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Review article

Computer assisted sperm morphometry in mammals: A review

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ABSTRACT

Computer-assisted sperm morphometry analysis (CASMA or ASMA) systems were developed to reduce the subjectivity of sperm morphology assessement. This review focuses on a complete description of the CASMA technique, including recent developments, factors of variation, results in the different species and possible applications. Techniques to study sperm morphometry include light microscopy, phase-contrast microscopy and, more recently, fluorescence microscopy. Most published studies on sperm morphometry have been centered on the whole sperm heads, although some of them also measured other parts of the sperm structure, such as the nucleus, acrosome, midpiece or flagellum. The independent study of sperm components may be more informative than the traditional assessment of the whole sperm head. Morphometric data provided by the CASMA system may be analyzed using classical statistics although, given the heterogeneity of spermatozoa in the ejaculates, the study of sperm subpopulations using clustering procedures may be more informative. Morphometric results may vary depending on factors intrinsic and extrinsic to the semen donor. Intrinsic factors may include, among others, genetic factors, age and sexual maturity. Extrinsic factors may include those related to the influence of environment on the donor, as well as those related with sample processing and the morphometric analysis itself. Once standardized, this technique may provide relevant information in studies focused on evolutionary biology, sperm formation, sperm quality assessment, including prediction of the potential fertility, semen cryopreservation, or the effect of reprotoxicants.

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1. Introduction

Despite the apparent simplicity of sperm, this specialized cell presents enormous diversity in size and shape in the different species. Given its importance in the reproductive process, a battery of techniques has been developed to study sperm quality and function. Among these techniques, the study of sperm morphology is considered an essential part of the semen analysis that reflects, more than motility, the genetic and DNA characteristics of the cell (Thurston et al., 2001; Murphy et al., 2013). Poor sperm morphology has been associated with low fertility rates (Chandler et al., 1988; Jasko et al., 1990; Barth et al., 1992). However, subjective estimates of sperm morphology suffer from lack of precision, repeatability and accuracy (Hidalgo et al., 2006). For example, quality control programs in human samples have shown inter-technician coefficient of variation ranging from 10 to 80% (Cooper et al., 2002; Alvarez et al., 2005; Filimberti et al., 2013). These variations have limited the usefulness of such morphological evaluation as a quality predictor of semen for reproduction (Ombelet et al., 1995). To reduce the subjectivity of sperm morphology assessment, computer-assisted sperm morphometry analysis systems (CASMA or ASMA, generically CASA) were developed and first appeared on the market in the 1990s (Davis et al., 1992; Kruger et al., 1993).

CASA systems provided a series of objective parameters that have facilitated the standardization of morphological semen evaluation. This review focuses on a complete description of the CASMA technique, including recent developments, factors of variation, results in the different species and possible applications.

2. Sperm morphometry assessment techniques

The earliest attempts at sperm morphometry assessment were based on manual measurement of sperm images in photographs (Schmassmann et al., 1982). The first review of mammalian sperm dimensions is from this period (Cummins and Woodall, 1985). However, the levels of variation between males and within ejaculates, that have been described subsequently, could not be presented in this article. Later application of video image analysis using computers provided the opportunity to evaluate morphometric parameters more critically (Davis and Gravance, 1993; Davis and Katz, 1993; Davis et al., 1994). Using these computer-assisted systems, subtle differences between individuals were revealed which were not previously detected by subjective evaluation (Verstegen et al., 2002).

2.1. CASMA methods

Techniques used to examine sperm morphometry have included electron microscopy, light microscopy, phase-contrast (positive and negative) microscopy and fluorescence microscopy. The use of electron microscopy is limited because it is costly and time consuming, whereas light microscopy, in combination with specific sperm morphometry analysis software, is the technique most frequently used in sperm morphometry assessment.

2.1.1. Conventional CASMA methods

Conventional CASMA methods involve three different steps. The first concerns specimen preparation (specimen dilution/washing, smearing, fixing, staining); the second involves image acquisition by the microscope operating under bright field illumination; and the last one is about the image processing and analysis with specific software (Sancho et al., 1998). Fixatives may be aldehydes (glutaraldehyde, formaldehyde), alcohols (ethanol, methanol) or combinations (ethanol–ether, ethanol–acetic acid). Different staining protocols have been evaluated in different species, from single stains (Harris hematoxylin, Giemsa, toluidine blue, rose Bengal), to combinations (Harris hematoxylin/rose bengal, aniline blue/crystal violet, Mayer's hematoxilyn/eosin, etc.), or commercial kits containing fixatives and stains (Haemacolor, Sperm Blue, Diff-Quik, Papanicolaou) (Tables 1–3). Specimen staining technique and optics are chosen to maximize the image contrast and definition before the input to a computer (Verstegen et al., 2002). Several of these parameters are known to be able to significantly affect the morphometric traits of spermatozoa using conventional CASMA (see Section 3). In consequence, the results of the different studies cannot be directly compared and there is a high dispersion in the morphometric traits of the sperm provided by different works for a given species.