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The Effects of Fetal Number on Maternal Serum Progesterone and Estradiol of Ewes During Pregnancy

W. Manalu[1,2], M.Y. Sumaryadi[1,3] and N. Kusumorini[1]

Faculty of Veterinary Medicine, Bogor Agricultural University.

ABSTRACT: Twenty nine ewes with similar weight and age were used to study the contributions of fetal number to circulating concentrations of progesterone or estradiol during whole pregnancy. The ewes were injected with 1 ml synthetic prostaglandin $PGF_{2\alpha}$ (Prosolvin, Intervet) i.m. and were repeated on d 11 from the first injection to synchronize the estrous cycle. Based on the number of lambs born at parturition date, the ewes were assigned into 5 groups (0, 1, 2, 3, and 4) with n = 3, 12, 9, 4, and 1, respectively. Blood samples were collected every other week for progesterone and estradiol analyses using radioimmunoassay. Simple correlation was used to determine the relationship between the fetal number and serum progesterone or estradiol concentrations. The results of the experiment showed that fetal number correlated with the maternal serum progesterone concentrations either during the first half of pregnancy ($P<0.01$) with $r = 0.70$, the second half of pregnancy ($P<0.01$) with $r = 0.75$, or during the whole period of pregnancy ($P<0.01$) with $r = 0.77$. Fetal number did not correlate with maternal serum concentrations of estradiol during the first half pregnancy. However, fetal number correlated with the estradiol concentrations during the second half of pregnancy ($P<0.01$) with $r = 0.49$ and during the whole period of pregnancy ($P<0.05$) with $r = 0.46$. It was concluded that fetal number strongly affected maternal serum concentrations of progesterone, and to a lesser extend those of estradiol, during pregnancy.

Key Words: Sheep, Pregnancy, Fetal Number, Estradiol, Progesterone.

Introduction

Increased litter size means increased number of corpus luteum (Piper and Bindon, 1984; Bradford, 1985; Bradford et al., 1986) and placental mass secreting progesterone and estradiol during gestation period (Rattray et al., 1974; Hayden et al., 1979; Butler et al., 1981). Progesterone and estradiol are hormones produced by corpus luteum and placenta, and their concentrations increase during pregnancy (MacDonald, 1980). The hormones involve in stimulating mammary growth and development during pregnancy (Knight and Peaker, 1982; Anderson, 1986; Tucker, 1986; Tucker, 1987) in preparations of milk synthesis for the newborn lambs. In addition, progesterone was well known for its effects on maintenance of pregnancy (MacDonald, 1980).

There were a lot of studies on the effects of progesterone and estradiol on mammary growth and development (Harness and Anderson, 1977a; Harness and Anderson, 1977b; Wright and Anderson, 1982; Wahab and Anderson, 1989). It was hypothesized that secretion of endogenous progesterone and estradiol correlated with the increased placental mass (as glands secreting progesterone and estradiol) with fetal number during pregnancy to stimulate mammary gland growth and development in preparation for more milk synthesis for the newborn lambs. The objective of this experiment was to study the profile of maternal serum concentrations of progesterone and estradiol in relation to the number of fetus bored during pregnancy.

[1]Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University,

[2]Corresponding author: W. Manalu, Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University, Jalan Taman Kencana No. 3, Bogor 16151.

[3]PhD candidate in Animal Physiology, Bogor Agricultural University Graduate School. Permanent address: Laboratory of Reproductive Physiology, Faculty of Animal Sciences, Jenderal Soedirman University.

Materials and Methods

Experimental design and protocol

Twenty nine ewes with similar body weight and age 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 the estrous cycle. Blood samples were drawn every other week beginning one day after the last $PGF_{2\alpha}$ injections. After parturition, the experimental ewes were assigned into 5 groups of fetal number (0, 1, 2, 3, and 4) with n = 3, 12, 9, 4, and 1, 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 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.

Hormone analyses

Progesterone. 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. 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 first half of pregnancy, the second half of pregnancy and during the whole period of pregnancy were correlated with the number of fetus using simple regression and correlation analysis (Neter et al., 1985).

Results and Discussion

Averages of maternal serum progesterone concentrations during the first half of pregnancy, the second half of pregnancy and during the whole period of pregnancy are presented in Table 1. The results of the experiment showed that fetal number correlated with maternal serum progesterone concentrations either during the first half of pregnancy (P<0.01) with r = 0.70, the second half of pregnancy (P<0.01) with r = 0.75, or during the whole period of pregnancy (P<0.01) with r = 0.77. Regardless of fetal number, maternal serum progesterone concentrations during the second half of pregnancy increased by 142% as compared to those during the first half of pregnancy. Variations in maternal serum progesterone concentrations during the whole period of pregnancy were 59% due to the fetal number.

Averages of maternal serum estradiol concentrations during the first half of pregnancy, second half of pregnancy, and during the whole period of pregnancy are presented in Table 2. Fetal number did not correlate with maternal serum estradiol concentrations during the first half of pregnancy (r 0.06). However, the increased maternal serum concentrations of estradiol during the second half of pregnancy and during the whole period of pregnancy correlated with the fetal number with r = 0.49 (P<0.01) and 0.46 (P<0.05), respectively.

Maternal serum estradiol concentrations during the second half of pregnancy increased by 256% as compared to those during the first half of pregnancy.

The strong correlation between the number of fetus and maternal serum progesterone, and to a lesser extend maternal serum estradiol, concentrations during pregnancy indicated that the placental mass was an important factor in determining the circulating levels of progesterone

Table 1. Maternal serum concentrations of progesterone (ng/ml) during the first half of pregnancy, the second half of pregnancy and during the whole period of pregnancy of ewes with different fetal number.[a]

Fetal number		Stages of reproduction		
		First half of pregnancy	Second half of pregnancy	The whole pregnancy
0	Mean ± SE	2.6580 ± 0.1313	2.9553 ± 0.7449	2.8066 ± 0.4192
1	Mean ± SE	5.4437 ± 0.3601	12.5740 ± 0.9898	9.0089 ± 0.6004
2	Mean ± SE	6.6708 ± 0.3442	17.9124 ± 0.8191	12.2917 ± 0.4739
3	Mean ± SE	6.7957 ± 0.8250	17.4932 ± 1.4094	12.0602 ± 0.9363
4		8.9087	19.9555	14.4321
r		0.70**	0.75**	0.77**

[a]Presented as means and SE of 3, 12, 9, 4, and 1 ewes with 0, 1, 2, 3, and 4 fetal number, respectively, during pregnancy.
** P<0.01.

Table 2. Maternal serum concentrations of estradiol (pg/ml) during the first half of pregnancy, the second half of pregnancy and during the whole period of pregnancy of ewes with different fetal number.[a]

Fetal number		Stages of reproduction		
		First half of pregnancy	Second half of pregnancy	The whole pregnancy
0	Mean ± SE	2.7039±0.2301	4.0329±1.0693	3.3684±0.6373
1	Mean ± SE	2.0031±0.2772	7.9509±1.1781	5.4420±0.6647
2	Mean ± SE	3.0244±0.3060	14.7531±2.5135	8.8888±1.3741
3	Mean ± SE	3.1048±0.2225	12.8233±2.0717	7.9640±1.0105
4		2.4706	12.8711	7.6709
r	0.34[a]	0.06	0.49**	0.46*

[a]Presented as means and SE of 3, 12, 9, 4, and 1 ewes with 0, 1, 2, 3, and 4 fetal number, respectively, during pregnancy.
** P<0.01, * P<0.05.

and estradiol in maternal serum. The results found in this study agreed with those found in previous reports (Hayden et al., 1979; Butler et al., 1981; Refsal et al., 1991).

Placenta started secreting progesterone and estradiol after placentation had been completed around the second half of gestation period (MacDonald, 1980). This could be the reason for dramatic increased progesterone and estradiol concentrations during the second half of pregnancy (Sumaryadi and Manalu, 1994). The inflection of progesterone and estradiol concentrations during the second half of pregnancy could explain the observations that mammary gland growth and development in sheep and goat increased dramatically during the second half of pregnancy (Rattray et al., 1974; Anderson, 1975; Anderson et al., 1981).

Even though placenta was not functional during the first half of pregnancy, contributions of fetal number to the progesterone and estradiol concentrations were high. This probably due to the high correlation between litter size and ovulation rate (Piper and Bindon, 1984; Bradford, 1985;

Bradford et al., 1986). Therefore, ewes with higher fetal number tended to have higher number of corpora lutea. Corpora luteal number had been reported to correlate with maternal serum progesterone and estradiol concentrations either during the luteal phase of estrous cycle or during pregnancy (Sumaryadi and Manalu, 1994).

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 fetus from 1 to 4. Contributions of the increased number of fetus to the increased maternal serum progesterone concentrations only 50, 56, and 59% during the first half of pregnancy, the second half of pregnancy, and the whole period of pregnancy, respectively, with far less contribution to the increased concentrations of estradiol (24 and 21% during the second half pregnancy and during the whole period of pregnancy, respectively).

The results indicated that the increased number of fetus did not arithmetically increase synthetic activities of the placental cells secreting the hormones. It was hypothesized that there were some other factors other than the number of fetus and corpora lutea that affected the expression of synthetic activities of the placental cells. These factors could be blood flow, growth factors, or hormones controlling the growth and development of placental glands secreting the hormones. The increased fetal number could probably reduced substrate and blood flow to each placenta, thereby decreasing synthetic activities of the placental cells secreting the hormones. These phenomena were assumed to be analogous with the common observations on decreasing individual lamb birth weight with increased litter size.

The results suggested that the number of fetus bored correlated more to the concentrations of progesterone than to those of estradiol either during the first half of pregnancy, the second half of pregnancy or during the whole period of pregnancy. This probably related partly to the vital function of progesterone in maintenance of pregnancy (MacDonald, 1980). Furthermore, different responses in synthetic activities of cells secreting progesterone and cells secreting estradiol probably exists in keeping a proper balance between progesterone and estradiol concentrations in maintenance of pregnancy. Contributions of fetal number to progesterone and estradiol were greater as compared to those of corpora luteal number (Sumaryadi and Manalu, 1994).

Conclusions

It was concluded that the fetal number affected maternal serum concentrations of progesterone, and to a lesser extend those of estradiol, during pregnancy. Contributions of fetal number were greater to maternal serum concentrations of progesterone as compared to those of estradiol. Endogenous secretion of progesterone and estradiol as mammogenic hormones could be increased by increasing the number of placental mass.

Suggestions

Correlation between the increased fetal number and maternal serum progesterone or estradiol concentrations with the mammary gland growth and development as well as milk production merits further studies for more understanding on hormones controlling mammary gland growth and development. In addition, the experiment needs to be extended to evaluate the profiles of other mammogenic hormones in relation to the increased fetal number.

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