ABSTRACT

Background: Currently infertility of couples is a major problem, whereas the share of man and woman in infertility disorder is alike. It is important to discover the cause of infertility as soon as possible. The diagnostic possibilities of male infertility detection include the examination of sperm by flow cytometry and microscopic examination of semen.

Aims: The aim of our work is to draw up an overview of the possible causes of male infertility and a summary of possible examination of semen using flow cytometry. Next aim is to evaluate the data measured by flow cytometry obtained from men over the years 2008, 2009 and 2010 at the Institute of Clinical Immunology and Allergology University Hospital Hradec Králové, Czech Republic, and determine interdependencies between the measured sperm parameters. Further, the work deals with the methodology optimization in examination of semen using flow cytometry.

Methods: Determination of sperm parameters (sperm count and leukocyte count, sperm vitality, acrosom status and presence of intra-acrosomal protein) was performed using flow cytometer Cytomics FC 500 with CXP analytic software. The method used is based on the test SpermFlow from the company Exbio. Semen samples were obtained from 163 men.

Results: Statistical analysis of our data set showed that men with low sperm count in ejaculate have a larger proportion of sperm with impaired acrosome. A group of men with oligospermia also have a higher proportion of sperm with insufficient quantity of intra-acrosomal protein required for successful fertilization process. An interesting finding is that men with poor sperm vitality also have a higher proportion of sperm with impaired acrosome. During, further analysis we documented neither a link between reduced vitality and leukospermia nor between reduced vitality and oligospermia. We have not found any dependence even when examining the impact of age of man on sperm count in ejaculate.

Methodology optimization in examination of semen using flow cytometry showed that the method of dilution of semen does not significantly affect the calculation of the concentration of sperm in the sample of normospermatic man. On the other hand, with

oligospermatic or azoospermatic men the way of diluting should be considered. When comparing the ways of determination of the leukocyte count in semen using flow cytometry and microscopic methods we found similar results. Staining the sperm with propidium iodide and 7-aminoaktinomycine D, we found that propidium iodide is preferable to determine sperm vitality. The result of this test is most depending on the method of preservation and processing of the sample. We agree with the methodical recommendation SpermFlow by Exbio, that the best is 30min incubation of diluted semen with propidium iodide. As to washing the sample to determine the acrosomal protein, we consider triple washing of the sample the best, prior to staining with the antibody Hs-14.

Conclusions: Although flow cytometry is still not able to evaluate all the examined sperm parameters such as morphology, its introduction to the diagnosis of male infertility and the progressive development enables fast, accurate and precise determination of sperm parameters. Currently, efforts are made to optimize the new methodologies to determine other parameters of sperm quality, either directly or indirectly. This was successful in the case of motility, where the discovery of mitochondrial membrane potential related to sperm motility allows the determination of this parameter using flow cytometry.