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Differences in boar sperm head shape and dimensions recorded by computer-assisted sperm morphometry are not related to chromatin integrity

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Abstract

Although sperm head shape and relative dimensions are considered reliable indicators of sperm quality, their quantification is most often operator-driven, e.g., subjective. Artificial insemination semen doses from 35 mature stud boars of known fertility and belonging to three breeds and two hybrid breeds (Duroc, Large White, Landrace, respectively, Yorker and Risco) were used in this study. Sperm samples were extended to 100×10^6 cells per mL and 10 μL of the sperm suspension used to made smears which, stained, were examined using phase contrast microscopy interfaced with an automated sperm morphology analyzer (ASMA, ISAS®). Each sperm head was measured for four primary parameters [area (A) μm^2, perimeter (P) μm, length (L) μm, width (W) μm], and four derived parameters of head shape [(L/W), ($4\pi A/P^2$), (($L - W$)/($L + W$)), ($\pi LW/4A$)]. Definition of head size was statistically performed. The threshold for each class was established on the basis of the area values, considering the 25th percentile as small and the 75th percentile as large spermatozoa. In a second step, sperm head shape was determined as normal, elliptic, abnormal (rugose) contour, long or irregular and percentiles set as above to define spermatozoa with normal values for each shape parameter. Significant differences were found among breeds in the size of morphologically normal spermatozoa, which were significantly larger and more elliptic ($P < 0.001$) in the Duroc breed. Sperm chromatin integrity was studied using the SCSA-assay, with significant differences observed in the degree of fragmentation intensity (DFI) although this value was consistently low in all animals studied. The hereby-validated ASMA was able to determine significant differences in sperm shape and dimensions among breeds, which were not accompanied by deviations in chromatin structure neither within nor between fertile AI-boars.

Keywords: Semen analysis; Sperm morphometry; Chromatin structure; Boar

1. Introduction

Owing to its relationship to fertility of the ejaculate/male [1], assessment of sperm morphology is considered a major component of the spermiogramme. Thus, aliquots of semen are fixed, for instance, in a buffered solution of formalin or by the production of

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air-dried smears, to be later examined for the presence and quantity of morphological sperm abnormalities, using light microscopy on wet or stained samples, respectively. The accounting includes the localization of abnormalities present in the different segments of each spermatozoon, i.e., the head (including the acrosome), neck, mid-piece (including the presence of cytoplasmic droplets) and tail. The accounting of all detected abnormalities allows the determination of the magnitude of the affection that originates them (including deviations of spermatogenesis, sperm maturation, etc.), as well as the presence of specific defects [2]. In species such as pigs, largely selected for sperm quality for artificial insemination (AI), a frame of ''normality'' has been described when the frequency of abnormal sperm heads do not surpass 10% and when none of the other parameters (acrosomes, mid-piece, tails, proximal cytoplasmic droplets) do not surpass 5% each or totals 10–15% [3]. Certain abnormalities are considered without relevance for fertility, such as the presence of distal cytoplasmic droplets. However, the presence of specific defects in spermatogenesis (generally hereditary) that result in the typical morphological abnormalities (such as nuclear vacuoles [diadem defect], acrosomal plicae [knobbed defect], decapitated spermatozoa, short or mutilated tails [tail stump], coiled tails [Dag defect] or corkscrewed mid-pieces [corkscrew defect]; seen in pigs are very serious defects, since they interfere with fertilization and can lead to sterility. Sperm morphology determinations, particularly of those defects that appear to have a clear relation to fertility for their uncompensable nature such as pyriform sperm head shape (as an expression of a defective chromatin condensation during spermiogenesis [4]), are only reliable when a large number of spermatozoa per sample are examined, often when >500 sperm heads are accounted for [5]. This imposes a large amount of work on the technicians that usually perform these evaluations manually, making the evaluations time-consuming and solely relying on the expertise, usually acquired by years of experience, of the operators.

Computed-based equipment for morphological analyses is available on the market since more than a decade [6] but most of these instruments and softwares have been inadequate to properly measure sperm head dimensions. Recently, new and more affordable, computer assisted light microscopy systems have been incorporated to the market to objectively measure sperm motility. However, computerized systems to objectively estimate sperm head dimensions are not widely used.

In the present year, a new software has been developed and incorporated to the market, this software is more affordable than other systems and allows an objective measurement of sperm head shape and dimensions. The development of objective protocols for computerized analysis of sperm morphology has been considered a high priority for the investigation of human semen [7], and also is considered a priority for domestic animals [8].

Spermatozoa from otherwise normal individuals differ in shape and dimensions among species [9] and even between individuals [10,11] but the dimensions studied have concerned length (of tails particularly [12]). Differences among spermatozoa from different breeds of pigs have not been explored. Moreover, some defects (as pyriform sperm head shapes, see [13]) have been related to the chromatin status, although proper measurements of chromatin structure have not routinely been linked to sperm shape (see [14]). Boar spermatozoa are characterized by having a highly condensed chromatin where protamines tightly pack and protect the haploid DNA [15]. Optimal sperm DNA packing seems essential for full expression of male fertility potential [16] and handling of spermatozoa including short-term storage has been discussed as to whether it causes deterioration of the nuclear DNA, with varying results (compare [17] with [18]). Cryopreservation has particularly been related to a deterioration of DNA integrity [19] and even related to the ability of boars to sustain sperm freezing [20]. Over the past 25 years, several methods have been designed to determine DNA damage including the Comet assay, TUNEL, the acridine orange test (AOT), the tritium-labelled 3H-actinomycin D (3H-AMD) incorporation assay, the in-situ nick translation (ISNT) assay, the DNA breakage detection-fluorescence in-situ hybridizations (DBD-FISH) and the sperm chromatin structure assay (SCSA) [21]. While most assays are basically microscopy methods, the Tunel and the SCSA assays can advantageously use flow cytometry [22]. Although all methods indicate DNA strand breaks, the SCSA is used most commonly. The SCSA characterizes sperm nuclear chromatin stability based on the increased susceptibility of altered DNA to *in situ* denaturation when exposed to very low pH. Denaturation is detected by staining with the metachromatic dye acridine orange which results in green fluorescence for native DNA and red fluorescence for denatured DNA. The degree of denaturation within each sperm nucleus is quantified by flow cytometry [23]. The SCSA provides data of spermatozoa with or without DNA fragmentation, the extent of DNA fragmentation and the proportion of