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THE PROFILES OF WEEKLY PROGESTERONE AND STRADIOL CONCENTRATIONS DURING PREGNANCY IN EWES : 2. THEIR CORRELATIONS WITH MAMMARY GROWTH INDICES AT PARTURITION

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ABSTRACT

Fifteen pregnant (9 and 6 carrying a single and multiple fetuses, respectively) and 5 nonpregnant ewes, as a control, were used to study the correlations of weekly maternal serum progesterone and estradiol concentrations during pregnancy with mammary growth indices at parturition. Blood samples were drawn weekly (weeks 0 to 20) during gestation period for determination of progesterone and estradiol concentrations. The experimental ewes were sacrificed at parturition to determine mammary growth indices (mammary dry fat-free tissue [DFFT], DNA, RNA, and collagen). Concentrations of progesterone at week 1 and weeks 3 to 19 of pregnancy positively correlated with mammary DFFT, DNA, and RNA at parturition. Maternal serum progesterone concentrations at weeks 1, 3 and weeks 6 to 20 of pregnancy positively correlated with mammary collagen at parturition. Concentrations of estradiol at weeks 5, 7, 9, and 11 to 20 positively correlated with the mammary DFFT, DNA, RNA, and collagen at parturition. The higher the maternal serum progesterone and estradiol concentrations during pregnancy the greater the mammary growth and development at parturition.

Keywords : progesterone, estradiol, mammary growth, pregnancy, sheep

Secretions of estradiol and progesterone increase dramatically with the changes in ovarian activity during the estrous cycle and pregnancy. In ovine, secretion of estradiol increases during proestrous along with the maturation of the follicle in the ovary, then decreases significantly during the embryonal stage of pregnancy, and increases precipitously during the fetal stage of pregnancy until parturition (Umo *et al.*, 1976; Pant *et al.*, 1977; Manalu *et al.*, 1996). Progesterone increases 2 days after ovulation, and it shows a marked rise from day 5 to a peak between days 7 and 13 (Umo *et al.*, 1976; Pant *et al.*, 1977). It remains almost stable during the first 7 weeks of pregnancy and increases dramatically after week 8 of pregnancy (Butler *et al.*, 1981; Manalu *et al.*, 1996).

During the luteal phase of estrous cycle and the embryonal stage of pregnancy, maternal serum progesterone concentrations are positively correlated with the number of corpora lutea (Quirke *et al.*, 1979). During the fetal stage of pregnancy in goats and sheep (when the placenta is functional), progesterone and estradiol (Manalu *et al.*, 1996), and placental lactogen (Hayden *et al.*, 1979; Hayden *et al.*, 1980; Butler *et al.*, 1981) also increases with the increased fetal number.

Mammary gland growth and development during pregnancy in ovine starts during the estrous cycle, slowly progresses until the first three months of gestation and dramatically increases during the last two months of gestation (Anderson, 1975; Anderson *et al.*, 1981) around the time when the placenta significantly secretes progesterone (Ricketts & Flint, 1980; Sheldrick *et al.*, 1981), and placental lactogen (Hayden *et al.*, 1979; Hayden *et al.*, 1980; Butler *et al.*, 1981).

Temporal changes in mammogenic hormones secretions relating to pregnancy seem to correlate well with the mammary growth pattern in ovine. The present study was designed to determine temporal changes in maternal serum progesterone concentration during pregnancy and mammary gland growth and development at parturition in ewes.

METHODS

Experimental Conditions and Animals

This experiment was conducted during the hot (25 to 32°C) and wet (70 to 80% relative humidity) season in the humid tropics of Indonesia. Experimental animals were 20 Javanese thin-tail ewes (5, 9, 4, and 2 ewes carrying 0, 1, 2, and 3 fetuses, respectively) with similar body weight (20 to 22 kg) and age (2 to3 years) at breeding. Javanese thin-tail sheep is a meat-type indigenous breed well recognized for its high prolificacy (Bradford *et al.*, 1986; Sutama *et al.*, 1988). The experimental ewes were injected twice with $PGF_{2\alpha}$ (i.m) at an 11-day interval. Three days after the last injection, 15 ewes were mated naturally by group breeding.

Blood Sampling and Processing

Ten ml of blood samples were drawn with plain vacutainer or sterile syringes from the jugular vein between 0900 and 1000 h. The first blood samples were taken one day after the last prostaglandin injection (week 0 of pregnancy), and 10 days later (seven days after the predicted ovulation, as the end of week 1 of pregnancy). Additional blood samples were drawn weekly on Thursday until parturition. Blood samples were allowed to clot in a cool ice box, centrifuged to separate serum, which was then frozen for estradiol and progesterone analyses. At parturition the experimental ewes were sacrificed for determination of mammary gland growth and development at the beginning of lactation.

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Progesterone, Estradiol, and Mammary Gland Analyses

The concentration of serum progesterone was measured in duplicate by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) using I^{125}-progesterone as a tracer, with a slight modification to accommodate wide ranges of progesterone concentrations in pregnant ovine (Manalu *et al.*, 1996). The progesterone assay used the whole serum without prior ether extraction. The radioactivities of I^{125}-progesterone-bound tubes were counted with an automatic gamma counter. The lowest and highest limits of sensitivity of assay were 0.1 and 20 ng/ml, respectively. Therefore, the concentrations of standard progesterone used to construct the standard curve ranged from 0.1 to 20 ng/ml. A sample volume of 100 ml serum was used in the assay of samples with progesterone concentrations ranged from 0.1 to 20 ng/ml. For samples with progesterone concentrations lower than 0.1 ng/ml, sample volume was increased to 200 ml. For samples with progesterone concentrations higher than 20 ng/ml, sample volume was reduced to 50 ml. Inter- and intra-assay coefficients of variation were 6. and 4%, respectively. Concentrations of progesterone were parallel in the sample volumes of 50, 100, and 200 ml.

The concentration of serum estradiol was measured in duplicate by the solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) using I^{125}-estradiol as a tracer, with a slight modification to accommodate wide ranges of estradiol concentrations in pregnant ovine (Manalu *et al.*, 1996). The estradiol assay used the whole serum without prior ether extraction. The radioactivities of I^{125}-estradiol-bound tubes were counted with an automatic gamma counter. The lowest and highest limits of sensitivity of assay were 20 and 150 pg/ml, respectively. Therefore, the concentrations of standard estradiol used to construct the standard curve ranged from 20 to 150 pg/ml. A sample volume of 100 ml serum was used in the assay for samples with estradiol concentrations ranged from 20 to 150 pg/ml. For samples with estradiol concentrations lower than 20 pg/ml, sample volume was increased to 200 to 300 ml to bring the estradiol concentrations to the range of standard used. For samples with estradiol concentrations higher than 150 pg/ml, sample volume was decreased to 50 ml to bring the estradiol concentrations to the range of standard used. All samples' estradiol concentrations were within the range of concentrations of standard estradiol used to construct the standard curve. Inter- and intra-assay variations coefficients were 7 and 5.0%, respectively. The concentrations of estradiol were parallel in the sample volumes of 50, 100, 200 and 300 ml.

Dry fat-free tissue (DFFT), DNA, RNA and collagen are indices used to determine mammary gland growth and development. Dry fat-free tissue (DFFT) was measured by modification of method described by Anderson (1975). Half the udder was excised and the mammary gland was isolated by trimming skin and subcutaneous fat and removing milk inside the gland. The isolated mammary gland was frozen for easy slicing. The thinly sliced mammary gland was soaked in ethanol for 48 hr and then with diethyl ether (48 hr) until the glands became free of fat, and then dried at 50°C for 24 h to obtain DFFT. The DFFT was then ground to make a fine powder to be used for mammary chemical indices determinations. Mamamary DNA was determined by p-nitrophenylhydrazine reaction (Webb & Levy, 1955), RNA by orcinol reaction (Albaum & Umbreit, 1947), collagen by measuring hydroxyproline (Woessner, 1961).

Statistical Analyses

Weekly estradiol and progesterone concentrations during pregnancy were regressed with mammary DFFT, DNA, RNA and collagen at parturition using simple regression and correlation analyses (Neter *et al.*, 1985).

RESULTS

The profile of maternal serum progesterone concentration during pregnancy in the experimental ewes is setout in Figure 1. Progesterone concentration slowly increased during the first 8 weeks of pregnancy. After that, it increased rapidly and reached peak concentrations around weeks 12 to 16 of pregnancy and then it decreased near parturition. In the ewes carrying multiple fetuses, concentrations of progesterone were distinctly higher than in those carrying a single fetus, and the greatest differences occurred during weeks 12 to 16 of pregnancy.

The profile of estradiol concentrations in the maternal serum during pregnancy in the experimental ewes is depicted in Figure 2. The concentrations of estradiol were constantly low during the first 8 weeks of pregnancy. After week 8 of pregnancy, estradiol concentration in the pregnant ewes constantly increased until parturition, and being higher in the higher litter size. In the ewes carrying multiple fetuses, the concentrations of estradiol were distinctly higher than in those carrying a single fetus, and the greatest differences occurred during weeks 12 to 20 of pregnancy.

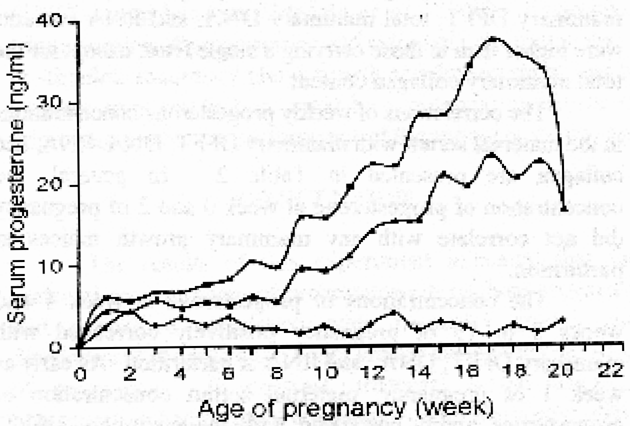

Figure 1. Weekly serum progesterone concentrations during pregnancy in the nonpregnant ewes (■), ewes carrying a single (D) and multiple (■) fetuses in Javanese thin-tail sheep.

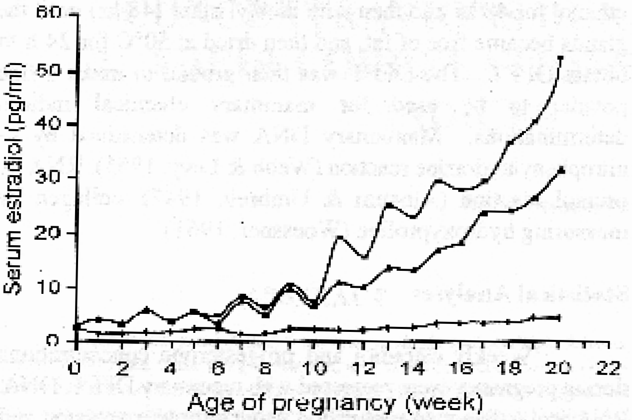

Figure 2. Weekly serum estradiol concentrations during pregnancy in the nonpregnant ewes (u), ewes carrying a single (D) and multiple (n) fetuses in Javanese thin-tail sheep.

Table 1. Mean (±SE) mammary DFFT and total mammary DNA, RNA, and collagen contents at parturition in the nonpregnant ewes, ewes carrying a single and multiple fetuses

Litter size	N	Total mammary chemical indices contents (g)			
		DFFT	DNA	RNA	Collagen
0	5	2.34^{c}	0.10^{c}	0.03^{c}	0.11^{b}
		0.21	0.01	0.003	0.01
1	9	26.39^{b}	0.92^{b}	0.30^{b}	0.30^{a}
		2.02	0.09	0.05	0.04
>1	6	45.88^{a}	1.51^{a}	0.94^{a}	0.35^{a}
		10.56	0.30	0.23	0.04

[abc]Means with different superscripts in the same column are significantly different ($P < 0.05$).

Average mammary DFFT and its component were absolutely lower in nonpregnant than in pregnant ewes (Table 1). However, in pregnant ewes carrying multiple fetuses, mammary DFFT, total mammary DNA, and RNA contents were higher than in those carrying a single fetus, except for the total mammary collagen content.

The correlations of weekly progesterone concentrations in the maternal serum with mammary DFFT, DNA, RNA, and collagen are presented in Table 2. In general, the concentration of progesterone at week 0 and 2 of pregnancy did not correlate with any mammary growth indices at parturition.

The concentrations of progesterone at week 1 and weeks 3 to 19 of pregnancy positively correlated with mammary DFFT, DNA, and RNA at parturition. As early as week 1 of pregnancy, maternal serum concentration of progesterone highly correlated with the mammary DFFT, DNA, and RNA at parturition. The higher the maternal serum concentrations at weeks 3 to 19 the higher mammary DFFT, DNA, and RNA contents indicating a greater mammary growth and development, mammary cell number and cellular activities at parturition. The highest correlations of mean serum progesterone concentrations with mammary DFFT, DNA, and RNA were occurred at week 12 of pregnancy.

The concentrations of progesterone at weeks 1 and 3, and weeks 7 to 20 of pregnancy positively correlate with mammary collagen at parturition. As early as week 1 of pregnancy, maternal serum concentration of progesterone was highly correlated with the mammary collagen at parturition. The higher the maternal serum concentrations at weeks 1 and 3, and weeks 7 to 20 of pregnancy the higher mammary collagen contents implying the greater mammary ductal growth and branching at parturition. The highest correlation of mean serum progesterone concentrations with mammary collagen was occurred at week 14 of pregnancy.

Table 2. Coefficients of correlation between weekly maternal serum progesterone concentrations during pregnancy and mammary gland indices (DFFT, DNA, RNA and collagen) at parturition in Javanese thin-tail ewes

Weeks of pregnancy	Mammary gland indices			
	DFFT	DNA	RNA	Collagen
0	0.01	0.04	0.03	0.21
1	0.66**	0.64**	0.65**	0.54*
2	0.04	0.11	0.15	0.08
3	0.65**	0.68**	0.56*	0.49*
4	0.47*	0.51*	0.44*	0.24
5	0.46*	0.51*	0.47*	0.26
6	0.58*	0.69**	0.59*	0.49*
7	0.67**	0.77**	0.73**	0.57*
8	0.62*	0.70**	0.60*	0.68**
9	0.78**	0.84**	0.78**	0.62*
10	0.73**	0.76**	0.68**	0.67**
11	0.77**	0.82**	0.75**	0.67**
12	0.85**	0.89**	0.84**	0.60*
13	0.69**	0.74**	0.72**	0.61*
14	0.57*	0.60*	0.51*	0.84**
15	0.79**	0.81**	0.66**	0.74**
16	0.72**	0.76**	0.78**	0.69**
17	0.80**	0.84**	0.78**	0.80**
18	0.71**	0.76**	0.70**	0.82**
19	0.74**	0.74**	0.69**	0.72**
20	0.43	0.41	0.44	0.78**

* $P < 0.05$.
** $P < 0.01$.

The correlations of weekly estradiol concentrations in the maternal serum with mammary DFFT, DNA, RNA, and collagen are presented in Table 3. In general, the concentration of estradiol at early stage of pregnancy (weeks 0 to 4) did not correlate with any mammary growth indices at parturition. The correlations of estradiol with DFFT, DNA and RNA, and with collagen, became evident from weeks 11 to 20 and weeks 12 to 20 of pregnancy, respectively, as concentrations of estradiol in the maternal circulation became significantly increase. These data indicated that the higher the concentrations of estradiol in the maternal circulation during weeks 11 to 20 of pregnancy, the greater the mammary gland growth and development at parturition.

Table 3. Coefficients of correlation between weekly maternal serum estradiol concentrations during pregnancy and mammary gland indices (DFFT, DNA, RNA and collagen) at parturition in Javanese thin-tail ewes

Weeks of pregnancy	Mammary gland indices			
	DFFT	DNA	RNA	Collagen
0	0.13	0.24	0.08	0.09
1	0.13	0.06	0.12	0.35
2	0.12	0.15	0.16	0.09
3	0.25	0.31	0.26	0.39
4	0.07	0.09	0.10	0.32
5	0.48*	0.48*	0.44*	0.57**
6	0.10	0.08	0.28	0.02
7	0.49*	0.46*	0.39	0.31
8	0.39	0.38	0.16	0.42
9	0.55**	0.53*	0.56**	0.55**
10	0.16	0.16	0.02	0.29
11	0.65**	0.65**	0.71**	0.38
12	0.63**	0.68**	0.61**	0.57**
13	0.50*	0.60**	0.52*	0.55**
14	0.67**	0.68**	0.63**	0.55**
15	0.63**	0.62**	0.59**	0.69**
16	0.61**	0.62**	0.57**	0.76**
17	0.60**	0.56**	0.49*	0.63**
18	0.48*	0.50*	0.42*	0.71**
19	0.63**	0.58**	0.57**	0.66**
20	0.62**	0.64**	0.57**	0.64**

* $P < 0.05$
** $P < 0.01$

DISCUSSION

Progesterone and estradiol are traditionally considered as hormones function in maintaining of pregnancy and parturition, respectively. The patterns of progesterone and estradiol profiles during pregnancy in sheep and goats have been explained previously (Manalu *et al.*, 1996; Manalu & Sumaryadi, 1998). The increased maternal serum progesterone and estradiol with the advance of pregnancy, especially during the fetal stage of pregnancy, is far beyond the levels required to maintain pregnancy. Previous report states that the increased maternal serum progesterone, and probably estradiol, with the advance of pregnancy has some farther functions in stimulation of mammary growth during pregnancy in preparation of nutrients secretion required by the newborn offsprings (Manalu & Sumaryadi, 1996).

Observations in sheep and goats (Anderson, 1975; Anderson *et al.*, 1981) suggest that mammary gland growth and development during pregnancy are parallel with the temporal changes in hormonal secretion during gestation. Among hormones that their secretions increase during pregnancy are estradiol, progesterone, relaxin, and placental lactogen (Hayden *et al.*, 1979; Hayden *et al.*, 1980; Ricketts & Flint, 1980; Butler *et al.*, 1981; Sheldrick *et al.*, 1981). These hormones are generally grouped as mammogenic hormones i.e., hormones stimulating mammary growth and development. Exogenous administrations of progesterone, estradiol and relaxin in ovariectomized and nonpregnant mice have been shown to stimulated mammary gland growth and development as indicated by the increase in DFFT, DNA, RNA and collagen contents of the glands (Harness & Anderson, 1977a; Harness & Anderson, 1977b; Hayden *et al.*, 1979; Wright & Anderson, 1982; Wahab & Anderson, 1989).

The results of this experiment showed that the increased maternal concentrations of progesterone and estradiol during pregnancy had a positive correlation with mammary growth and development at parturition. Higher concentrations of progesterone and estradiol during pregnancy associated with the greater mammary growth and development at parturition. The correlation of maternal serum progesterone with mammary gland growth and development was evident since the beginning of pregnancy. However, the correlation of maternal serum estradiol with mammary gland growth and development was more evident during the fetal stage of pregnancy. The difference in temporal correlation of maternal serum concentrations of estradiol and progesterone during pregnancy with mammary gland growth at parturition was probably related to the temporal difference in secretion of both hormones, and the fluctuation in the secretion of the hormones during pregnancy. Maternal serum progesterone concentration increased slowly during weeks 1 to 8 of pregnancy, then increased dramatically until week 17, and then slowly decreased approaching parturition. Maternal serum estradiol tended to decrease from estrous to pregnancy (not shown in the Figure 2) and relatively stable during the first 7 weeks of pregnancy, and slowly increased until week 10 of pregnancy. Estradiol increased dramatically at week 11 of pregnancy until parturition.

Regardless of the difference, the results implied that increasing maternal serum progesterone and estradiol during pregnancy, either by exogenous administration or by increasing their endogenous secretion by superovulation, could be utilized to improve mammary gland growth and development at the beginning of lactation and milk production during lactation. Preliminary result shows that superovulated ewes have higher progesterone concentrations and greater mammary growth and development during pregnancy (Manalu *et al.*, 1999) and correspondingly higher milk production (60%) during lactation (Manalu *et al.*, 2000). Exogenous administration of progesterone and estradiol during pregnancy to stimulate mammary gland growth and development, and to increase milk production could be a potential technique in improving production performance of the animal in the tropics.

CONCLUSION

The results of this experiment indicated that the maternal concentrations of progesterone throughout pregnancy had a positive correlation with mammary growth and development at parturition; being greater during the fetal stage of pregnancy. In contrast, maternal serum estradiol concentration had positive correlations with mammary gland growth and development during the fetal stage of pregnancy. The results suggested that increasing maternal serum progesterone and estradiol during pregnancy, either by exogenous administration or by increasing their endogenous secretion by superovulation, could be used to improve mammary gland growth and development at the beginning of lactation and milk production during lactation.

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