Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 69 (2008) 1129–1138
Theriogenology
www.theriojournal.com

Assessment of motility of ejaculated, liquid-stored boar spermatozoa using computerized instruments

F. Tejerina [a,b], K. Buranaamnuay [a,c], F. Saravia [a], M. Wallgren [a], H. Rodriguez-Martinez [a,*]

[a] *Division of Reproduction, Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences (SLU), Ullsväg 14C, Box 7054, SE-750 07 Uppsala, Sweden*
[b] *Department of Medicine, Surgery, and Veterinary Anatomy, Section of Medicine and Surgery, Faculty of Veterinary Medicine, University of Leon, CP 24071 Leon, Spain*
[c] *Department of Obstetrics, Gynecology, and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, 10330 Bangkok, Thailand*

Received 14 September 2007; received in revised form 19 November 2007; accepted 12 January 2008

Abstract

Visual-motility assessment is a tool used to determine the quality of boar ejaculates. This method is subjective by nature, and consequently, computer-assisted sperm analysis (CASA), with different software designs, has been developed for more objective assessment using conventional image analysis or particle counting. In the present study, we compared the results of sperm analysis using a conventional CASA system (Cell Motion Analyzer, SM-CMA^{TM}), with those using a novel software (QualiSperm^{TM}) and those of visual assessment performed by two operators. Ejaculates were collected weekly from four Swedish Landrace boars for 4 weeks. Each ejaculate was divided into three aliquots of different sperm concentration (300, 125, and 40 million spermatozoa/mL) and stored at ~17 °C for 96 h. Only samples at 40 million spermatozoa/mL concentration were analyzed using both automated systems; for the remaining concentrations, the SM-CMA^{TM} was not used due to its inability to examine higher sperm concentrations. The number of spermatozoa analyzed was highest for the QualiSperm^{TM} (~300–5000 spermatozoa), followed by the SM-CMA^{TM} (~200 spermatozoa), and lastly, by subjective motility evaluation (~100 spermatozoa). There was a time-course decrease in motility of the liquid-stored semen, detectable by either computerized method. Although the percentage of motile spermatozoa measured by the two automated systems correlated well ($r \geq 0.75$), there was disagreement between operators. In conclusion, because of the lower degree of variation, the numbers of spermatozoa analyzed, and the speed of analysis (~1 min per sample), QualiSperm^{TM} appears to be a suitable instrument for routine work, provided it maintains stability and is available at an affordable price.

Keywords: Computer-assisted sperm analysis (CASA); QualiSperm; Boar; Motility; Sperm storage

1. Introduction

Many methods are used to estimate the viability of a semen sample and thus evaluate potential fertility of the male from whom the semen has been collected, in order to eliminate male animals with substandard fertility and also, to avoid the use of substandard samples that may

* Corresponding author at: Division of Reproduction, Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences (SLU), Ullsväg 14C, Box 7054, SE-750 07 Uppsala, Sweden. Tel.: +46 1867 2172; fax: +46 1867 3545.
E-mail address: heriberto.rodriguez@kv.slu.se (H. Rodriguez-Martinez).

0093-691X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2008.01.027

result in lower fertility after artificial insemination (AI) [1]. Most methods of semen assessment *in vitro* measure general characteristics of the spermatozoa (morphology, motility patterns, membrane and organelle integrity, etc.), all essential to fertility, provided these attributes are maintained until the spermatozoa are confronted with the oocyte. More complicated methods (which are therefore mostly used for research purposes, rather than being intended for routine use by the AI industry) attempt mimicking *in vitro* the interactions between the spermatozoa and the female genital tract, the oocyte vestments, and the fertilization process *in vivo*. The outcomes of these explorations relate differently to fertility, depending on the method used, as well as on the number of spermatozoa evaluated at one time. For instance, maintenance of membrane integrity appears to be more closely related to semen fertility than does sperm motility, but only when a large number of spermatozoa are examined (using a fluorescent-activated cell sorter (FACS) or fluorometry [2,3]).

The use of AI in the swine industry has increased exponentially, particularly in Europe, where some countries such as Spain artificially inseminate more than 80% of sows [4]. Most of the semen is still used as liquid, extended and stored at temperatures slightly below room temperature (17–20 °C), although a certain percentage is also used deep-frozen for gene-banking purposes or for export of genetics. Consequently, there is an increasing interest in the diagnostic methods used for semen analysis in AI. However, the battery of diagnostic methods used by the industry is as yet restricted. Under routine conditions, only sperm concentration and sperm motility are assessed, as indicators of sperm production and viability. Sperm morphology is rarely checked, when boars enter the production line, or where pathologies are suspected.

Assessment of sperm motility is usually done by visual evaluation of sperm movement under phase contrast microscopy. This visual evaluation is rapid and cheap, but its accuracy depends on the experience of the operator, which explains the large intra- and inter-assay variation documented in the literature [5–8]. To overcome this problem, different evaluation techniques, such as turbidimetry, laser Doppler spectroscopy, photometric systems, and computer-aided instrumentation, have been developed [9]. Among them, the most successful systems have been grouped into what has been termed ‘‘computer-assisted sperm analysis (CASA)’’ instrumentation. A CASA instrument records, by means of a video camera, the path followed by spermatozoa placed on a wet smear over a certain time interval. The signal picked up by the camera is digitized and the information processed by a computer which reconstructs, for a certain number of frames, each individual spermatozoon’s fixed (most CASA instruments) or summary (Hobson instrument) path trajectory. The different systems locate a certain point in each spermatozoon (often the head) for the signal, and also check for presence of a tail, so that spermatozoa are separated from the neighboring debris, based on size, presence of tail, and speed of translation. The computer is, thereafter, able to use a series of variables considered absolute kinematic parameters, such as sperm velocities and the lateral displacement of the sperm head (LDH). Using these, it recalculates other derived parameters, such as proportions of spermatozoa with various patterns of movement (e.g., linear, nonlinear, circular, or even local, non-translational motility), their degree of linearity, dance, etc. [10]. The CASA examination is considered fast and more ‘‘objective’’, yielding a large amount of data, but analyzing a restricted number of spermatozoa (often of the order of hundreds per sample), which in most instruments are followed for a restricted time. Computer-assisted sperm analysis instruments are, therefore, useful for research purposes, since they provide data the human eye cannot register. Moreover, CASA analysis make it possible to determine the presence of sperm subpopulations coexisting in an ejaculate [11,12], the effect of cryopreservation [13,14], and the appearance of sperm changes such as hyperactivation [15,16].

On the other hand, CASA instruments are not widely used in commercial practice. There are several reasons for this. Firstly, CASA requires a certain degree of calibration and validation [17], and proper programming of species-specific settings [18]. Also, it is costly [9]. It does not always show a relationship between motility and fertility [1,19]. Furthermore, CASA instruments can present errors of measurement, caused by too many spermatozoa in a sample, the crossing of trajectories, or collisions of spermatozoa, all of which produce variation in the results [20]. Moreover, under certain conditions (such as during handling and storage, and particularly, post-cryopreservation), the data gathered show some seemingly paradox results when compared with the initial readings, due to the fact that the system only records the surviving spermatozoa, whose number and motility patterns may differ from those of the original sperm population [10].

Alternative systems have been tried, and newer computerized sperm analysis systems (such as QualiSperm™; Biophos, Pfäffikon, Switzerland; http://www.biophos.com) work on a different principle.