

## SUMMARY

Puberty is a multifactorial and complex process determined, among others, by the attainment of an appropriate body weight and a critical adipose tissue mass and the proper functioning of endocrine regulatory mechanisms. In ruminants, the timely attainment of sexual maturity contributes to increased reproductive performance of the flock and to the economic efficiency of breeding. One of the problems observed during the first year of life in animals is delayed puberty, which is associated with metabolic and neurohormonal disturbances.

KNDy (K – kisspeptin, N – neurokinin B, Dy – dynorphin) are neuropeptides produced and secreted, among others, by the arcuate nucleus of the hypothalamus. They are involved in the neuroendocrine regulation of the development and functioning of the reproductive system. Kisspeptins, including kisspeptin-10 (KiSS-10), play an important role in the initiation of puberty; they activate GnRH neurons, thereby stimulating the production and secretion of gonadoliberein and, consequently, the secretion of LH and FSH from the pituitary gland. Limited reports indicate that neurokinin B (NKB) and dynorphin A (Dyn A) may also participate in the regulation of reproductive processes in ruminants. However, to date, there have been no data on the relationship between the KiSS, NKB, and Dyn A neurohormone system and the timing of puberty initiation in sheep. Moreover, the available reports on the effects of NKB and Dyn A on gonadotropin secretion by pituitary cells *in vitro* are ambiguous and do not relate to sheep. There are also no data on the direct effects of NKB and Dyn A on KiSS-10 secretion at the pituitary level in ewes during puberty.

The experiment was conducted in two stages. In the first stage, carried out under *in vivo* conditions, twenty-four Uhruska ewe lambs were used. The first group consisted of lambs derived from singleton pregnancies (S; n = 13), whereas the second group comprised ewe lambs from twin pregnancies and thus predisposed to delayed puberty (T; n = 11). The aim of the study was to determine changes in plasma concentrations of KiSS-10, NKB, and Dyn A in ewe lambs from the fourth to the tenth month of life and to analyse the relationship between the concentrations of these neuropeptides and the timing of the first ovulation in both groups of sheep. The aim of the second stage of the experiment, conducted under *in vitro* conditions, was to investigate the effects

of KiSS-10, NKB, and Dyn A on LH and FSH secretion and to analyse the influence of NKB and Dyn A on KiSS-10 secretion by anterior pituitary cells in ewe lambs.

The *in vivo* studies demonstrated that the age at which ewe lambs attain sexual maturity depends on the type of pregnancy from which they originate (singleton/multiple), the rate of body weight gain, and the concentrations of the analysed neuropeptides in blood plasma. In sheep originating from singleton pregnancies, the first ovulation was observed at 8 months of age, whereas in those derived from twin pregnancies it occurred 2 months later. The mean body weight of ewes from singleton pregnancies was higher throughout the entire experimental period than in the group coming from twin pregnancies. In both the S and T groups, a positive correlation was demonstrated between changes in plasma KiSS-10 and NKB concentrations with age (S:  $r = 0.86$ , T:  $r = 0.89$ ). At the same time, under the same conditions, a negative correlation was found between KiSS-10 and Dyn A (S:  $r = -0.55$ , T:  $r = -0.98$ ), as well as between NKB and Dyn A (S:  $r = -0.89$ , T:  $r = -0.94$ ). In singleton ewes, the initiation of ovarian activity was associated with a statistically significant ( $P \leq 0.05$ ) increase in KiSS-10 (S:  $95.28 \pm 5.21$  ng/l) and NKB (S:  $384.19 \pm 38.36$  ng/l) concentrations after 8 months of age, whereas in sheep coming from twin pregnancies, it was associated with a significant increase in KiSS-10 concentration (T:  $95.80 \pm 7.4$  ng/l) in the 10th month of life, preceded by an increase in NKB concentration (T:  $364.70 \pm 47.52$  ng/l) after 9 months of age. Delayed puberty in sheep from group T was associated with a persistently higher level of Dyn A up to 8 months of age, accompanied by lower plasma concentrations of KiSS-10 and NKB compared with group S (publication no. 3).

In the second stage of the study, conducted under *in vitro* conditions, a direct effect of KiSS-10, NKB, and Dyn A on gonadotropin secretion by ovine anterior pituitary cells was demonstrated. After 4, 12, and 24 hours of exposure to KiSS-10, an increase in LH secretion ( $10^{-11}$ – $10^{-8}$  M KiSS-10) and FSH secretion ( $10^{-11}$ – $10^{-7}$  M KiSS-10) was found, compared with the control. The highest level of LH secretion was recorded after 24-hour exposure of cells to  $10^{-8}$  M KiSS-10. A statistically significant ( $P \leq 0.05$ ) increase in FSH secretion was observed after 12 and 24 hours at a concentration of  $10^{-9}$  M KiSS-10 compared with the control. NKB and Dyn A, at all concentrations used ( $10^{-11}$ – $10^{-7}$  M), stimulated the secretion of both LH and FSH. The strongest stimulatory effect ( $P \leq 0.05$ ) on LH secretion was observed after 12- and 24-hour exposure to  $10^{-8}$  M NKB. FSH secretion reached its maximum after 12 and 24 hours of cell exposure to the highest concentration ( $10^{-7}$  M) of NKB. These values were statistically significantly higher

( $P \leq 0.05$ ) than those in the control. Dyn A at concentrations of  $10^{-10}$ – $10^{-7}$  M significantly increased LH secretion ( $P \leq 0.05$ ), whereas at concentrations of  $10^{-11}$ – $10^{-9}$  M, it significantly increased FSH secretion ( $P \leq 0.05$ ), compared with the control (publication no. 1). The effect of NKB on KiSS-10 secretion by anterior pituitary cells depended on the duration of exposure and its concentration in the culture medium. NKB, unlike Dyn A, had a significant effect ( $P \leq 0.05$ ) on KiSS-10 secretion from ovine pituitary cells after 6, 12, 18, and 24 hours at a concentration of  $10^{-8}$  M, and after 24 hours at a concentration of  $10^{-7}$  M. Dyn A, at all concentrations used ( $10^{-11}$ – $10^{-7}$  M), had no significant effect on KiSS-10 secretion throughout the duration of the experiment (publication no. 2).

The obtained pioneering results indicate a significant role of the studied neuropeptides in the control of initiation of reproductive activity in sheep. A direct effect of KiSS-10, NKB, and Dyn A on gonadotropin secretion at the pituitary level was demonstrated. Moreover, the stimulatory effect of NKB on KiSS-10 secretion by ovine anterior pituitary cells indicates the possibility of an indirect effect of this neuropeptide on gonadotropin secretion, mainly LH.

**Keywords:** dynorphin A, gonadotropins, kisspeptin-10, neurokinin B, puberty