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Original Research

Computer-Assisted Sperm Analysis of Head Morphometry and Kinematic Parameters in Warmblood Stallions Spermatozoa

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ABSTRACT

The present study was conducted to determine the seminal characteristics of warmblooded stallions and to assess a relationship between motility parameters of spermatozoa and sperm head dimensions using computer-assisted sperm analysis. In total, 35 ejaculates were collected during the breeding season 2016 from 10 clinically healthy and fertile warmblooded stallions (age 3–22 years). The volume of ejaculate (mL), sperm concentration (M/mL), motility of sperm with kinematic parameters, and sperm morphometry were evaluated. The motility characteristics, sperm concentration, and morphometric parameters of sperm were determined objectively using Sperm Class Analyzer (SCA, MICROPTIC SL). The overall mean ± standard deviation values for volume of ejaculate, concentration, total motility (MOT), progressive motility, curvilinear velocity, straight-line velocity (VSL), average path velocity (VAP), straightness, linearity, amplitude of lateral head displacement, beat-cross frequency, elongation, and area were 50.29 ± 29.88 mL, 210.64 ± 90.98 × 10^6/mL, 88.68 ± 10.8%, 36.64 ± 15.36%, 63.23 ± 15.98 μm/s, 31.02 ± 9.39 μm/s, 47.53 ± 14.32 μm/s, 65.24 ± 5.72%, 48.8 ± 8.34%, 2.68 ± 0.41 μm, 7.29 ± 0.84 Hz, 36.2 ± 3.59%, 15.49 ± 1.54 μm^2, respectively. There were significant differences among stallions in all evaluated parameters of sperm ($P < .05$). There were significant positive correlations between area and velocity parameters (VSL, VAP, and wobble coefficient [WOB]). The elongation positively correlated with MOT. More elongated sperms demonstrated a lower frequency of curvilinear path intersections with the average path.

1. Introduction

Artificial insemination with cooled-shipped semen is the most commonly used method in horse reproduction [1]. However, the efficiency of cooled stallion semen remains limited, as indicated by suboptimal pregnancy rates achieved during artificial insemination with cooled semen (45%–70%) [2,3]. Stallion fertility holds an important place in this area as it is an economically important trait supported by a complex environment and a genetic background [4]. The evaluation of sperm quality is useful in predicting the fertility of stallions and is of a great importance in maximizing the reproductive efficiency in programs of assisted reproduction [5,6]. For the evaluation of semen quality, the most important parameters are the concentration, motility, and morphology of spermatozoa [7,8]. The evaluation of motility is one of the most used parameters for the evaluation of sperm quality [9,10].

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Morphology is also considered to be a reliable indicator of fertility, and the fertilization capacity of an individual positively correlates with the percentage of sperm cells with normal morphological structure [11,12]. There is a higher frequency of morphometric sperm defects at stallions with low fertility [13]. Fertilizing ability of spermatozoa depends on their shapes and sizes, which affect the course of the acrosomal reaction and sperm penetration of the ovum. Some authors mention a correlation between the morphometric characteristics of spermatozoa and sperm concentration in the ejaculates of stallions [14]. It is also possible that sperm concentration in an ejaculate influences the sperm shape and dimensions, and therefore influencing the motility characteristics and fertilizing ability [15,16]. It has been found that sperm dimensions are highly variable in males belonging to different animal species, breeds within a species, and within individuals of one population [17]. The variation among stallions was generally greater than the variation of ejaculates of one stallion [18]. Some studies have indicated an existence of an association between the sperm head morphometry and stallion fertility. The increase in morphological abnormalities of sperm heads was correlated with a reduction of pregnancy rates [19] and also negatively correlated with the fertility of stallions [20]. Spermatozoa head dimensions of males with decreased fertility differ from the head dimensions of spermatozoa of highly fertile stallions [21,22].

A number of studies indicate that variation in sperm head morphology may be a sensitive biomarker of abnormal chromatin structure and thus of fertility [23,24]. Although sperm head shapes and relative dimensions are considered reliable indicators of a sperm quality, their quantification is the most subjective [25]. However, subjective estimates of a sperm morphology suffer from lack of precision, repeatability, and accuracy [26,27]. The introduction of automated computer-assisted sperm analysis (CASA) systems attempted to overcome the problem of the subjectivity of visual-based assessment methods [25,27–29]. Computer-assisted sperm analysis allows the objective determination of ejaculate parameters (motility parameters, sperm concentration, newest models also have a morphology module) [27]. Computer-assisted sperm analysis has an important and increasing role for assurance of semen quality. Most of today's CASA systems offer the advantage of reduced bias compared with visual (subjective) evaluations. More, they provide outcome measures which could serve as a reliable basis for further semen samples processing used for distribution, and provide an assurance that the semen meets an insemination dose quality requirements [30].

Computer-assisted sperm analysis is the only objective method for motility, progressivity, and hyperactivity sperm sample analysis, and therefore provides an information on fertility potential of the ejaculate. The CASA is based on the capture of successive microscopic images which are then digitalized. The motile spermatozoa observed in these images are subsequently identified in the successive images, thus allowing for the establishment of their trajectories. Finally, the obtained trajectories are mathematically processed, which allows the definition of these trajectories in a numerical form. The results of this processing are reflected in a series of parameters, which precisely define the exact movement for each individual spermatozoon [31]. In the 1990s, the automated sperm morphometry analysis (ASMA) systems were introduced. Although this technology was originally designed for a human sperm, it has been progressively adapted to some animal species [25,28,29,32]. Automated sperm morphometry analysis systems have improved the assessment of sperm morphology with accurate and repeatable measurements of sperm head dimensions of different species including stallions [18,33–35]. Automated sperm morphometry analysis systems provided a series of objective parameters that have facilitated the standardization of morphological semen evaluation [36]. In addition, these automated systems can be used to prove subtle differences in the sperm head dimensions between fertile and subfertile stallions [29]. Currently, the precision of ASMA systems depends on the standardization of analytical variables, on the appropriate sample preparation (washing, fixation, and staining) and on the proper microscopic image analysis [37]. Errors are often the results of differences between ASMA systems, therefore, appropriate settings of CASA play a significant role in the accuracy of the outcomes [38].

The present study was conducted to determine the seminal characteristics of warmblooded stallions and assessment of the relationship between motility, parameters of spermatozoa, and sperm head dimensions including CASA. Sperm Class Analyzer (SCA) was used for objective determination of sperm concentration, motility, and velocity of sperm and morphometric characteristics.

2. Material and Methods

2.1. Stallions

Semen samples were obtained from 10 clinically healthy and fertile warmblooded stallions (age 3–22 years). These stallions were owned by ZH Písek and they have been used in the breeding season 2016 in artificial insemination with fresh semen. The stallions were stabled in the same conditions. All stallions were stabled in boxes at least 5 × 5 m. The feed dose of stallions contained 11 kg of hay, 1 kg of straw, 3 kg of oats, and mineral and vitamin supplementation (PREMIN for breeding stallions, STARFIT granulated feed). The exercise was an integral part of management, 1–4 hours a day, 6 days a week. The exercise was provided in a combination of turn-out in paddock, riding, and mechanical walker.

2.2. Semen Collection and Evaluation

Thirty-five ejaculates were collected in total during the breeding season 2016 (April–July) from stallions (April eight ejaculates, May nine ejaculates, June 10 ejaculates and July eight ejaculates; three, four, three, four, two, four, four, three, four, and four ejaculates from stallion S1, S2, S3, S4, S5, S6, S7, S8, S9, and S10, respectively). The volume of ejaculate (mL), sperm concentration (M/mL), motility of sperm with kinematic parameters, and sperm morphometry were evaluated. The evaluation of fertility was based on the data from the whole breeding season. It is