Validation of computer-assisted sperm morphology analysis using SpermVision™ Automorph

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Introduction

Worldwide, third generation CASA systems have become an integral part of semen processing in many farm animal AI stations. Sperm motility and concentration is assessed simultaneously with high precision, if the system is used by well-trained personnel (Amann & Katz 2004, Ehlers et al. 2011). Only recently, a new generation of CASA systems offers automated, fast assessment of distinct sperm morphology abnormalities in parallel to motility measurements. Since sperm morphology is an essential standard semen quality parameter with high relevance for fertility, the recognition of morphological abnormal spermatozoa presents a mile-stone in the development of CASA systems. To determine the use of such development in AI laboratories, careful validation of the system by experienced personnel under standardized conditions is required. Commonly, new diagnostic tests or instruments are evaluated for their capability of classifying samples with known characteristics correctly. One criterion in such an evaluation is the sensitivity of the new test. This value describes to what extend the new method identifies abnormal ("positive") samples within a given set of samples. In turn, samples known to be negative for a certain attribute should also be judged negative by the test method. This is indicated by the specificity of the test. In addition, reliability of result is important. Therefore, the positive predictive value indicates how many of the positive tagged samples are truly positive. Similarly, the negative predictive value indicates how many of the negative test results are correct. For morphology analysis with a CASA system the afore-mentioned estimates should be evaluated on two different levels. When the evaluation is based on the classification of the individual spermatozoa, the potential of the software for the detection of morphological normal and abnormal spermatozoa per se is evaluated. This first level evaluation must be passed with a reasonable result before further evaluation based on individual samples becomes meaningful. On the base of single sperm cells these estimates give an indication about the general potential of the software to detect abnormal spermatozoa. Based on semen samples the potential for implementation in daily quality control standards can be judged.

The aim of the present study was to assess the suitability of the CASA system Sperm Vision™ Automorph (Minitube, Verona, USA) to assess distinct sperm morphological abnormalities, i.e. proximal cytoplasmic droplets, distal cytoplasmic droplets and bent tails in boar semen samples. The potential of the system for simultaneous assessment of sperm motility, concentration and morphology was studied.

Material & Methods

Evaluation of sperm morphology assessment

The Sperm Vision™ Automorph system was equipped with an Olympus BX41 microscope, 20x objective, 0.63 tv-adapter and camera with a resolution of 900 x 900 pixel. A total of 210 diluted boar semen samples were analysed. The samples were shipped overnight from different AI studs to the University of Veterinary Medicine in Hannover and used for the experiment 24 to 48 hours after collection. Prior to the measurements an aliquot of the samples was incubated for 15 minutes at 38°C in a water bath to activate motility of the spermatozoa. Samples were agitated after incubation to ensure homogeneous distribution of the cells and 1.8 µl were filled into a chamber of a 4-chamber slide (Leja, Nieuw Vennep, The Netherlands) with a chamber depth of 10 µm. Four analysis fields were used for evaluation of sperm morphology. An automated stage assured that the same positions in the central axis within each chamber were assessed.

In parallel, an aliquot of the incubated sperm suspension was fixed in a formol citrate solution according to Hancock (1957). A total of 200 spermatozoa were judged for cytoplasmic droplets (proximal and distal) and bent tails in a phase contrast microscope (100x objective, oil immersion) by a well experienced lab technician.

To assess the general quality of the system the results from 48 of 210 samples were reviewed independently by two investigators being experienced in the assessment of sperm morphology. They recorded whether all defect spermatozoa visible on the screen were tagged by Sperm Vision™ Automorph and whether the classification of tagged spermatozoa was correct. A sample based evaluation of was done on all 210 samples.

Comparison of motility between 10 and 20 µm Leja chambers

Since a Leja chamber of 10 µm depth was used for morphology evaluation instead of the so far commonly used 20 µm chamber, motility parameters were compared between the two chamber types in a separate trial. Samples were incubated for 15 minutes at 38°C in a water bath and ten analysis fields per sample assessed in each chamber.

Comparison of sperm concentration between 10 µm Leja chamber and Thoma 'neu' haemocytometer

In a third experiment the sperm concentration of 25 samples was assessed with Sperm Vision™ Automorph using a Leja chamber with 10 µm depth and in parallel using a Thoma 'neu' chamber. Whole ejaculates or fractions of ejaculates were mixed in a ratio of 1 + 9 with BTS extender and incubated for 15 minutes in a water bath at 38°C. Assessments were based on ten fields per sample. Subsequently sperm concentration was determined with both methods.

Results & Discussion

Sperm morphology

Based on the visual assessment of 12,568 individual spermatozoa from 48 samples the software Sperm Vision™ Automorph detected 83 % of spermatozoa that had a proximal droplet, distal droplet or a bent tail (sensitivity). About 96 % of the intact spermatozoa were recognized as intact (specificity). In 89 % of the cases when Sperm Vision™ Automorph addressed a sperm cell as defect the system was right (positive predictive value). In turn, 94 %

of spermatozoa tagged as intact were correctly identified as being intact (negative predictive value).

In a sample based evaluation, 210 semen samples were judged according to quality standards from the German Umbrella Organisation for Pig Production (ZDS, 2005). According to this standard, samples would fail quality control either by exceeding the threshold value for total amount of abnormal spermatozoa (25 %) or due to elevated percentages (> 15 %) of spermatozoa with solely cytoplasmic droplet or a bent tail. On average 307 ± 104 spermatozoa per sample were analyzed for morphology with Sperm Vision™ Automorph. The CASA system detected 83 % of the samples that exceeded more than 20 % of spermatozoa with either a proximal droplet, distal droplet or a bent tail. We used a lower threshold in our evaluation compared to the German AI standard as we focussed only on a subset of abnormalities and others might be also present in the samples. In the subcategories of samples that contained more than 15 % spermatozoa with a bent tail or with cytoplasmic droplets, Sperm Vision™ Automorph identified 58 % (18 of 31) and 89 % (93 of 104) of the samples, respectively. Although the sensitivity for semen samples with elevated numbers for spermatozoa with bent tail was relatively low as compared to cytoplasmic droplets, all of the 13 samples judged false negative for this criterion would have been correctly rejected for production due to above-threshold values of the total number of abnormal spermatozoa.

Sperm concentration and motility

The comparison of motility parameters between measurements using chambers with 20 µm and 10 µm depths showed no differences in total and progressive motility ($p > 0.05$). However, assessments in chambers with 10 µm depth resulted in higher values for descriptors of sperm velocity ($p < 0.05$). This might to some extend be attributed to the narrower chamber geometry which limits three dimensional movement patterns of the spermatozoa, thereby facilitating that spermatozoa cover a longer distance per time in the narrow chamber.

The estimates of sperm concentration with Sperm Vision Automorph™ and Thoma 'neu' chamber showed a good congruency. No significant difference between the outcomes of both methods could be observed ($p > 0.05$). However, samples with an original concentration of more than 600 x 10^6 sperm/ ml tended to be underestimated by Sperm Vision™ Automorph. It was apparent, that viscosity of such samples at a dilution ratio of 1 + 9 impaired optimal filling of the Leja chamber with 10 µm depth. A higher dilution ratio for very dense samples may diminish such influences.

Conclusion

The CASA system Sperm Vision™ Automorph combines the evaluation of sperm concentration, motility and relevant morphology traits in one step procedure. The detection algorithm allows reliable detection of most of the spermatozoa with proximal cytoplasmic droplets, distal cytoplasmic droplets and bent tails. When applying classification criteria of German pig AI standards, the majority of samples with a considerable degree of abnormal spermatozoa were classified correctly by the system. Therefore, Sperm Vision™ Automorph offers the opportunity to implement continuous assessment of sperm morphology in semen production and quality control programs of boar AI studs.

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