Contributions of Maternal Serum Progesterone and Estradiol Concentrations or Corpora Luteal and Fetal Number to Mammary Growth and Development of Ewes During Pregnancy

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

Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University.

ABSTRACT: Sixteen ewes with similar weight and age were used in an experiment, and injected twice with PGF_2 before being bred. Number of corpora lutea was determined 5 d after the onset of estrous cycle by laparoscopy. Number of ewes having 0, 1, 2, 3, and >3 corpora lutea were 3, 2, 5, 4, and 2, respectively. At parturition date, the number of ewes gave birth to 0, 1, 2, 3, and 4 lambs were 3, 6, 4, 2, and 1, respectively. Blood samples were collected every other week during pregnancy for progesterone and estradiol analyses with radioimmunoassay. At parturition date, the experimental ewes were sacrificed and the mammary glands were excised for determination of dry-fat-free tissue (DFFT). Multiple regression and correlation was used to determine the relationships of serum progesterone and estradiol concentrations and of corpora luteal and fetal number with mammary gland DFFT. The results of the experiment showed that the increased progesterone and estradiol concentrations during pregnancy simultaneously contributed 46.3% to the increased mammary gland DFFT at parturition ($P < 0.05$). The increased concentrations of progesterone or estradiol alone contributed 46.13% ($P < 0.01$) and 28.98% ($P < 0.05$), respectively, to the increased mammary gland DFFT. Both the number of corpora lutea and fetus simultaneously contributed 47.0% to the increased mammary gland DFFT ($P < 0.05$). Number of corpora lutea or fetus alone contributed 46.4 ($P < 0.01$) and 39.0% ($P < 0.01$), respectively, to the increased mammary gland DFFT. It was concluded that the increased number of corpora lutea and fetus, through their effects on mammogenic hormones secretions, markedly increased mammary gland growth and development during pregnancy.

Keywords: Sheep, Corpus luteum, Fetal number, Progesterone, Estradiol, Mammary Growth.

Introduction

A major amount of mammary development is associated with hormonal stimuli of pregnancy, and the absence of these stimuli reduces the amount of mammary tissue (Anderson, 1975; Anderson et al., 1981; Anderson, 1986). Mammary gland growth and development is under the control of mammogenic hormones i.e. progesterone, estradiol, placental lactogen, somatotropin, prolactin, relaxin etc. (Harness and Anderson, 1977a; Harness and Anderson, 1977b; Wright and Anderson, 1982; Knight and Peaker, 1982; Anderson, 1986; Tucker, 1986; Tucker, 1987; Wahab and Anderson, 1989). In small sheep and goat mammary gland growth and development increased dramatically during the last two months of gestation period (Rattray et al., 1974; Anderson, 1975; Anderson et al., 1981) along with the increased maternal serum progesterone and estradiol concentrations.

It was hypothesized that concentrations of maternal serum progesterone and estradiol, and corpora luteal and fetal number through their effects on progesterone and estradiol secretions, contributed to mammary gland growth and development. The objective of this experiment was to quantify the contribution of serum progesterone and estradiol

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

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

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

concentrations, corpora luteal and fetal number to mammary gland growth and development.

Experimental Procedures

General. Sixteen ewes with similar body weight and age were used to study the contributions of maternal serum progesterone and estradiol, and of corpora luteal and fetal number to the growth and development of mammary glands. 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 $PGF_{2\_}$ i.m. twice to synchronize the estrous cycle. Five days after the onset of estrous cycle number of corpora lutea was counted by laparoscopy. Distribution of ewes with 0, 1, 2, 3, and >3 was 3, 2, 5, 4, and 2, respectively. After parturition, the experimental ewes were assigned into 5 groups of fetal number (0, 1, 2, 3, and 4) with n = 3, 6, 4, 2, and 1, respectively. Blood samples were drawn biweekly beginning one day after the last $PGF_{2\_}$ injections for progesterone and estradiol analyses.

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.

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.

Mammary gland dry-fat-free tissue analyses. Half of the mammary glands was extracted with ethanol for 48 h with twice replacement and then with diethylether with the same duration and replacement until the tissues was free of fat. The extracted mammary gland was then dried in oven at 55°C for 72 h for obtaining dry-fat-free tissue.

Statistical analyses. Progesterone and estradiol concentrations during the whole period of pregnancy, and the number of corpora lutea and fetus were correlated with the mammary gland DFFT with multiple regression and correlation analysis (Neter et al., 1985).

Results and Discussion

The individual data of progesterone and estradiol and mammary gland DFFT of the experimental ewes are presented in Table 1. The results of the experiment showed that the increased progesterone and estradiol concentrations during pregnancy simultaneously contributed 46.3% to the increased mammary gland DFFT at parturition (P < 0.05). The increased concentrations of progesterone or estradiol alone contributed 46.13% (P < 0.01) and 28.98% (P<0.05), respectively, to the increased mammary gland DFFT.

Averages of mammary gland DFFT of the experimental ewes in each corpora luteal and fetal number are presented in Table 2. Both the number of corpora lutea and fetus contributed 47.0% to the increased mammary gland DFFT (P<0.05). Number of corpora lutea or fetus alone contributed 46.4 (P<0.01) and 39.0% (P<0.01), respectively, to the increased mammary gland DFFT. It was concluded that the increased number of corpora lutea and fetus, through their effects on mammogenic hormone secretions, markedly increased mammary gland growth and development during pregnancy.

Table 1. Correlation between maternal serum concentrations of progesterone and estradiol during the whole period of pregnancy with mammary glands dry-fat-free (DFFT) of ewes.

Aimal #	Stages of reproduction			
	Progesterone	Mammary gland DFFT	Number of fetus	Mammary gland DFFT
1	1.9988	2.5134	2.6753	2.5134
2	3.0378	2.3658	2.7887	2.3658
3	3.3833	1.4921	3.0869	21.2338
4	7.2804	26.1199	3.9550	22.6888
5	7.4353	27.2250	4.5460	26.1199
6	7.4674	15.4560	4.6413	1.4921
7	7.7494	21.2338	5.6712	15.4560
8	9.3358	39.4697	7.4688	27.2250
9	10.5287	22.6888	7.6709	37.9469
10	11.6348	29.2826	7.7472	34.7633
11	12.4378	79.6930	8.4958	29.2867
12	13.0998	34.7633	8.7678	29.2826
13	14.4321	37.9469	9.2805	79.6930
14	14.7747	84.2944	10.3382	84.2944
15	14.8495	29.2867	10.6466	39.4697
16	15.3352	24.5491	15.8366	24.5491
	$r_{p\text{-}dfft}$= 0.68		$r_{pe\text{-}dfft}$ = 0.68	$r_{e\text{-}dfft}$= 0.54

$r_{p\text{-}dfft}$ = correlation of maternal progesterone alone with mammary gland DFFT.
$r_{e\text{-}dfft}$ = correlation of maternal estradiol alone with mammary gland DFFT.
$r_{pe\text{-}dfft}$ = correlation of maternal progesterone and estradiol simultaneously with mammary gland DFFT.

Table 2. Correlation between corpora luteal and fetal number during the whole period of pregnancy with mammary glands dry-fat-free tissues (DFFT) of ewes.

No	Stages of reproduction			
	Number of corpora lutea	Mammary gland DFFT	Number of fetus	Mammary gland DFFT
1	0	2.1238±0.3187	0	2.1238±0.3187
2	1	21.3405±5.8846	1	26.4652±3.2982
3	2	30.8377±2.7760	2	40.4236±13.3562
4	3	39.3749±15.0752	3	56.7885±27.5062
5	>3	58.8206±20.8733	4	37.9469
		$r_{cl\text{-}dfft}$= 0.68	$r_{clf\text{-}dfft}$= 0.69	$r_{f\text{-}dfft}$= 0.63

$r_{cl\text{-}dfft}$ = correlation of corpora lutea alone with mammary gland DFFT.
$r_{f\text{-}dfft}$ = correlation of fetal number alone with mammary gland DFFT.
$r_{clf\text{-}dfft}$ = correlation of corpora luteal and fetal number simultaneously with mammary gland DFFT.

The strong correlation between the number of corpora lutea (Sumaryadi and Manalu, 1994), fetal number (Manalu et al., 1994) and maternal serum progesterone, and to a lesser extend maternal serum estradiol, concentrations during pregnancy indicated that the magnitude of contributions of those parameters to mammary gland growth and development were similar (around 47%). Previous studies reported (Harness and Anderson, 1977a; Harness and Anderson, 1977b; Wright and Anderson, 1982; Wahab and Anderson, 1989) that mammary gland growth and development was under the control of mammogenic hormones.

Mammary gland growth and development in sheep and goat dramatically increased during the second half of gestation period (Rattray et al., 1974; Anderson, 1975; Anderson et al., 1981). The dramatic increased mammary gland growth and development during this period agreed with the inflection of maternal serum progesterone and estradiol concentrations during the second half pregnancy (Manalu et al., 1994; Sumaryadi and Manalu, 1994). However, the increased progesterone and estradiol concentrations, in conjunction with the increased corpora luteal and fetal number, during pregnancy contributed only around 47% to the increased mammary gland growth and development during pregnancy. Therefore there were some other factors, that were not measured in this study, affecting the growth and development of mammary glands.

It was reported that several hormones called mammogenic hormones controlled mammary gland growth and development during pregnancy i.e., progesterone, estradiol, placental lactogen, somatotropin, prolactin, relaxin (Harness and Anderson, 1977a; Harness and Anderson, 1977b; Wright and Anderson, 1982; Wahab and Anderson, 1989). Placental lactogen is secreted by placenta. Some studies reported correlation of litter size with maternal serum concentrations of placental lactogen in sheep (Butler et al., 1981) and goat (Hayden et al., 1979) and with mammary gland growth and development (Rattray et al., 1974; Hayden et al., 1979; Butler et al., 1981). Relaxin is produced by corpus luteum and its concentration should be affected by the number of corpora lutea.

Corpus luteum and placenta secrete progesterone and estradiol, and placental lactogen and relaxin (MacDonald, 1980). The other mammogenic hormones are produced by pituitary glands. Therefore, there are some other factors, outside corpus luteum and placenta, that involve in controlling mammary gland growth and development. In term of corpus luteum and placenta, still there are some other hormones that were not measured in this experiment. Considering the numerous hormones and factors involved in regulating mammary gland growth and development, 50% contributions of corpus luteum, placenta, and progesterone and estradiol to the mammary growth and development was considered to be reasonably high.

Even though contributions of corpora luteal and fetal number, and maternal serum concentrations of progesterone and estradiol only around half to the increased mammary gland growth and development during pregnancy, these phenomena could be used as alternative means of stimulating mammary gland growth and development to increase milk production either for consumption of the newborn lambs or for product to be harvested by dairymen.

Implications

It was concluded that the increased number of corpora lutea and fetus, through their effects on progesterone and estradiol or probably other mammogenic hormones secretions, markedly increased mammary gland growth and development during pregnancy. The contributions of these parameters around half to the total increased in the mammary gland growth and development.

Contributions of corpora luteal and fetal number to other mammogenic hormones in relation to mammary gland growth and development merit further studies.

Acknowledgements

Part of this experiment was funded by grants provided to The Life Sciences Center, Inter University Center, Bogor Agricultural University by Proyek Peningkatan Penelitian dan Pengabdian pada Masyarakat, Directorate of Higher Education with the contract # 005/P4M/DPPM/L.3311/PAU/1992 and # 007/P4M/DPPM/L.3311/1993. The authors extend a deep appreciation to Asmarida, Edy, Sardju and Supriawan for technical assistance during the experiment. The last but not least, the great expertise of Mr. I. Inounu in counting the corpus luteum, and an exemplary excellent cooperation provided by Dr. A. Djajanegara and Dr. K.

Diwyanto of Balai Penelitian Ternak, Bogor, in using laparoscopy, were greatly appreciated.

Literature Cited

Anderson, R.R. 1975. Mammary gland growth in sheep. J. Anim. Sci. 41:118.

Anderson, R. R. 1986. Mammary gland. In: B. Larson (Editor). Lactation. p. 3. Iowa State University Press, Ames. .

Anderson, R.R., J.R. Harness, A.F. Sinead and M.S. Salah. 1981. Mammary growth pattern in goats during pregnancy and lactation. J. Dairy Sci. 64:427.

Butler, W.R., S.M. Fullenkamp, L.A. Capiello and S. Handwerger. 1981. The relationship between breed and litter size in sheep and maternal serum concentrations of placental lactogen, estradiol and progesterone. J. Anim. Sci. 53:1077.

Harness, J.R. and R.R. Anderson. 1977a. Effect of relaxin in combination with prolactin and ovarian steroids on mammary growth in hypophysectomized rats. Proc. Soc. Exp. Biol. Med. 156:354.

Harness, J.R. and R.R. Anderson. 1977b. Effect of relaxin and somatotropin in combination with ovarian steroids on mammary glands in rats. Biol. Reprod. 17:599.

Hayden, T.J., C.R. Thomas and I.A. Forsyth. 1979. Effect of number of young born (litter size) on milk yield of goats: role of placental lactogen. J. Dairy Sci. 62:53.

Knight, C.H. and M. Peaker. 1982. Development of the mammary gland. J. Reprod. Fertil. 65:521-536.

Neter, J., W. Wasserman and M.H. Kutner. 1985. Applied linear Statistical Model, (2nd Ed). Irwin, Homewood, Illinois.

MacDonald, L.E. 1980. Veterinary Endocrinology and Reproduction. (3rd Ed.). Lea & Febriger, Philadelphia.

Manalu, W., M. Y. Sumaryadi, and N. Kusumorini. 1994. The effects of fetal number on maternal serum progesterone and estradiol of ewes during pregnancy.

Rattray, P.V., W.N. Garret, N.E. East and N. Hinman. 1974. Growth, development and composition of the ovine conceptus and mammary gland during pregnancy. J. Anim. Sci. 38:613.

Sumaryadi, M.Y. and W. Manalu. 1994. The effects of corpora luteal number on serum progesterone and estradiol of ewes during luteal phase of estrous cycle and pregnancy.

Tucker, H.A. 1986. Endocrine and neural control of the mammary gland. In: B. Larson (Ed.). Lactation. p. 39. Iowa State University Press, Ames.

Tucker, H.A. 1987. Quantitative estimates of mammary growth during various physiological states: A review. J. Dairy Sci. 70:1958.

Wahab, I.M. and R.R. Anderson. 1989. Physiologic role of relaxin on mammary gland growth in rats. Proc. Soc. Exp. Biol. Med. 192:285.

Wright L.C. and R.R. Anderson. 1982. Effect of relaxin on mammary growth in the hypophysectomized rat. In: R.R. Anderson (Editor). Relaxin. p. 341. Plenum Press, New York.