NEW METHOD OF VISUALIZATION OF NATIVE SPERM INTRACELLULAR ORGANELLES FOR SPERM SELECTION AND DIAGNOSTICS OF MALE INFERTILITY


Abstract

The new microscopy method for native assessment of sperm ultramorphology (NASUM) was developed. NASUM gives possibility to visualize chromatin, acrosome and its granularity, vacuoles, mitochondria, and nuclear membrane pores, microfibrils of the tail, and chromocenter, as well as evaluate the degree of chromatin condensation in native spermatozoa.

Introduction

Various authors have demonstrated that selection of vacuole-free spermatozoa for intracytoplasmic sperm injection (ICSI) leads to higher pregnancy rates and lower rates of pregnancy loss. Bartoov et al. (2001) developed the intracytoplasmic morphologically selected sperm injection (IMSI) method which included the microscope for vacuole visualization and specific morphological criteria of spermatozoa selection for ICSI. It was shown that selection of vacuole-free spermatozoa for intracytoplasmic sperm injection (ICSI) leads to higher pregnancy rates and lower rates of pregnancy loss (Berkovitz et al., 2006). The relationship between vacuolization and DNA fragmentation has also been established. However, IMSI does not provide the scientists with the possibility of visualization of the intracellular structures other than vacuoles.

Objectives

The aim was developing a new microscopy method of sperm morphology evaluation, allowing us to visualize the intracellular organelles of spermatozoa.

Results

The developed method of observation and the constructed microscope system allows for studying sperm morphological features at subcellular level and detect structural anomalies invisible during the usual light optical microscopy (see picture). The subcellular organelles can be morphologically classified on the basis of the presence of specific malformations defined according to the descriptive approach reported by Bartoov et al. (2002): acrosome: absent, partial or vesiculated; post-acrosomal lamina: absent or vesiculated; neck: abaxial, disordered or showing cytoplasmic droplet; tail: absent, coiled, broken, multi or short. For the nucleus, the morphological normal state can be defined by the shape and content of the chromatin. It is proposed to name the new method as native assessment of sperm ultramorphology (NASUM).

Methods

The microscope system developed by us employed both Hoffman and Nomarski contrast techniques. Resolution increase was attained with light interference suppression via using circularly polarized light. The introduction of additional lenses into the optical system allowed us to reach 20000X total magnification (including video zoom). Also we used green 500 mW laser lighting (wavelength 532±10 nm) with grain suppression and resulting uniform field of light. We obtained 12348 microphotographs of native immobilized spermatozoa from in vitro fertilization (IVF) program patients with male factor of infertility.

Conclusions

NASUM allows us to visualize chromatin, acrosome and its granularity, vacuoles, mitochondria, nuclear membrane pores, microfibrils of the tail, and chromocenter, as well as evaluate the degree of chromatin condensation in native spermatozoa. The microphotographs substantiating the feasibility of observation of the aforementioned subcellular structures in native spermatozoa demonstrated the NASUM technique resolution 0.05 μm. Together with electron microscopy of fixed spermatozoa, NASUM might be used efficiently in male infertility diagnostics. Also, further study of human sperm nucleus chromatin architecture using NASUM will fill the gaps existing at present in the scientific understanding of the non-random arrangement of chromosomes and make the future clinical application of data obtained feasible. We expect that NASUM would assist in improving fertilization rates, embryo quality, blastocyst development rates, rates of implantation and pregnancy, as well as decreasing the incidence of pregnancy loss in assisted reproduction programs using the micromanipulation techniques.