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Profile of Gonadotropic Hormone Secretion in Sheep with Disturbed Rhythm of Seasonality

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ABSTRACT

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The effect of artificial conditions of a short daylight period (16 h darkness (D): 8 h light (L)) and exogenous melatonin on milk yield parameters of sheep during spring and summer was examined to determine the impact of using sheep for milk on the secretion level of gonadotrophic hormones. The research was conducted on 60 sheep lambed in February. After raising the lambs, the sheep were divided into 3 groups and assigned for dairy use (May–September). The mothers in the control Group 1 (G1) were maintained under natural daylight conditions. The sheep in Group 2 (G2) were maintained under conditions of an artificial photoperiod (16 h D : 8 h L). Meanwhile, the mothers in Group 3 (G3) were given melatonin implants. A 6-hour collection of blood from 6 sheep of each group was performed every 4 weeks. The concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in plasma were measured using radioimmunoassay. The average LH concentration in G1 gradually increased since May (5.32 ± 0.2 ng/ml), reaching the highest value in August (6.70 ± 0.2 ng/ml). In G2, the increase in LH occurred 4 weeks after the introduction of the 16 h D : 8 h L condition (6.26 ± 0.2 ng/ml). The maximum LH concentration in G3 was noted in August (7.31 ± 0.2 ng/ml). The average FSH concentration in G1 gradually increased since May (6.59 ± 0.2 ng/ml), reaching the highest value in August (10.50 ± 2.6 ng/ml). In G2, there was a significant increase in the FSH concentration in June (9.00 ± 0.3 ng/ml). In the final period during lactation, the FSH concentrations in G2 (13.51 ± 1.3 ng/ml) and G3 (13.60 ± 1.9 ng/ml) were higher than in G1. The results indicate that using sheep for milk does not inhibit the secretion of gonadotropic hormones induced by the simulation of short daylight conditions and exogenous melatonin.

Keywords: sheep; lactation; FSH; LH

Successive physiological processes in animals are synchronized with the changes in the conditions of the external environment, and they are also subject to the actions of the endogenous biological clock. A biochemical signal that informs the body of the changing lighting conditions is the synthesis of melatonin. The main source of melatonin for the rhythmic nature of its 24-hour fluctuations in blood is the pineal gland (Lincoln et al. 1982; Lincoln 1992). In short-day breeders, the long signal and high concentrations of melatonin during the short daylight conditions in the autumn–winter season (September–January) stimulate the secretion of gonadotropic hormones synthesized by the pituitary gland. The significance of melatonin in the control of the reproductive cycle in sheep was proven by keeping animals in conditions of an artificial photoperiod and by supplying exogenous melatonin (Molik et al. 2007, 2013). The study showed that the introduction of

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short daylight conditions (16 h D : 8 h L) in sheep or the administration of exogenous melatonin initiates the activity of the gonads and the instance of the rutting season, which happened earlier than under natural conditions. The administration of exogenous melatonin increases the activity of the hypothalamic–pituitary axis and the secretion of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) (Goodman et al. 2010). The observed increase in the secretion of LH from the pituitary gonadotropic cell in response to the prolonged administration of melatonin results from the increase in the GnRH pulse frequency released by the hypothalamus (Clarke and Arbabi 2016; Ciechanowska et al. 2017). The effects of melatonin on the hypothalamic–pituitary axis are related to its diffusion into the cerebrospinal fluid (CSF) in the third ventricle of the brain, which enables the direct effect of melatonin on the secretion of hormones from the pituitary gland (Tricoire et al. 2002; Christian et al. 2015).

For complete synchronization of the reproductive cycle, it appears that the melatonin signal is necessary for a long photoperiod. In the experiments, which were performed on sheep with a surgically removed pineal gland (pinealectomized), to which melatonin was supplied intravenously to imitate a signal of varying length, a seasonal increase in sexual activity is most effectively synchronized by the spring and summer melatonin signal (Woodfill et al. 1994). In sheep, the phenomenon of seasonality refers not only to the reproductive activity; the lactation and milk yield parameters during their dairy life are closely linked to the length of the day. The period of rearing lambs and the accompanying lactation are an integral part of the sheep reproductive cycle. The length of the period of sexual activity, from late summer to early winter, allows for breeding in two extremes under conditions in relation to the season and length of day. In the most commonly used model, birthing and the beginning of sheep lactation coincide with the winter period (short daylight period), and extending the length of day is a stimulating factor for milk yield (Molik et al. 2007). One study also attempted to prolong lactation and increase the efficiency of dairy in longwool sheep, which were lambed in June by keeping the mothers in artificial conditions of a long daylight period (16 h L : 8 h D) until mid-November (8 h L : 16 h D) (Molik et al. 2009). The milk yield indicators in this group of sheep were higher during the milking period than in the sheep that were lambed in June and kept under natural light conditions. The lactation of the sheep that lambed in June and were kept in conditions of an artificially extended photoperiod lasted several days longer, and ewes produced by 26% more milk during the milking period than the group of ewes subjected to natural changes in the length of day. However, artificial maintaining a long photoperiod in the autumn months does not prevent the rapid reduction of milk production and the termination of lactation in November. Hormonal changes, which were observed in the sheep under artificial conditions of a long daylight period, showed that despite the reduced concentration of melatonin in the blood, the secretion of prolactin also deteriorated similarly to the milk yield parameters (Molik et al. 2007).

The main aim of the study was to verify whether a disturbed rhythm of seasonality in milking sheep due to exogenous melatonin implants, and artificial conditions of daylight (16 h D : 8 h L) will affect the secretion of gonadotropic hormones LH and FSH (follicle-stimulating hormone).

MATERIAL AND METHODS

Animals. The Local Agricultural Animal Care and Use Committee in Krakow, Poland, approved all procedures performed on the animals in this study (Protocol 25/2010). The study was conducted at the Experimental Station of the Department of Animal Biotechnology, Agricultural University in Krakow. Sixty Polish Longwool ewes, a breed showing seasonality of reproduction, were investigated. The animals were 4–5 years of age and weighed 60 ± 5 kg. All animals were housed in individual pens under a natural photoperiodic and thermoperiodic environment (longitude: 19°57'E, latitude: 50°04'N). Ewes recorded an average Body Condition Score (BCS) of 3 (on a scale 0–5, where 0 = emaciated, 5 = obese; Russel et al. 1969).

In Poland, the long day photoperiod lasts from March to May, when the average day length is approximately 15 h, and the short day photoperiod lasts from September to December, when the day length averages 9 h.

Dietary treatments. Throughout the experiment, the sheep were fed according to their physiological status. From the preparation for mating to the end of the 4th month of pregnancy, they were fed in conformity with the standards of the National

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Research Institute of Animal Production, Krakow-Balice (Norms 1993) based on the forage pasture, silage, and hay. From the 5th month of pregnancy to drying off, the sheep received 1.5 kg of pelleted concentrate (7.5 MJ net energy and 220 g crude protein per kg of concentrate) and a hay supplement meeting the standard (Norms 1993). All animals had free access to water and mineral licks.

Experimental design. The ewes were randomly allocated to three groups, per 20 animals each. Estrus was synchronized using a 14-day treatment with intravaginal progestogen-impregnated sponges (40 mg fluorogestone acetate (FGA) Chronogest; Intervet International, The Netherlands). Ewes were also intramuscularly injected with a single dose of 500 IU of pregnant mare serum gonadotropin (Serogonadotropin; Biowet, Poland) on the day of sponge removal. Estrus detection was performed twice daily with an adult ram equipped with an apron. Ewes were presented individually to the male. Mating after estrus synchronization was performed on the 5th of September for all groups of sheep (Groups 1–3). Polish Longwool rams weighing 80 ± 5 kg were used for natural mating. The ewes lambed within the 20th–25th of February. Lambs were raised with mothers up to 56 days of age; then they were separated, and the mothers were assigned for milking. Since the start of milking on day 57, the sheep of the first group (G1 – control) were kept under natural conditions, and mothers of the second group (G2) were subcutaneously implanted melatonin implants (18 mg; Ceva Aimal, France). Meanwhile, throughout the milking period Group 3 (G3) remained in a 20 m^2 room in the artificial conditions of a short daylight period (16 h D : 8 h L). During the course of the experiment the sheep of Groups 1 and 2 were maintained indoors with the possibility to run. After separation from the lambs, the ewes were milked until the dry period twice a day using an Alpha-Laval milking machine. Individual milk checks were performed every 10 days. Once a month (every 4 weeks), 6 sheep from each group were subjected to a 6-hour collection of blood. Blood samples were collected once every 30 min from 10:00 h until 16:00 h by a catheter attached to the jugular vein.

Analytical techniques

LH assay. The plasma LH concentration was determined in duplicate 100-µl aliquots by a routine double-antibody radioimmunoassay (RIA), using anti-ovine LH, and anti-rabbit gammaglobulin antisera, and ovine LH standard (teri.oLH, Tucker Endocrine Research Institute LLC, USA). A complete characterization of the antibodies and method were described by Stupnicki and Madej (1976). The range of the calibrated curve was 0.3–40 ng/ml, and the working dilution of antibodies was 1 : 18 000. The sensitivity of an assay was 0.06 ng/ml, and the intra- and inter-assay coefficients of variation were 9 and 12%, respectively.

FSH assay. The plasma FSH concentration was assayed in duplicate 100-µl aliquots by the RIA double-antibody method using antiovine-FSH, and antirabbit-gammaglobulin antisera, according to a previously validated RIA (Stupnicki and Madej 1976), modified in the laboratory of The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences. The antiserum against FSH (teri.anti-oFSH) and the ovine FSH standards of immunochemical grade for radiolabelling (teri.FSHig) and a reference preparation calibrated in terms of NIH-FSH-S1 (teri.oFSH) were obtained from Dr. Reichert Jr. (Tucker Endocrine Research Institute LLC, USA). The range of the calibrated curve was from 1.56 to 200 ng/ml, and the working dilution of antibodies was 1 : 15 000. The assay sensitivity was 1.5 ng/ml, and the intra- and interassay coefficients of variation were 3.3 and 11.3%, respectively.

Statistical analysis. The results of the plasma hormone (LH, FSH) concentrations were analyzed by one-way analysis of variance followed by a post-hoc comparison using Scheffé's test (Statistical Analysis System, Version 9.1).

RESULTS AND DISCUSSION

Studies have shown that during the first blood collection in May the highest LH concentration was found in the group of sheep kept under conditions of short daylight period 16 h D : 8 h L (G3) (6.26 ± 0.2 ng/ml), whereas the lowest concentration of the hormone was found in the control group (G1) (5.32 ± 0 2 ng/ml) with a significant difference ($P \leq 0.01$) (Figure 1). In June, the LH concentration increased in all groups, and the highest values were reached in the group of sheep with melatonin implants (G2) (6.48 ± 2 ng/ml) and in G3 (6.25 ± 0.1 ng/ml). The lowest LH concentration in June was found in G1 (6.18 ± 0.2 ng/ml). During the third collection in July the secretion of LH declined, the

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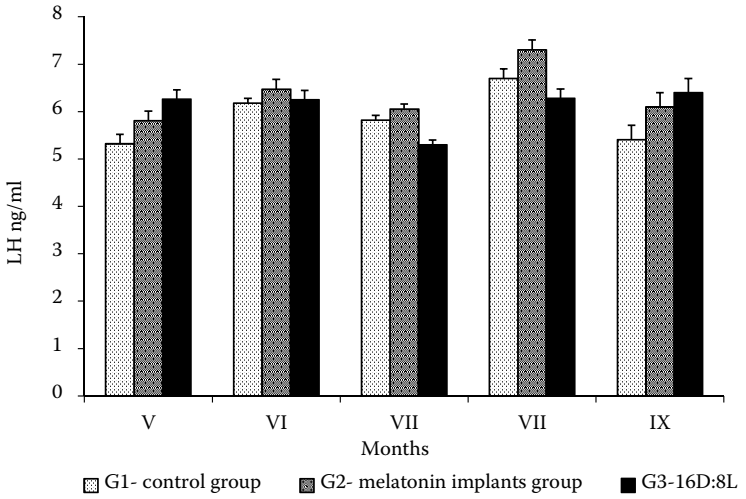

Figure 1. Means (± SEM) of luteinizing hormone (LH) concentrations in plasma of sheep (G1) kept under natural lighting conditions, (G2) with exogenous melatonin implants, and (G3) kept under a short artificial photoperiod (16 h darkness : 8 h light); for statistical comparisons see the text
means in months differ significantly at $^{*}P \leq 0.05$, $^{**}P \leq 0.01$

highest concentration of this hormone was found in G2 (6.05 ± 0.1 ng/ml), and the lowest level of LH was found in G3 (5.3 ± 0.1 ng/ml) with significant differences ($P \leq 0.01$). The average LH concentration in G1 (5.82 ± 0.1 ng/ml) was significantly ($P \leq 0.05$) higher than in G3. The secretion of LH increased with lactation and the shortening day length (extension of the dark phase). In August, the highest LH concentration was found in G2 (7.31 ± 2 ng/ml) and the lowest LH concentration was found in G3 (6.28 ± 0.1 ng/ml) with significant differences ($P \leq 0.01$). In September, during the last month of the experiment, the highest LH concentration was found in G3 (6.40 ± 0.1 ng/ml) followed by G2 (6.10 ± 0.1 ng/ml). At this time the lowest significant ($P \leq 0.01$) LH concentration was found in the sheep of G1 (5.41 ± 0.9 ng/ml). In summary, the profile changes in the LH-tested groups of sheep showed that the mean LH concentration in the control group G1 gradually increased from May (5.32 ± 0.2 ng/ml), reaching the highest value in August (6.70 ± 0.2 ng/ml). In G2, compared with G1, a significant ($P < 0.01$) increase in the LH concentration was observed 4 weeks after the introduction of 16 h D : 8 h L conditions (6.26 ± 0.2 ng/ml). The maximum LH concentration in G2 was found in August (7.31 ± 0.2 ng/ml), and it was higher ($P < 0.01$) than the concentration in groups G1 and G3. The profiles of the LH concentration in the individual collections are presented in Figure 1 and Table 1.

By analyzing the changes in the FSH secretion in the tested groups of sheep, the highest concentration of this hormone was in May for the sheep of G3 held under 6 h D : 8 h L conditions (8.36 ± 0.2 ng/ml), whereas the lowest significant ($P \leq 0.01$) FSH concentration was found in the sheep of the control group G1 (6.59 ± 0.2 ng/ml). During the first blood collection in the G2 group of sheep with melatonin implants the FSH concentration was slightly lower (7.61 ± 0.3 ng/ml) than in G3.

Table 1. Luteinizing hormone secretion profile in experimental sheep (in ng/ml)

Groups	Months				
	May	June	July	August	September
G1	5.32 ± 0.2**	6.18 ± 0.1	5.82 ± 0.1	6.70 ± 0.2**	5.41 ± 0.3
G2	5.81 ± 0.2**	6.48 ± 0.2	6.06 ± 0.1	7.31 ± 0.2**	6.10 ± 0.3
G3	6.26 ± 0.2	6.25 ± 0.2	5.30 ± 0.1*	6.28 ± 0.2	6.40 ± 0.3

G1 = control, G2 = melatonin implants, G3 = 16 h darkness : 8 h light
values are means ± standard deviation
significant difference at $^{*}P \leq 0.05$, $^{**}P \leq 0.01$

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In June, the highest FSH concentration was still found in the G3 group (9.0 ± 0.2 ng/ml), whereas the lowest was found in G1 (7.36 ± 0.2 ng/ml) with significant differences ($P \leq 0.01$). At this time the concentration of FSH in G2 (8.19 ± 0.3 ng/ml) was significantly ($P \leq 0.05$) higher than that observed in sheep from the control group (G1). During the third blood collection in July the significantly ($P \leq 0.01$) highest FSH concentration was found in the G3 sheep maintained under conditions of 16 h D : 8 h L (9.31 ± 0.2 ng/ml), whereas the sheep in the control group G1 and in the G2 group with melatonin implants showed FSH concentrations in the range of 7.70 ± 0.2 ng/ml and 7.5 ± 0.1 ng/ml, respectively. During the shortening daylight period the FSH concentration increased in all groups of sheep. In August, the highest FSH concentration was found in the G2 sheep with melatonin implants (11.31 ± 1.3 ng/ml). A lower value showed the G3 sheep maintained under the conditions of 16 h D : 8 h L (11.00 ± 1.2 ng/ml), whereas the lowest FSH concentrations were found in the control group G1 (10.50 ± 1.6 ng/ml). During the last collection in September the secretion of FSH increased in G2 sheep (13.6 ± 1.9 ng/ml) as well as in G3 sheep (13.51 ± 1.3 ng/ml). Significant ($P \leq 0.01$), the lowest FSH concentration was found in the control group G1 (8.80 ± 0.9 ng/ml).

The examinations demonstrated that the average FSH concentration in G1 increased gradually from May onward (6.59 ± 0.2 ng/ml), reaching the highest value in August (10.5 ± 2.60 ng/ml, $P < 0.05$). In G2, compared with G1, a significant ($P < 0.01$) increase in FSH concentration was in June (9.00 ± 0.3 vs 7.32 ± 0.2 ng/ml). The increase in FSH concentration was also observed during this period in G3, but its value (8.18 ± 0 3 ng/ml) did not differ significantly from the values noted for G1. During the final lactation period the FSH concentrations in G2 (13.51 ± 1.3 ng/ml) and G3 (13.60 ± 1.9 ng/ml) were significantly ($P < 0.01$) higher than in G1 (8.80 ± 0.9 ng/ml) (Figure 2, Table 2).

Changes in the day length play a very important role for the livestock, deciding of their productivity. The photoperiod in seasonally breeding animals e.g. in the short-day breeders (sheep) and/or in the long-day breeders (hamster or mare) is particularly the most important signal affecting many physiological processes, it is associated closely with changes in the concentration of melatonin (Misztal et al. 2004). In mature, sexually active sheep, melatonin is a hormone that modulates their sexual activity. The period of increased concentration of melatonin in the bloodstream depends on the length of the night and is subject to seasonal changes; hence, the highest concentration of melatonin in the bloodstream of sheep is found during the autumn–winter season in the northern hemisphere (Malpaux et al. 1993; Tortonese 2016).

The experiments indicated that the introduction of exogenous melatonin in the form of implants and the simulation of the artificial light conditions

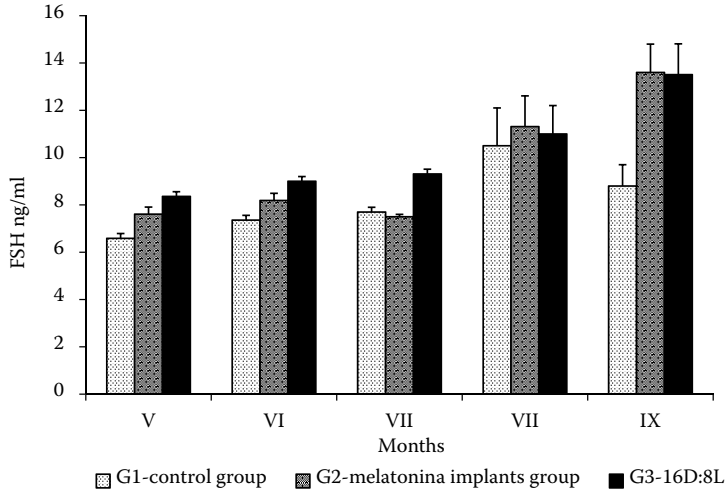

Figure 2. Means (± SEM) of follicle stimulating hormone (FSH) concentrations in plasma of sheep (G1) kept under natural lighting conditions, (G2) with exogenous melatonin implants, and (G3) kept under a short artificial photoperiod (16 h darkness : 8 h light); for statistical comparisons see the text
means in months differ significantly at $^{*}P \leq 0.05$, $^{**}P \leq 0.01$

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Table 2. Follicle stimulating hormone secretion profile in experimental sheep (in ng/ml)

Groups	Months				
	May	June	July	August	September
G1	6.59 ± 0.2**	7.36 ± 0.2	7.70 ± 0.2	10.50 ± 1.6**	8.80 ± 0.9
G2	7.61 ± 0.3**	8.19 ± 0.3*	7.50 ± 0.1**	11.31 ± 1.3*	13.60 ± 1.2**
G3	8.36 ± 0.2**	9.00 ± 0.2	9.31 ± 0.2	11.00 ± 1.2	13.51 ± 1.3**

G1 = control, G2 = melatonin implants, G3 = 16 h darkness : 8 h light
values are means ± standard deviation
significant difference at *$P \leq 0.05$, **$P \leq 0.01$

of a short day (16 h D : 8 h L) cause an increase in the basic concentrations of LH and FSH in sheep. These results confirm the observations of seasonal sheep, for which a long signal of melatonin stimulates the secretion of LH (Thiery et al. 2002). The biotechnological methods currently used in animal breeding allow breeders to induce the reproductive period of sheep, as confirmed by the experiments, in which the supply of exogenous melatonin in the pasture or in the form of a subcutaneous injection, intravenous infusion or a vaginal or subcutaneous implant, induced the breeding season. However, these processes are not as clear in the case of lactating sheep as data by Misztal et al. (2002) has shown that the GnRH–LH axis response to induced melatonin depends on the physiological status of the animal and the photoperiod. During the anestrous period, the perfusion of melatonin to the hypothalamus and the infusion of melatonin to the third ventricle of the brain do not significantly influence the secretion of GnRH and LH. However, in the reproductive season, the same infusion of melatonin induced a significant increase in LH secretion characterized by an increased amplitude of pulses. These results suggest that pineal hormone can demonstrate the synchronization effect on the daily rhythm of LH release, which is observed during the sexual activity period. The characteristic rhythm of LH is that it occurs in sheep in the breeding season and that the rise in LH concentration in the bloodstream occurs usually in the evening or during night hours (Misztal et al. 2002; Tortonese 2016). It may be presumed that additional introduction of melatonin to the body, especially in the afternoon hours, induces some physiological processes, which are characteristic for the period of darkness. The obtained results indicate that the use of sheep for milking does not inhibit the secretion growth of gonadotropic hormones induced by the simulation of a short day length and melatonin.

The release of FSH is subjected to smaller fluctuations depending on the season than the release of LH. However, the synthesis and secretion of gonadotropins, LH and FSH, are regulated by hypothalamic GnRH (Knobil 1980); the secretion of FSH is less dependent upon GnRH control, and it is instead regulated largely by GnRH-independent factors that include gonadal activins, inhibins, and follistatins (Kovacs et al. 2002). In addition to the role of GnRH and gonadal hormones in the control of FSH secretion, the existence of a separate, highly specific FSH-releasing hormone (FSH-RH) of hypothalamic origin has been proposed (Amstalden et al. 2003). Experimental evidence to support this theory has included the identification of the separation fraction FSH-RH and LH-releasing hormone activity in sheep hypothalamic extracts as well as the selectively impaired release of FSH after lesioning of the dorsal anterior hypothalamic area and posterior/mid infundibulum (Marubayashi et al. 1999). Moreover, GnRH-independent pulses of FHS have been detected by determining the FSH concentration in the hypothalamic–hypophyseal portal circulation (Padmanabhan et al. 1995).

CONCLUSION

In the Polish Longwool sheep (seasonally reproductive breed) in the environmental conditions of Poland, the period of sexual activity occurs during the growth of melatonin secretion period (shortening day). The studies demonstrated that the introduction of exogenous melatonin and artificial conditions of the short day caused an increase in the secretion of LH and FSH in sheep. In addition, it is worth noticing that the present research was carried out on sheep used for milking. These studies therefore showed an interesting fact that the process of lactation and sustained at this time a high concentration of prolactin as we noticed in the previous studies (Molik et al. 2013) did not contribute to the inhibition of secretion of gonadotropic hormones (LH, FSH) in sheep.

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