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

W. Manalu[1] and M. Y. Sumaryadi[2]
Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University
Bogor, Indonesia

ABSTRACT : An experiment was conducted to investigate correlations of litter size and average serum progesterone concentrations during pregnancy with mammary gland growth and development at parturition. Twenty ewes (5, 9, 4, and 2 ewes carrying 0, 1, 2, and 3 lambs, respectively) were used to measure weekly serum progesterone concentration during pregnancy. At parturition, the experimental ewes were slaughtered for determination of mammary gland growth and development at parturition (mammary dry fat-free tissue [DFFT], DNA, RNA, collagen, protein, and glycogen). Correlation of mammary DFFT with litter size and averages serum progesterone concentrations were 0.75 and 0.72, respectively. Litter size or maternal serum progesterone concentrations did not correlate with the mammary DNA concentration. However, litter size or maternal serum progesterone concentrations positively correlated ($p < 0.01$) with the mammary RNA and protein concentrations, but negatively correlated with the mammary collagen ($p < 0.01$) and glycogen ($p < 0.05$) concentrations. Litter size or maternal serum progesterone positively correlated ($p < 0.01$) with the total mammary DNA, RNA, collagen, protein and glycogen contents. These results implied that the increased concentrations of progesterone with the increased litter size during pregnancy improved mammary gland growth and development at parturition.

(**Key Words**: Progesterone, Mammary Growth, Pregnancy, Sheep)

INTRODUCTION

In the gonadally intact ovine, a major amount of mammary development occurrs during pregnancy (Anderson, 1975), which is associated with dramatic changes in secretions of mammogenic hormones. The main sources of these mammogenic hormones are the luteal ovary (mainly estrogen, progesterone, and relaxin) and the placenta (estradiol, progesterone and placental lactogen) (Ricketts and Flint, 1980).

Mammary gland growth and development during pregnancy in the ovine starts during the estrous cycle, and progress slowly during the first three months of gestation and dramatically increase during the last two months of gestation period (Rattray et al., 1974) around the time when the placenta significantly secretes progesterone (Ricketts and Flint, 1980), and placental lactogen (Hayden et al., 1980).

In the ovine, estradiol increases during estrous and decreases around 12 h after the onset of estrous (Pant et al., 1977), it continues to decrease until week 7 of pregnancy, and dramatically increases during the rest of pregnancy (Umo et al., 1976). Progesterone starts increasing 2 days after ovulation and shows a marked rise from day 5 to a peak between days 7 and 13, and remains stable during the rest of the luteal phase of pregnancy and again increases dramatically after week 8 of pregnancy (Manalu and Sumaryadi, 1997). During early pregnancy, maternal serum progesterone concentrations are positively correlated with the number of corpora lutea (Jarrell and Dziuk, 1991). After the second half of pregnancy, progesterone and estradiol (Manalu and Sumaryadi, 1997), and placental lactogen (Hayden et al., 1979; Hayden et al., 1980) also increase with the increased fetal number in the goats and sheep.

The profile of hormonal changes seems to correlate

[1] Address reprint requests to W. Manalu.
[2] Present address: M. Y. Sumaryadi, Laboratory of Physiology and Reproduction, Faculty of Animal Sciences, Jenderal Soedirman University, P. O. Box. 110, Purwokerto 53123, Central Java, Indonesia.
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well with the mammary gland growth pattern during pregnancy in the ovine (Manalu and Sumaryadi, 1997). However, there is no comprehensive study to determine quantitative correlation among litter size, pregnancy-related mammogenic hormone profiles, and mammary growth indices in a gonadally intact animal. Few studies report a positive correlation between litter size and mammary growth in ewes (Rattray et al., 1974; Butler et al., 1981). This present study was conducted to study the correlations of litter size and maternal serum progesterone concentrations during pregnancy with mammary gland growth and development indices at parturition in sheep.

MATERIALS AND METHODS

Environmental conditions and animal description

This experiment was conducted during the hot and wet season of the year in the humid tropic of Indonesia. Average daily temperature and relative humidity ranged from 25 to 32℃ and from 70 to 80%, respectively. The Javanese thin-tail sheep used in the experiment is an indigenous breed used mainly for meat production and is well known for its high prolificacy.

Experimental design and sampling protocol

Experimental animals were twenty 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 to 3 years) at breeding. The experimental ewes were injected twice with $PGF_2\alpha$ (i.m) with an 11-day interval. Three days after the second prostaglandin injection, the experimental ewes, except the control ewes, were mated naturally by colony breeding. Blood samples were drawn on days 1 and 10 after the last prostaglandin injection. Afterwards, blood samples were drawn weekly until parturition i.e., week 20 of pregnancy. Weeks of pregnancy were calculated from the predicted ovulation days (three days after the second prostaglandin injection). At parturition, the experimental animals were slaughtered for determination of mammary gland growth and development at parturition. The experimental ewes were groupped according to the number of fetal carried (number of born lambs).

Progesterone analyses

Concentration of serum progesterone were measured in duplicate by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) with a slight modification to accomodate wide ranges of progesterone concentrations in pregnant ovine (Manalu and Sumaryadi, 1997). Radioactivity of progesterone-bound tubes was counted with an automatic gamma counter. Concentrations of standard progesterone used to construct standard curve ranged from 0.1 to 20 ng/ml. A sample volume of 100 μl serum was used in the assay with progesterone concentrations ranging from 0.1 to 20 ng/ml. When concentrations of progesterone were below 0.1 or above 20 ng/ml, the measurements were repeated by increasing or decreasing sample volume, respectively, to bring the the sample progesterone concentrations to the range of standard used. Inter- and intra-assay coefficients of variations were 9, and 4.0%, respectively. Concentrations of progesterone were parallel in the sample volumes of 50, 100, and 200 μl.

Mammary gland growth and development indices measurements

Dry fat-free tissue (DFFT) of the mammary gland was measured by modification of method described by Anderson (1975). Half the udders 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 in 50℃ for 24 h to obtain DFFT. The DFFT was ground to make a fine powder to be used for measurement of mammary DNA by p-nitrophenilhydrazine reaction (Webb and Levy, 1956), RNA by orcinol reaction (Albaum and Umbreit, 1947), collagen by measuring hydroxyproline (Woessner, 1961), protein by Lowry method (Lowry et al., 1951), and glycogen by anthrone method (Seifter et al., 1950).

Statistical analyses

Data were examined using analysis of variance technique. Average progesterone concentration and litter size were correlated with mammary gland growth and development indices by using multiple linear regression and correlation analyses (Snedecor and Cochran, 1982).

RESULTS

Average serum progesterone concentrations during the whole gestation in the ewes carrying 0 (nonpregnant), 1, 2, and 3 fetuses were 2.89 ± 0.27, 12.02 ± 1.44, 18.66 ± 1.68, and 18.34 ± 2.61 ng/ml, respectively. Ewes carrying 2, and 3 fetuses had higher averages serum progesterone

concentrations by 55 and 53%, respectively, as compared to those carrying a single fetus ($p < 0.01$). However, there was no difference ($p > 0.05$) in averages serum progesterone concentrations during pregnancy between the ewes carrying two and three fetuses in this experiment (table 1).

Mammary DFFT during pregnancy increased dramatically ($p < 0.01$) in the pregnant ewes as compared to nonpregnant ewes. Ewes carrying multiple fetuses had significantly higher mammary DFFT than those carrying a single fetus ($p < 0.01$). There was no difference in mammary DFFT between ewes carrying twin and triplet fetuses. Mammary DFFT in nonpregnant ewes, ewes carrying 1, 2, and 3 fetuses were 2.34 ± 0.21, 26.39 ± 2.02, 40.42 ± 11.57, and 56.79 ± 15.88 g, respectively (table 2).

There was no difference in mammary DNA concentrations (μg/mg DFFT) between nonpregnant and pregnant ewes, and among litter sizes in pregnant ewes (39.73 ± 2.56, 35.18 ± 2.53, 32.56 ± 5.42, and 41.56 ± 4.19 μg/mg DFFT, in nonpregnant ewes, ewes carrying 1, 2, and 3 fetuses, respectively (table 1). Total mammary DNA contents, however, increased dramatically ($p < 0.01$) in the pregnant ewes as compared to those in nonpregnant ewes.

There was no difference in total mammary DNA contents between ewes carrying 1 and 2 fetuses. Ewes carrying 3 fetuses had higher total mammary DNA content ($p < 0.01$) as compared to those carrying 1 and 2 fetuses (0.10 ± 0.01, 0.92 ± 0.09, 1.19 ± 0.24 and 2.16 ± 0.42 g in nonpregnant ewes, ewes carrying 1, 2, and 3 fetuses, respectively (table 2).

Table 1. Average maternal serum progesterone concentrations during pregnancy and mammary DNA, RNA, collagen, protein and glycogen concentrations at parturition in Javanese thin-tail sheep[1]

Litter size		Progesterone (ng/ml)	Mammary indices concentrations				
			DNA	RNA	Collagen	Protein	Glycogen
			(μg/mg DFFT)				
0	Mean	2.89[c]	39.73[a]	11.71[b]	46.22[a]	529.34[c]	8.44[a]
	SE	0.27	2.56	0.99	2.43	24.47	0.72
1	Mean	12.02[b]	35.18[a]	11.49[b]	11.61[b]	729.88[b]	3.55[b]
	SE	1.44	2.53	1.70	1.11	19.69	0.31
2	Mean	18.66[a]	32.56[a]	20.42[a]	11.04[b]	728.70[b]	4.68[b]
	SE	1.68	5.42	0.53	2.77	19.06	1.05
3	Mean	18.34[a]	41.56[a]	19.64[a]	8.73[b]	833.35[a]	3.94[b]
	SE	2.16	4.19	1.33	3.22	36.44	0.14

[1] Means and SE of 5, 9, 4, and 2 ewes carrying 0, 1, 2, and 3 fetuses, respectively.
[a,b,c] Different superscripts in the same column refer to difference between litter size ($p < 0.01$).

Table 2. Mammary DFFT and total mammary DNA, RNA, collagen, protein, and glycogen contents at parturition in Javanese thin-tail sheep[1]

Litter size		Total mammary chemical indices content					
		DFFT	DNA	RNA	Collagen	Protein	Glycogen
		g					
0	Mean	2.34[c]	0.095[c]	0.027[c]	0.107[b]	1.261[c]	0.020[c]
	SE	0.21	0.013	0.003	0.008	0.154	0.002
1	Mean	26.39[b]	0.916[b]	0.297[b]	0.304[a]	19.349[b]	0.092[b]
	SE	2.02	0.087	0.046	0.035	1.743	0.008
2	Mean	40.42[a]	1.186[b]	0.816[a]	0.360[a]	30.209[a]	0.231[a]
	SE	11.57	0.236	0.225	0.059	9.578	0.107
3	Mean	56.79[a]	2.160[a]	1.178[a]	0.342[a]	49.061[a]	0.217[a]
	SE	15.88	0.421	0.387	0.044	15.303	0.055

[1] Means and SE of 5, 9, 4, and 2 ewes carrying 0, 1, 2, and 3 fetuses, respectively.
[a,b,c] Different superscripts in the same column refer to difference between litter size ($p < 0.01$).

Mammary RNA concentrations (μg/mg DFFT) in ewes carrying multiple fetuses were significantly higher than those carrying a single fetus and non pregnant ewes ($p < 0.01$). There was no difference in mammary RNA concentrations between ewes carrying a single fetus and nonpregnant ewes, and between ewes carrying twin and triplet fetuses. Mammary RNA concentrations in nonpregnant ewes, ewes carrying 1, 2, and 3 fetuses were 11.71 ± 0.99, 11.49 ± 1.70, 20.42 ± 0.53, and 19.64 ± 1.33 μg/mg DFFT, respectively (table 1). Total mammary RNA contents of the pregnant ewes increased dramatically ($p < 0.01$) as compared to those of nonpregnant ewes. Ewes carrying multiple fetuses had higher total mammary contents ($p < 0.01$) than those carrying a single fetus. There was no difference in total mammary contents between ewes carrying twin and triplet fetuses. Total mammary RNA contents in the nonpregnant ewes, ewes carrying 1, 2, and 3 fetuses were 0.03 ± 0.003, 0.30 ± 0.05, 0.82 ± 0.23, and 1.18 ± 0.39g, respectively (table 2).

Mammary collagen concentrations of the pregnant ewes were dramatically decreased as compared to those nonpregnant ewes ($p < 0.01$). There was no difference in mammary collagen concentrations among litter sizes in the pregnant ewes. Mammary collagen concentrations in nonpregnant ewes, ewes carrying 1, 2, and 3 fetuses were 46.22 ± 2.43, 11.61 ± 1.11, 11.04 ± 2.77, and 8.73 ± 3.22 μg/mg DFFT, respectively (table 1). Total mammary collagen contents in the pregnant ewes were higher than those in nonpregnant ewes ($p < 0.01$). There was no difference in total mammary collagen contents among litter sizes in the pregnant ewes. Mammary collagen contents in the nonpregnant ewes, ewes carrying 1, 2, and 3 fetuses were 0.11 ± 0.01, 0.30 ± 0.04, 0.36 ± 0.06, and 0.34 ± 0.04 g, respectively (table 2).

Mammary protein concentrations in the pregnant ewes were significantly higher than those in nonpregnant ewes ($p < 0.01$). There was no difference in mammary protein concentrations between ewes carrying 1 and 2 fetuses. Ewes carrying 3 fetuses had higher mammary protein concentrations ($p < 0.01$) as compared to those nonpregnant ewes, ewes carrying 1, and 2 fetuses. Mammary protein concentrations in nonpregnant ewes, ewes carrying 1, 2, and 3 fetuses were 529.34 ± 24.47, 729.88 ± 19.69, 728.70 ± 19.06, and 833.35 ± 36.44 μg/mg DFFT, respectively (table 1). Total mammary protein contents in the pregnant ewes were dramatically increased as compared to those nonpregnant ewes ($p < 0.01$). Ewes carrying multiple fetuses had higher total mammary protein contents than those carrying a single fetus ($p < 0.01$). There was no difference in total mammary protein contents between ewes carrying 2 and 3 fetuses. Total mammary protein contents of nonpregnant ewes, ewes carrying 1, 2, and 3 fetuses were 1.262 ± 0.154, 19.349 ± 1.743, 30.209 ± 9.578, and 49.061 ± 15.303 g, respectively (table 2).

Mammary glycogen concentrations of the pregnant ewes were dramatically decreased as compared to nonpregnant ewes ($p < 0.01$). There was no difference in mammary glycogen concentrations among litter sizes in the pregnant ewes. Mammary glycogen concentrations of the nonpregnant ewes, ewes carrying 1, 2, and 3 fetuses were 8.44 ± 0.72, 3.55 ± 0.31, 4.68 ± 1.05, and 3.94 ± 0.14 μg/mg DFFT, respectively (table 1). Total mammary glycogen contents in the pregnant ewes were higher than those in nonpregnant ewes ($p < 0.01$). There was no difference in total mammary glycogen contents between ewes carrying 2 and 3 fetuses. Ewes carrying multiple fetuses had higher mammary glycogen contents than those carrying a single fetus ($p < 0.01$). Mammary glycogen contents in the nonpregnant ewes, ewes carrying 1, 2, and 3 fetuses were 0.02 ± 0.002, 0.09 ± 0.01, 0.23 ± 0.11, and 0.22 ± 0.06 g, respectively (table 2).

Correlations of litter size and average maternal serum progesterone concentration during pregnancy with mammary DFFT at parturition were 0.75 and 0.72, respectively ($p < 0.01$). Litter size and average maternal serum progesterone concentrations during pregnancy, however, did not correlate with mammary DNA concentrations at parturition. Litter size and average maternal serum progesterone concentration during pregnancy positively correlated ($p < 0.01$) with the mammary RNA concentrations ($r = 0.60$ and 0.59, respectively) and protein concentrations ($r = 0.75$ and 0.69, respectively). However, litter size and average maternal serum progesterone concentrations during pregnancy negatively correlated with the mammary collagen concentrations ($p < 0.01$) ($r = -0.73$ and -0.70, respectively) and glycogen concentrations ($p < 0.05$) ($r = -0.51$ and -0.55, respectively). Mammary DNA, RNA, collagen, protein, and glycogen concentrations at various averages serum progesterone concentrations during pregnancy are presented in figure 1.

Litter size positively correlated with total mammary DNA, RNA, collagen, protein ($p < 0.01$) and glycogen ($p < 0.05$) contents at parturition ($r = 0.82$, 0.76, 0.60, 0.74, and 0.57, respectively). Averages maternal serum progesterone concentrations during pregnancy positively

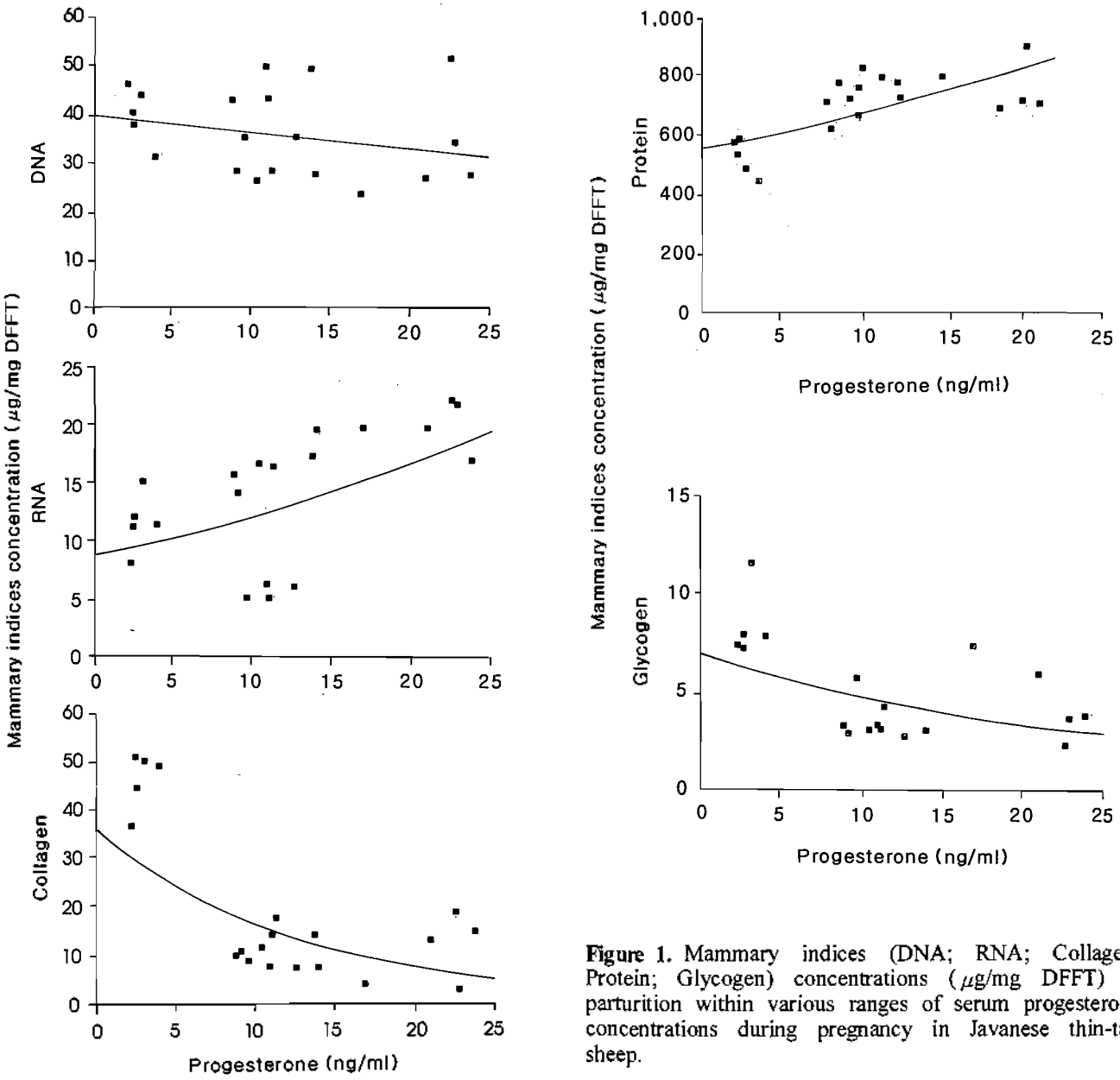

Figure 1. Mammary indices (DNA; RNA; Collagen; Protein; Glycogen) concentrations (μg/mg DFFT) at parturition within various ranges of serum progesterone concentrations during pregnancy in Javanese thin-tail sheep.

correlated ($p < 0.01$) with total mammary DNA, RNA, collagen, protein and glycogen content (r were 0.74, 0.70, 0.76, 0.68, and 0.53, respectively). Mammary DFFT, total mammary DNA, RNA, collagen, protein, and glycogen contents at various averages serum progesterone concentrations during pregnancy are presented in figure 2.

DISCUSSION

Profiles of progesterone concentrations during pregnancy and litter size in Javanese thin-tail ewes were explained elsewhere. Maternal serum progesterone concentrations correlated well with litter size. Ewes carrying multiple fetuses had higher average serum progesterone concentrations and better developed mammary glands (Manalu and Sumaryadi, 1997). These data and those reported in this result indicated that mammary growth and development, as indicated by the mammary DFFT, increased with the increased progesterone concentrations and litter size.

Data analysis showes that increased average maternal serum progesterone concentrations during pregnancy and litter size did not correlate with mammary DNA concentrations per unit weight of mammary DFFT. These data indicated that the number of cells per unit weight of mammary glands did not change with the increased

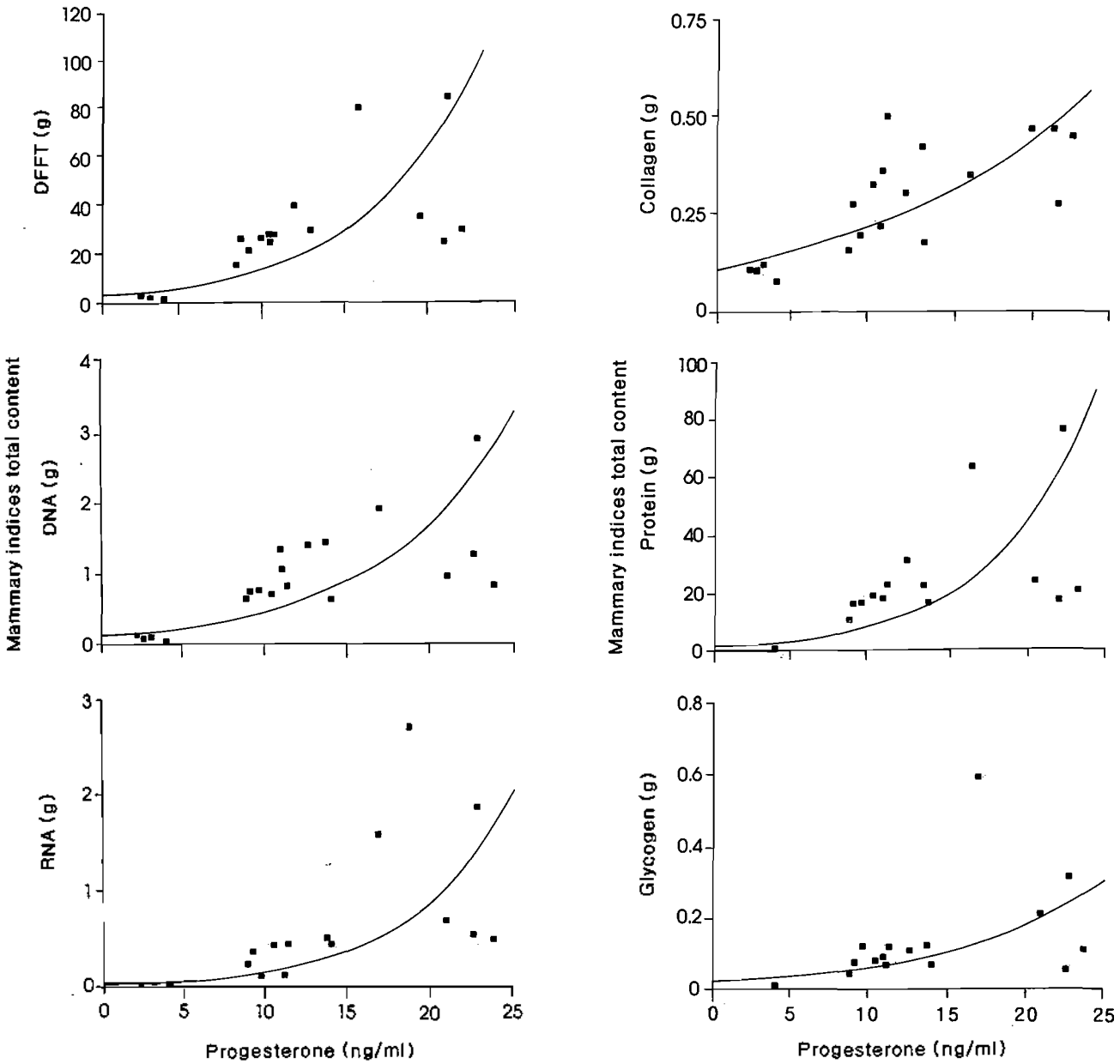

Figure 2. Mammary indices total (DFFT; DNA; RNA; Collagen; Protein; Glycogen) contents at parturition within various ranges of serum progesterone concentrations during pregnancy in Javanese thin-tail sheep.

averages serum progesterone concentrations and litter size. However, total cell number in the mammary gland, as indicated by the total DNA content, was indeed increase in the ewes carrying higher number of fetuses and having higher averages serum progesterone concentrations during pregnancy.

While cell number per unit weight of mammary tissue did not change, synthetic activities, as indicated by RNA concentrations, increased with the increased average serum progesterone concentrations and litter size. The positive correlation of litter size and averages maternal serum progesterone during pregnancy with mammary protein concentrations corroborated the increased synthetic activities per unit weight of mammary gland. The negative correlation of litter size and average serum progesterone concentration during pregnancy with mammary collagen and glycogen concentrations indicated that stromal fraction per unit weight of DFFT became less as compared to the mammary parenchymal fraction, although total collagen and glycogen contents increased.

The increased other mammogenic hormones secretion in addition to progesterone with the increased litter size

could explain the close correlation of mammary gland growth and development with litter size. Placental lactogen as a potent mammogenic hormone during late pregnancy was reported increased with the increased litter size (Hayden et al., 1980; Butler et al., 1980).

The similar contribution of average serum progesterone concentrations during pregnancy as that of litter size to mammary gland growth at parturition, without additive effects, indicated that endogenous progesterone secretion itself, to the most part, was represented in the effects of litter size and its related factors. In conclusion, mammary growth and development indices increased with the increased endogenous mammogenic hormones secretions during pregnancy due to the increased number of fetuses carried. The results suggest that growth and development of the mammary gland during pregnancy could be improved by increasing endogenous secretion of mammogenic hormones.

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