Summary

Sheep are short-day breeders, i.e. mate at times of the year when days are shorter and nights are longer. In these animals, the reproductive onset in the appropriate time of year is synchronized by lengthening days between the winter and summer solstices. The relatively long days around the summer solstice restrain reproductive activity and forestall the start of the breeding season until late summer/early autumn. Whereas during the shortening days between the summer solstice and autumnal equinox a normal intensity and duration of reproductive neuroendocrine induction during the impending breeding season are sustained. This adaptation to the changing seasons restricts the reproductive activity to the best time of the year. So, offspring are born and raised during spring and summer when the opportunity for growth and development is maximal due to the abundant amounts of milk from mother. Besides the seasonal changes, the diurnal fluctuations in the activity of the hypothalamic-pituitary-gonadal (HPG) axis, which control the process of reproduction, were also identified. It was found that in nocturnal species a luteinizing hormone (LH) surge occurs during the late afternoon or early evening whereas in the diurnal animals the sexual behavior and the LH surge take place very early in the morning, just before the sunrise. Because the day/night changes in the HPG axis activity are important for the generation of the LH surge on the optimal day time for the reproductive habits of animals, the factors disturbing the day/night pattern of the gonadotropins secretion may significantly decrease the reproduction success. It is postulated that both seasonal and diurnal changes in the activity of the HPG axis may be dependent upon the circulating level and duration of the melatonin, a hormone synthesized mainly by pinealocytes of the pineal gland during the disappearance of the light signal. However, the mechanism through

which melatonin affects reproduction has not yet been fully understood. Although in some reports it is suggested that in the in seasonal breeders, both seasonal and circadian changes of reproduction process may result from the central action of melatonin, it is rather postulated that melatonin influences the reproduction mainly through affecting the gonadotropins secretion in the tubular part of the pituitary called *pars tuberalis* (PT) – an exclusive part of the pituitary expressing melatonin receptors. It is postulated that the secretory activity of the *pars distalis* is regulated and supported by the PT, so any changes in the local gonadotropins secretion in the PT may significantly influence the secretory activity of the anterior pituitary gland.

In our previous study it was shown that inflammation disturbs the activity of HPG axis in ewes both at the hypothalamic and pituitary levels. It was found that an immune/inflammatory challenge may influence the secretion of gonadotropins, particularly LH, *via* suppression of gonadotropin-releasing hormone (GnRH) secretion in the hypothalamus, as well as that inflammatory mediators may directly affect LH secretion in the anterior pituitary gland. Moreover, the fact that pituitary cells express both proinflammatory cytokines and their corresponding receptors supports the thesis that both circulating and locally synthesized inflammatory mediators may directly disturb the secretory activity of the pituitary. This may profoundly influence endocrine system and affect many processes including reproduction. Having in mind the important role of the PT in the photoperiodic control of reproduction, examining the effect of inflammation on the secretory activity of this pituitary region seems to be reasonable.

Therefore, the present study was designed to determine the effect of inflammation induced by peripheral administration of bacterial endotoxin – lipopolysaccharide (LPS) on the LH synthesis and GnRH receptor expression in the PT of ewes and to examine whether this effect depends upon the time of the day (day/night)

and reproductive status of animals (anestrous season/follicular phase of the oestrous cycle).

A research hypothesis assumed that inflammation influences the secretion of LH in the ovine PT. In order to verify the hypothesis three experiments on blackhead female sheep were performed:

- **Experiment I** – In the *ex vivo* study the effect of melatonin on LH secretion in the PT explants of female sheep taken from animals in the follicular phase of the oestrous cycle differing in immunological status was determined (***publication 1***).
- **Experiment II** – The effect of inflammation on nocturnal expression of proinflammatory cytokines: interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)α, and their receptors in the ovine PT, taking into account different photoperiodic conditions (long day/short day) was found (***publication 2***).
- **Experiment III** – The inflammatory effects on LH and GnRH receptor expression in the ovine PT, during anestrous season and follicular phase of the oestrous cycle, taking into account daytime/nighttime were studied (***publication 3***).

It was found that the PT may be a gateway for immune-melatonin-reproductive interactions. In the *ex vivo* experiment it was shown that the PT explants collected from LPS-treated ewes were characterized by lower GnRH-dependent response in LH release. It was also found that inflammation reduced the gene expression of GnRH receptor and MT1 melatonin receptors in the PT. In the first *in vivo* experiment it was shown that the transcripts encoding all examined proinflammatory cytokines and their receptors were expressed in the PT. Moreover, the photoperiod influenced the gene

expression of certain proinflammatory cytokines and their receptors in the PT. It was also determined that the PT collected from LPS-treated ewes during short day photoperiod were characterized with higher expressions of IL-6, glycoprotein 130, TNF and TNF type I and type II receptors genes as compared with the tissues collected during long day photoperiod. Furthermore, in the second *in vivo* experiment it was found that LPS administration inhibited nocturnal increase in the gene and protein expression of LH in the PT of ewes during the follicular phase. Since in day active species the nocturnal accumulation of LH protein in the pituitary precedes LH surges, these surges may be profoundly delayed or their occurrence may be disturbed. Suppression of LH secretion may result from the decreased sensitivity of the PT on the action of GnRH as the inflammation reduced the GnRH receptor expression.

Concluding, it is suggested that systemic inflammation induced by the administration of bacterial endotoxin disturbs the synthesis of LH in the ovine PT. However, the effect of inflammation on the secretory activity of the PT may be dependent upon the photoperiodic condition. At least partially, the inflammatory-dependent changes in the secretory activity of the PT may be induced by locally synthesized proinflammatory cytokines. Also, another mechanism by which inflammation induced by the administration of bacterial endotoxin affects the reproduction process in seasonal breeders may be a reduction in the sensitivity of PT to both melatonin and GnRH, leading to a disruption of LH biosynthesis in this part of the pituitary gland.