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PHYSICAL PROPERTIES OF STALLION SEMEN IN RELATION TO SOME QUALITATIVE AND QUANTITATIVE CHARACTERISTICS

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Abstract

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A growing number of diagnostic methods lead to a perfection of the semen evaluation of stud animals. An elementary semen examination and a careful health assessment have always been the most important part of a stud stallion evaluation. The aim of this experiment was to find the relationship between some qualitative and quantitative semen characteristics and the electrical conductivity and dynamic viscosity of semen. The possible use of the semen physical properties as an additional measure of the semen evaluation in stallions was considered. The electrical conductivity ranged between 0.32 $S.m^{-1}$ and 0.55 $S.m^{-1}$ with the total mean value 0.38 ± 0.04 $S.m^{-1}$. The dynamic viscosity varied between 88.0 $mPa.s^{-1}$ and 189.0 $mPa.s^{-1}$ and the mean value was 130.19 ± 37.3 $mPa.s^{-1}$. The analysis revealed a highly significant positive linear correlation between the electrical conductivity of semen and the sperm concentration in the ejaculate ($r_p = 0.69$; $P \leq 0.01$). Significant negative correlations were found between the electrical conductivity and the ejaculate volume ($r_p = -0.38$;$P \leq 0.05$). The electrical conductivity was positively linearly correlated to the sperm motility ($r_p = 0.42$; $P \leq 0.05$). The dynamic viscosity of the semen was negatively correlated to the hydrogen ion concentration only ($r_p = -0.46$; $P \leq 0.05$).

ejaculate, stud stallion, electrical conductivity, viscosity

1 INTRODUCTION

An essential prerequisite for a successful artificial insemination (AI) in horse breeding is a selection of premium stud stallions that produce an adequate amount of a good quality semen. In order to secure these requirements, a number of semen evaluation methods were created. The elementary methods are essential for the semen evaluation with respect to its freezing and subsequent utilisation in the artificial insemination. For the elementary parameters we evaluate both fresh semen immediately after its collection and the frozen semen intended for AI (Kliment *et al.*, 1989).

A growing number of diagnostic methods lead to a perfection of the semen evaluation of stud animals (Brito, 2007; Ball, 2008). An elementary semen examination (the ejaculate volume, sperm concentration, total sperm count, sperm motility etc.) and a careful health assessment of stallions have always been the most important part of a stud stallion evaluation (Angela *et al.*, 2002; Quientero-Moreno *et al.*, 2002; Morrell *et al.*, 2008). The electric charge of a sperm cell is associated with the electric potential. The stability or variability of the sperm electric potential are affected by numerous factors. The sperm electric charge gradually decreases with semen aging and with growing concentration of lactic acid. The loss of the electric charge diminishes the sperm motility and leads to a formation of agglutinations. Viscosity is a typical characteristic of a real fluid. It arises from the shear stress between the layers of the fluid flow

(Máchal *et al.*, 1977; Máchal and Křivánek, 2002). The issue of the electrical conductivity and dynamic viscosity were rarely studied (Garnica *et al.*, 1993; Bravo *et al.*, 2000; Andrade-Rocha, 2005; Esfandiari *et al.*, 2006). The possible use of the semen physical properties as an additional measure in the semen evaluation in stallions can be considered. The aim of this experiment was to determine relationships between some qualitative and quantitative semen characteristics and the electrical conductivity and dynamic viscosity of semen.

2 MATERIAL AND METHODS

2.1 Biological material and experimental environment

The experiment employed 11 clinically healthy stud stallions from the reproduction centre of Tlumačov Regional Stud s.e., where they took part in the breeding programme of the Czech Warmblood horse. The stallions were of a different breed and age (ranging from 10 to 26 years). Their semen was collected on regular bases and subsequently processed into short-term-preserved insemination doses. The stallions were housed under invariable conditions over the whole period of the experiment. The feed ration consisted of forage (grass hay and alfalfa-grass haylage) and concentrate (oat and granulated mixture enriched with minerals and vitamins). The quantity of concentrate varied according to the stallions' physical activity.

2.2 Semen collection

A total number of 95 samples of ejaculate were collected over the experimental period. The samples were analysed (following the methodology) for the sperm electrical conductivity, dynamic viscosity and for the basic qualitative and quantitative characteristics. The semen from the stallions was collected on a phantom mare into an artificial vagina of Missouri type (Minitüb comp.). The interior of the artificial vagina was lined with a disposable PVC sheath replaced at each collection. The artificial vagina was warmed up to 40 °C–42 °C before the collection. Inside it was lubricated with White Vaseline to ensure natural lubricity. Immediately after the collection, the semen was examined in the laboratory. The major quantitative and qualitative characteristics were evaluated and the physical properties measured.

2.3 Qualitative and quantitative examination of the ejaculate

The semen volume was measured in a calibration beaker, pre-warmed up to the temperature of 39 °C ± 1 °C. The volume was determined with the accuracy of ± 5 cm^3. The hydrogen ion concentration in semen was determined by CyberScan Ion 510 with a probe Polilyte Lab. Alongside the probe a temperature sensor was applied, which monitored the temperature and thus the pH value was automatically compensated. The sperm motility was determined subjectively by a microscopic observation. A drop of freshly collected semen was pipetted on a microscope slide, pre-warmed up to 39 °C ± 1 °C, and then a thin cover slip was laid over it. The sperm motility was assessed at 200-300-fold magnification. A percentage of progressively motile sperm cells was subjectively estimated in three fields of view at minimum. Beside the desirable progressive motility (straight-forward motion), other forms of motion were noted (the non-progressive motility – rolling, moving in circles, on the spot, backwards), and were considered defective. Another unwanted phenomenon was a sperm clustering, so called agglutination. A sperm concentration in ejaculate was determined hematocytometrically, by counting cells in Bürker chambers.

2.4 Determination of the semen electrical conductivity

The electrical conductivity of the semen was assessed according to the methodology by Máchal and Křivánek (2002). It was measured by bioimpedance spectroscopy analyser, which allows measuring electrical conductivity as a function of frequency in the range of 10 Hz to 100 kHz. Thus it was feasible to determine a possible phase shift of an electric signal in a sample and a capacity part of conductivity. In order to avoid an error caused by transitional impedance, a 4-plate conductivity probe was used.

A formula for the conductivity probe:

$$\sigma = K \times G,$$

where K (m^{-1}) is a cell constant of the electrode, determined by measuring a calibration solution of known conductivity (20 % solution NaCl with conductivity 1961 $S.m^{-1}$). The principle of a 4-point probe measuring is in passing a current of known frequency through two outer probes and measuring the voltage through the inner probes. In our experiment the probe was equipped with the fifth, earth electrode with zero potential. The inner electrodes, which measure voltage in the sample, were connected to an alternating electronic voltmeter with input electric resistance of about hundreds of MΩ. This eliminated the effect of transition resistance of a sample-electrode contact. The samples were analysed for the frequency dependence of the electrical conductivity by the inner electronics of Z01 device, which automatically alters frequency at 10s steps. Most of the commercially available devices operate by direct current and voltage which is not suitable for fluids and biological materials because electrolysis occurs at the probe contacts during measuring and the transition resistance increases.

2.5 Determination of the semen dynamic viscosity

The dynamic viscosity of the stallion semen was determined in the rotational viscosimeter TR9.

In this type of viscosimeter the sample is dragged between two defined surfaces, one of which rotates (disc). The torque required to rotate a disk in a fluid is a function of the viscosity of that fluid and is assessed at a known speed, which in our case ranged between 0.3 and 200 rpm.

2.6 Statistical analysis

The obtained values were statistically analysed using STATISTICA 9.0. Comparison of individual populations was done with the use of Scheffe's test. Linear dependences were counted using Pearson's correlation coefficient.

3 RESULTS

The results were based on a total of 95 samples of fresh semen collected from 11 clinically healthy stud stallions which were used in the breeding programme of the Czech Warmblood horse.

3.1 Qualitative and quantitative semen characteristics

The results of the laboratory analyses of semen are presented in Tab. I. The ejaculate volume ranged from 10 cm^3 to 160 cm^3, with the total mean value 50.39 ± 25.5 cm^3. The sperm motility ranged between 50% and 85%, with the total mean value 68.4 ± 7.92%. The hydrogen ion concentration varied between 6.64 and 7.43, with the mean value 7.04 ± 0.13. The sperm concentration ranged between 0.02 $.10^9 \cdot cm^{-3}$ and 0.4 $.10^9 \cdot cm^{-3}$ with the mean value 0.11 $.10^9$ ± 0.08 $.10^9 \cdot cm^{-3}$. The total sperm count in the ejaculate ranged between 0.49 $.10^9$ and 15.60 $.10^9$ and the mean value was 4.65 $.10^9$ ± 2.87 $.10^9$ spermatozoa. The total motile sperm count varied between 0.29 $.10^9$ and 10.92 $.10^9$ with the mean of 3.21 $.10^9$ ± 2.05 $.10^9$ motile spermatozoa.

3.2 Physical semen properties

The mean values of the electrical conductivity and dynamic viscosity of the stallion semen are presented in Tab. I. The electrical conductivity ranged between 0.32 $S.m^{-1}$ and 0.55 $S.m^{-1}$ with the total mean value 0.38 ± 0.04 $S.m^{-1}$. The dynamic viscosity varied between 88 $mPa.s^{-1}$ and 189 $mPa.s^{-1}$ and the mean value was 130.19 ± 37.3 $mPa.s^{-1}$.

3.3 The relationships between physical properties and qualitative and quantitative semen characteristics

The relationships between qualitative and quantitative characteristics of semen and its physical properties were described by Pearson correlation coefficients and are presented in Table II. The analyses revealed a highly significant positive linear correlation between the electrical conductivity of semen and the sperm concentration in ejaculate (r_p = 0.69; P ≤ 0.01). Significant negative correlations were found between the electrical conductivity and the volume of ejaculate (r_p = –0.38; P ≤ 0.05). The electrical conductivity was positively linearly correlated to the sperm motility (r_p = 0.42; P ≤ 0.05). The dynamic viscosity of semen was negatively correlated to the hydrogen ion concentration only (r_p = –0.46; P ≤ 0.05).

4 DISCUSSION

The reproductive management is one of the major issues in horse breeding industry today. An elementary semen examination and a careful health

I: *Mean values of the qualitative, quantitative and physical characteristics of semen*

	volume [cm^3]	motility [%]	pH	sperm concentration [$10^9 \cdot cm^{-3}$]	TSC [$\cdot 10^9$]	TMSP [$\cdot 10^9$]	conductivity [$S \cdot m^{-1}$]	viscosity [$mPa \cdot s^{-1}$]
x	50.39	68.4	7.04	0.11	4.65	3.21	0.38	130.19
s_x	25.50	7.92	0.13	0.08	2.87	2.05	0.04	37.30

TSC – total sperm count
TMSC – total motile sperm count

II: *Phenotypic correlations between the observed semen characteristics*

	volume	motility	pH	concentration	TSC	TMSC	conductivity	viscosity
volume		–0.05	0.13	–0.42$^+$	0.35	0.34	–0.38$^+$	0.14
motility			–0.03	0.44$^+$	0.16	0.33	0.42$^+$	–0.38
pH				–0.34	–0.19	–0.19	–0.21	–0.46$^+$
concentration					0.50^{++}	0.56^{++}	0.69^{++}	–0.14
TSC						0.98^{++}	0.28	0.15
TMSC							0.34	0.09
conductivity								–0.24
viscosity								

TSC – total sperm count; TMSC – total motile sperm count
Level of significance: + P ≤ 0.05; ++ P ≤ 0.01

assessment of a stallion have been, and still continue to be, the most important part of a stud stallion evaluation. A number of authors looked into an elementary semen examination (Kliment, 1989; Parlevliet *et al.*, 1991; Angela *et al.*, 2002; Sieme *et al.*, 2003; Morrell *et al.*, 2008). The sperm cell motility is a semen characteristic most frequently described in literature. It is commonly assessed by the standardised method of CASA (computer-assisted semen analysis). The CASA combines a microscope, video camera and computer software to determine the sperm motility. The mean values of the sperm motility determined by CASA were considerably higher (88.9%) in research of Quientero-Moreno *et al.* (2002) than our values (68.4 ± 5 %). The sperm concentration is also a common semen characteristic often described in literature. Gamboa *et al.* (2006) claimed that the sperm concentration was affected by season. In their study they observed that the sperm concentration dropped from 228 $.10^6 \cdot ml^{-1}$ in September to 210 $.10^6 \cdot ml^{-1}$ in May and further to 164 $.10^6 \cdot ml^{-1}$ in July. The mean concentration of sperm cells in our experiment was 0.11 $.10^9$ ± 0.08 $.10^9 \cdot cm^{-3}$. Our value was similar to that presented by Sieme *et al.* (2003), which was 0.15 $.10^9 \cdot cm^{-3}$.

Not a great number of scientists studied physical properties of semen (Garnica *et al.*, 1993; Bravo *et al.*, 2000; Máchal and Křivánek, 2002; Andrade-Rocha, 2005; Esfandiari *et al.*, 2006). Máchal and Křivánek (2000) determined the electrical conductivity of a bull semen (0.84 $S.m^{-1}$) in their study. In their subsequent experiment the authors evaluated the physical properties of a rooster semen. Its electrical conductivity ranged from 0.84 $S.m^{-1}$ to 1.02 $S.m^{-1}$ (Máchal and Křivánek, 2002). In our experiment, the electrical conductivity of a stallion semen was lower (0.38 ± 0.04 $S.m^{-1}$) than those of the bull and rooster semen.

We determined the dynamic viscosity of the stallion semen (130.19 ± 37.3 $mPa.s^{-1}$). Hunter *et al.* (2011) suggested that the semen viscosity was closely related to the motile sperm percentage and the type of sperm motion. However, we have not found a significant correlation between viscosity and motility to support their theory.

CONCLUSIONS

Since not much research has been done in the field of physical properties of stallion semen, the comparison of our results with others was somewhat restricted. Our experiment offered an insight into the issue of a possible assessment of semen qualitative and quantitative characteristics, based on the determination of semen physical properties. However, the results of this pilot study need to be repeated and re-confirmed before any serious implications and recommendations for this alternative examination can be made.

SUMMARY

The aim of this study was to determine the relationship between some qualitative and quantitative semen characteristics and physical semen properties (electrical conductivity and dynamic viscosity) and to consider its use as an additional measure in semen evaluation in stallions.

The experiment included 95 semen samples of 11 clinically healthy stud stallions which took part in the breeding programme of the Czech Warmblood horse. Their semen was evaluated immediately after the collection. The semen volume was measured in a calibration beaker. The hydrogen ion concentration in semen was determined by CyberScan Ion 510 with probe Polilyte Lab. The sperm motility was determined subjectively by a microscopic observation. The sperm concentration in the ejaculate was determined hematocytometrically, by counting cells in Bürker chambers. The electrical conductivity of semen was assessed according to the methodology by Máchal and Křivánek (2002). It was measured by bioimpedance spectroscopy analyser, which allows measuring electrical conductivity as a function of frequency in the range of 10 Hz to 100 kHz. The dynamic viscosity of the stallion semen was determined in the rotational viscosimeter TR9. The electrical conductivity ranged between 0.32 $S.m^{-1}$ and 0.55 $S.m^{-1}$ with the total mean value 0.38 ± 0.04 $S.m^{-1}$. The dynamic viscosity varied between 88.0 $mPa.s^{-1}$ and 189.0 $mPa.s^{-1}$ and the mean value was 130.19 ± 37.3 $mPa.s^{-1}$. The analyses revealed a highly significant positive linear correlation between the electrical conductivity of semen and the sperm concentration in the ejaculate ($r_p = 0.69$; $P \leq 0.01$). Significant negative correlations were found between the electrical conductivity and the ejaculate volume ($r_p = -0.38$; $P \leq 0.05$). The electrical conductivity was positively linearly correlated to the sperm motility ($r_p = 0.42$; $P \leq 0.05$). The dynamic viscosity of the semen was negatively correlated to the hydrogen ion concentration only ($r_p = -0.46$; $P \leq 0.05$).

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