

The reaction of phosphorylation and dephosphorylation of proteins is one of the most common posttranslational modification and it affects approximately 30% of all proteins of animal organisms. Phosphorylation is a process of regulating the activity and function of the polypeptides involved in many biological processes. Phosphorylated proteins act as the regulators of cell activity, control their metabolism, growth or the “death path”, called apoptosis. In the study of proteins profiles and proteins expression of the male reproductive system special attention is paid to the importance of proteins phosphorylation as a initiatory and regulatory factor for such important reactions as capacitation, acrosome reaction and sperm maturation processes.

Research conducted on mouse sperm has shown that the proteins acting chaperones such as endoplasmic reticulum protein 99 (erp99) and heat shock protein 60 (Hsp 60) located in the plasma membrane of sperm tail are phosphorylated. These proteins enable the sperm to recognize the oocyte zona pellucida, and hence a greater success during fertilization of the ovum. In contrast, phosphorylation of tyrosine residues of the sperm tail induces the hyperactivation of the sperm. As a result of such a process acquires the ability to move and penetrate the oocyte. The developing aspect of the proteomic research concerning the male reproductive system is the maturation of gametes in the epididymis. Changes processing in the constituent parts of the sperm plasmalemma and correlations between the components of the reproductive system's environment and the maturing cells are important in the fertilizing capacity of sperm generally. The sperm found in the testes is immature, immobile and deprived of the oocyte fertilizing capacity. Only with passage through the epididymal tract sperm undergo the process of maturing. The occurrence of biochemical changes specific to individual epididymal sections determines the sperm ability to capacitate and undergo the acrosome reaction. The epididymes of mammals fulfill the most important function in the sperm maturation. They regulate the concentration of gametes, they are responsible for their functional maturation and quantity of substances released into the lumen of the epididymis. Moreover, they serve as a storage facility for the mature sperm until the moment of ejaculation, they protect cells against inflammation and oxidative stress and participate in the removal of inactive, injured or dead cells. Modifications in the sperm structure during its maturation in the epididymal duct are based on the reorganization of lipids within the sperm head plasmalemma and proteolytic changes, which are strictly correlated with the environment provided by the epididymal fluid. No research has been carried out on the phosphorylation of protein in the epididymal fluid of stallion, nor analyses of the epididymal sperm phosphoproteome in various stages of the maturation process. The results of proteomic analysis of epididymal sperm of rodents have shown a relationship between the sperm protein phosphorylation, and the level of maturation resulting in the ability to fertilize an oocyte. These changes are associated with mitochondrial phosphorylation of many proteins and production of mitochondrial membrane potential. During the passage of sperm through the epididymal tract, the first process that is taking place is the phosphorylation of the proteins in the head and tail of sperm. Once the sperm is located in the cauda of the epididymis proteins phosphorylations of the full length of tail takes place to enable them to change the nature of their movement and thus a successful fertilization of the oocyte.

In recent years, a growing interest in the artificial insemination of mares has been observed among horse breeders. The use of the epididymal sperm for this purpose has become more widely used and beneficial. Often breeders are faced with situations in which stallion is not able to give semen or there is a need to euthanize it, while the genetic material of an individual is very valuable. In such cases, the only possibility to use the epididymal semen that is previously conserved in the liquid state or cryopreserved. It should be emphasized that the first foal being a result of in-vitro fertilization with use of sperm collected from the epididymis born over 50 years ago. Further studies on the efficiency of the epididymal sperm fertilization, either fresh, preserved in liquid or frozen state indicate their effective use in the artificial insemination of mares. Experiments concerning the effectiveness of mares artificial insemination with the epididymal semen indicate a rate of 65% fertilized mares. A comparative analysis of motility and morphology parameters of epididymal and ejaculated sperm did not show significant differences. Such results give hope for popularizing the usage of epididymal sperm in the artificial insemination of domestic animals, by creating sperm banks, which will allow for their long-term preservation. The proteomic analysis of the epididymal semen will help to determine the role of proteins phosphorylation-dephosphorylation reactions in stallion's epididymal sperm maturation processes. The research will also provide with knowledge of seasonal changes specificity in protein phosphorylation of the epididymal sperm and fluid.