# SUMMARY

Ovarian cysts (CJ) cause 15-30% of cases of intermittent or permanent infertility in cows. Their primary form is follicular cyst. The etiology of follicular cysts is complex, multifactorial and heterogeneous, and the pathogenesis is multidirectional and still not fully elucidated. The development of cysts can be caused by a number of hormonal, metabolic, environmental and hereditary conditions.

The aim of this study was to determine the significance of changes in the concentration of kisspeptin in blood and follicular fluid and the expression of *kiss-1* and *kiss-1r* mRNA in ovarian cells in the pathogenesis of follicular cysts in cows. In the first stage of the study (*in vivo*), clinical specimens were used from healthy cyclic cows (group I, n=12), from cows classified at the first examination after parturition as healthy cyclic that did not ovulate after the initial blood sampling in the following cycle and were found to have follicular cysts (group II, n=12), and from cows with follicular cysts found during pregnancy diagnosis min. 120 days postpartum (group III, n=10). The second stage of the study, in *vitro*, was conducted using ovaries isolated from healthy cows at slaughter. Under *in vivo* conditions, the concentration of kisspeptin-10 in plasma and follicular fluid collected from cows with follicular cysts and cyclic cows was compared, and an attempt was made to establish marker values of kisspeptin-10 concentration indicating the presence of a follicular cyst on the ovary. In order to establish the predictive values of kisspeptin-10 concentrations, with regard to the risk of cyst development, the plasma levels of KiSS-10 in cows during the late follicular phase of the estrous cycle preceding cyst formation were analyzed. The mRNA expression of kisspeptin and its receptor KiSS1R in granulosa cells and theca cells isolated from ovarian follicles and follicular cysts was also compared. Moreover, the effect of leptin on KiSS-10 secretion by ovarian follicle granulosa cells was analyzed *in vitro*, and the effect of KiSS-10 on 17β-estradiol secretion was determined. The effect of leptin on the expression of leptin receptor mRNA (OB-Rb), KiSS-1 mRNA, KiSS1R receptor mRNA in granulosa cells *in vitro* was also investigated . As a result of the study, KiSS-10 plasma concentrations in cows between days 18 and 21 of the estrous cycle preceding follicular cyst formation were found to be more than twice lower (12.65 +/- 3.60 ng/ml; range: 9.07 - 17.49 ng/ml) than in healthy cows (28.78 +/- 2.61 ng/ml; range: 25.26-31.56 ng/ml). KiSS-10 concentration values in the range of 9.00 - 17.50 ng/ml on day 18-21 of the estrous cycle can therefore be considered prognostic - indicating an increased risk of anovulation and follicular cyst formation in the following cycle. In contrast, plasma KiSS-10 levels in cows with diagnosed follicular cysts 1-4 days before puncture are more than twice as high (64.03 +/- 3.86 ng/ml, range 59.97-68.56 ng/ml) as in healthy cows (28.78 +/- 2.61 ng/ml; range: 25.26-31.56 ng/ml). KiSS-10 concentration values in the range of 60.00-68.50 ng/ml can therefore be considered as markers - indicating the presence of a follicular cyst on the ovary. In healthy cyclic cows, the mean KiSS-10 concentration was 28.78 +/- 2.61 ng/ml (range: 25.26-31.56 ng/ml). KiSS-10 concentration values in this range can therefore be considered predictive - indicating the occurrence of ovulation in a given estrous cycle. It was also found that KiSS-10 concentrations in follicular cyst-derived fluid are about 1.6 times higher than in follicular fluid derived from preovulatory follicles. In addition, the mRNA expression level of kisspeptin was shown to be significantly higher (p≤0,05) in granulosa cells derived from follicular cysts compared to cells isolated from preovulatory follicles. Analysis of kisspeptin receptor mRNA expression levels showed an inverse relationship. As a result of *in vitro* experiments, leptin was found to affect the secretion of kisspeptins

by granulosa cells of ovarian follicles of cows in a time- and concentration-dependent manner. A positive correlation was found between cell exposure to leptin at concentrations of $10^{-10}$ - $10^{-9}$ M and KiSS-10 secretion.

At the same time, a negative correlation was shown between leptin in the concentration range of $10^{-8}$ - $10^{-5}$ M and KiSS-10 secretion from ovarian follicle granulosa cells. It was also established that kisspeptin-10 affects the secretion of 17β-estradiol by granulosa cells of ovarian follicles of cows in a time- and concentration-dependent manner. A positive correlation was found between cell exposure to kisspeptin-10 at concentrations of $10^{-10}$ - $10^{-9}$ M and 17β-estradiol secretion. At the same time, a negative correlation was found between kisspeptin-10 in the concentration range of $10^{-9}$ - $10^{-5}$ M and the secretion of 17β-estradiol from granulosa cells of the ovarian follicle. In addition, it was found that exposure of ovarian follicle granulosa cells to leptin at concentrations of $10^{-10}$ - $10^{-7}$ M results in an increase in *kiss1* mRNA expression in *vitro* compared to controls. In contrast, leptin at the highest concentrations used ($10^{-6}$ - $10^{-5}$ M) significantly ($p \leq 0.05$) decreases *kiss1* mRNA levels. Analysis of the mRNA expression level of kisspeptin receptors under the influence of leptin showed an inverse relationship.

The results indicate that the KiSS1/ KiSS1R system may be an important element in the pathogenesis of follicular cysts in cows not only at the level of the hypothalamus and pituitary gland, but also at the level of the ovaries. Significantly ($p \leq 0,05$) lower plasma concentrations of KiSS-10 than in healthy cows between days 18 and 21 of the estrous cycle preceding follicular cyst formation, as a result of insufficient signal stimulating 17β-estradiol secretion from granulosa cells of the ovarian follicle, may be responsible for the lack of normal pre-ovulatory 17β-estradiol secretion conditioning ovulation and, thus, cause follicular cyst formation. In contrast, significantly ($p \leq 0,05$) higher KiSS-10 concentrations in plasma and  cyst-derived fluid than in healthy cows, and significantly higher levels of kisspeptin mRNA expression in granulosa cells derived from follicular cysts compared to cells isolated from preovulatory follicles, by reducing the expression level of kisspeptin receptors in granulosa cells, may cause a similar effect related to the lack of normal pre-ovulatory ovulation-conditioning 17β-estradiol secretion and, thus, preserve the hormonal status responsible for cyst maintenance and lack of ovulation in subsequent estrous cycles. However, the exact role of the KiSS1/ KiSS1R system in the pathogenesis of follicular cysts in cows requires further studies under *in vivo* conditions , taking into account not only clinical and slaughter material, taking into account not only clinical and slaughterhouse material, but also experimental samples obtained from cows exposed to different doses of KiSS-10 in order to assess their effect on ovarian activity.