The Effects of Corpora Luteal Number on Serum Progesterone and Estradiol of Ewes During Luteal Phase of Estrous Cycle and Pregnancy

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ABSTRACT: Two experiments were conducted to study the effects of corpora luteal number on maternal serum concentrations of progesterone and estradiol during luteal phase of estrous cycle and pregnancy. The experimental ewes were injected twice with 1 ml $PGF_{2\alpha}$ to destroy previously existed corpora lutea. The number of corpora lutea was counted by laparoscopy 5 days after the onset of estrous cycle. Blood samples were collected weekly during the estrous cycle and biweekly during pregnancy for hormone analyses using radioimmunoassay. Simple correlation was used to determine the relationship between the number of corpora lutea and progesterone or estradiol concentrations. Progesterone concentrations increased with the number of corpora lutea during the luteal phase of estrous cycle ($P<0.05$) ($r = 0.34$), the first half of pregnancy ($P<0.01$) ($r = 0.48$), the second half of pregnancy ($P<0.01$) ($r = 0.63$), and during the whole gestation period ($P<0.01$) ($r = 0.63$). However, serum estradiol concentrations decreased slightly with the number of corpora lutea ($r = -0.09$) during the luteal phase of the estrous cycle, then slightly increased during the first half of pregnancy ($r = 0.02$), the second half of pregnancy $r = 0.33$), and during the whole pregnancy ($r = 0.30$). The results suggested that the number of corpora lutea correlated more with the concentrations of progesterone than with those of estradiol during the luteal phase of estrous cycle, the first and the second half of pregnancy or during the whole period of pregnancy. It was concluded that endogenous secretion of progesterone and estradiol as mammogenic hormones could be increased by increasing the number of ovulating follicle during the estrous cycle.

Key Words: Sheep, Estrous Cycle, Pregnancy, Corpus Luteum, Estradiol, Progesterone.

Introduction

During the luteal phase of estrous cycle and pregnancy, progesterone and estradiol are primarily produced by corpus luteum and placenta. Concentrations of the hormones increased during the luteal phase of estrous cycle and pregnancy (MacDonald, 1980). The hormones are involved in stimulating mammary growth and development (Harness and Anderson, 1977a; Harness and Anderson, 1977b; Wright and Anderson, 1982; Anderson, 1986; Tucker, 1986; Tucker, 1987; Wahab and Anderson, 1989) during pregnancy in preparations of milk synthesis for the newborn lambs. In addition, progesterone has been well known for its effects on maintenance of pregnancy through its effects on growth and development of the uterine gland and tissue to support the survival of the embryo (MacDonald, 1980; Anderson, 1986; Tucker, 1986).

It was hypothesized that secretion of endogenous progesterone and estradiol correlated with the number of ovulating follicles during the estrous cycle in preparation of uterine environments for further conception, and mammary gland growth and development for milk synthesis. The objective of this experiment was to study the profile of maternal serum concentrations of progesterone and estradiol in relation to the number of corpora lutea either during the luteal phase of estrous cycle or during pregnancy.

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Experimental Procedures

General. Two experiments were conducted to study the correlation between the number of corpora lutea and serum concentrations of progesterone and estradiol. Forty seven ewes with similar weight (20 - 22 kg) and age (2 - 3 years) were used to study the correlation between the number of corpora lutea and maternal serum progesterone and estradiol during the luteal phase of estrous cycle. Twenty nine ewes were used to study the correlation between the number of corpora lutea and maternal serum concentrations of progesterone and estradiol during pregnancy. The experimental ewes were maintained in the experimental pen with a month adaptation to the experimental conditions prior to mating period. Prior to mating period, the ewes were injected with $PGF_{2\alpha}$ i.m. twice to synchronize estrous cycle and to destroy previously existed corpora lutea. The number of corpora lutea formed during the estrous cycle was counted 5 d after the onset of estrous cycle by laparoscopy. Blood samples were drawn weekly during the estrous cycle and biweekly during pregnancy beginning one day after the last $PGF_{2\alpha}$ injections. During the estrous cycle experiment, the experimental ewes were assigned into 4 groups according to the number of corpora lutea (1, 2, 3, and >3) formed during the estrous cycle with n = 12, 19, 13, and 3, respectively. During the period of pregnancy, the experimental ewes were assigned into 5 groups according to the number of corpora lutea (0, 1, 2, 3, and >3) formed during the estrous cycle with n = 3, 4, 13, 6, and 3, respectively.

Blood sampling and processing. Ten milliliters of blood samples were drawn with plain vacutainer or sterile syringes from jugular vein prior to morning feeding at around the same time weekly or biweekly. The blood samples were allowed to clot in an cool ice box and transported to the laboratory for further separation of serum by centrifugation. The serum samples were then kept frozen for further hormone analyses.

Progesterone analyses. Concentration of serum progesterone was measured by the solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The radioactivities of progesterone-bound tubes were counted with an automatic gamma counter (Aloka, Model ARC 503, Aloka Co., Ltd., Japan). The concentrations of standard progesterone used to construct a standard curve ranged from 0.1 to 20 ng/ml. All samples progesterone concentrations were within the range of concentrations of the standard progesterone used to construct the standard curve. A sample volume of 100 µl serum was used in the assay.

Estradiol analyses. Concentration of serum estradiol was measured by the solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The radioactivities of estradiol-bound tubes were counted with an automatic gamma counter (Aloka, Model ARC 503, Aloka Co., Ltd., Japan). The concentrations of standard estradiol used to construct a standard curve ranged from 20 to 500 pg/ml. All samples estradiol concentrations were within the range of concentrations of the standard estradiol used to construct the standard curve. A sample volume of 100 µl serum was used in the assay.

Statistical analyses. Averages of progesterone and estradiol concentrations during the estrous cycle, and first and the second half of pregnancy, and during the whole period of pregnancy were correlated with the number of corpora lutea using simple regression and correlation analysis (Neter et al., 1985).

Results and Discussion

Averages of maternal serum progesterone concentrations during the luteal phase of estrous cycle, the first half of pregnancy, the second half of pregnancy, and the whole period of pregnancy are presented in Table 1.

Maternal serum progesterone concentrations increased with the number of corpora lutea during the luteal phase of estrous cycle ($P<0.05$) with r = 0.34. During the first half of pregnancy the number of corpora lutea strongly correlated with the concentrations of maternal serum progesterone ($P<0.01$) with r = 0.48. During the second half of pregnancy, when placentation had been completed, correlation between the number of corpora lutea and maternal serum progesterone concentrations was even greater ($P<0.01$) with r = 0.63. Maternal serum progesterone concentrations during the whole pregnancy strongly correlated with the number of corpora lutea ($P<0.01$) with r = 0.63. Regardless of corpora luteal number, progesterone concentrations during the first and the second half of pregnancy increased by 40 and 240%, respectively, as compared to those during the luteal phase of estrous

Table 1. Maternal serum concentrations of progesterone (ng/ml) during luteal phase of estrous cycle, first half of pregnancy, second half of pregnancy and during the whole period of pregnancy of ewes with different number of corpora lutea.[1]

Number of corpus luteum	Stages of reproduction			
	Luteal phase of estrous cycle[2]	First half of pregnancy	Second half of pregnancy	The whole pregnancy
0	ND	2.6580±0.1313	2.9553±0.7449	2.8066±0.4192
1	3.21±0.15	5.2991±0.5790	11.6699±1.0917	8.4845±0.6062
2	4.21±0.22	6.1070±0.4191	16.0122±1.2631	11.0713±0.6943
3	4.10±0.36	6.7564±0.5700	14.9122±1.7418	10.8338±1.0887
>3	5.17±1.15	6.6688±1.1205	19.2569±0.4139	12.9578±0.7477
r	0.34[a]	0.48[b]	0.63[b]	0.63[b]

[1]Presented as means and SE of 12, 19, 13, and 3 ewes with 1, 2, 3, and >3 corpora lutea, respectively, during the luteal phase of estrous cycle, and 3, 4, 13, 6, and 3 ewes with 0, 1, 2, 3, and >3 corpora lutea, respectively, during the period of pregnancy.

[2]There was no group of ewes with 0 corpus luteum during the luteal phase of estrous cycle.

[a] $P<0.05$. [b] $P<0.01$,

Table 2. Maternal serum concentrations of estradiol (pg/ml) during luteal phase of estrous cycle, first half of pregnancy, second half of pregnancy and during the whole period of pregnancy of ewes with different number of corpora lutea.[1]

Number of corpus luteum	Stages of reproduction			
	Luteal phase of estrous cycle[2]	First half of pregnancy	Second half of pregnancy	The whole pregnancy
0	ND	2.7039	4.0329	3.3684
		0.2301	1.0693	0.6373
1	4.90	3.0883	7.4305	5.2594
	1.41	0.5865	1.1172	0.8468
2	4.09	2.8964	12.4790	6.9954
	1.14	0.2735	2.4205	1.1844
3	4.45	3.2160	9.7141	6.4650
	1.29	0.4462	2.2253	1.1421
>3	2.57	2.6679	14.0401	8.3540
	0.04	0.2319	0.7472	0.4803
r	-0.09	0.002	0.33	0.30

[1]Presented as means and SE of 12, 19, 13, and 3 ewes with 1, 2, 3, and >3 corpora lutea, respectively, during the luteal phase of estrous cycle, and 3, 4, 13, 6, and 3 ewes with 0, 1, 2, 3, and >3 corpora lutea, respectively, during the period of pregnancy.

[2]There was no group of ewes with 0 corpus luteum during the luteal phase of estrous cycle.

cycle. Maternal serum progesterone concentrations during the second half of pregnancy increased by 142% as compared those during the first half of pregnancy.

Maternal serum concentrations of estradiol during the luteal phase of estrous cycle, the first half of pregnancy, the second half of pregnancy, and the whole period of pregnancy are presented in Table 2.

Maternal serum estradiol concentrations decreased slightly with the number of corpora lutea (r = -0.09) during the luteal phase of estrous cycle. Maternal serum concentrations of estradiol slightly increased with the increased number of corpora lutea during the first half of pregnancy with r = 0.02. During the second half of pregnancy, however, the increased number of corpora lutea positively correlated, but not significant, with maternal serum concentrations of estradiol with r = 0.33. Maternal serum estradiol concentrations during the whole period of pregnancy increased (P>0.05) with the increased number of corpora lutea (r = 0.30). Regardless of corpora luteal number, estradiol concentrations during the first and the second half of pregnancy decreased by 27% and increased by 162%, respectively, as compared to those during the luteal phase of estrous cycle. Maternal serum progesterone concentrations during the second half of pregnancy increased by 256% as compared those during the first half of pregnancy.

Progesterone concentrations slowly increased and reached peak 15 days after PGF_2 injections during the luteal phase of estrous cycle and then returned to the basal level. On the other hand, maternal serum concentrations of estradiol tended to decrease during the luteal phase of estrous cycle. When the ova were fertilized, maternal serum progesteron and estradiol concentrations slowly increased during the first half of pregnancy and then dramatically increased and finally reached plateau during the second half of pregnancy. Then estradiol tended to increase during the weeks approaching the parturition date.

The strong correlation between the number of corpora lutea and maternal serum progesterone, and to a lesser extend maternal serum estradiol, concentrations during the luteal phase of estrous cycle and during pregnancy indicated that the number of corpora lutea was an important factor in determining the circulating level of progesterone and estradiol in maternal serum. The inflection in progesterone and estradiol secretions after the first half of pregnancy probably due to the functional placenta as glands secreting the hormones during this period (Manalu et al., 1994), since ovulation rates litter size are closely related (Piper and Bindon, 1984; Bradford, 1985; Bradford et al., 1986).

There was no available reports at this time about the correlation of corpora luteal number with maternal serum concentrations of progesterone and estradiol. However, the ranges found in these studies agreed with those reported in sheep (Pant et al., 1977; Butler et al., 1981).

The increased progesterone and estradiol concentrations with the increased number of corpora lutea was regarded as biological signals for uterine growth and development in preparation of suitable environments for implantation and embryonic growth and development (Rattray et al., 1974; MacDonald, 1980) even though all ova released would not be necessarily fertilized.

When the data were observed closely, however, the increased concentrations of maternal serum progesterone and estradiol did not arithmetically increase with the increased number of corpora lutea from one to greater than three. Contributions of the increased number of corpora lutea to the increased maternal serum progesterone concentrations were only 12, 23, 40, and 40% during the luteal phase of estrous cycle, the first half of pregnancy, the second half of pregnancy, and the whole period of pregnancy, respectively, with far less contributions to the increased concentrations of estradiol (-08, 0.004, 10.9 and 9%, respectively).

The results of the experiment indicated that the increased number of corpora lutea probably did not arithmetically increase synthetic activities of the cells secreting the hormones. It was hypothesized that there were some other factors, other than the number of corpora lutea, that affected the expression of synthetic activities of the cells. These factors could be blood flow, growth factors, or hormones controlling the growth and development of the theca cells and the granulosa cells of corpus luteum secreting the hormones. The increased number of corpora lutea would decrease blood flow to each corpus luteum thereby reducing substrates and growth factors inflows to sustain maximum growth and development of each corpus luteum. This hypothesis analogue with the common observations that birth weight of individual lamb would decreased as litter size increased.

The results suggested that the number of corpora lutea correlated more to the concentrations of progesterone than those of estradiol either during

the luteal phase of estrous cycle, the first half and the second half of pregnancy or during the whole period of pregnancy. These phenomena could be related to the primary function of progesterone as a hormone required for uterine growth and development (MacDonald, 1980) in preparation of suitable environment for implantation, while the function of estradiol was assumed to be secondary (MacDonald, 1980). In addition, different responses in synthetic activities of the cells secreting progesterone and the cells secreting estradiol probably exists in keeping proper balance between progesterone and estradiol in maintenance of pregnancy.

Implications

The results of this experiment showed that progesterone and estradiol concentrations increased with the increased number of corpora lutea. Contributions of corpora lutea to maternal serum concentrations of progesterone were greater than to those of estradiol. Endogenous secretions of progesterone and estradiol as mammogenic hormones could be increased by increasing the number of ovulating follicle during the estrous cycle.

The contributions of the increased maternal serum progesterone and estradiol concentrations during pregnancy to mammary gland growth and development merit further studies.

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