal phase has been shown to be associated with low embryo survival rate (Diskin et al., 2002). Whether the effects of ovulatory follicle size on subsequent embryo survival are mediated directly or indirectly through its effect on subsequent CL size and P4 secretion is not yet fully elucidated (Lynch et al., 2010).

The objective of the present study was conducted to determine the growth and development of CL in Thai-native goats throughout the estrous cycle.

MATERIALS AND METHODS

Animals preparation

All experimental procedures were conducted under protocols approved by the Khon Kaen University Animal Care and Use Advisory Committee (Reference No. 0514.1.12.2/87). Non pregnant Thai-native goats (n=12), 8 months of age, were used exhibiting at least one estrous cycle of normal duration (20-21 days) were randomly assigned for estrous synchronization and superovulation protocols Treatment 1 (progesterone 14 days+PMSG): Animals were randomly treated with controlled internal drug release (CIDR) devices contain the hormone progesterone (EAZI-BREED™ CIDR®) for 14 days, and i.m. injection of 300 IU PMSG on day 13 (1 day prior to sponge removal). Treatment 2 (progesterone 14 days+FSH): Animals were randomly treated with CIDR (EAZI-BREED™ CIDR®) for 14 days, and i.m. injection of FSH twice daily (5, 4, and 3 mg per injection on day 12, 13, and 14, respectively), with the average body weight of 16.9±3.7 kg (mean±SE) respectively. Animals were fed with 1% BW of concentrate (16% CP) and ad libitum feeding of fresh grass. Clean water and mineral block were provided for animals ad libitum. Animals were vaccinated against foot and mouth disease (FMD), and brucellosis, according to the standard farm requirement of the Department of Livestock Development and the University farm.

Detection onset of estrus

Day 0 of the estrous cycle (standing estrus) was determined as the onset of estrus at by using vasectomized buck. The does were observed visually twice daily for estrus detection at 6.00 a.m. and 6.00 p.m. The vasectomized buck was introduced in the female goats and for at least 30 minutes.

Sample collection

Goats underwent laparotomy to collect the ovary and CL using bilateral ovariectomy. Briefly, goats were injected with 0.075 mg xylazine (Rompun®; L.B.S. Laboratory, Thailand) and bilateral ovariectomy surgery was performed to determine numbers of CL at 24, 48, 72, and 96 h after CIDR removal as previously described for ewes (Luther et al., 2007). For each goat, both ovaries weighed, and CL were enucleated, counted, weighed, and collected according to the procedure of Fields and Fields (1996).

Progesterone (P4) assay

A blood sample for P4 analysis was collected from each doe in the morning when onset of estrus was detected. Blood samples were collected via jugular venipuncture into 7 mL into EDTA solution, then immediately centrifuged at 1500 x g for 15 min. Blood plasma samples were harvested and freeze stored at -20°C until assay. P4 concentrations were determined by Enzyme-linked Immunosorbant Assay or ELISA (Cushwa et al., 1992). Goat anti-mouse IgG was made by using a P4-horse radish peroxidase conjugate, obtained from National Center for Genetic Engineering and Biotechnology, Thailand (BIOTEC). The intraassay coefficients of variation was 8.75%, and assay sensitivity was 0.025 ng/mL. A P4 concentration > 1.0 ng/mL was used to verify the presence of a functional CL (Lassala et al., 2004).

Statistical analyses

The data were expressed as means±SEM. Data on the numbers of CL were analyzed by analysis of variance using the GLM procedure of SAS (2001). A probability of $P<0.05$ was considered to be significant different.

RESULTS

Laparotomy was performed at 24, 48, 72 and 96 h after CIDR removal. Number of CL were recorded (Table 1). Number of CL in goats receiving the protocols were not significantly different at 48, 72, 96 h after ovulation ($P>0.05$) except for at 24 h ($P=0.04$).

Table 1. The effects of progestin protocols on number of CL in Thai-native goats.

Duration	14 days CIDR+PMSG	14 days CIDR+FSH	*P*-value
CL at 24 h	0.00 ± 0.00^{b}	1.00 ± 0.37^{a}	0.04
CL at 48 h	0.83 ± 0.17	1.50 ± 0.62	0.55
CL at 72 h	2.17 ± 0.40	2.17 ± 0.83	0.90
CL at 96 h	3.33 ± 0.49	2.50 ± 0.89	0.67

^{a,b}different superscripts in the same row indicate significant difference among treatment groups ($P< 0.05$)

The ovulatory response of goats receiving 14 days CIDR+FSH occurred sooner than goats in receiving 14 days CIDR+PMSG by which ovulation rates at 24 were significantly different (data from number of CL at 24 h after sponge removal; ($P=0.04$).

The overall mean P4 concentration in Thai-native goats observed during estrus was 3.2±0.1 ng/mL. P4 levels during periods of the estrous cycle are summarized in Figure 1. Concentration of plasma P4 were not significantly different among goats difference in CL numbers (1, 2 and 3), with the concentration of 6.36, 5.45 and 5.72, respectively.

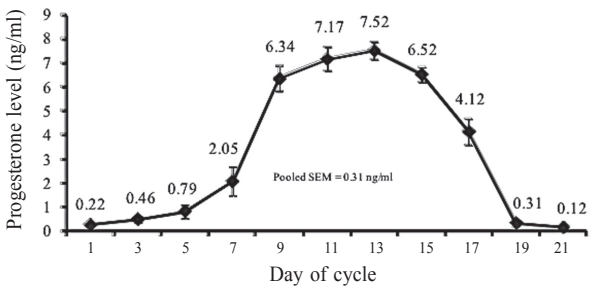

Figure 1. Plasma P4 concentration of Thai-native goats throughout the estrous cycle.

Plasma P4 concentrations of goats were low (<1 ng/ml) during the first 5 days of the estrous cycle, but significantly increased by day 7 (2.05 ng/ml), due to the presence of the developing CL. By day 19, the P4 concentration was less than 1 ng/ml due to the regression of CL.

Table 2. Fresh weight of ovaries and CL, and percentage of ovarian weight represented by luteal and nonluteal tissues on day 13 of the estrous cycle.

Day of the estrous cycle	No. of goat	Fresh weight		% of ovarian weight	
		Ovaries (mg)	CL (mg)	Luteal cell[1]	Non-luteal cell[2]
13	3	841.8±164.47	311.75±63.59	43.93±7.21	56.07±7.21

[1]CL were enucleated from ovaries.
[2]Ovaries without CL.

Goats underwent laparotomy to collect the ovary and CL using bilateral ovariectomy. Fresh weight of ovaries and CL were 841.8±164.47 and 311.75±63.59 mg, respectively. Percentages of ovarian weight represented by luteal and non-luteal tissues were 43.93 and 56.07%, respectively (Table 2).

DISCUSSION AND CONCLUSION

These results of are in agreement with many reports of previous studies that have shown that progestin with exogenous FSH or PMSG can promote ovarian activity and follicular development in does (Greyling and Van Niekerk, 1990a, b; Menegatos et al., 1995; Greyling and Van der Nest, 2000). Additionally, greater doses of PMSG would likely have promoted greater ovarian activity, which could have decreased the interval to onset of estrus and ovulation (Baril et al., 1993).

P4 levels of the female goats followed a pattern consistent with follicular and luteal phases and with ovarian inactivity postpartum. It was recommended that P4 levels should be monitored in the practical application of clinical endocrinology in caprine reproduction (Perkins and Fitzgerald., 1994). The peripheral plasma P4 profile during the reproductive cycle of the Thai-native goat in this study was similar to the West African Dwarf and indigenous Damascus does (Zarkawi and Soukouti, 2001).

The study was also indicated that estrous synchronization and superovulation protocols were not affected the number of CL. In addition, growth and development of CL gradually increased in early, but rapidly increased in mature or growing CL. Until day 13 or 14 due to the peak of P4 concentration. The regression of CL or luteolysis occurred between day 16 or 17 due to the secretion of $PGF_{2\alpha}$.

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