Maternal Serum Concentrations of Total Triiodothyronine, Tetraiodothyronine and Cortisol in Different Status of Pregnancy During Late Pregnancy in Ettawah-Cross Does

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ABSTRACT : Fifteen Ettawah-cross does were used to study maternal serum concentrations of total triiodothyronine (T_3), teraiodothyronine (T_4) and cortisol in different status of pregnancy (nonpregnant, aborted, single and twin-bearing does) during late pregnancy.

Analysis of the data indicated that there was no significant changes in total T_3, T_4, and cortisol concentrations with the advance of pregnancy. Concentrations of T_3, T_4, and cortisol decreased by 38.9, 34.9, and 32.6%, and 12.0, 15.7 and 27.6%, and 41.6, 44.0, and 43.7% in the aborted, single and, twin-bearing, respectively, as compared to those nonpregnant does. There was no significant difference in concentrations of T_3 and cortisol between aborted, single and twin-bearing does, and in those of T_4 between aborted and single-bearing does. However, T_4 concentrations in twin-bearing were lower by 17.7 and 14.1% than those in aborted and single-bearing does, respectively.

The decreased concentrations of thyroid hormones in pregnant does suggested that fetus could have increased iodine uptake from maternal circulation causing a decrease in the availability of this nutrient for synthesis of maternal thyroid hormones. The decreased concentrations of cortisol could have been associated with the increased metabolism of the hormone to regulate nutrients influx into the placenta of pregnant does.

(**Key Words**: Triiodothyronine, Tetraiodothyronine, Cortisol, Pregnancy, Abortion, Goat)

INTRODUCTION

Indonesian sheep and goats are prolific (Bradford et al., 1986), but their overall reproductive efficiency and productivites are substantially low (Sutama, 1992.) The low reproductive efficiency and productivity, in part, due to the high embryonic and fetal mortalities, in addition to the reported high mortality rate of preweaned lambs and kids (Obst et al., 1982) which increased with the increased litter size.

The increased preweaned mortality was assumed to be, mostly, due to the low birth weight and insufficiency of milk production (Tiesnamurti, 1992) to nourish the newborn lambs and kids. Poor prenatal growth could be affected by uterine and placental growth (Dziuk, 1992), and nutritional availability in the maternal circulation (Bell, 1984). Extra uterine factor such as heat stress could impair blood flow and nutrient availability during pregnancy (Roman-Ponce et al., 1978).

In the ovine the growth and development of the fetus is dramatically increase during the second half of gestation. During this period, nutrients extraction from the maternal circulation for energy sources and for synthetic new tissues, and maternal tissue energy mobilization would be increased (Annison et al., 1984).

Increase in synthetic activities during pregnancy requires a greater maternal hormonal changes to regulate nutrient flow to the gravid uterus. During this period of high synthetic activities, maternal concentrations of hormones regulating nutrient flow for synthetic and energy-yielding processes such as insulin, glucagon, growth hormone, and placental lactogen are dramatically change (Annison et al., 1984).

Thyroid hormones and cortisol are well known for their effects on energy metabolism and gluconeogenesis, respectively (Utiger, 1987; Baxter and Tyrrell, 1987). These hormones are rarely measured in relation to the metabolic load during pregnancy in ovine, especially in relation to the state of pregnancy and litter size in does. The objectives of this present study were to measure

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serum concentrations of triiodothyronine, tetraiodothyronine and cortisol in different state of pregnancy and litter size in does raised in traditional small ruminant management practice in Indonesia.

MATERIALS AND METHODS

Experimental design and protocol

Fifteen Ettwah-cross does with similar weight (20-22kg) and age (2-3 years) were maintained in the experimental pen with 2 months adaptation to the experimental conditions prior to mating period. During the mating period, the experimental does were mated naturally with 5 bucks for a month and the bucks were removed from the flock and the experimental does were maintained in a group of three per pen. Blood samplings were conducted weekly after the breeding period. In the end of experimental period, only 8 does gave birth with normal kids (5 singles and 3 twins). Three does aborted and the others 4 were nonpregnant during the experimental period (as was verified by progesterone analysis [Manalu et al., 1996]). The experimental does were then grouped according to the status of pregnancy i. e., nonpregnant, aborted, single and twin-bearing does with n = 4, 3, 5, and 3, respectively.

Environmental conditions and animal management

The experiment was conducted in the village site. Average daily ambient temperature and relative humidity ranged from 25 to 32℃ and from 70 to 80%, respectively. Experimental does were kept in a pen with a raised floor as a typical small ruminant housing in the region. Fresh field grass was fed as libitum with supplementation of table salt block. The animal were provided drinking water as libitum. This feeding regiment was adopted to simulate prevailing management practice in the small-holder small-ruminant farmer in the region.

Blood sampling and processing

Ten milliliters of blood samples were drawn with plain vacutainer of sterile syringes from jugular vein prior to morning feeding at around the same time weekly. The blood samples were allowed to clot in a 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. Weeks prior to parturition or abortion were counted back from the last blood sampling prior to parturition or abortion date.

Hormone analyses

Total triiodothyronine (T_3). Concentration of total serum T_3 in duplicate was measured by solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The radioactivity of triiodothyronine-bound tubes were counted with an automatic gamma counter (Aloka, Model ARC 503, Aloka Co., Ltd., Japan). The concentrations of standard triiodothyronine used to construct standard curve ranged from 20 to 200 ng/dl. All samples triiodothyronine concentrations were within the range of concentrations used to construct the standard curve. A sample volume of 100 μl serum was used in the assay. Coefficiency of intra and inter-assay variations were 4 and 3%, respectively.

Total tetraiodothyronine (T_4). Concentration of total serum T_4 in duplicate was measured by solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The radioactivity of tetraiodothyronine-bound tubes were counted with an automatic gamma counter (Aloka, Model ARC 503, Aloka Co., Ltd., Japan). The concentrations of standard tetraiodothyronine used to construct standard curve ranged form 10 to 100 ng/ml. All samples tetraiodothyronine concentrations were within the range of concentrations used to construct the standard curve. A sample volume of 25 μl serum was used in the assay. Coefficiency of intra and inter-assay variations were 5 and 3%, respectively.

Cortisol. Concentration of serum cortisol in duplicate was measured by solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The radioactivity of cortisol-bound tubes were counted with an automatic gamma counter (Aloka, Model ARC 503, Aloka Co., Ltd., Japan). The concentrations of standard cortisol used to construct standard curve ranged from 10 to 200 ng/ml. All samples cortisol concentrations were within the range of concentrations used to construct the standard curve. A sample volume of 50 μl serum was used in the assay. This volume was doubled from the volume recommended by the manufacturer to accommodate lower cortisol concentrations in ruminant animal. Coefficiency of intra and inter-assay variations were 3 and 4%, respectively.

Data analyses

Data during the 7-week measurement were analyzed for main effect (week prior to parturition or abortion, and status of pregnancy: nonpregnant, aborted, single and twin-bearing) and their interactions with randomized design with factorial arrangement with unequal n. The difference among means of main effects were tested by using least significant difference (Snedecor and Cochran,

1982).

RESULTS

Average weekly serum triiodothyronine concentrations of experimental does are presented in table 1. There was no difference in the concentrations of triiodothyronine among weeks during the 7-week measurement prior to parturition or abortion within the same status of pregnancy. Average maternal serum concentraions of T_3 decreased ($p < 0.01$) by 38.9, 34.9, and 32.6% in aborted, single, and twin-bearing does, respectively, as compared to those of nonpregnant does (68.25, 72.71, and 75.26 vs 111.62 ng/dl). There was no difference in average maternal serum concentrations of T_3 of aborted, single and twin-bearing does. However, aborted does had a numerically lower concentrations of T_3 as compared to those single and twin-bearing does. The number of fetuses carried in pregnant does did not afftect maternal serum concentrations of T_3 during late pregnancy.

Table 1. Concentrations of serum triiodothyronine in nonpregnant, aborted, single- and twin-bearing does 7 weeks prior to parturition or abortion[1]

Status[2]	Weeks prior to parturition or abortion[3]							Mean[4]
	7	6	5	4	3	2	1	
	(ng/ml)							
Nonpregnant								
Mean	143.23	101.70	116.78	100.49	118.48	103.04	97.58	111.62[a]
SE	12.00	17.00	15.49	9.68	14.89	14.32	15.17	6.11
Aborted								
Mean	59.92	83.29	57.99	48.10	76.74	63.08	88.67	68.25[b]
SE	14.55	8.06	3.76	0.03	20.67	16.92	25.01	5.75
Single								
Mean	74.48	63.92	62.42	84.46	58.36	64.13	101.20	72.71[b]
SE	14.81	14.84	6.39	12.11	4.95	10.39	14.53	5.81
Twin								
Mean	86.80	75.60	85.77	64.71	61.29	71.82	80.82	75.26[b]
SE	9.21	10.11	13.94	5.72	5.14	8.05	9.91	3.76

[1] Means and SE of 4, 3, 5, and 3 does for nonpregnant, aborted, single- and twin-bearing does, respectively.
[2] Status are status of pregnancy i. e., nonpregnant, aborted, single- and twin-bearing does, respectively.
[3] Weeks prior to parturition only applied to single-and twin-bearing does. For aborted does this term means weeks prior to abortion. For nonpregnant does, this term means nothing but as a control for hormonal profile at the same period of blood sampling.
[4] Different superscripts in this column refer to difference between status of pregnancy ($p < 0.01$).

Average weekly serum tetraiodothyronine concentrations of experimental does are presented in table 2. There was no difference in the concentrations of tetraiodothyronine among weeks during the 7-week measurement prior to parturition or abortion within the same status of pregnancy. Average maternal serum concentrations of T_4 decreased by 12.0, 15.7, and 27.6% in aborted ($p < 0.05$), single ($p < 0.01$), and twin-bearing ($p < 0.01$) does, respectively, as compared to those of nonpregnant does (47.76, 45.78, and 39.31 vs 54.30 ng/dl). There was no difference in average maternal serum concentrations of T_4 of aborted and single-bearing does. Increased number of fetuses carried in pregnant does (from 1 to 2) significantly decreased ($p < 0.05$) maternal serum concentrations of T_4 by 14.1%. Pregnant does carrying 2 fetuses had consistently lower serum concentrations of T_4 during the whole weeks of measurement than those of nonpregnant and single-bearing does.

Average weekly serum cortisol concentrations of experimental does are presented in table 3. There was no difference in the concentrations of cortisol among weeks during the 7-week measurement prior to parturition or abortion within the same status of pregnancy. Average maternal serum concentrations of cortisol decreased ($p < 0.01$) by 41.6, 44.0, and 43.7% in aborted, single, and

Table 2. Concentrations of serum tetraiodothyronine in nonpregnant, aborted, single-and twin-bearing does 7 weeks prior to parturition or abortion[1]

Status[2]	Weeks prior to parturition or abortion[3]							Mean[4]
	7	6	5	4	3	2	1	
	(ng/ml)							
Nonpregnant								
Mean	57.50	55.52	57.32	48.57	65.55	52.50	43.13	54.30[a]
SE	1.99	3.53	2.24	1.10	9.10	4.45	5.99	2.71
Aborted								
Mean	45.13	50.43	50.99	48.62	54.02	34.96	50.15	47.76[b]
SE	5.93	5.86	4.65	6.25	8.81	4.36	6.22	2.28
Single								
Mean	47.05	46.17	44.86	47.12	48.67	48.63	37.99	45.78[b]
SE	5.73	5.00	3.94	3.48	5.65	4.49	5.36	1.39
Twin								
Mean	44.21	39.88	43.83	39.20	35.25	40.05	32.72	39.31[c]
SE	5.82	2.44	3.85	1.25	1.04	2.96	1.77	1.58

[1] Means and SE of 4, 3, 5, and 3 does for nonpregnant, aborted, single- and twin-bearing does, respectively.
[2] Status are status of pregnancy i. e., nonpregnant, aborted, single-and twin-bearing does, respectively.
[3] Weeks prior to parturition only applied to single-and twin-bearing does. For aborted does this term means weeks prior to abortion. For nonpregnant does, this term means nothing but as a control for hormonal profile at the same period of blood samping.
[4] Different superscripts in this column refer to difference between status of pregnancy ($p < 0.01$).

Table 3. Concentrations of serum cortisol in nonpregnant, aborted, single- and twin-bearing does 7 weeks prior to parturition or abortion[1]

Status[2]	Weeks prior to parturition or abortion[3]							Mean[4]
	7	6	5	4	3	2	1	
	(ng/ml)							
Nonpregnant								
Mean	21.45	26.21	22.03	19.85	17.67	14.20	11.76	19.02[a]
SE	2.77	5.52	5.61	5.89	4.98	2.83	2.44	1.86
Aborted								
Mean	9.09	6.89	12.77	15.40	8.41	11.65	13.56	11.11[b]
SE	1.33	1.63	2.33	1.76	0.85	3.42	5.35	1.45
Single								
Mean	6.66	10.54	16.87	8.70	11.61	8.93	11.25	10.65[b]
SE	1.53	1.35	8.54	2.33	1.92	1.46	1.75	1.22
Twin								
Mean	17.16	7.14	5.23	10.05	8.77	8.67	17.97	10.71[b]
SE	7.96	3.08	0.60	2.99	1.52	0.79	6.42	1.86

[1] Means and SE of 4, 3, 5, and 3 does for nonpregnant, aborted, single- and twin-bearing does, respectively.
[2] Status are status of pregnancy i. e., nonpregnant, aborted, single-and twin-bearing does, respectively.
[3] Weeks prior to parturition only applied to single and twin-bearing does. For aborted does this term means weeks prior to abortion. For nonpregnant does, this term means nothing but as control for hormonal profile at the same period of blood sampling.
[4] Different superscripts in this column refer to difference between status of pregnancy ($p < 0.01$).

nonpregnant does (11.11, 10.65, and 10.71 vs 19.02 ng/dl). There was no difference in average maternal serum concentrations of cortisol of aborted, single and twin-bearing does. The increased fetal number carried in pregnant does did not affect maternal serum concentrations of cortisol during late pregnancy.

DISCUSSION

The increased metabolic load of the pregnant does in this experiment consistently decreased serum thyroid hormones and cortisol concentrations. This result was contrary to the common knowledge that high metabolic activity is associated with the high concentrations of these hormones (McDonald, 1980). Why did pregnant does have lower circulating T_3, T_4, and cortisol concentrations than those nonpregnant ones?

The reduction in total circulating T_3 and T_4 could be due to the increased turnover rate or the decreased secretion rate of the hormones from the thyroid glands, or combination of both. It was reported that thyroxine binding globulin (TBG) increased during pregnancy, and high estradiol concentration caused a reduction in free circulating hormones but an increase in total circulating T_3 and T_4 (Utiger, 1987).

The iodine required for thyroid hormones synthesis in the fetal thyroid glands were taken up from the maternal circulation. Extraction of iodine from the maternal circulation, which would increase with the higher fetal number, would decrease iodine availability for the maternal thyroid glands that ultimately reduced maternal thyroid hormones synthesis and their total circulating concentrations. The results of this experiment clearly demonstrated that the degree of decrease in serum T_4 concentrations in twin-bearing was almost twice as those in single-bearing does (15.7 vs 27.6%). Even though the reduction of T_3 in pregnant does was greater than that of T_4 (34.9 and 35.3% in single and twin-bearing, respectively, as compared to nonpregnant does), the effect of fetal number on the reduction of T_3 was not observed. This could have been accomplished by increasing conversion of T_4 to T_3 (Braverman et al., 1970) in the twin-bearing does. The presumably increased conversion of T_4 ot T_3 could have contributed, in part, to the greater decrease in T_4 in twin bearing than single-bearing does.

Averaged maternal serum concentrations of T_3 and T_4 in aborted does during 7 week-period prior to abortion were not different from those in single-bearing does, and still higher than those in nonpregnant does. This data indicated that the abortion case in this experiment was not due to the deficiency of iodine.

The decreased concentrations of cortisol in pregnant does in this experiment agrees with other reports that levels of cortisol were unchanged during pregnancy or throughout gestation in the ewe (Wintour et al., 1976). However, Boulfekhar and Brudieux (1980) reported that cortisol concentrations in the maternal circulation of ewes decreased by 64.9% around 57 days before parturition as compared to those during the first half of pregnancy, and then slightly rising approaching parturition date (Rice et al., 1984). Given that cortisol concentration during the first half of pregnancy was similar to those during cycling, the decreased cortisol concentrations reported by Boulfekhar and Brudieux (1980) agreed with our results.

The decreased serum concentrations of cortisol in pregnant does might not relate to the uptake of the hormone by the placenta, but could have been associated with the increased metabolism of the hormone in the maternal tissues (Annison et al., 1984).

The increased fetal number and stage of pregnancy could have increased metabolism of this hormone that ultimately decreased its maternal concentrations. However, the increased fetal number and stage of pregnancy did not affect maternal serum concentrations cortisol. Does carrying two fetuses could have obtained extra cortisol from fetal secretion (Currie and Thorburn, 1977) that ultimately restored maternal cortisol concentration that would be otherwise lower due to the increased cellular utilization in the maternal tissues.

The nonsignificant difference in maternal cortisol concentrations of aborted from pregnant does suggested that nutrients deficiency related to the modulating effects of cortisol was not the reason for the abortion case. There should be some other factors other than cortisol and thyroid hormones such as heat stress (which is common in humid-tropical climate of Indonesia) that could impair blood flow and nutrient uptake by the fetus (Roman-Ponce et al., 1978) that probably indirectly cause abortion.

In conclusion, pregnancy decreased maternal serum concentrations of T_3, T_4, and cortisol. Serum T_4 concentrations in the twin-bearing does had a greater reduction as compared to those in single-bearing does. These results suggested a requirement to differentiate feeding management based on litter size in pregnant does or ewes. Neither thyroid hormones nor cortisol deficiency could be associated with the abortion case in the experimental does.

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