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Flow cytometric evaluation of sperm parameters in relation to fertility potential

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Abstract

Most laboratory methods used to evaluate semen quality have not correlated highly with fertilizing capacity. The discovery of a variety of fluorochromes and compounds conjugated to fluorescent probes has enabled a more widespread analysis of sperm attributes, and in conjunction with the flow cytometer, permit the evaluation of a large number of spermatozoa.

A number of characteristics of sperm integrity, viability and function can be assessed by flow cytometry. The DNA status of spermatozoa has been determined using the metachromatic properties of acridine orange (AO). AO staining, when used in the sperm chromatin structure assay (SCSA®), correlates with fertility in a number of species. DNA fragmentation can also be assessed using the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay, which identifies DNA strand breaks by labeling free 3′-OH termini with modified nucleotides. The status of the sperm acrosome can be determined using fluorescently labeled lectins and LysoTracker Green DND-26, a fluorescent acidotropic probe. Capacitation status has been observed through calcium-mediated changes using chlortetracycline (CTC) or by changes in membrane fluidity monitored by the binding of the fluorescent amphiphilic probe, Merocyanine 540. Fluorescently labeled annexin-V, C6NBD and Ro-09-0198 can also be used to detect changes in membrane phospholipid distribution. Cell viability can be determined using the propensity of propidium iodide (PI), ethidium homodimer-1 (EthD-1) or Yo-Pro-1 to permeate damaged membranes. These are generally more adaptable to clinical flow cytometry than the bisbenzimide membrane impermeable stain, Hoechst 33258, which excites in the ultraviolet range and requires

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UV laser equipment. Mitochondrial function can be determined using rhodamine 123 (R123) and MitoTracker Green FM (MITO) and 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolyl-carbocyanine iodide (JC-1). Flow cytometry is a tool that may be used in the future to monitor many new potential markers of sperm function.

Keywords: Flow cytometry; Spermatozoa; Fertility; Boar sperm; Sperm parameters

1. Introduction

Traditionally, laboratory assays used to assess the quality of a semen sample prior to its use for artificial insemination have involved an evaluation of the percentage of motile spermatozoa (either subjectively or computer-assisted), the percentage of spermatozoa with normal morphology and the concentration in a unit dose. The main aim of all semen analyses is to accurately, objectively, rapidly and inexpensively predict the fertility of a sample. Unfortunately, these commonly used laboratory assays often do not fit these criteria and have not correlated well with the fertilizing capacity of the spermatozoa.

Spermatozoa are intricate cells that require a number of criteria to be met in order to achieve fertilization, for example, the attainment of the hyperactivated state, an intact acrosome, the ability to capacitate, a normal complement of DNA and the ability to bind to the zona pellucida, just to mention a few. The discovery of a variety of fluorochromes and compounds conjugated to fluorescent probes has made possible a more widespread analysis of semen quality at a biochemical, ultrastructural and functional level. Most functional assays using fluorochromes have been developed by staining the spermatozoa with the fluorochrome of interest and examining the cells with fluorescent microscopy to verify the accuracy of the parameter being assessed. However, microscopic analysis only measures a small number of spermatozoa within a population, is time-consuming, can be subjective and generally measures sperm attributes individually. By adapting these assessments for use with a flow cytometer, fluorescent markers can be accurately and rapidly used to measure sperm attributes on a large scale.

Flow cytometry is a system for making measurements on single cells as they travel in suspension one by one past a sensing point. In a few minutes, the flow cytometer can acquire data on all subpopulations within a sample, making it ideal for assessment of heterogenous populations, such as spermatozoa. The adaptation of flow cytometry to sperm assessment began when it was used for measuring their DNA content [1] and its application to semen analysis has gradually increased over the last 10–15 years. Flow cytometry is now applied to semen evaluation of traits such as cell viability, acrosomal integrity, mitochondrial function, capacitation status, membrane fluidity and DNA status. New fluorescent stains and techniques are continuously being developed that have potential application to the flow cytometric evaluation of spermatozoa. In this paper, the main semen parameters that can be analyzed with fluorochromes and adapted for use with a flow cytometer will be reviewed and the relationship of these tests to fertility will be discussed.