



The Trumorph[®] system: The new univ the morphology of living sperm



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ABSTRACT

Evaluation of sperm morphology is a fundamental component of semen analysis, but its real significance has been obscured by a plethora of techniques that involve fixation and staining procedures that induce artefacts. Here we describe Trumorph[®], a new method for sperm morphology assesment that is based upon examination of wet preparations of living spermatozoa immobilized by a short 60 °C shock using negative phase contrast microscopy. We have observed samples from five animals of the following species: bull, boar, goat and rabbit. In every case, all the components of the sperm head and tail were perfectly defined, including the acrosome and midpiece (in all its length, including cytoplasmic droplets). A range of morphological forms was observed, similar to those found by conventional fixed and stained preparations, but other forms were found, distinguishable only by the optics used. The ease of preparation makes it a robust method applicable for analysis of living unmodified spermatozoa in a range of situations. Subsequent studies on well-characterized samples are required to describe the morphology of potentially fertilizing spermatozoa.

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1. Introduction

Sperm morphology is an essential part of semen analysis (Mortimer and Menkveld, 2001; Yaniz et al., 2015). It is superior to analysis of motility for evaluating the genetic and DNA characteristics of the cell (Meyer and Barth, 2001; Rodríguez-Martínez, 2006; Menkveld et al., 2011). Abnormal sperm may represent disturbances in spermatogenesis that affect not only abnormal sperm but also sperm in the same ejaculate that are otherwise normal or near-normal appearing (Dale et al., 1994; Saacke et al., 2000).

Poor sperm morphology has been correlated with a decrease in fertility rate (Chandler et al., 1988; Jasko et al., 1990; Barth et al., 1992; Irvine et al., 1994; Holroyd et al., 2002), in intrauterine insemination (Van Waart et al., 2001)

in the formation of the pronucleus after in vitro fertilization (Jeulin et al., 1986; Krüger et al., 1986; Tanghe et al., 2002), and in prolificacy (Alm et al., 2006). Species can be classified as heterospermic or homospermic based on their sperm morphology, but sperm abnormalities are strongly associated with semen quality and fertility in both cases (Lee et al., 1996; Thurston et al., 1999). Heterospermic species (human, great apes, horse, bull, camelid, dog, etc.) have semen with high proportions of structurally morphologically abnormal spermatozoa, while homospermic species (rabbit, boar, goat, ram, etc.) exhibit patterns of sperm morphology that are largely consistent, and where it is difficult to observe clear morphological abnormalities, particularly in the head. In both types of species, however, morphometry analysis has revealed that there is important variation within and among individuals (Gravance and Davis, 1995; Sancho et al., 1998; Gago et al., 1999; Soler et al., 2000, 2005; Bellastella et al., 2010; Buendía et al., 2002). These differences were related with different fertility rate both

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in ejaculates of the same individual and among individuals (Söderquist et al., 1991; Oettlé, 1993; Hirai et al., 2001; Soler et al., 2006).

Therefore, morphometric analysis of sperm can provide important information for establishment of semen quality. Moreover, sperm morphology is also used to indicate actions of genotoxic agents (Rubeš et al., 1991; Gago et al., 2000) and other stressful conditions (Rathore, 1968; Barth and Bowman, 1994; Said et al., 2005) on the organism.

The greatest handicap to assessing the sperm morphology is the dependence of morphometric analysis on a plethora of fixation and staining techniques that involve a process of air drying that changes the actual hydration level of the cell and alters cell morphology and dimensions (Katz et al., 1986). The staining process can induce additional artefactual images that results in an assessment that is incongruent with the real characteristics of the cells (Cooper et al., 2007).

Previously, we have validated the use of Trumorph® system for human semen (Soler et al., 2015). The aim of the present work is to use this new technique for the establishment of a universal protocol for analysis of sperm morphology based on the observation of living cells in different animal species.

2. Materials and methods

2.1. Samples

Boar ejaculates were obtained by the gloved-hand method from adult animals two times a week with alternating 3–4 day intervals. The sperm rich fraction was diluted in Duragen medium (Magapor S.L., Zaragoza, Spain). Boars from Hypor España strains were housed in NUTRIVALL farm (Tordesillas, Valladolid, Spain) and sperm doses were sent to our laboratory in an insulated bag at 4 °C the day before the analysis.

Bull semen was collected from adult (4 years) Holstein males, housed in Xenética Fontao (Lugo, Spain). Semen was collected two times a week with alternating 3–4 day intervals. Ejaculates were collected by artificial vagina, diluted with Biladyl® (Minitüb, Tiefenbach, Germany), packaged in 0.25 mL French-straws (25 × 10⁶ spz/straw), and cryo-preserved following a standard protocol in a programmable freezer. All the samples were sent to our laboratory in liquid nitrogen container. Thawing was done in a waterbath at 37 °C for 20 s.

Dog semen was collected by masturbation from mature, trained English bulldogs (12–17 months). Raw samples were immediately used in the laboratory facilities (Reprovalcan, València, Spain).

Blue fox ejaculates were obtained by masturbation during the breeding season (March). Ejaculates were diluted 1:5 (v/v) in Fox-extender (Kubus, Las Rozas, Spain). Samples were obtained by masturbation in different farms from adult animals in the Vaasa region of Finland. Analyses were done immediately in the laboratory of each farm.

Semen collection from male goats was done as described previously (Salvador et al., 2006). Goat semen was collected by artificial vagina from adult Murciano-Granadina males, maintained under uniform nutritional

conditions and regular semen extraction (CITA-IVIA, Segorbe, Spain). Immediately after collection ejaculates were immersed in a warm water bath at 30 °C until their assessment in the laboratory. Semen was diluted in milk-based extender, and sent to the laboratory at room temperature.

Samples from mice were obtained in our laboratory (Proiser R+D, S.L., Paterna, Spain) from the tail of the epididymis, after the sacrifice of the animals by ether anaesthesia. Following exposure, the epididymis was cut and placed into a tube containing 1.5 mL of Ham's medium where it was cut open with scissors to facilitate sperm removal. Aliquots were collected after 5 min.

Rabbit semen was collected by artificial vagina from mature hybrid rabbit bucks housed at a commercial AI centre (INRASAT, Tarragona, Spain). After semen collection and removal of any gel plug, semen was diluted in an extender containing gelatin (1.4 g/100 mL, Speermy, Tortosa, Spain) and transported to the laboratory at 15 °C.

Stallion samples were obtained from Spanish Purebred horses individually housed at the Veterinary Teaching Hospital of the University of Extremadura (Cáceres, Spain). Animals were collected on a regular basis (two collections/week) during the breeding season, using a pre-warmed, lubricated Missouri model artificial vagina with an inline filter to eliminate the gel fraction. The semen was immediately transported to the laboratory for evaluation and processing.

2.2. Procedure

Samples of five individuals from each species were used. Samples were gently mixed with a Vortex before morphological analysis. A 3-μL-drop of the sample was placed on a cleaned glass slide, covered with a 22 mm × 22 mm coverslip, and placed into the Trumorph® system (Proiser R+D, S.L., Paterna, Spain, based on Soler and Blasco, 2013) for a light pressure of 6 kp (Fig. 1). Under these conditions, the depth between slide and coverslip is of ~6 μm, where



Fig. 1. Trumorph® system prototype. See the heating stage with the pressure mechanism.

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