ABSTRACT

Huntington's disease (HD) is one of the incurable and fatal diseases. HD belongs to the monogenic neurodegenerative diseases. According to the number of the CAG repetitions in the gene coding huntingtin, the onset of the disease is in childhood (5%), in the middle age, which is the most common (90%) and in the older age (5%). Beginning of the disease is manifested by changes in behavior; including problems with coordination and movement. Later, there is a psychological change. The disease leads to death. Until now, there is no effective curative treatment.

In 2009, we created a model of the transgenic minipigs (TgHD) carrying the N - terminal part of the human mutant huntingtin (mtHtt) at our Institute in Liběchov. The number of offsprings and the resemblance in physiology and morphology between the pig (*Sus scrofa*) and humans (*Homo sapiens*) give us opportunities not only to study changes not only in central nerve organs, but also in peripheral tissues. The reproductive problems of TgHD boars were observed as the first phenotypic changes. Therefore, my work focuses at first on a study of the reproduction parameters of TgHD boars as well as ultrastructural, immunocytochemical and biochemical changes in testes and spermatozoa.

In PhD thesis, I described in details the reproductive defects in TgHD boars of F1 and F2 generations. All gained data were always compared between the wild-type (WT) and TgHD boars from the same litter. The reproductive changes started at the age of 13 months in both generations of TgHD boars. Significant changes in number of spermatozoa per ejaculate, in their movement and in their progressive movement were confirmed at the age of 24 and 36 months, too. Transmission electron microscopy (TEM) revealed numerous morphological abnormalities, first of all at sperm tails and testicular epithelium at the age of 24 and 36 months. These morphological observations were supported by immunocytochemical approaches that confirmed the significantly lower proliferation activity in spermatogonia of TgHD boars. The key result is that the high expression of mtHTT occurs in all cells of the seminiferous tubules and also in all parts of sperm tail, in the midpiece especially. The described changes were also confirmed by the noninvasive approach, using ^{31}P magnetic spectroscopy (MRS) for examination of testicular tissue of siblings (WT and TgHD boars). The significant decrease in ratio of phosphodiesterases (PDE/γ-ATP) in the testicular parenchyma of TgHD boars was documented.

All morphological and functional changes described above were confirmed in the detailed study of mitochondrial metabolism in spermatozoa of WT and TgHD boars. The key advantage of this biological material is that it can be collected noninvasively and longitudinally. The oxidative function was measured by polarography and metabolism of mitochondria was also measured after oxidation of radioactive substrates. These methodological approaches proved the significant decrease in the activity of respiratory complex I and they also revealed four parameters indicating the serious impairment of glycolytic activity in spermatozoa of TgHD boars. These experiments described in details biomarkers monitoring progress of HD and fully confirmed the negative effect of mtHTT on mitochondrial metabolism leading to testicular degeneration and to substantial functional changes of spermatozoa. .

The experiments in this PhD thesis clearly demonstrated that TgHD minipigs, with the preclinical development of the HD phenotype, represent the suitable, biomedical model for testing of all current methods of gene therapy of HD.