

Effect of Superovulation Prior to Mating on Milk Production Performance During Lactation in Ewes

W. Manalu,* M. Y. Sumaryadi,† Sudjatmogo,*¹

and A. S. Satyaningtjas*

*Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University, Jalan Taman Kencana I No. 3, Bogor 16151, Indonesia

†Laboratory of Physiology and Reproduction, Faculty of Animal Sciences, Jenderal Soedirman University, P.O. Box 110, Purwokerto 53123, Indonesia

ABSTRACT

Thirty lactating ewes were used to evaluate the effect of superovulation on milk production. Twelve ewes had been injected, prior to mating, with 700 IU of pregnant mare serum gonadotropin; 18 ewes were injected with saline as a control. Thirteen ewes (nine control and four superovulated ewes) were fed at low plane of nutrition; the other ewes (nine control and eight superovulated ewes) were fed at high plane of nutrition. Superovulated ewes, fed at both low and high planes of nutrition, had dramatically higher milk yields (59%), and their milk composition was not changed. Plane of nutrition increased milk lactose and P contents without significant effect on milk production. The increased milk yields in the superovulated ewes were accompanied by increases in dry matter, gross energy intakes, and gross efficiency of milk synthesis. At the end of lactation, superovulated ewes had higher mammary dry fat-free tissue, total DNA, and total RNA. The results demonstrated that superovulation prior to mating dramatically increased milk production and efficiency regardless of plane of nutrition. Increased milk production and efficiency in the superovulated ewes were due to the increased mammary secretory cell numbers and their synthetic activities presumably through the increased endogenous hormonal stimulation of mammary growth and development during pregnancy.

(**Key words:** superovulation, milk yields, sheep)

Abbreviation key: DFFT = dry fat-free tissue, PMSG = pregnant mare serum gonadotropin.

INTRODUCTION

Milk production is affected by the number of secretory cells in the mammary glands of lactating mammals,

synthetic activity per secretory cell, and the availability of substrates for milk constituents synthesis in the secretory cells. The number of secretory cells and synthetic activity per secretory cell during lactation are influenced by the degree of mammary growth and development attained at parturition (at the beginning of lactation) and the rate of secretory cell loss (mammary involution) as lactation advances. Hormonal control of mammary gland growth and development has been studied extensively (8). However, researchers rarely incorporate this research when they stimulate mammary growth and development by endogenous mammogenic hormones during pregnancy to increase secretory cells, synthetic activity per cell, and milk production during lactation.

In sheep and goats, extensive mammary gland growth and development occurs during pregnancy (2, 3, 22), and milk synthetic capacity is attained near parturition. Mammary growth and development during pregnancy is associated with a dramatic change in mammogenic hormone secretions beginning during the estrous cycle until parturition. These pregnancy-related mammogenic hormones are secreted by the ovary and corpora lutea during the estrous cycle and early pregnancy (mainly estrogen, progesterone, and relaxin) (14, 21) and the placenta during the placental phase of pregnancy (estradiol, progesterone and placental lactogen) (5, 12, 13, 23, 29, 31).

During the luteal phase of the estrous cycle and early pregnancy, maternal serum progesterone concentrations are positively correlated with the number of corpora lutea (14, 21, 25). Ewes carrying multiple fetuses have heavier placenta and higher serum progesterone and placental lactogen during late pregnancy (5, 12, 13, 15, 28, 31), have better developed mammary glands at parturition (12, 15, 17), and have correspondingly higher milk yields during lactation (12).

A series of hormonal changes during pregnancy is initiated by the maturation of the follicle(s) leading to ovulation and luteogenesis. Several hormones and growth factors secreted by the ovary and corpora lutea (35) act on the uterus and placenta to stimulate their

Received October 19, 1998.

Accepted October 27, 1999.

Corresponding author: W. Manalu, Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University, Jalan Taman Kencana I No. 3, Bogor 16151, Indonesia. Telephone: 62 251 328487. Fax: 62 251 323161.

¹Permanent address: Faculty of Animal Sciences, Diponegoro University, Semarang, Indonesia.

growth and further secretions of hormones and growth factors required for maintenance of pregnancy (4, 20) and to stimulate mammary gland growth and development (8) in preparation of milk synthesis for the newborn offspring. Ovaries of mammals have many follicles that, for the most part, never ovulate. The number of follicles that ovulate to form corpora lutea can be increased by superovulation as dispensable sources of endogenous hormones and growth factors augmenting hormonal changes during pregnancy that, in turn, stimulate mammary gland growth and development.

Superovulation of ewes prior to mating increases corpora lutea, placental weight, and serum progesterone concentrations during pregnancy (18), which results in better mammary ductal growth at early pregnancy and multiplied epithelial cells during late pregnancy (19). This experiment was designed to demonstrate and evaluate milk production performance for one full lactation (84 d) of superovulated ewes; the ewes were fed at low and high planes of nutrition.

MATERIALS AND METHODS

Experimental Design and Protocol

Thirty Javanese thin-tail ewes with similar weight (12 to 15 kg) and age (1 to 1.5 yr) at the beginning of experiment, were maintained for 2 mo to adapt to the experimental conditions prior to mating. After the adaptation period, the experimental ewes were injected twice with 7.5 mg of PGF_{2α} i.m. at 11-d intervals. Twelve ewes were injected with 700 IU of pregnant mare serum gonadotropin (**PMSG**) (Folligon, Intervet, North Holland, The Netherlands) at the time of the last prostaglandin injection (about the end of diestrus) to stimulate superovulation, and the other 18 ewes were injected with saline as a control. Two days after the last prostaglandin injections, at the onset of the estrous cycle, the experimental ewes were mated in a group.

After mating, 13 experimental ewes (nine nonsuperovulated and four superovulated) were fed at a low plane of nutrition (diet contained 12% CP and 65% TDN), and the others (nine nonsuperovulated and eight superovulated ewes) were fed at high plane of nutrition (diet contained 15% CP and 75% TDN). The composition of diets used is presented in Table 1. Rations were mixed and provided as pellet. Chemical analyses of the diets are presented in Table 2.

During pregnancy, the experimental ewes were fed twice a day, feeding was restricted at early pregnancy and increased gradually with the advance of pregnancy. During the first 7 wk of pregnancy, the experimental ewes were fed at maintenance level (average consumption was 0.4 kg/d). The amount of feed provided was increased gradually until wk 15 of pregnancy (average

Table 1. Composition of the experimental diets.

Constituent (%)	Low plane (12% CP and 65% TDN)	High plane (15% CP and 75% TDN)
Dry elephant grass	49.21	19.67
Corn	27.29	35.07
Ricebran	12.27	10.01
Soybean meal	5.13	14.44
Coconut meal	5.62	19.95
Bone meal	0.15	0.19
Fish meal	0.15	0.49
Premix	0.18	0.18
Total	100.00	100.00

consumption was 0.56 kg/d). From wk 15 to parturition, the ewes were freely allowed to consume feed (average consumption was 1.0 kg/d). During lactation, the experimental ewes were fed twice a day, and the ewes had free access to feed and water. Feed consumption was recorded daily and presented as a total for 84 d of lactation. Samples from daily feeding were taken and composited for the whole lactation for chemical analyses. At parturition, the number of lambs born was recorded per ewe.

During the 1st wk postpartum, milk was not harvested; the ewes were allowed to nurse their respective lambs. One week after parturition, milk was harvested twice a day for 84 d postpartum by hand milking with a prior injection of 3 IU of oxytocin (i.m.). Milk production weekly and for the whole lactation (84 d) was determined from daily milking. Samples were taken from each milking and were composited for 1 wk and for the whole lactation. A sample of composited milk was used to determine milk solid, fat, protein, lactose, gross energy, Ca, and P contents. Milk SCC was determined weekly (26). Body weights of ewes were determined at the beginning and at the end of lactation.

On d 92 postpartum, the experimental ewes were slaughtered by a standard procedure adopted in local slaughterhouse. The mammary glands were excised to further determine mammary chemical indices. During the experiment, the experimental ewes were main-

Table 2. Chemical composition of the experimental diets.¹

Component	Low plane (12% CP and 65% TDN)	High plane (15% CP and 75% TDN)
CP, %	12.12	15.20
Crude fiber, %	15.91	11.88
Calculated TDN, %	65.00	75.00
Ether extract, %	5.51	5.80
N-free extract, %	43.75	43.71
Ash, %	9.48	9.50
Ca, %	0.88	1.02
P, %	0.61	0.76
Gross energy, kcal/kg	4161.09	3855.16

¹Based on DM.

tained and treated according to local laws and regulations and to Guidelines for the Care and Use of Animals in Research stipulated by the National Research Council of the Republic of Indonesia.

Mammary Gland Indices Analysis

Dry fat-free tissue (DFFT) of the mammary gland was measured by a modified method of Anderson (2). Half 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 h and then with diethyl ether (48 h) 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 determine mammary chemical indices. Mammary DNA was determined by *p*-nitrophenylhydrazine reaction (34), and RNA was determined by orcinol reaction (1).

Milk Composition and Energy Analysis

Milk solids were measured by drying the milk samples for 48 h at 50°C. Milk fat percentage was determined by measuring triglycerides in the sample with a commercial kit (Triglycerides Kit, catalog #339-50, Sigma Chemical Co., St. Louis, MO). Milk protein percentage was measured with a commercial kit (Total Protein Kit, catalog #690A, Sigma Chemical Co., St. Louis, MO). Lactose was determined by the procedure described by Teles et al. (32). Milk gross energy was measured by combustion of dry milk solids with a bomb calorimeter. Calcium and P contents of the dry milk solids were determined by the procedure used to determine feed Ca and P.

Statistical Analysis

Data were analyzed as a completely randomized design with a factorial arrangement. Analysis of variance was performed by using general linear model procedure of SAS (24) to test the effects of main factors (superovulation, plane of nutrition, and litter size) and their interactions. Since litter size did not significantly affect all parameters measured, the data were pooled for litter size and the analyses were performed to test the effects of superovulation, plane of nutrition, and their interactions.

RESULTS

Superovulation did not significantly affect litter size. At parturition, of nine nonsuperovulated ewes fed at a

low plane of nutrition, six and three ewes gave birth to a single and twin lambs, respectively. Of four superovulated ewes fed at a low plane of nutrition three and one ewes gave birth to a single and quadruplet lambs, respectively. Of nine nonsuperovulated ewes fed at high plane of nutrition seven and two ewes gave birth to a single and twin lambs, respectively. Of eight superovulated ewes fed at high plane of nutrition five and three ewes gave birth to a single and twin lambs, respectively. Litter size (single and multiple) did not affect total milk production during 84-d lactation period ($P > 0.05$).

Total milk yields, mean milk compositions, and their respective yields and SCC for 84 d of lactation are presented in Table 3. Superovulated ewes, either fed at low or high planes of nutrition, had dramatically higher (by 59%) total milk yields ($P = 0.0001$) (24.90 vs. 39.49 kg in the control and superovulated ewes, respectively). Total weekly milk yields in the control and superovulated ewes for 12 wk of lactation are presented in Figure 1. Superovulated ewes had consistently higher milk production during the whole lactation.

Milk yields of the ewes fed at high plane of nutrition tended to be higher ($P = 0.0594$) than those of fed at low plane of nutrition (27.01 vs. 33.59 kg in the ewes fed at low and high planes of nutrition, respectively). Total weekly milk yields in the ewes fed at low and high planes of nutrition for 12 wk of lactation are presented in Figure 2. During 12 wk of measured lactation, ewes fed at high plane of nutrition had slightly higher milk yields than those fed at a low plane of nutrition.

The increased milk yields in the superovulated ewes did not change milk composition (milk solids, lactose, fat, protein, Ca, and P) and SCC (Table 3). As a result, superovulated ewes had higher solids, lactose, butterfat, protein, Ca, and P yields ($P < 0.01$) by 56 (4.64 vs. 7.22 kg), 60 (1.14 vs. 1.82 kg), 60 (2.30 vs. 3.67 kg), 76 (0.70 vs. 1.23 kg), 40 (11.28 vs. 15.76 g), and 58% (7.4 vs. 11.72 g), respectively, as compared to nonsuperovulated ewes. Plane of nutrition did not affect milk solids, fat, and protein percentage; however, milk lactose and P contents were higher in the ewes fed at high plane of nutrition ($P < 0.05$ and 0.01, respectively). Milk Ca percentage tended to be higher in the ewes fed at high plane of nutrition ($P = 0.0559$). Nonsuperovulated ewes fed at high plane of nutrition had numerically higher SCC compared with other groups. Because of the tendency toward higher milk yields, ewes fed at high plane of nutrition had more solids ($P < 0.05$), lactose ($P < 0.05$), protein ($P < 0.05$), Ca ($P < 0.01$), and P ($P < 0.01$) yields by 29, 32, 30, 41, and 58%, respectively, as compared to those fed at low plane of nutrition (4.87 vs. 6.28 kg, 1.20 vs. 1.58 kg, 0.79 vs. 1.03 kg, 10.60 vs. 14.97 g, and 6.86 vs. 10.86 g, respectively). Ewes fed at

Table 3. Milk yield, milk composition, milk constituent yields, and SCC during 84-d lactation period in the control and superovulated ewes fed at low or high plane of nutrition.

	Plane of nutrition				Level of significance		
	Low ¹		High ²		Super-ovulation	Plane of nutrition	Interaction
	Control ³ (n = 9)	Superovulation ⁴ (n = 4)	Control ³ (n = 9)	Superovulation ⁴ (n = 8)			
Milk yield, kg	22.65 ± 1.49	36.82 ± 1.87	27.15 ± 2.36	40.83 ± 1.94	**	NS	NS
Milk solid, %	18.45 ± 0.73	17.56 ± 0.60	18.93 ± 0.31	18.57 ± 0.57	NS	NS	NS
Solid yield, kg	4.16 ± 0.28	6.45 ± 0.34	5.11 ± 0.41	7.60 ± 0.47	**	*	NS
Lactose, mg/ml	43.97 ± 0.51	44.94 ± 1.40	47.17 ± 0.76	46.72 ± 0.77	NS	*	NS
Lactose yield, kg	0.99 ± 0.07	1.65 ± 0.06	1.28 ± 0.12	1.91 ± 0.11	**	*	NS
Milk fat, %	9.12 ± 0.26	9.19 ± 0.12	9.23 ± 0.19	9.33 ± 0.15	NS	NS	NS
Butterfat yield, kg	2.09 ± 0.19	3.38 ± 0.19	2.51 ± 0.23	3.81 ± 0.20	**	NS	NS
Milk protein, %	2.86 ± 0.09	3.02 ± 0.20	3.09 ± 0.14	3.13 ± 0.07	NS	NS	NS
Protein yield, kg	0.64 ± 0.04	1.11 ± 0.09	0.84 ± 0.05	1.29 ± 0.07	**	*	NS
Ca, %	0.23 ± 0.01	0.20 ± 0.02	0.25 ± 0.01	0.23 ± 0.01	NS	NS	NS
Ca yield, g	9.54 ± 0.97	12.98 ± 1.37	13.02 ± 1.19	17.17 ± 1.35	**	**	NS
P, %	0.15 ± 0.01	0.13 ± 0.01	0.17 ± 0.01	0.18 ± 0.02	NS	**	NS
P yield, g	6.13 ± 0.60	8.50 ± 0.85	8.67 ± 0.64	13.33 ± 1.61	**	**	NS
SCC ⁵	5.01 ± 0.02	5.02 ± 0.07	5.10 ± 0.03	5.00 ± 0.04	NS	NS	NS

¹Ewes fed with diet contained 12% CP and 65% TDN.²Ewes fed with diet contained 15% CP and 75% TDN.³Ewes injected with saline prior to mating.⁴Ewes injected with 700 IU of pregnant mare serum gonadotropin (PMSG) prior to mating.⁵Log₁₀-transformed.**P* < 0.05.***P* < 0.01.

high plane of nutrition tended to have higher butterfat yields (*P* = 0.0734) (2.47 vs. 3.13 kg) (Table 3).

Body weights at the beginning and at the end of lactation were not different between control and superovulated ewes (22.1 vs. 22.7 kg, and 23.4 vs. 25.0 kg, respectively) and between ewes fed at low and high planes of nutrition (21.0 vs. 23.5 kg, and 22.5 vs. 25.2 kg, respectively), leading to a nonsignificant difference in BW gain of the experimental ewes during lactation (Table 4).

The increased milk yields in the superovulated ewes were associated with increases in DM and gross energy

intakes (8 and 10%) (*P* < 0.01) (61.27 vs. 65.92 kg, and 247.10 vs. 270.90 Mcal, in the nonsuperovulated and superovulated ewes, respectively). However, ewes fed at low plane of nutrition had higher DM and gross energy intakes (15 and 21%) (*P* < 0.01) during lactation (68.08 vs. 59.34 kg, and 284.00 vs. 234.89 Mcal, in the ewes fed at low and high planes of nutrition, respectively).

Comparable to the increased milk and milk component yields, total milk gross energy in the superovulated ewes was higher by 53% (*P* < 0.01) than in nonsuperovulated ewes (26.40 vs. 40.50 Mcal). Even though

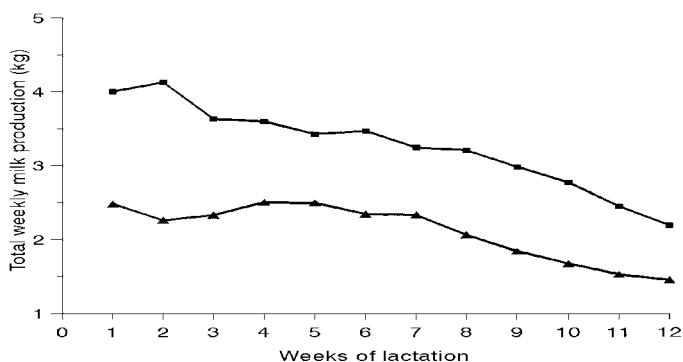
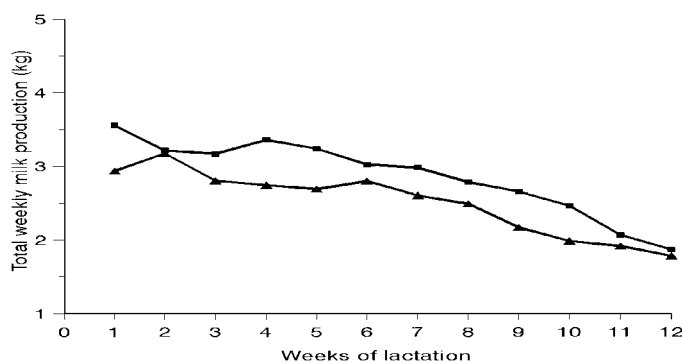
**Figure 1.** Total weekly milk production of control (▲) and superovulated ewes (■) during 12 wk of lactation, regardless of plane of nutrition.**Figure 2.** Total weekly milk production of ewes fed at low (▲) and high (■) planes of nutrition during 12 wk of lactation, regardless of superovulation.

Table 4. Body weights at the beginning and end of lactation, BW gain, DM and gross energy intakes, milk gross energy, and gross efficiency of milk synthesis during 84-d lactation, and mammary indices at the end of lactation in the control and superovulated ewes fed at low or high plane of nutrition.

	Plane of nutrition				Level of significance		
	Low ¹		High ²		Super-ovulation	Plane of nutrition	Interaction
	Control ³ (n = 9)	Superovulation ⁴ (n = 4)	Control ³ (n = 9)	Superovulation ⁴ (n = 8)			
BW at the start of lactation, kg	20.61 ± 0.98	21.88 ± 0.72	23.61 ± 1.39	23.44 ± 1.28	NS	NS	NS
BW at the end of lactation, kg	21.56 ± 0.72	24.63 ± 1.38	25.22 ± 1.26	25.25 ± 1.71	NS	NS	NS
BW gain, kg/84 d	0.94 ± 0.59	2.75 ± 0.83	2.42 ± 0.55	1.81 ± 0.76	NS	NS	NS
Total DMI, kg	66.17 ± 1.48	72.39 ± 0.83	56.37 ± 1.32	62.68 ± 2.31	**	**	NS
Total gross energy intake, Mcal	276.36 ± 6.52	301.28 ± 3.44	214.17 ± 4.51	255.72 ± 13.21	**	**	NS
Total milk gross energy, Mcal	24.32 ± 2.42	40.06 ± 2.80	28.85 ± 3.40	40.68 ± 2.38	**	NS	NS
Milk efficiency, %	8.88 ± 0.90	13.32 ± 1.01	13.46 ± 1.57	16.12 ± 1.07	*	**	NS
Mammary DFFT, ⁵ g	9.86 ± 0.52	15.84 ± 1.38	12.04 ± 1.27	14.26 ± 1.23	**	NS	NS
Total mammary DNA, g	0.33 ± 0.05	0.79 ± 0.06	0.43 ± 0.07	0.62 ± 0.07	**	NS	NS
Total mammary RNA, g	0.14 ± 0.02	0.25 ± 0.02	0.19 ± 0.04	0.25 ± 0.03	**	NS	NS

¹Ewes fed with diet contained 12% CP and 65% TDN.²Ewes fed with diet contained 15% CP and 75% TDN.³Ewes injected with saline prior to mating.⁴Ewes injected with 700 IU of pregnant mare serum gonadotropin (PMSG) prior to mating.⁵DFFT = Dry fat-free tissue.**P* < 0.05.***P* < 0.01.

gross energy intake increased, superovulated ewes had higher gross efficiency of milk synthesis ($P < 0.05$) than nonsuperovulated ewes (11.0 vs. 15.2%). Similar to total milk yields, milk gross energy was not different between ewes fed at low and high planes of nutrition (29.20 vs. 34.80 Mcal); however, ewes fed at high plane of nutrition had higher gross efficiency of milk synthesis ($P < 0.01$) than those fed at low plane of nutrition (10.2 vs. 14.8%) (Table 4).

Mammary gland analysis at the end of lactation indicated that superovulated ewes had 37% higher mammary DFFT ($P < 0.01$) (10.82 vs. 14.86 g). The higher mammary DFFT at the end of lactation in the superovulated ewes was accompanied by 79 and 56% increases in total DNA and RNA ($P < 0.01$) (0.38 vs. 0.68 g and 0.16 vs. 0.25 g, respectively) as compared to nonsuperovulated ewes. Plane of nutrition did not, however, affect mammary DFFT, total DNA, and RNA (Table 4).

DISCUSSION

The results of this experiment demonstrated that, regardless of plane of nutrition, superovulation of ewes prior to mating increased milk and milk component yields and gross efficiency of milk synthesis without significant changes in milk composition and SCC. Somatic cell counts of milk in the superovulated and control ewes were similar to those found in the nonmastitis ewes (9), and even lower than those found in somatotropin-treated lactating ewes (7). Distribution of single

and twin litter size in the superovulated ewes was similar to that found in nonsuperovulated ewes in this breed of sheep (15, 17). Therefore, the effect of superovulation on milk production was not related to litter size, as litter size did not significantly affect total milk production during 84-d lactation period. The nonsignificant effect of litter size on milk production in this breed of sheep is also reported (16).

Milk production is affected by the number of secretory cells in the mammary glands during the whole lactation and synthetic activity per secretory cells (8), and nutrient availability in the secretory cells. Although DMI in the superovulated ewes was increased, the milk yield increase (14.17 vs. 13.68 kg in the ewes fed at low and high planes of nutrition, respectively) was far greater than that of DMI (6.22 and 6.31 kg in the ewes fed at low and high planes of nutrition, respectively). The greater increase in milk production than in DMI in the superovulated ewes, without significant changes in BW, suggests an increase in the rate of substrate incorporation into milk components (milk synthesis). The increased rate of milk synthesis in the superovulated ewes could be caused by the increase in the number of mammary secretory cells and their synthetic activities, leading to maximum utilization of substrates available. This indication was supported by the increased gross efficiency of milk synthesis and the higher total mammary DNA and RNA at the end of lactation in the superovulated ewes.

Previous study in our laboratory showed that superovulated ewes have greater mammary ductal growth during early pregnancy and multiplied epithelial cells during late pregnancy (19). Lactating superovulated ewes used in the present experiment were most likely to have more secretory cells and increased synthetic activity. Chemical analysis of the mammary glands at the end of lactation in the present experiment indicated that superovulated ewes maintained more cells and higher synthetic activity at the end of lactation than did nonsuperovulated ewes. Ewes with higher progesterone concentrations during pregnancy have better-developed mammary glands at parturition (15, 17), and maintain more cells and higher synthetic activity at the end of lactation (16). How did superovulation increase the number of secretory cells in the mammary glands?

Estradiol, progesterone, relaxin, and placental lactogen (10, 11, 33, 37) along with other mammogenic hormones and growth factors (8) are known to stimulate mammary growth and to initiate mammary epithelial cells proliferation (36) through mediation of some growth factors (8). These hormones are secreted by the ovary, corpus luteum, and placenta during pregnancy. Animals with higher litter size have more corpora lutea (14, 21) and heavier placental mass (5, 12, 28). Ewes carrying multiple fetuses have higher progesterone and placental lactogen concentrations during pregnancy (5, 12, 13, 15, 17, 28, 31), greater mammary growth and development (12, 15, 17, 22), and higher mammary content of DNA and RNA as indicators of epithelial cells and synthetic activities at parturition, respectively (15, 17).

The increased endogenous secretions of estradiol prior to ovulation, relaxin, and progesterone during the luteal phase of pregnancy and progesterone and placental lactogen during the placental phase of pregnancy could have stimulated a greater mammary growth. Superovulated animals have more functional corpora lutea and dramatically higher concentrations of estradiol and progesterone and have heavier placental mass during pregnancy (18, 25). Progesterone has a role in directing gene expression in uterine stromal cells (20), and the higher progesterone in the superovulated ewes is associated with a greater uterine and placental weights (18). Placental weight is positively correlated with the placental lactogen concentration in maternal circulation (28). The increased endogenous secretion of progesterone, and probably other hormones and growth factors secreted by the corpus luteum and placenta, in the superovulated ewes is associated with the greater ductal growth of mammary glands during early pregnancy and mammary cell growth and synthetic activity

during late pregnancy (19), leading to a dramatic increase in milk production during lactation.

In conclusion, the effects of superovulation on mammary gland growth and development, milk production, and efficiency of milk synthesis were obvious. As a simple comparison, the improved plane of nutrition during lactation in the nonsuperovulated ewes produced 27.15 kg of milk (with 13.46% efficiency), while superovulation in the ewes fed at low plane of nutrition produced 36.82 kg of milk (with 13.32% efficiency). Feeding a high plane of nutrition to the superovulated ewes produced 40.83 kg of milk (with 16.12% efficiency) as compared to 22.65 kg of milk (with 8.88% efficiency) and 27.15 kg of milk (with 13.46% efficiency) in the unsuperovulated ewes fed at low and high planes of nutrition, respectively. The superovulation approach, in combination with improvement in plane of nutrition, is attractive to the dairy industry as an alternate technique in improving milk production. As a comparison, continuous administration of exogenous somatotropin in lactating dairy cows increases milk production from 15 to 25% (6). The effect of single injection of pregnant mare serum gonadotropin prior to mating on milk production was consistent during lactation, regardless of ration quality (increased by 59%, from average 24.9 kg in the nonsuperovulated ewes to 39.49 kg in the superovulated ewes).

The results were expected to stimulate further studies with different breeds of sheep and other species of domestic mammals to better evaluate the potential use of superovulation technique in increasing milk production. Some hormonal data, primarily on mammogenic hormones profiles in the superovulated animals, however, are still to be determined. In addition, improvement of superovulation techniques to obtain a similar stage of follicle maturation is crucial in the maintenance of pregnancy in the superovulated animals. Different stages of follicle maturation during superovulation can result in a premature luteolysis (27, 30) that could impair the conception.

ACKNOWLEDGMENTS

This experiment was funded by grant provided by The Office of the State Ministry of Research and Technology (RISTEK), Indonesian Institute of Sciences (LIPI), and National Research Council (DRN) of The Republic of Indonesia through the Riset Unggulan Terpadu III (Contract #: 132/FT/RUT/BPPT/IV/96).

REFERENCES

1. Albaum, H. G., and W. W. Umbreit. 1947. Differentiation between ribose 3-PO₄ and ribose 5-PO₄ by means of the orcinol-pentose reaction. *J. Biol. Chem.* 167:369-373.

- 2 Anderson, R. R. 1975. Mammary gland growth in sheep. *J. Anim. Sci.* 41:118–123.
- 3 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.* 64:427–432.
- 4 Ashworth, C. J. 1992. Synchrony embryo-uterus. *Anim. Reprod. Sci.* 28:259–267.
- 5 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.* 53:1077–1081.
- 6 Chalupa, W., and D. T. Galligan. 1989. Nutritional implications of somatotropin for lactating cows. *J. Dairy Sci.* 72:2510–2524.
- 7 Fernandez, N., M. Rodriguez, C. Peris, M. Barcelo, M. P. Molina, A. Torres, and F. Adriaens. 1995. Bovine somatotropin dose titration in lactating dairy ewes. 1. Milk yield and milk composition. *J. Dairy Sci.* 78:1073–1082.
- 8 Forsyth, I. A. 1996. The insulin-like growth factor and epidermal growth factor families in mammary cell growth in ruminants: Action and interaction with hormones. *J. Dairy Sci.* 79:1085–1096.
- 9 Gonzalez-Rodriguez, M. C., and P. Carmenes. 1996. Evaluation of the California mastitis test as a discriminant method to detect subclinical mastitis in ewes. *Small Ruminant Res.* 21:245–250.
- 10 Harness, J. R., and R. R. Anderson. 1977. Effect of relaxin and somatotropin in combination with ovarian steroids on mammary glands in rats. *Biol. Reprod.* 17:599–604.
- 11 Harness, J. R., and R. R. Anderson. 1977. Effects of relaxin in combination with prolactin and ovarian steroids on mammary growth in hypophysectomized rats. *Proc. Soc. Exp. Biol. Med.* 156:354–360.
- 12 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.* 62:53–57.
- 13 Hayden, T. J., C. R. Thomas, S. V. Smith, and I. A. Forsyth. 1980. Placental lactogen in the goat in relation to stage of gestation, number of fetuses, metabolites, progesterone and time of day. *J. Endocrinol.* 86:279–290.
- 14 Jarrell, V. L., and P. J. Dziuk. 1991. Effect of number of corpora lutea and fetuses on concentrations of progesterone in blood of goats. *J. Anim. Sci.* 69:770–773.
- 15 Manalu, W., and M. Y. Sumaryadi. 1998. Correlations of litter size and maternal serum progesterone concentration during pregnancy with mammary gland growth and development indices at parturition in Javanese thin-tail sheep. *Asian-Australasian J. Anim. Sci.* 11:300–306.
- 16 Manalu, W., and M. Y. Sumaryadi. 1998. Mammary gland indices at the end of lactation in Javanese thin-tail ewes with different litter sizes. *Asian-Australasian J. Anim. Sci.* 11:648–654.
- 17 Manalu, W., and M. Y. Sumaryadi. 1998. Maternal serum progesterone concentration during gestation and mammary gland growth and development at parturition in Javanese thin-tail ewes carrying a single or multiple fetuses. *Small Ruminant Res.* 27:131–136.
- 18 Manalu, W., M. Y. Sumaryadi, Sudjatmogo, and A. S. Satyaningtiyas. 1998. Effect of superovulation on maternal serum progesterone concentration, uterine and fetal weights at weeks 7 and 15 of pregnancy in Javanese thin-tail ewes. *Small Ruminant Res.* 30:171–176.
- 19 Manalu, W., M. Y. Sumaryadi, Sudjatmogo, and A. S. Satyaningtiyas. 1999. Mammary gland differential growth during pregnancy in superovulated Javanese thin-tail ewes. *Small Ruminant Res.* 33:279–284.
- 20 Mulholland, J., D. Roy, and S. R. Glasser. 1994. Progesterone directed gene expression in rat uterine stromal cells. Pages 33–39 in *Endocrinology of Embryo-Endometrium Interactions*. S. R. Glasser, J. Mulholland, and A. Psychoyos, eds. Plenum Press, New York, NY.
- 21 Quirke, J. F., J. P. Hanrahan, and J. P. Gosling. 1979. Plasma progesterone levels throughout the oestrous cycle and release of LH at oestrous in sheep with different ovulation rates. *J. Reprod. Fertil.* 55:37–44.
- 22 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.* 38:613–626.
- 23 Ricketts, A. P., and A.P.F. Flint. 1980. Onset of synthesis of progesterone by ovine placenta. *J. Endocrinol.* 86:337–347.
- 24 SAS User's Guide: Statistics, Version 5 Edition. 1985. SAS Inst., Cary, NC.
- 25 Saumande, J. 1980. Concentrations of luteinizing hormone, oestradiol-17 β and progesterone in the plasma of heifers treated to induce superovulation. *J. Endocrinol.* 84:425–437.
- 26 Schalm, O. W., E. J. Carrol, and N. C. Jain. 1971. *Bovine Mastitis*. Lea and Febiger, Philadelphia, PA.
- 27 Schiewe, M. C., T. A. Fitz, J. L. Brown, L. D. Stuart, and D. E. Wildt. 1991. Relationship of oestrus synchronization method, circulating hormones, luteinizing hormone and prostaglandin F-2 α receptors and luteal progesterone concentration to premature luteal regression in superovulated ewes. *J. Reprod. Fertil.* 93:19–30.
- 28 Schoknecht, P. A., S. N. Nobrega, J. A. Petterson, R. A. Ehrhardt, R. Slepatis, and A. W. Bell. 1991. Relations between maternal and fetal plasma concentrations of placental lactogen and placental and fetal weights in well-fed ewes. *J. Anim. Sci.* 69:1059–1063.
- 29 Sheldrick, E. L., A. P. Ricketts, and A.P.F. Flint. 1981. Placental production of 5 β -pregnane-3 α ,20 α -diol in goats. *J. Endocrinol.* 90:151–158.
- 30 Stubbing, R. B., W.T.K. Bosu, C.A.V. Barker, and G. J. King. 1986. Serum progesterone concentrations associated with superovulation and premature corpus luteum failure in dairy goats. *Can. J. Vet. Res.* 50:369–373.
- 31 Taylor, M. J., G. Jenkin, J. S. Robinson, G. D. Thorburn, H. Friesen, and J.S.D. Chan. 1980. Concentrations of placental lactogen in chronically catheterized ewes and fetuses in late pregnancy. *J. Endocrinol.* 85:27–34.
- 32 Teles, F.F.F., C. K. Young, and J. W. Stull. 1978. A method for rapid determination of lactose. *J. Dairy Sci.* 61:506–508.
- 33 Wahab, I. M., and R. R. Anderson. 1989. Physiologic role of relaxin on mammary gland growth in rats. *Proc. Soc. Exp. Biol. Med.* 192:285–289.
- 34 Webb, J. M., and H. B. Levy. 1955. A sensitive method for the determination of deoxyribonucleic acid in tissues and microorganisms. *J. Biol. Chem.* 213:107–113.
- 35 Wiltbank, M. C., and G. D. Niswender. 1992. Functional aspects of differentiation and degeneration of steroidogenic cells of the corpus luteum in domestic ruminants. *Anim. Reprod. Sci.* 28:103–110.
- 36 Woodward, T. L., W. E. Beal, and R. M. Akers. 1993. Cell interactions in initiation of mammary epithelial proliferation by oestradiol and progesterone in prepubertal heifers. *J. Endocrinol.* 136:149–157.
- 37 Wright, L. C., and R. R. Anderson. 1982. Effect of relaxin on mammary growth in the hypophysectomized rat. Pages 341–353 in *Relaxin*. R. R. Anderson, ed. Plenum Publishing Corporation, New York, NY.