

VII. SUMMARY

Both kisspeptins (KiSS-1) and leptin play a significant role in the course of puberty in sheep by initiation of the hypothalamic-pituitary-ovarian axis activity. Also hormones of thyrotropic axis participate in the regulation of reproductive processes in ruminants. However, there is no reports about relationships between KiSS-1/GPR54 (G protein-coupled receptor) system, leptin, hormones of thyrotropic axis and the time of the achievement of sexual maturity in sheep. Therefore, the aim of the present study was to analyse the relationships between daily weight gains, pituitary expression of KiSS-1/GPR54 system, plasma concentration of leptin, kisspeptin-10 (KiSS-10), thyroid-stimulating hormone (TSH), free thyroxin (fT4) and the time of the first ovulation in ewe lambs predisposed to delayed puberty and control sheep. Moreover, the impact of leptin on KiSS-10 secretion as well as on KiSS-1 mRNA and GPR54 mRNA expression in ovine pituitary cells *in vitro* was determined. Also the *in vitro* influence of kisspeptin-10 and leptin on TSH secretion was investigated.

The experiments were carried out on the high prolific SCP sheep: 114 ewes and 64 ewe lambs selected according to the birth type and body weight of their mothers. The newborn ewe lambs were divided into four groups: IS – singleton offspring of normally weighing sheep (without predisposition to the delayed puberty); IT – twin offspring of the normally weighing sheep (predisposed to the delayed puberty due to the low birth weight resulting from the multiple pregnancy); IIS – singleton offspring of the fatty sheep (predisposed to the delayed puberty due to the low birth weight resulting from the obesity of mothers during pregnancy) and IIT – twin offspring of the fatty sheep (predisposed to the delayed puberty due to the low birth weight resulting from the multiple pregnancy as well as from the obesity of mothers). All ewe lambs were weighed at birth and every two weeks thereafter, until eight months of postnatal age. From four to eight months of postnatal age, blood samples from jugular vein of ewe lambs were collected. Then, daily weight gains were recorded and plasma KiSS-10, leptin, TSH and fT4 concentrations were analysed by ELISA using species-specific antibodies. At the same time, the activity of ovaries was estimated using laparoscopy. In pituitary glands obtained from ewes aged six, seven and eight months, KiSS-1 and GPR54 mRNA expression was determined by Real-Time PCR. Moreover, pituitary glands were isolated from ewe lambs to establish pituitary cell cultures *in vitro*. Pituitary cells were cultured in the McCoy 5A medium: 1) without hormones (the control); 2) with leptin (10^{-10} - 10^{-5} M); 3) with KiSS-10 (10^{-11} - 10^{-5} M); 4) with peptide

234 (potent neutral antagonist of GPR54, 10^{-7} M); 5) with KiSS-10 (10^{-11} - 10^{-5} M) and peptide 234 (10^{-7} M). The media were collected to estimate the concentration of KiSS-10 and TSH by ELISA. At the same time cell proliferation index was determined for calculation of secretion values of above mentioned hormones. The expression of KiSS-1 mRNA and GPR54 mRNA by Real-Time PCR in the pituitary cells was also analysed.

The results obtained from *in vivo* studies show that the time of the achievement of sexual maturity in ewe lambs was dependent on the birth type (singleton/twin), the mother's body mass and plasma leptin level. Multiple pregnancy as well as high mother's body mass and plasma leptin concentration were factors that predispose ewe lambs to the delayed puberty. It was observed that increase in plasma leptin concentration in ewe lambs (up to the value: 3.35 ± 0.26 - 3.60 ± 0.19 ng/mL) was positively correlated with the elevation of plasma kisspeptin-10 concentration (up to the value: 31.26 ± 1.54 - 32.24 ± 2.25 ng/mL) and pituitary KiSS-1 mRNA expression. Such hormonal status was associated with the occurrence of the first ovulation. However, GPR54 mRNA expression in pituitary gland has decreased around the time of the first ovulation. The lack of ovulation during the first breeding season in ewe lambs, which were twin offspring of the fatty sheep, was connected with low birth weight, high daily weight gains from the 110th to 240th day of postnatal life, hyperphysiological leptin levels, low plasma KiSS-10 concentration and low pituitary KiSS-1 mRNA expression. The obtained results also show that secretory activity of thyroid gland in pubertal ewe lambs was dependent on the birth type (singleton/twin), the mother's body mass and plasma leptin level. Elevation of free thyroxin concentration and decrease in plasma thyroid-stimulating hormone concentration was correlated with occurrence of the first ovulation.

Moreover, *in vitro* studies have shown that TSH secretion from ovine pituitary cells was dependent on time of exposure and the dose of leptin. Treatment of the cells with 10^{-10} to 10^{-6} M of leptin resulted in elevated TSH secretion. However, the introduction of 10^{-5} M of leptin reduced thyroid-stimulating hormone secretion, compared to the control. The obtained results also show that 10^{-10} to 10^{-7} M of leptin stimulated KiSS-1 expression as well as KiSS-10 secretion from anterior pituitary cells of ewe lambs. In contrast, the highest concentration of leptin (10^{-5} M) significantly decreased KiSS-1 expression and KiSS-10 secretion compared to the control. Furthermore, kisspeptin-10 did not change TSH secretion *in vitro*, except exerting a short-term influence after 2 h. Treatment of the cells with 10^{-11} to 10^{-8} M of KiSS-10 significantly increased TSH secretion from ovine pituitary cells after 2 h of the experiment.