Changes in plasma concentrations of leptin in ewes during pregnancy

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Abstract

Previous research has demonstrated circulating concentrations of leptin increase in ewes during mid pregnancy then decline in late pregnancy and early lactation. This study was designed to more narrowly define the timing of changes in circulating concentrations of leptin with pregnancy in ewes. Katahdin ewes (n = 19) located at latitude 34.275 and longitude -85.183 (Mount Berry, GA) were utilized. Blood samples were collected weekly via jugular veinpuncture beginning immediately before ram exposure on September 23 and continuing until 4 weeks post-lambing. Ewes were exposed to a ram fitted with a marking harness for a 63 day breeding season. Breeding marks were recorded daily. Lambing date and number of lambs born was recorded. Week of gestation was calculated by breeding mark. The blood sample collected prior to breeding was considered week 0. Plasma concentration of leptin was determined by radioimmunoassay. Data were tested for effects of date of sample, pregnancy status, and date of sample by pregnancy status interaction using procedures for repeated measures *(JMP ver. 7 ~ SAS Institute Inc., Cary, NC)*. Data were also tested for effects of week of gestation and number of lambs. Pregnancy had an effect on plasma concentrations of leptin (P = 0.0407; 6.06 ± 0.19 vs 4.67 ± 0.64 ng/ml in pregnant vs non-pregnant ewes, respectively). There was also an effect of date of sample (P < 0.0001) on plasma concentrations of leptin. Week of gestation had an effect of plasma concentrations on leptin (P < 0.0001) with ewes having lower plasma concentrations of leptin during weeks 12, 13, 16, and 18-21 of gestation as well as four weeks after lambing when compared to before breeding. Plasma concentrations of leptin were higher weeks 1 to 11, 14, 15, and 17 of gestation than after lambing, but did not differ from values before lambing. These data confirm a decline in circulating concentrations of leptin in the last third of gestation and continuing into early lactation in ewes.

Keywords: Sheep, Pregnant, Leptin

Introduction

- Ehrhardt, *et al.* demonstrated an increase in maternal circulating concentration of leptin mid-pregnancy in sheep (2001). However, Ehrhardt et al. only reported maternal leptin concentrations at pre-breeding (20-40 days pre-breeding), mid-pregnancy (d 50-60), late pregnancy (d 125-135) and early lactation (d15-22 post partum).
- Thomas, *et al.* measured maternal circulating concentrations of leptin weekly throughout pregnancy and reported higher maternal circulating concentrations of leptin in high intake adolescent ewes than in moderate intake ewes (2001). Maternal circulating concentrations of leptin did not appear to increase in moderate intake ewes in mid pregnancy.
- Thomas, *et al.* attributed differences in maternal circulating concentrations of leptin to differences in dietary intake and body fatness.
- Determining the cycle of leptin in pregnant ewes will help better understand metabolic changes with pregnancy.

Objective: *To characterize changes in maternal circulating concentrations of leptin in ewes.*

Materials and Methods

In this study, 18 ewes were bled once a week to determine changes in leptin hormone levels during pregnancy. Blood samples were taken from the 18 ewes beginning on September 23, 2008 at 9:30 am. After collection of the initial blood sample, 16 ewes were exposed to a ram fitted with a marking harness, while two ewes were kept for a control group. Breeding marks were recorded daily. The ram remained with the 16 ewes for 60 days, beginning on September 23, 2008. On October 28, one of the control ewes was exposed to the ram and bred. On November 22, 2009, the ram was removed. The following Tuesday (November 25), a second, non-pregnant control ewe was added to the study. Thus, after November 25, there were 17 pregnant ewes and 2 non-pregnant ewes in the study. Beginning four weeks prior to ram exposure and continuing until ram removal, ewes were supplemented with 0.11 kg/day/head of corn gluten feed. Beginning on November 25, ewe body condition scores and weights were recorded every four weeks **(Table 1)**. On January 6, pregnant ewes began supplementation with 0.22 kg/day/head of corn gluten feed until the end of the study. Ewes were supplied with free choice bermudagrass hay at all times.

Blood samples were collected weekly on Tuesdays at approximately 9:30 am until four weeks after parturition. At parturition, number of lambs born per ewe was recorded. The order in which the ewes were bled was random. Blood samples were collected into 6-mL chilled tubes containing EDTA (10.8 mg), aprotinin (150 µl at 1.5 mg/ml) and bacitracin (150 µl of 50 mM solution). The collected samples were stored on ice for transport to the laboratory, and then centrifuged at 1,500 g for 15 minutes at 4ºC to harvest the plasma. Plasma was stored at -20ºC until assayed. Plasma concentrations of leptin were determined in triplicate by radioimmunoassay as described previously (Delavau et al., 2000). The intra-assay CV was 7.06%.

For analysis of data, the Tuesday preceding each breeding date which resulted in conception was considered week zero, each subsequent week was then numbered until lambing, and the first Tuesday following lambing was considered week A1. Plasma concentrations of leptin were then tested for effect of week of gestation using the univariate split-plot method for repeated measures analysis with JMP Software *(JMP, ver. 6 ~ SAS Institute Inc., Cary, NC)*. Data was also analyzed for effect of pregnancy, date, and pregnancy by date interaction on plasma concentration of leptin using the univariate split-plot method for repeated measures analysis with JMP Software. Dates which included only one pregnant or one open ewe were excluded. We also tested for effects on the number of lambs (0-4) on plasma concentrations of leptin throughout gestation using the univariate split-plot method for repeated measures analysis with JMP Software. When appropriate, means separation was performed using student's t test. Body condition score and weight were also tested for correlation with plasma concentration of leptin using the multivariate pairwise correlations option with JMP Software.

Results

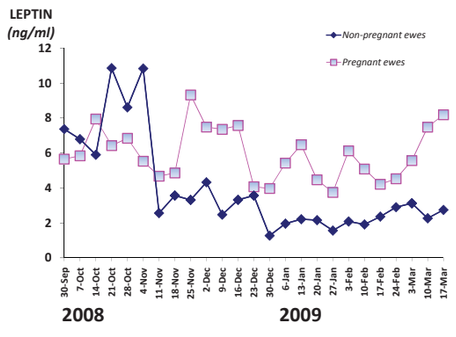
Pregnancy had an effect on plasma concentrations of leptin (P = 0.0407; 6.06 ± 0.19 vs 4.67 ± 0.64 ng/ml in pregnant vs non-pregnant ewes, respectively). There was also an effect of date of sample (**Figure 1**; P < 0.0001) on plasma concentrations of leptin. Week of gestation had an effect of plasma concentrations of leptin (**Figure 2**; P < 0.0001) with ewes having lower plasma concentrations of leptin during weeks 12, 13, 16, and 18-21 of gestation as well as four weeks after lambing than before breeding. Plasma concentrations of leptin were higher weeks 1 to 11, 14, 15, and 17 of gestation than after lambing, but did not differ from values before lambing. There was no effect of the number of lambs on plasma concentrations of leptin throughout pregnancy (P = 0.3080). Body condition and plasma concentrations of leptin were correlated (r = 0.651; P < 0.0001).

Table 1. Mean Ewe Body Condition Score and Weight *(± standard error)*

[a] Body Condition Score on a scale of 1 to 5 with 1 being thin and 5 being obese. Score performed by a single individual.

Date	Condition Score[a]	Weight (kg)
11/25/08	2.74 ± 0.09	52.1 ± 2.2
12/23/09	2.53 ± 0.11	52.6 ± 2.2
1/20/09	2.37 ± 0.08	56.0 ± 2.4
2/17/09	2.58 ± 0.09	62.4 ± 2.8
3/17/09	2.21 ± 0.07	51.6 ± 1.8
4/14/09	2.08 ± 0.08	50.0 ± 1.4

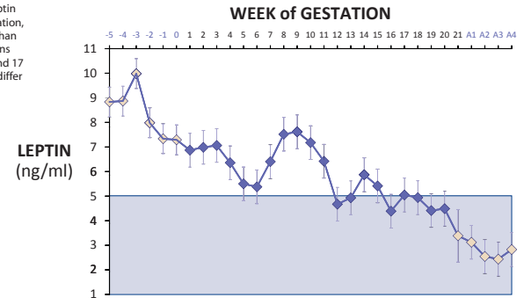
Figure 1. Effect of date of sample and pregnancy on plasma concentrations of leptin.



Numbers of ewes in each group were one pregnant and 17 non-pregnant on 9/30/2008; 10 pregnant and 8 non-pregnant on 10/7/2008; 15 pregnant and 3 non-pregnant on 10/14/2008; 16 pregnant and two non-pregnant on 10/21 and 10/28/2008; 17 pregnant and one non-pregnant 11/4 to 11/18/2008. From 11/25/2008 to 2/10/2009 there were 17 pregnant and two non-pregnant; 16 pregnant and three non-pregnant 2/17/2009; 14 pregnant and five non-pregnant 2/24/2009; four pregnant and 15 non- pregnant 3/3/2009; one pregnant and 18 non-pregnant on both 3/10 and 3/17/2009. Mean standard error = 1.27 ng/ml. Pregnancy had an effect on plasma concentrations of leptin (P = 0.0407). There was also an effect of week on plasma concentrations of leptin (P < 0.0001).

Figure 2. Week of gestation on plasma concentrations of leptin.

Ewes had lower plasma concentrations of leptin during weeks 12, 13, 16, and 18 to 21 of gestation, as well as four weeks after lambing (A1-A4) than before breeding (-5- 0). Plasma concentrations of leptin were higher weeks 1 to 11, 14, 15, and 17 of gestation than after lambing, but did not differ from values before lambing.



Discussion

Leptin was higher in pregnant than non-pregnant ewes as expected.

Although maternal circulating concentrations of leptin appeared to increase in mid-gestation, the dramatic increase in leptin during pregnancy observed by Ehrhardt, *et al.* (2001) was not seen during this trial.

Possible influencing factors include differences in breed, environment, diet, and frequency of samples. Diet and the resulting changes in body condition is the most likely explanation as Thomas, *et al.* (2001) observed differences in dietary intake and level of body fat account for most of the change in circulating concentrations of leptin in adolescent ewes.

In this study we observed circulating concentrations of leptin were correlated with body condition score. Both the pregnant and the control ewes showed a similar decrease in leptin through the winter months when diet quality is likely at its lowest due to decreased availability of fresh forage.

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Megan Gafnea bleeding a ewe.
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