Infertility affects approximately 10-18% of couples at reproductive age and - according to World Health Organization (WHO), is known as one of the social diseases. It is estimated that approximately 7% of men worldwide is affected with infertility and about 40-60% of all infertility cases originates from the male side. Male factor etiology is associated with abnormal semen parameters, such as abnormal sperm concentration, motility, and/or morphology. Oligozoospermia (low sperm number) accounts for approx. 30% of cases and is the most frequent semen abnormality in infertile males.

It is suggested that spermatozoa of oligozoospermic males may have altered DNA methylation pattern of selected genes playing an important role in spermatogenesis. Methylation is one of the most important epigenetic factors playing crucial role in gametic imprinting, chromosome X inactivation, gene silencing and protein conformation. Together with acetylation, this epimodification is responsible for regulation of transcriptomic gene's activity. Previous research in spermatozoa provided speculations that affected health of the offspring may be caused by epigenetic defects resulted from aberrant sperm DNA methylation. Hypomethylation may also alter the process of cell differentiation, so the embryo genomic expression may reveal disturbed synchronization in its development.

We hypothesize that alterations in specific methylation patterns of particular genes may be linked to some cases of oligozoospermia. This points out a strong need of a great number of new experiments that would determine and clarify methylation role in disruption of spermatogenesis. Beside of standard seminological analysis (concentration, morphology, motility), hormones evaluation and karyotyping, and the genetic factors (microdeletions of chromosome Y, CFTR mutations), diagnosis of oligozoospermia needs also a deep look into spermatozoa in genomic and epigenetic manner. What is important, most of men that decided to apply for a help of assisted reproductive technologies (ART), are oligozoospermic. It has to be pointed out, that some immature gametes with not fully established methylation pattern can be taken for fertilization in ART. Additionally, selection criteria of spermatozoa for IVF rely only on the motility and morphology of the sperm - status of the genetic and epigenetic content of the sperm remains unknown. Thus, a basic knowledge concerning the mechanisms and meaning of the gametic epigenome disturbances seems to be important because of the relatively high frequency of ART-births today (approximately, 1-3% of all live-births).The unique epigenetic marks in spermatozoa may be crucial facilitating proper mature gamete function and being responsible for poising specific genes activation in the early embryo. Therefore, understanding the paternal epigenetic ‘landscape’ seems to be of a paramount importance.

In this Project we want to delineate methylation patterns for selected genes in DNA from spermatozoa of infertile men with decreased number of spermatoza in ejaculate (oligozoospermia). The goal of the Project will be supported by the analyzes of sperm chromatin integrity (including sperm DNA fragmentation and chromatin deprotamination evaluations), sperm aneuploidy level at least for six chromosomes and analysis of histone H4 lysines' acetylation. All those elements are important for proper functioning of sperm genome. We are going to use variety of methods to evaulate full description of sperm status in oligozoospermic males, suc as: pyrosequencing, thin-layer chromatography (TLC), immunofluorescence, chromomycin A3 and aniline blue stainings, TUNEL test, acridine orange staining and fluorescent *in situ* hybridization (FISH).

In case of spermatozoa, methods used for seminal diagnostic base on separate analyzes, that are not sufficient for delineation of the reasons and mechanisms that may underlie infertility. In this Project we performed complex analysis of results obtained with different methods in spermatozoa, including evaluation not only of genetic content of spermatozoa but also analyzis of epigenetic factors that are not neutral for proper spermatozoa production process. Ultimately, results obtained in this Project will facilitate the development of new diagnostic procedures, leading to greater improvement of the prognosis for assisted reproductive medicine where the risk of transmitting of genetic defects to offspring is of a great concern.