Project title: *A comprehensive characteristics of candidate genomic regions for canine monogenic disorders of sex development, analyzed with the use of next generation sequencing (NGS) and droplet digital PCR (ddPCR)*

Disorders of sexual development (DSD), formerly called also intersexuality or hermaphroditism, usually lead to sterility or infertility. Identification of a healthy carrier of a mutation causing hereditary disease or disorder, including DSD, is an important issue in livestock and companion animals breeding programs. In the last years, studies of canine hereditary diseases have been intensively developed and numerous causative mutations were characterized on a molecular level. Thus, the dog is presently considered as an important large animal model in studies on molecular background of genetic diseases and disorders. Unfortunately, knowledge concerning monogenic canine DSD is very scarce, although they are quite often diagnosed in different dog breeds. In this project proposal we plan to extend this knowledge by the use of new techniques applied to study DNA sequence variation. We hypothesize that due to breed differences between gene pools, causative mutations responsible for phenotypically similar hereditary forms of canine DSD can be breed-specific. The aim of this project is identification of new causative mutations in selected regions of the dog genome, which harbour genes and their regulatory sequences crucial for mammalian sex determination and differentiation. In these regions we will search for mutations changing amino acid sequence or influencing gene expression, as well as copy number variation (CNV) affecting number of entire gene copies or their regulatory sequences. The alterations of DNA sequence found in DSD (with a normal set of male sex chromosomes – XY DSD or female sex chromosomes – XX DSD) dogs (n=38) will be compared with sequence of the same regions in control dogs (n=20). Altogether, 15 genomic regions, which span approx. 5.3 Mb and harbour 15 genes *(WNT4*, *RSPO1*, *CTNNB1*, *FOXL2*, *GATA4*, *NR5A1*, *AR*, *MIS*, *MISR2*, *SRD5A2*, *FGF9*, *HSD17B3*, *SOX3*, *DAX1* and *DMRT1)* with a known function in sex determination and differentiation will be studied. Two crucial genes for testis development (*SRY* and *SOX9)* will be not included since they have been already analyzed in our previous studies. Novel SNP and indel variants will be identified with the use of targeted next generation sequencing (tNGS) and their distribution in dog breeds will be analyzed with the use of Sanger sequencing technique. The CNV polymorphism will be studied by a new, highly sensitive technology – droplet digital PCR (ddPCR). The expected effect of the proposed project is identification of new mutations responsible for different types of canine DSD. Identification of such mutations will give an opportunity for effective eradication of the undesired alleles from gene pools of dog breeds in which a given DSD form is observed. We also assume that the obtained results will be helpful in searching for causative mutations for DSD in livestock species.