The effects of leptin on plasma concentrations of prolactin, growth hormone, and melatonin vary depending on the stage of pregnancy in sheep¹

Malgorzata Szczesna,^{*,2} Katarzyna Kirsz,^{*} Tomasz Misztal,[†] Edyta Molik,^{*} and Dorota A. Zieba^{*}

^{*}Agricultural University, Faculty of Animal Sciences, Department of Animal Biotechnology, 31–274 Krakow, Poland; and [†]The Kielanowski Institute of Animal Physiology and Nutrition, Department of Animal Physiology, Polish Academy of Sciences, 05-110 Jablonna, Poland

ABSTRACT: The effects of hyperleptinemia and leptin resistance during gestation are unclear. Leptin, an important neuroendocrine regulator, has anorexic effects, but its interactions with other metabolic hormones during pregnancy are unclear. We examined potential roles of leptin in regulating prolactin (PRL), GH, and melatonin plasma concentrations during pregnancy in Polish Longwool ewes. Twelve estrus-synchronized ewes carrying twins after mating were randomly assigned to receive i.v. injections of saline or recombinant ovine leptin (2.5 or 5.0 µg/kg BW). Blood samples were collected (15-min intervals over 4 h) immediately before the first injection at dusk and kept under red light. Treatments were repeated at 2-wk intervals, starting before mating and continuing from days 30 to 135 of gestation. Concentrations of plasma PRL, GH, and melatonin were determined using a validated RIA. The effects of leptin on hormone plasma concentrations varied depending on pregnancy

stage and leptin dose. PRL plasma concentrations were affected at most stages of pregnancy and before gestation. In non-, very early- (day 30), and late- (day 120 and 135) pregnant ewes, exogenous leptin stimulated PRL ($P < 0.001$) plasma concentrations, while during the second month of gestation, it decreased PRL concentrations ($P < 0.01$). Leptin affected GH plasma concentrations ($P < 0.05$) only during the first 2 mo of pregnancy, with no effects during the second part of gestation or before pregnancy. In early-pregnant ewes (day 30 and 45), leptin decreased melatonin plasma concentrations ($P < 0.05$), but at day 60, leptin stimulated melatonin plasma concentrations at low ($P < 0.01$) and high doses ($P < 0.05$), with no effects in ewes after 105 d of gestation. These data indicate specific pregnancy-induced endocrine adaptations to changes in energy homeostasis, supporting the hypothesis that leptin affects PRL, GH, and melatonin release during gestation.

Key words: growth hormone, leptin, leptin resistance, pregnancy, prolactin, sheep

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INTRODUCTION

The maintenance of energy homeostasis is a key element that ensures the proper functioning of an organism. This is especially important with

regard to the processes associated with reproduction. It is generally accepted that a close relationship exists between leptin concentrations in the blood and female reproductive processes. Inappropriate concentrations of this adipokine negatively affect puberty onset (Ahima et al., 1997; Cheung et al., 1997), fertility, and the course of pregnancy. Inversely, reproductive status including the phase of the estrus cycle (Popovic and Casanueva, 2002) and stage of pregnancy (Chien et al., 1997; Hardie et al., 1997; Tomimatsu

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²Corresponding author: m.szczesna@ur.krakow.pl

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et al., 1997) are important factors that determine the amount of leptin in the bloodstream. In sheep, which are seasonally breeding animals, the plasma leptin concentration increases in the first half of pregnancy but decreases during the second half of gestation (Ehrhardt et al., 2001). Pregnancy and lactation are enormous challenges faced by females, and the maintenance of energy homeostasis during these periods requires many adaptations related to the physiological and endocrinological status of the animal. These involve specific adjustments not only in leptin production but also in leptin-mediated actions. In various species, gestation is characterized by physiological hyperphagia despite a coexisting hyperleptinemia, which suggests that a specific state of leptin resistance occurs during this period. The assumption is that pregnancy-induced hyperleptinemia is required for actions other than the hypothalamic regulation of appetite, such as modulation of the endocrine system. The work reported here was designed to determine how leptin modulates prolactin (PRL), GH, and melatonin plasma concentrations in sheep during pregnancy to broaden our knowledge about interactions between these hormones, which we demonstrated in non-pregnant seasonally breeding females in previous studies (Zieba et al., 2008; Szczesna et al., 2011a).

MATERIALS AND METHODS

All of the animal-related procedures used in these studies were approved by the First Local Ethical Committee on Animal Testing in Krakow.

Animals

Studies were performed at the Experimental Station in the Department of Animal Biotechnology, Agricultural University in Krakow (longitude: 19°57'E, latitude: 50°04'N). These experiments were carried out on a total of 12 adult 3- to 4-year-old female Polish Longwool sheep in a condition rated 3 on a five-point scale (Russel et al., 1969) and weighing 55.0 ± 6.0 (SD) kg (before mating) and 67.4 ± 6.1 (SD) kg (before parturition). Ewes were fed twice daily at 0700h and 1600h with a diet formulated to provide 100% of the National Research Institute of Animal Production recommendations for maintenance (Norms, 1997) according to their physiological status. Water was available ad libitum. Ewes were group-housed under natural photoperiodic and thermoperiodic conditions. During sample collection, ewes were placed in individual carts.

Procedures and Treatments

In a reproductive season, the estrus cycles of each ewe were synchronized using a 14-d treatment with intravaginal progestogen-impregnated sponges (40-mg fluorogestone acetate; Chronogest, Intervet International, Boxmeer, The Netherlands). On the day of sponge removal, ewes were injected intramuscularly with a single dose of 500 IU of pregnant mare serum gonadotrophin (PMSG or serogonadotropin, Biowet, Drwalew, Poland). Estrus detection was performed twice daily (at 0800 h and 2000 h) with an adult ram equipped with an apron. Estrus was defined as an acceptance of mounting. After estrus detection, ewes were presented individually to the male and mated naturally. All ewes were mated 36 ± 12 h after PMSG injection in the same term of year (October). Pregnancy was verified 40 d after mating using an ultrasonographic scanner (Aloka SSD 500 Micrus, Equine Therapy System, Inc., Greenwood Village, CO). All ewes used in this experiment had twin pregnancies.

During the experiment, the animals were randomly divided into three experimental groups: 1) control group—animals ($n = 4$) were given i.v. injections of saline; 2) leptin 1 group—animals ($n = 4$) were given i.v. injections of a solution of leptin ($2.5 \mu\text{g/kg BW}$) in saline; 3) leptin 2 group—animals ($n = 4$) were given i.v. injections of a solution of leptin ($5.0 \mu\text{g/kg BW}$) in saline. The dose of leptin for the i.v. study was determined based on our previous experience (Zieba et al., 2003, 2004), theoretical calculations, and a published study of i.v. injections in sheep (Muñoz-Gutiérrez et al., 2004).

The recombinant ovine leptin used in these experiments was purchased from PLR Laboratory (Rehovot, Israel). The injections of saline/leptin were administered on days -15 , 30, 45, 60, 75, 90, 105, 120, and 135 of gestation. The experimental timeline and procedures applied are shown in Fig. 1.

In the morning of the sample collection period, sheep were fitted with jugular catheters for intensive blood sampling. Subsequently, females from each group were restrained into individual sampling carts to familiarize them with the experimental conditions. Carts were constructed of wood with solid floors and allowed the animals to stand or lie down freely during the sampling procedures. All sample collection periods began at sunset, and all procedures were conducted under illumination of a dim red light. This light was insufficiently bright (<3 lux at 20 cm) and of an inappropriate wavelength (622 to 780 nm) to influence melatonin production

Figure 1. Timeline for experimental procedures. Twelve Polish Longwool ewes were assigned randomly to one of three groups ($n = 4/\text{group}$) during pregnancy (sample collection performed at $-15, 30, 45, 60, 75, 90, 105, 120$ and 135 d of pregnancy). Treatments consisted of single i.v. injections of 1) saline (control); 2) a low dose of leptin ($2.5 \mu\text{g/kg BW}$; leptin 1 group), and 3) a high dose of leptin ($5.0 \mu\text{g/kg BW}$; leptin 2 group) at the beginning (time-zero) of the 4-hour experiment. Arrows indicate the 17 time points for blood sampling at 15-min intervals. No sample collection was performed at 0 or 15 d of pregnancy (these time points are indicated in gray font).

(Reiter, 1985). Exogenous leptin/saline was administered in single i.v. injections at the beginning of the sample collection period. Blood samples (5 mL) were collected at 15-min intervals for 4 h, beginning immediately before the leptin/saline injection at sunset (time-zero). The blood samples were dispensed into tubes containing $150 \mu\text{L}$ of a heparin solution ($10,000 \text{ IU/mL}$). The plasma was separated by centrifugation at $3,000 \times g$ at 4°C for 10 min and stored at -20°C for later measurement of leptin, PRL, GH, and melatonin concentrations.

Hormone Assays

RIA for leptin. Circulating leptin concentrations were determined using a highly specific ovine leptin RIA with the double-antibody method, using a specific, high-affinity rabbit antibody generated against recombinant ovine leptin and anti-rabbit- γ -globulin antisera and a recombinant ovine leptin standard, as described by Delavaud et al. (2000). The intra-assay and interassay coefficients of variation of the leptin assay were 3.2% and 11.0%, respectively, and the assay sensitivity was 0.3 ng/mL .

RIA for PRL. Prolactin plasma concentrations were assayed by the RIA using double-antibody method using anti-ovine-PRL and anti-rabbit- γ -globulin antisera, as described by Wolińska et al. (1977). The PRL standard was synthesized and kindly provided by Professor Kazimierz Kochman from The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences (Kochman and Kochman, 1977). The assay sensitivity was 2.0 ng/mL , and the intra-assay and interassay

coefficients of variation were 9.1% and 12.0%, respectively.

RIA for GH. Growth hormone plasma concentrations were determined by a validated RIA using anti-bovine GH and anti-rabbit γ -globulin antisera and a bovine GH standard (NIDDK-GH-B-1003A), as previously described by Dvorak et al. (1978). The assay sensitivity was 0.4 ng/mL , and the intra-assay and interassay coefficients of variation were 6.1% and 11.4%, respectively.

RIA for melatonin. Melatonin concentrations in unextracted plasma were determined according to the method of Fraser et al. (1983) and modified by Misztal et al. (1996). Ovine anti-melatonin serum (AB/S/01, Stockgrand Ltd., UK), synthetic melatonin (Sigma Chemical Co., St. Louis, MO), and [O-methyl- ^3H]-melatonin (Amersham Biosciences, UK) served as the first antibody, reference standard, and tracer, respectively. Melatonin-free plasma for curve calibration and blanks was obtained from blood samples collected from sheep and stripped of endogenous melatonin using activated charcoal (Norit-A; Sigma Chemical Co.). The sensitivity of the assay was 3.9 pg/mL , and the intra-assay and interassay coefficients of variation were 10.5% and 13.2%, respectively.

Statistical Analysis

All data are expressed as the mean $\pm$ SEM. Values determined for all collection points at indicated stages of gestation were averaged to calculate the means used for comparisons among treatment

groups. Hormone data were analyzed by a series of two-way ANOVA using SigmaPlot statistical software (version 11.0; Systat Software Inc., Richmond, CA) for repeated measures. The statistical models included the main effects of leptin treatment and the stage of gestation. After determining a significant F -value, the means were contrasted using the Holm-Sidak method. Statistical significance was set at $P < 0.05$.

RESULTS

Gestational Changes in Concentrations of Leptin, PRL, GH, and Melatonin

Intravenous treatment with leptin increased ($P < 0.05$) the mean concentrations of circulating leptin compared to controls at all stages of pregnancy in a dose-dependent manner (Fig. 2).

Endogenous leptin concentrations increased during midpregnancy in the control group. Plasma leptin concentrations determined between 45 and 90 d of pregnancy were greater ($P < 0.01$) than in pregravid or periparturient ewes at 135 d of gestation (d.g.). Leptin concentrations during the course of the first half of pregnancy were increased and then, after peaking at day 60, began to decline. The concentration of leptin observed in ewes before delivery was comparable ($P \geq 0.05$) to the concentration before mating and during the first month of pregnancy. There were no differences ($P \geq 0.05$) in PRL concentrations between periparturient and nonpregnant ewes; however, we observed that PRL concentrations changed ($P < 0.05$) during pregnancy, reaching a minimum in the middle of

gestation (at day 75). Growth hormone concentrations were increased ($P < 0.05$) during early gestation (on days 30 and 45) in relation to concentrations from days 60 to 120 of gestation. In addition, we observed greater ($P < 0.05$) concentrations of melatonin during midpregnancy than during early- and late-pregnancy.

Effects of Leptin on Plasma PRL Concentrations

The effects of leptin on PRL plasma concentrations varied depending on the day of pregnancy and dose of leptin (Fig. 3). Before (day 15 of gestation) and at the beginning of pregnancy (30 d.g.), leptin 1 treatment increased ($P < 0.001$) plasma concentrations of PRL, but leptin 2 treatment had no effect. At day 45 of gestation, leptin 2 treatment reduced ($P < 0.001$) plasma concentrations of PRL, and at day 60 of gestation, this effect was observed for both doses of leptin. During late-pregnancy (120 d), leptin 2 treatment stimulated an increase ($P < 0.001$) in PRL plasma concentrations, whereas at day 135 of gestation, plasma concentrations of PRL were elevated in response to both leptin 1 ($P < 0.01$) and leptin 2 ($P < 0.001$) treatments.

Effects of Leptin on Plasma GH Concentrations

In the current study, the effect of leptin on GH plasma concentrations was observed only during the first half of pregnancy (Fig. 4). Leptin 1 increased ($P < 0.001$) GH plasma concentrations at 30 d.g., leptin 2 decreased ($P < 0.05$) GH concentrations at 45 d.g., and both leptin 1 and leptin 2 decreased GH concentrations ($P < 0.05$) at 60 d. Leptin had

Figure 2. Mean ($\pm$ SEM) plasma concentrations of leptin in pregnant sheep treated with single i.v. injections of saline (control) or leptin at a dose of 2.5 μ g/kg BW (leptin 1) or 5.0 μ g/kg BW (leptin 2) at the indicated stages of gestation. Differences between experimental groups are depicted with *** $P < 0.001$, ** $P < 0.01$, or * $P < 0.05$.

Figure 3. Mean ($\pm$ SEM) plasma concentrations of prolactin (PRL) in pregnant sheep treated with single i.v. injections of saline (control) or leptin at a dose of 2.5 μ g/kg BW (leptin 1) or 5.0 μ g/kg BW (leptin 2) at the indicated stages of gestation. Differences between experimental groups are depicted with *** P < 0.001, ** P < 0.01, or * P < 0.05.

Figure 4. Mean ($\pm$ SEM) plasma concentrations of GH in pregnant sheep treated with single i.v. injections of saline (control) or leptin at a dose of 2.5 μ g/kg BW (leptin 1) or 5.0 μ g/kg BW (leptin 2) at the indicated stages of gestation. Differences between experimental groups are depicted with *** P < 0.001, ** P < 0.01, or * P < 0.05.

no effects on GH plasma concentrations during the second half of gestation or in the perigravid state.

Effects of Leptin on Plasma Melatonin Concentrations

The results showed that during pregnancy, leptin influenced melatonin plasma concentrations, and its effects changed depending on the day of gestation (Fig. 5). In early-pregnant ewes, leptin 1 decreased (P < 0.05) melatonin plasma concentrations at 30 d.g., and both doses of leptin decreased melatonin plasma concentrations at 45 d.g. At 60 d.g., both the leptin 1 (P < 0.01) and leptin 2 (P < 0.05) stimulated melatonin plasma concentrations in comparison with controls. During midpregnancy, leptin 1 decreased (P < 0.05) melatonin concentrations at

75 d.g. and had no effect at 90 d.g., while leptin 2 increased (P < 0.05) and decreased (P < 0.05) melatonin concentrations at 75 and 90 d.g., respectively. We did not observe any effects of leptin on melatonin plasma concentrations in late-pregnant ewes (after 105 d.g.). Thus, the gestation effects of leptin were dose-dependent: leptin 1 increased (P < 0.05) melatonin plasma concentrations, while leptin 2 (P < 0.05) decreased melatonin plasma concentrations in relation to control animals.

DISCUSSION

The effects of leptin on plasma hormone concentrations depend on the stage of pregnancy and the leptin dose. The assumption was that hyperleptinemia accompanying gestation may be an

Figure 5. Mean ($\pm$ SEM) plasma concentrations of melatonin in pregnant sheep treated with single i.v. injections of saline (control) or leptin at a dose of 2.5 μ g/kg BW (leptin 1) or 5.0 μ g/kg BW (leptin 2) at the indicated stages of gestation. Differences between experimental groups are depicted with *** $P < 0.001$, ** $P < 0.01$, or * $P < 0.05$.

important factor in the mechanism that prepares an organism for the new challenges of pregnancy and subsequent lactation.

The source of elevated leptin concentrations during pregnancy seems to be species-specific. In humans (Henson and Castracane, 2006) and mice (Gavrilova et al., 1997), the concentration depends on placenta-derived factors, while in sheep (Thomas et al., 2001), placental leptin production occurs on a very small scale. In Karakul ewes carrying a single fetus, maternal plasma leptin concentrations rose approximately 180% from prebreeding to midpregnancy (60 d.g.) and then declined progressively over the course of late pregnancy (Ehrhardt et al., 2001). The present experiments show that those changes may be even greater. As reported here, leptin concentrations gradually increased during the first half of the pregnancy, reaching a 2.8-fold increase on day 60, and then gradually decreased, reaching concentrations comparable to the pregravid state. The results obtained in the current study are consistent with the finding that in midpregnancy ewes, a 2.3-fold increase in leptin mRNA levels in maternal white adipose tissue was noticed in comparison with the nongravid or postgravid state (Ehrhardt et al., 2001). Additionally, in cattle, plasma leptin concentrations were elevated during late pregnancy, began to decrease at least 30 d before parturition and declined to a nadir at delivery, indicating that high leptin concentrations during gestation are associated with an increase in adiposity rather than the secretory activity of the placenta in ruminant species (Liefers et al., 2005). Although pregnancy-induced hyperleptinemia occurs in most species, the exact factors responsible for increases in leptin

concentrations in pregnant females remain unclear. It is most likely driven by hormonal changes associated with gestation, including mating-induced physiological alternations such as surges in PRL, corpus luteum graviditatis formation and maintenance through enhanced progesterone release and the loss of the cyclical elevations in serum estradiol or the creation of the placenta, which can secrete numerous hormones. Season- and/or dose-dependent effects on leptin synthesis/secretion have been demonstrated with reference to PRL, GH, insulin and melatonin (Szczesna et al., 2011b), sex steroid hormones (Machinal et al., 1999), progesterone (Stelmanska et al., 2012), and placenta-derived factors (Sivan et al., 1998).

Regardless of the source of pregnancy-induced hyperleptinemia and taking into account the lack of its typical influence on the reduction of food intake during pregnancy, questions arise regarding the functions this phenomenon fulfils in pregnant females. Although the hypothalamic response to leptin during pregnancy has been investigated repeatedly in various species, the effects of leptin on the plasma concentrations of PRL, GH, or melatonin (examined in the present experiment) have not been studied often. In addition to well-known central actions on the regulation of energy homeostasis in the hypothalamus, leptin is a pleiotropic hormone that also acts on many other targets. The multitude of organs in which the presence of leptin receptors has been identified confirms the diversity of its effects. Iqbal et al. (2000a) showed that in the ovine adenohypophysis, 34% of cells in the pars distalis and 94% of cells in the pars tuberalis are immunoreactive to leptin receptor antiserum.

Taking into account the importance of pituitary hormones in pregnancy- and lactation-related processes, two of them, GH and PRL, are of special interest, especially from point of view of ruminant physiology.

In ruminants, increased release of GH and decreased plasma leptin concentrations seem to be key adaptations of early lactation (Block et al., 2001). These endocrine changes may help the female adapt to the switch between pregnancy and lactation states and to adjust nutrient supplies and energy storage to levels adequate to the current requirements. Husted et al. (2008) reported that ewes had consistently low concentrations of GH during pregnancy, but GH concentrations peaked at lambing and then decreased gradually during lactation, while Wallace et al. (2006) did not find any significant difference in GH concentrations during pregnancy. Interestingly, in the current experiment, we noticed that during midpregnancy, GH concentrations decreased in comparison with earlier stages of gestation and the peripartum period.

Because some studies indicate that leptin may be involved in the regulation of GH release, in the current experiment, we focused on determining whether leptin might influence GH plasma concentrations during pregnancy. Within the pars distalis, immunoreactivity to leptin receptor antiserum was confirmed for 29% of gonadotropes and 69% of somatotropes (Iqbal et al., 2000a), suggesting that leptin may play an important role not only in the regulation of gonadotropin release, which has been repeatedly reported, but also may play a role in GH secretion to a greater extent. Involvement of leptin in the hypothalamic-pituitary-somatotropic axis has also been confirmed by the fact that in ovine dorsomedial and ventromedial hypothalamic nuclei, as well as in arcuate nuclei, 100% of somatostatin-immunoreactive neurons simultaneously express the long form of the leptin receptor (Iqbal et al., 2000b). Direct actions of leptin on the regulation of basal and GH-releasing hormone-stimulated secretion of GH from the bovine pituitary were proven by Zieba et al. (2003). A stimulatory role of leptin in GH release was also demonstrated in swine (Nonaka et al., 2006) and sheep (Roh et al., 2001). It was proven in sheep (Henry et al., 2001; Morrison et al., 2001) and cattle (Zieba et al., 2003) that the effect of leptin on GH secretion depends on nutritional status. Amstalden et al. (2002) confirmed that dietary restrictions or a negative energy balance cause increased sensitivity of tissues to the effects of leptin. Similarly, it may be expected that pregnant animals, which are particularly vulnerable

to a negative energy balance, adjust the leptin responsiveness of their tissues; however, information about the effects of leptin on GH release in pregnant animal is very limited. In the experiments reported here, exogenous leptin changed GH plasma concentrations only in ewes at the early stages of pregnancy, without any effects during other periods. This suggests that the ability of leptin to affect GH release varies during pregnancy and is dependent on additional factors. Interesting possibilities have been provided by research in which the treatment of pregnant ewes with exogenous GH during late, but not early, pregnancy not only influenced leptin concentrations but also modestly stimulated fetal growth and had a major effect on fetal body composition (Wallace et al., 2006). The authors suggest the existence of specific windows during the course of pregnancy when the sensitivity of the maternal endocrine system to humoral factors is more flexible (Wallace et al., 2006).

Only limited and divergent information is available on the influence of leptin on the secretion of PRL, another seasonal hormone particularly involved in reproduction. The link between these two factors is indicated by the fact that functional leptin receptors are present on lactotropic cells of the ovine pituitary (Iqbal et al., 2000a). A direct effect of leptin on PRL release was confirmed in vitro in rat (Yu et al., 1997) and bovine (Nonaka et al., 2005) pituitary cells. Nutritional restrictions influenced the effects of leptin on PRL secretion in rams (Celi et al., 2006). In relation to normally fed animals, our previous results in ewes demonstrated a dose-related, leptin-mediated stimulation of PRL release in a seasonally dependent manner (Zieba et al., 2008). These observations suggest that leptin responsiveness in the pituitary might be modulated in specific circumstances. Taking into consideration the changes that occur in the endocrine system and in metabolism in pregnant females, it can be expected that pregnancy would also induce specific changes in interactions between PRL and leptin. The current experiments in pregnant ewes proved that leptin is able to affect PRL plasma concentrations in almost all stages of pregnancy and even before gestation. In very early- and late-pregnant females, as well as in nonpregnant females, exogenous leptin stimulated PRL plasma concentrations, while during the second month of gestation, PRL concentrations decreased in leptin-treated groups. Experiments conducted on pregnant sows have shown a positive correlation between leptin and PRL during mid- and late-gestation (Salari et al., 2015). These observations confirm gestational

fluctuations in the effects of leptin action within the endocrine system, as discussed above.

Specific fluctuations in the biological effectiveness of leptin were especially noticeable in relation to the photoperiod. In our previous experiments, we showed that seasonally breeding sheep are a good model in which to study the phenomenon of leptin resistance, which we demonstrated during the long-day photoperiod together with season- and dose-dependent leptin actions on the secretion of PRL, GH, and melatonin (Zieba et al., 2008; Szczesna et al., 2011a). In this species, the concentration of leptin in the blood plasma during the long-day season is elevated by approximately 180% in comparison to concentrations in the short-day season, but this increase was not found to be related to the anorexic effect of this hormone (Marie et al., 2001). Interesting links also have been found in our previous studies, which indicate that a significant relationship exists between leptin and melatonin (Zieba et al., 2007). We previously demonstrated not only that exogenous leptin is able to modulate melatonin secretion from the ovine pineal gland in vitro (Zieba et al., 2007) and in vivo (Zieba et al., 2008), both in a seasonally dependent manner, but also that melatonin may participate in the regulation of leptin secretion from ovine white adipose tissue explants (Szczesna et al., 2011b). In seasonal mammals, melatonin also regulates photoperiodic changes in plasma concentrations of PRL through a melatonin-dependent oscillator located in the pars tuberalis, which is, as mentioned above, particularly rich in leptin receptors. This indicates the existence of a network of dependencies between these hormones. These interactions become more important during pregnancy, when the ability to integrate signals related to energy resources, food intake, and seasonal changes in the environment is crucial for achieving reproductive success. This is confirmed by the results of the current study, in which leptin affected the plasma concentrations of melatonin in pregnant ewes and the direction of this action was depended not only on the stage of pregnancy but also on the day length. High melatonin concentration was observed during pregravid stage during the short days period and low concentrations of that indolamine were noticed at the end of pregnancy when days became longer.

In the current experiment, we also noticed that there was a dose-dependent effect of leptin on some hormones. In nonpregnant and early-pregnant ewes (at 30 d.g.), the lower dose of leptin had a stimulatory effect on PRL plasma concentration, while at the same stage, the higher dose of leptin

had no effect. Various effects were also observed for different doses of leptin with respect to melatonin concentration. In ewes before pregnancy, leptin 1 increased melatonin concentrations, while leptin 2 caused an inhibitory effect on melatonin. In females during midpregnancy (at 75 d.g.), we also observed the dose-dependent action of leptin. In our previous experiment (Zieba et al., 2003), we described the leptin dose-dependent effects on the reproductive axis in ruminant species. Ovine recombinant leptin demonstrated an inverse, dose-dependent influence on the basal secretion of luteinizing hormone in fasted cows. We demonstrated the same inverse, dose-dependent effect of leptin on melatonin concentration in sheep after intracerebroventricular infusion of two doses of leptin: a low dose of exogenous leptin (0.5 µg/kg BW) and a high dose of leptin (1.0 µg/kg BW) (Zieba et al., 2008). Most likely, the inverse, dose-dependent effect may appear due to leptin receptor desensitization or inhibition of intracellular leptin signaling by suppressors of cytokine signaling-3 due to an auto-suppression mechanism (Bjorbak et al., 2000).

During gestation, both hyperleptinemia and a leptin-resistant state are present, but the exact physiological consequences of this phenomenon remain unclear. Certainly, it is an important adaptive mechanism to prevent a decrease in food intake and may prevent ovulation and adjust the female endocrine system to meet the requirements of pregnancy and lactation. In conclusion, the experiments reported here indicated that leptin is able to modulate PRL, GH, and melatonin plasma concentrations in pregnant sheep and that the ability of leptin to create these effects depends on the stage of pregnancy. These changes may be associated with leptin resistance induced by pregnancy, resulting in differential responsiveness of target tissues to leptin. A close association between leptin, PRL, GH, and melatonin may represent important mechanisms that form a regulatory node in a complicated dependence network linking nutritional status and neuroendocrine function. This is especially noticeable during pregnancy, when the biological complexity of the interplay between metabolism and the endocrine system is so significant.

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