Prediction of Litter Size Based on Hormones and Blood Metabolites Concentrations during Pregnancy in Javanese Thin-Tail Ewes

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ABSTRACT : Thirty nine pregnant Javanese thin-tail ewes (20 and 19 carried a single and multiple [2 to 3] fetuses, respectively), and six nonpregnant ewes as controls were used to measure maternal serum hormone and blood metabolite concentrations as predictors of number of fetuses carried during pregnancy. Serum hormones (progesterone, estradiol, triiodothyronine, and cortisol) and blood metabolites (b-hydroxy butyric acid [BHBA], and blood urea nitrogen [BUN]) were determined every four weeks during pregnancy and were used to predict litter size by discriminant analysis. The results of data analysis indicated that serum progesterone and estradiol concentrations at weeks 8, 12, 16 of pregnancy could be used to predict the number of fetuses carried with precision of 86.7 to 95.6%. Serum triiodothyronine, cortisol, BHBA, and BUN concentrations during pregnancy, however, were not good predictors of the number of fetuses carried. Serum progesterone and estradiol concentrations as early as 8 weeks of pregnancy in sheep could predict the number of fetuses carried with 86.7% precision. *(Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 5 : 682-688)*

Key Words : Litter Size, Progesterone, Estradiol, Triiodothyronine, Blood Urea Nitrogen, Beta-Hydroxy Butyric Acid, Pregnancy, Sheep

INTRODUCTION

The Javanese thin-tail sheep is a meat-type indigenous breed well recognized for its high prolificacy (Bradford et al., 1986; Sutama et al., 1988). However, the higher the litter size the lower the lamb birth weight with a final result in a higher preweaning lamb mortality (Obst et al., 1980; Sutama, 1988; Sutama, 1992; Tiesnamurti, 1992). Total lamb birth weight increases by 67 and 115% and average individual weight decreases by 16 and 28% with the increased litter size from 1 to 2 and 3, respectively, (Manalu and Sumaryadi, 1998c). Lower birth weight is due to the low prenatal weight (Dziuk, 1992). Lower prenatal weight with the increased number of fetuses carried is assumed to be partly caused by the inadequacy of nutrients supplies in the placenta from the maternal circulation, in addition to the space limitation in the uterus (Dziuk, 1992).

Improvement of prenatal weight by improving ewes' nutritional status during pregnancy requires prior knowledge of litter size to differentiate feeding regimen applied on ewes with different litter sizes. In Javanese thin-tail ewes and Ettawah-cross does, progesterone and estradiol increase with the increased litter size (Manalu et al., 1996; Manalu and Sumaryadi, 1998b; Manalu and Sumaryadi, 1998a). In goat, triiodothyronine concentrations during pregnancy are affected by litter size. However, cortisol and tetraiodothyronine concentrations are not affected by litter size (Manalu et al., 1997). Maternal serum beta-hydroxy butyric acid and blood urea nitrogen concentrations increase with the advance of pregnancy in sheep, even though there is no significant effect of litter size (Sumaryadi and Manalu, 1997).

The close association of maternal serum concentrations of progesterone, estradiol, triiodothyronine, and cortisol, and changes in maternal body metabolisms during pregnancy with the increased litter size makes possible to predict litter size based on those parameters. This present study tested the use of hormone and blood metabolite concentrations at weeks 4, 8, 12, and 16 of pregnancy to predict litter size in Javanese thin-tail ewes.

MATERIALS AND METHODS

Animals

This experiment was conducted during the hot (25 to 32℃) and wet (70 to 80% relative humidity) season in the humid tropics of Indonesia. Experimental animals were 45 Javanese thin-tail ewes (6, 20, and 19, ewes carrying 0, 1, and multiple (2 to 3) fetuses, respectively) with similar body weight (21.73 ± 1.55 kg) and age (2 to 3 years) at breeding. Prior to experiment, the ewes had been raised traditionally in a semigrazing system i.e., they grazed in the field during the day and were housed at night, without concentrate supplementation. In the laboratory, the experimental ewes were adapted to experimental conditions (experimental housing and ration) for one month, and were then injected twice with $PGF_{2\alpha}$ (i.m) at an 11-day interval to synchronize estrous cycle. Three

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days after the last injection, the experimental ewes were mated naturally by group breeding. During pregnancy the experimental ewes were fed a basal diet consisting of king grass (*Pennisetum purpureophoides*) hay (*ad libitum*) and 500 g concentrate (Indofeed, Bogor, Indonesia) per ewe per day. A complete mineral mixture (Lactamineral, Wonder Pharmaceutical Indonesia, Jakarta, Indonesia) was given 0.1 g/kg BW (around 2 g/ewe) daily in the morning prior to concentrate feeding, and water was available *ad libitum*. Compositions of king grass hay and concentrate used in the experiment are presented in table 1. The number of fetuses carried during pregnancy was determined by the number of lambs born at parturition. The pregnant ewes actually used in the analysis were in good conditions during pregnancy without any indication of abortion. Five days after the onset of estrous, the number of corpora lutea was examined by laparoscopy, and the number of lambs born was generally agreed with the number of corpora lutea.

Table 1. Compositions of king grass hay and concentrate used in the experiment (%)

Composition	Feed component	
	King grass hay	Concentrate
Moisture	10.7	15.0
Crude protein	11.7	16.0
Ether extract	4.9	4.0
Crude fiber	20.8	7.0
Nitrogen-free extract	44.5	50.0
Ash	7.5	8.0
Total digestible nutrient	65.9	68.0
Calcium	0.7	-
Phosphorus	0.5	-
Sodium chloride	0.3	-

Blood sampling and processing

Ten ml of blood samples were drawn with plain vacutainer or sterile syringes from the jugular vein between 0900 and 1000 h. The first blood sample was taken one day after the last prostaglandin injection (week 0 of pregnancy), and then at week 4, 8, 12, and 16 of pregnancy, respectively. Blood samples were allowed to clot in a cool ice box, centrifuged to separate serum, which was then frozen for progesterone, estradiol, triiodothyronine, cortisol, β-hydroxy butyric acid (BHBA), and blood urea nitrogen (BUN) analyses.

Hormone and metabolite analysis

Progesterone: The concentration of serum progesterone was measured in duplicate by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) using I^{125}-progesterone as a tracer, with a slight modification to accommodate wide ranges of progesterone concentrations in pregnant ovine (Manalu et al., 1996). The progesterone assay used the whole serum without prior ether extraction. The radioactivities of I^{125}-progesterone-bound tubes were counted with an automatic gamma counter. The lowest and highest limits of sensitivity of assay were 0.1 and 20 ng/ml, respectively. Therefore, the concentrations of standard progesterone used to construct the standard curve ranged from 0.1 to 20 ng/ml. A sample volume of 100 μl serum was used in the assay of samples with progesterone concentrations ranged from 0.1 to 20 ng/ml. For samples with progesterone concentrations lower than 0.1 ng/ml, sample volume was increased to 200 μl. For samples with progesterone concentrations higher than 20 ng/ml, sample volume was reduced to 50 μl. Inter- and intra-assay coefficients of variation were 6, and 4%, respectively. Concentrations of progesterone were parallel in the sample volumes of 50, 100, and 200 μl.

Estradiol: Concentration of serum estradiol was measured in duplicate by the solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) using I^{125}-estradiol as a tracer, with a slight modification to accommodate wide ranges of estradiol concentrations in pregnant ovine (Manalu et al., 1996). The estradiol assay used the whole serum without prior ether extraction. The radioactivities of I^{125}-estradiol-bound tubes were counted with an automatic gamma counter. The lowest and highest limits of sensitivity of assay were 20 and 150 pg/ml, respectively. Therefore, the concentrations of standard estradiol used to construct the standard curve ranged from 20 to 150 pg/ml. A sample volume of 100 ml serum was used in the assay for samples with estradiol concentrations ranged from 20 to 150 pg/ml. For samples with estradiol concentrations lower than 20 pg/ml, sample volume was increased to 200 to 300 μl to bring the estradiol concentrations to the range of standard used. For samples with estradiol concentrations higher than 150 pg/ml, sample volume was decreased to 50 μl to bring the estradiol concentrations to the range of standard used. All samples' estradiol concentrations were within the range of concentrations of standard estradiol used to construct the standard curve. Inter- and intra-assay variations coefficients were 7 and 5.0%, respectively. The concentrations of estradiol were parallel in the sample volumes of 50, 100, 200 and 300 μl.

Triiodothyronine (T_3): Concentration of total serum T_3 was measured in duplicate by solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The radioactivities of I^{125}-triiodothyronine-bound tubes were counted with an automatic gamma counter. The lowest and highest

limits of sensitivity of assay were 20 and 200 ng/ml, respectively. Therefore, the concentrations of standard triiodothyronine used to construct the 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. Inter- and intra-assay variations coefficients 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) using I^{125}-cortisol as a tracer, with a slight modification to accommodate low cortisol concentrations in the ruminant (Manalu et al., 1997). The cortisol assay used the whole serum without prior ether extraction. The radioactivities of I^{125}-cortisol-bound tubes were counted with an automatic gamma counter. The lowest and highest limits of sensitivity of assay were 10 and 200 ng/ml, respectively. Therefore, the concentrations of standard cortisol used to construct the 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. Inter- and intra-assay variations coefficients were 6 and 3%, respectively.

β-Hydroxy butyric acid (BHBA): Concentration of serum β-hydroxy butyric acid was determined by enzymatic method using commercial kit (Sigma Chemical Co., St. Louis, MO). Standard used in the determination ranged from 5 to 50 mg/dl. All samples BHBA concentrations were within the range of concentrations used to construct the standard curve.

Blood urea nitrogen (BUN): Concentration of blood urea nitrogen (BUN) was determined by enzymatic method using commercial kit (Sigma Chemical Co., St. Louis, MO). Standard used in the determination ranged from 15 to 75 mg/dl. All samples BUN concentrations were within the range of concentrations used to construct the standard curve.

Statistical analyses

Serum progesterone, estradiol, triiodothyronine, cortisol, BHBA, and BUN at weeks 4, 8, 12, and 16 of pregnancy were used to predict whether an ewe was nonpregnant, carried a single or multiple fetuses by using a discriminant function:

$$D_i = a_i + \sum b_{ij}H_j + \sum b_{ij}M_j$$

where,

- D_i = discriminant value for *i*th litter size (i=0, 1, and 2)
- a_i = a constant (intercept)
- $b_{ij(Cij)}$ = vector coefficient for hormone concentrations at week *j*th in ewe carrying *i* fetus for $H_j(M_j)$.
- H_j = hormone concentrations at week *j*th (j=4, 8, 12, and 16)
- M_j = blood metabolite concentrations at week *j*th (j= 4, 8, 12, and 16)

When:

1. $D_0<D_1$, and $D_0<D_2$, then litter size=0 (nonpregnant)
2. $D_1>D_0$, and $D_1<D_2$, then litter size=1 (single)
3. $D_2>D_0$, and $D_2>D_1$, then litter size=2-3 (multiple)

RESULTS

Serum concentrations of progesterone, estradiol, triiodothyronine (T_3), cortisol, and blood metabolites BHBA, and BUN during pregnancy in the nonpregnant ewes, ewes carrying a single and multiple fetuses are presented in table 2. Progesterone, estradiol, T3, cortisol, BHBA, and BUN concentrations during pregnancy increased with the increased litter size and the advance of pregnancy (figure 1). Serum progesterone, estradiol, T_3, cortisol, BHBA, and BUN concentrations during pregnancy in the ewes carrying

Table 2. Mean (SEM) maternal serum progesterone, estradiol, triiodothyronine, cortisol, beta-hydroxy butyric acid and blood urea nitrogen in nonpregnant ewes, ewes carrying a single fetus and multiple fetuses[1]

Hormones and metabolites	Litter size					
	Nonpregnant (n = 6)		Single (n = 20)		Multiple (n = 19)	
Progesterone (ng/ml)	2.82	0.46[a]	13.45	0.70[b]	19.79	0.95[c]
Estradiol (pg/ml)	2.61	0.12[a]	13.53	0.95[b]	16.63	1.05[c]
T_3 (ng/dl)	90.21	6.94[a]	94.36	3.08[a]	96.76	3.67[a]
Cortisol (ng/ml)	8.70	0.67[a]	10.81	0.59[b]	12.66	0.73[b]
BHBA (mg/dl)	9.56	0.56[a]	12.49	0.41[b]	14.66	0.64[c]
BUN (mg/dl)	23.38	1.13[a]	24.51	0.95[a]	25.84	0.76[a]

[1] Means of monthly measurements (0, 1, 2, 3, 4, and 5 months) during gestation period.

[a,b,c] Different superscripts in the same row refer to difference between litter size ($p<0.05$).

multiple fetuses increased by 47.1, 22.9, 2.5, 17.1, 17.4, and 5.4%, respectively, as compared to those in ewes carrying a single fetus.

Discriminant analysis (table 3) indicated that using serum progesterone and estradiol concentrations from weeks 8 to 16 of pregnancy or in combination with serum concentrations of triiodothyronine, cortisol, BUN and BHBA could exactly discriminate nonpregnant from pregnant ewes by 100% precision. However, concentrations of triiodothyronine, cortisol, BUN and BHBA without information on progesterone and estradiol concentrations were not good predictors of pregnancy status. Concentrations of progesterone and estradiol at week 8 of pregnancy were enough for determination of pregnancy status.

Progesterone and estradiol concentrations at week 4 of pregnancy could not discriminate ($p>0.05$) ewes with respective litter size, and therefore could not be used to predict litter size. Serum progesterone and estradiol concentrations at week 8 of pregnancy or greater were good predictors of litter size. Concentrations of progesterone and estradiol at week 8 of pregnancy, could predict whether an ewe carrying a single fetus or multiple fetuses with precisions of 85.0, and 84.2%, respectively. Of 20 ewes actually carrying a single fetus, 3 were predicted as carrying multiple

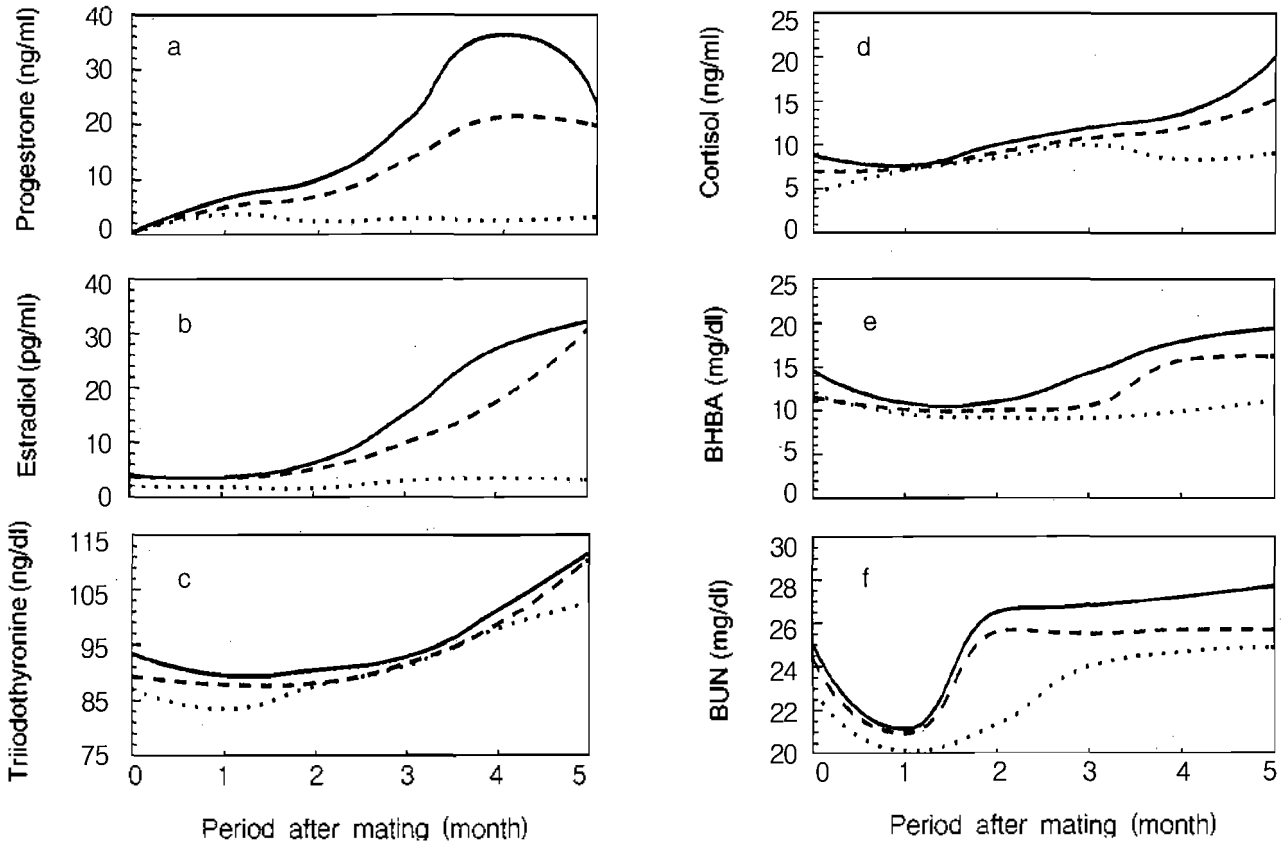

Figure 1. Profiles of progesterone (a), estradiol (b), triiodothyronine (c), cortisol (d), beta-hydroxy butyric acid (BHBA) (e), and blood urea nitrogen (BUN) (f) from 0 to 5 months after mating in nonpregnant ewes (•••), ewes carrying a single (----) and multiple fetuses (——) in Javanese thin-tail ewes. SEM of progesterone at week 0 (the lowest concentration) are 0.034 and 0.047 ng/ml and at week 17 (the highest concentration) are 2.59 and 2.66 ng/ml for ewes carrying a single and multiple fetuses, respectively. SEM of estradiol at week 0 (the lowest concentration) are 0.29 and 0.34 pg/ml and at week 20 (the highest concentration) are 5.14 and 5.75 pg/ml for ewes carrying a single and multiple fetuses, respectively. SEM of triiodothyronine at week 0 (the lowest concentration) are 4.43 and 12.85 ng/dl and at week 20 (the highest concentration) are 5.24 and 18.60 ng/dl for ewes carrying a single and multiple fetuses, respectively. SEM of cortisol at week 0 (the lowest concentration) are 0.93 and 3. 90 ng/ml and at week 20 (the highest concentration) are 1.72 and 6.12 ng/ml for ewes carrying a single and multiple fetuses, respectively. SEM of BHBA at week 0 (the lowest concentration) are 1.24 and 1.45 mg/dl and at week 20 (the highest concentration) are 1.23 and 2.97 mg/dl for ewes carrying a single and multiple fetuses, respectively. SEM of BUN at week 1 (the lowest concentration) are 1.27 and 1. 64 mg/dl and at week 20 (the highest concentration) are 1.01 and 2.33 mg/dl for ewes carrying a single and multiple fetuses, respectively.

fetuses. Of 19 ewes actually carrying multiple fetuses, 3 were predicted as carrying a single fetus (figure 2). Using serum progesterone and estradiol concentrations at week 12 of pregnancy did not increase precision of prediction of ewes carrying multiple fetuses as compared to that at week 8 of pregnancy (84.2%). Of 20 ewes actually carrying a single fetus, 1 ewe was predicted as carrying multiple fetuses. Of 19 ewes actually carrying multiple fetuses, 3 were predicted as carrying a single fetus. Addition of information on progesterone and estradiol concentrations at week 8 to that at week 12 of pregnancy did not increase precision of prediction of litter size. Using information on serum progesterone and estradiol concentrations at weeks 12 and 16 increased precision of prediction of ewes carrying multiple fetuses (94.7%).

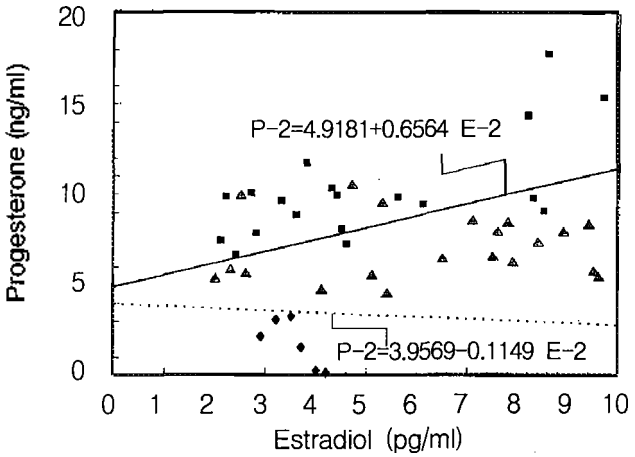

Figure 2. Discriminant lines separating nonpregnant ewes from ewes carrying a single fetus (•••), and ewes carrying a single fetus from those carrying multiple fetuses (——) based on progesterone and estradiol concentrations at week 8 of pregnancy, and distribution of litter sizes [(◆) nonpregnant ewes, (▲) ewes carrying a single fetus, and (■) ewes carrying multiple fetuses] around the discriminant lines.

Triiodothyronine, cortisol, BHBA, and BUN concentrations at any age of pregnancy were not good predictors of litter size. However, addition of serum T_3 and cortisol or BHBA and BUN concentrations to progesterone and estradiol concentrations at weeks 8, 12, and 16 of pregnancy could predict ($p<0.05$) litter size with precisions ranging from 80 to 100% (table 3). Using combination of T_3 and cortisol with BHBA and BUN at weeks 8, 12, and 16 of pregnancy, without progesterone and estradiol concentrations, still could predict litter size ($p<0.05$); however with a substantially lower precision of prediction (37.8 to 66.7%).

Based on the least number of hormones measured and sample required with high predictive precision, concentrations of progesterone and estradiol at week 8 of pregnancy were chosen as the best variables for prediction of litter size with the following equation:

$$D0 = -1.1962 + 0.1013\ PRO8 + 0.6056\ EST8$$
$$D1 = -5.7844 + 1.0081\ PRO8 + 0.7098\ EST8$$
$$D2 = -9.5020 + 1.7640\ PRO8 + 0.2136\ EST8$$

When $D_0<D_1$, and $D_0<D_2$, then litter size=0 (nonpregnant), $D_1>D_0$, and $D_1<D_2$, then litter size=1 (single), and $D_2>D_0$, and $D_2>D_1$, then litter size=2-3 (multiple).

DISCUSSION

The data of this study indicate that serum progesterone and estradiol concentrations at week 8 of pregnancy or greater are good predictors of litter size as compared to T_3, cortisol, BHBA and BUN. Concentrations of serum progesterone and estradiol at week 4 of pregnancy could not be used as a predictor of litter size. This is probably due to a nonsignificant difference in serum progesterone and estradiol concentrations among ewes carrying 1, 2, and 3 fetuses during the first 7 weeks of pregnancy in sheep (Manalu and Sumaryadi, 1998a,b). During this period of pregnancy, while concentrations of progesterone and estradiol of pregnant are distinct from nonpregnant ewes, pregnant ewes with different litter sizes have similar concentrations of these hormones (Manalu and Sumaryadi, 1998b).

The placenta of sheep and goats starts secreting progesterone around week 7 of pregnancy (Ricketts and Flint, 1980; Sheldrick et al., 1981), and at this phase of pregnancy, concentrations of this hormone became distinct between ewes and does carrying single and multiple fetuses (Manalu et al., 1996; Manalu and Sumaryadi, 1998a,b,c). Serum estradiol in the present experiment also increased after week 8 of pregnancy, and the effect of litter size was significant, as was also observed in the goat (Manalu et al., 1996).

Metabolic hormones such as triiodothyronine and cortisol have a closer association with the metabolic and nutritional status of the ewes than the number of fetuses carried (Manalu et al., 1997). Beta-hydroxy butyric acid and BUN concentrations are closely associated with the nutritional status of the ewes. The number of fetuses carried was assumed to influence the concentrations of these metabolites in the maternal circulation. However, profiles of these metabolites indicated that the effect of fetal number was evident during late pregnancy (figure 1). Therefore, these hormones and metabolites could not be used as strong predictor of litter size.

Since prediction of litter size is aimed to improve

Table 3. Precision of prediction of litter size (%) based on concentrations of hormones and metabolites in week 8, 12, and 16 of pregnancy

Variables used as predictors[1]	Precision of prediction of litter size (%)[2]			
	Nonpregnant (n = 6)	Single (n = 20)	Multiple (n = 19)	Total (n = 45)
1. a. PRO8, EST8	100.0 (6, 0)	85.0 (17, 3)	84.2 (16, 3)	86.7
b. PRO8, EST8, PRO12, EST12	100.0 (6, 0)	95.0 (19, 1)	84.2 (16,3)	91.1
c. PRO8, EST8, PRO12, EST12, PRO16, EST16	100.0 (6, 0)	95.0 (19, 1)	89.5 (17, 2)	93.3
d. PRO12, EST12	100.0 (6, 0)	95.0 (19, 1)	84.2 (16, 3)	91.1
e. PRO12, EST12, PRO16, EST16	100.0 (6, 0)	95.0 (19, 1)	94.7 (18, 1)	95.6
2. a. PRO8, EST8, TRI8, COR8	100.0 (6, 0)	80.0 (16, 4)	78.9 (15, 4)	82.2
b. PRO8, EST8, TRI8, COR8, PRO12, EST12, TRI12, COR12	100.0 (6, 0)	100.0 (20, 0)	84.2 (16, 3)	93.3
c. PRO8, EST8, TRI8, COR8, PRO12, EST12, TRI12, COR12, PRO16, EST16, TRI16, COR16	100.0 (6, 0)	100.0 (20, 0)	89.5 (17, 2)	95.6
3. a. PRO8, EST8, BHBA8, BUN8	100.0 (6, 0)	80.0 (16, 4)	73.7 (14, 5)	80.0
b. PRO8, EST8, BHBA8, BUN8, PRO12, EST12, BHBA12, BUN12	100.0 (6, 0)	100.0 (20, 0)	89.5 (17, 2)	95.6
c. PRO8, EST8, BHBA8, BUN8, PRO12, EST12, BHBA12, BUN12, PRO16, EST16, BHBA16, BUN16	100.0 (6, 0)	100.0 (20, 0)	100.0 (19, 0)	100.0
4. a. TRI8, COR8, BHBA8, BUN8	33.3 (2, 4)	25.0 (5, 15)	52.6 (10, 9)	37.8
b. TRI8, COR8, BHBA8, BUN8, TRI12, COR12, BHBA12, BUN12	66.7 (4, 2)	55.0 (11, 9)	57.9 (11, 8)	57.8
c. TRI8, COR8, BHBA8, BUN8, TRI12, COR12, BHBA12, BUN12, TRI16, COR16, BHBA16, BUN16	83.3 (5, 1)	55.0 (11, 9)	73.7 (14, 5)	66.7

[1] Concentrations of PRO=Progesterone; EST=Estradiol; TRI=Triiodothyronine, COR=Cortisol; BHBA=Beta-hydroxybutyric acid; and BUN=Blood urea nitrogen. Numbers after the abbreviation are weeks of pregnancy.

[2] The first number in the parenthesis refers to the number of ewes correctly predicted, and the second number refers to the number of ewes incorrectly predicted of the total number of ewes in the respective litter size.

the feeding regimen in the pregnant ewes, the benefit of knowing litter size is more meaningful prior to the period of fastest growth of the fetus when feeding improvement is generally practiced. Using maternal serum progesterone and estradiol concentrations as predictors of litter size as early as week 8 of pregnancy is superior to the other variables measured. The advantage of prediction of litter size at this age of pregnancy is the lowest number of blood samples and hormones measured. Concentration of progesterone alone gives a lower predictive power without information on estradiol concentration. Single sample determination of progesterone and estradiol at this age of pregnancy have a good predictive power of the test. In addition, prediction of litter size at this age of pregnancy is more useful in differentiating feeding strategy for respective litter size. Using maternal serum progesterone and estradiol concentrations at later age of pregnancy did not give a significant gain in the precision of prediction. Comparison of this method to ultrasonography, the standard method for the determination of fetal number in sheep and goats merits further study.

In conclusion, maternal serum progesterone and estradiol as early as week 8 of pregnancy (early placentation period) in sheep could be used to predict litter size (to differentiate ewes carrying a single and multiple fetuses) with a high precision. With this prior knowledge, feeding regimen for ewes carrying a single and multiple fetuses could be separated to obtain an optimal prenatal growth especially in the higher litter size.

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