JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

BIOCHEMICAL PARAMETERS OF SEMINAL PLASMA AFFECT MOTILITY TRAITS OF STALLION SPERMATOZOA

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doi: 10.15414/jmbfs.2018.7.5.516-518

ARTICLE INFO

Received 15. 12. 2017
Revised 18. 1. 2018
Accepted 6. 2. 2018
Published 1. 4. 2018

Regular article

OPEN ACCESS

ABSTRACT

The aim of this study was to observe the effect of selected biochemical parameters of seminal plasma on motility parameters of stallion spermatozoa. Fresh semen was collected from 12 breeding stallions at National Stud Farm in Topoľčianky in age of 5–26 years. Semen was divided in two aliquots. One part of ejaculate was centrifuged (15 minutes at 1000 x g) and the supernatant was separated. The supernatant was frozen and stored at −18°C until analysis. Other part of ejaculate was diluted with physiological solution in ratio 1:3 and stored at 7°C before CASA analysis. Later ejaculates were cultivated at temperature 38°C and total motility, progressive motility and velocity curved line were determined using CASA method at time intervals 0, 1, 2, 3, 6, 24 hours. Assessment of biochemical parameters was realized by Randox RX Monza analyzer. The correlation analysis showed a statistically significant ($P<0.05$) effect of Mg and Ca on spermatozoa motility. Further, positive significant ($P<0.05$) correlation was found between Mg and progressive motility. No correlation was found between biochemical parameters of stallion seminal plasma and velocity curved line (VCL). Results indicate that optimal concentration of calcium and magnesium in seminal plasma has beneficial effect on motility parameters of stallion spermatozoa. This phenomenon might be on focus, to improve the percentage of pregnant mares.

Keywords: spermatozoa, CASA, seminal plasma, biochemical composition, stallion

INTRODUCTION

Horses are unique among domestic animals because their selection for breeding is based on genetics, sport performance and fertility. With increased trend of reproductive technologies and transporting of semen is necessary to monitor stallion fertility **(Novak *et al.*, 2010)**. Application of assisted reproductive technologies in stallion reproduction, such as artificial insemination, increased its tendency in the last 25 years. A lot of factors can effect successful artificial insemination **(Waheed *et al.*, 2011)**. The primary factors affecting fertility using artificial insemination (AI) are number and quality of spermatozoa in the insemination dose, timing and frequency of insemination, seminal extenders, seminal handling and equipment **(Pickett and Shiner, 1994)**. Stallion ejaculates contain variable portion of motile and normal morphology spermatozoa along with spermatozoa unable of fertilization **(Krakowski *et al.*, 2015)**.
Seminal plasma stimulates spermatozoa motility during the ejaculation and takes care about protection against negative influence of environmental factors **(Błaszczyk *et al.*, 2013)**. However, during *in vitro* storage spermatozoa are not protected by seminal plasma. Addition of semen extender to dilute toxic elements in seminal plasma is frequent **(Morrel, 2011)**. The seminal fluid is composed of secretions of different glands of the male reproductive system such as the seminal vesicles, bulbourethral gland and prostate. Its biochemical constitution variously affects many sperm functions. Seminal plasma not only activates the spermatozoa, but also plays role of transport medium to carry the spermatozoa into the female reproductive tract **(Krakowski *et al.*, 2015)**. Further seminal plasma can affect spermatozoa morphology, motility, acrosome reaction and fertility. It is known that the seminal plasma include substances that support the sperm cells **(Asadpour, 2012)**. In seminal plasma is energy source often present in form of fructose, further contains proteins and various ions such as magnesium, calcium, zinc **(Morrel, 2011)**. Many components, have been characterised which have been associated with either positive or negative effects on reproductive system as well as on fertility **(Maxwell *et al.*, 2007, Halenár *et al.*, 2015, Halenár *et al.*, 2017)**. On the other hand, many environmental contaminants can also significantly affected male reproductive system **(Jambor *et al.*, 2016, 2017)**.
The aim of this study was to observe the effect of selected biochemical parameters of seminal plasma on motility parameters of stallion spermatozoa.

MATERIAL AND METHODS

Semen collection and processing

Fresh semen was collected from 12 breeding stallions at National Stud Farm in Topoľčianky in age of 5–26 years composed of following breeds: Hucul, Lipican, Arab thoroughbred, Selle francaise, Shagya-arab, Holsteiner. Horses were stabled in boxes with straw bedding and fed with oat and hay. Movement of stallions was provided by carousel, where they walked one hour a day as well as by individual field, where they stayed four hours a day. Semen was collected with lubricated pre-warmed artificial vagina (Colorado model, Minitüb, Landshut, Germany) after stimulation of stallion by mare situated close to the breeding phantom. Semen was divided in two aliquots. Part of ejaculate was after collection centrifuged (15 minutes at 1000 x g) and the supernatant was separated. The supernatant was frozen and stored at −18°C until analyses. Other part of ejaculate was diluted with physiological solution (NaCl 0.9% Braun, B. Braun Melsungen AG, Germany) in ratio 1:3. Consequently, fresh semen was stored at 7°C during the transport.

Motility analyses

Semen analyses were executed by Computer assisted semen analysis (CASA) method with SpermVision software (Minitube, Tiefenbach, Germany) and the microscope Olympus BX 51 (Olympus, Japan). Diluted semen samples were placed into Makler counting chamber (Sefi-Medical Instrumets, Germany) with volume of 10 µl heated to 37°C for each analysis. Evaluation of spermatozoa motility was performed in six time periods (0, 1, 2, 3, 6, 24 hours). Quality of

spermatozoa was measured by following parameters: motility (MOT), progressive motility (PRO) and velocity curved line (VCL). CASA system is able to make 30 pictures per second and all results are from 7 diverse sub-measurements of 7 different fields of Makler Counting Chamber **(Tirpák *et al.*, 2017; Del Gallego *et al.*, 2017)**.

Biochemical analyses

Calcium (Ca), phosphorus (P), magnesium (Mg), urea (U), total proteins (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TG) and cholesterol (CHOL) were measured using commercial kits DiaSys (Diagnostic Systems GmbH, Holzheim, Germany) on the Randox RX Monza analyzer (Crumlin, United Kingdom) **(Kováčik *et al.*, 2017)**.

Statistical analysis

All data were analyzed using the Statistical Analyses System (SAS 9.2. using of application Enterprise guide 5.1). Pearson's correlations between motility parameters and biochemical parameters were used. All statistical tests were carried out at levels of significance at P<0.05, P<0.01 and P<0.001 and results were interpreted as means and expressed with SD.

RESULTS AND DISCUSSION

One of the three aspects of present study was the spermatozoa motility. Total motility in the initially measured time was on level of 50%, but progressive motility percentage at the same time was only 10%. Results of stallion spermatozoa motility and progressive motility showed decreasing trend in each time interval. However, after 6 hours results showed rapid percentage drop-off in spermatozoa parameters. Results of the stallion spermatozoa motility parameters are summarized in the Figure 1.

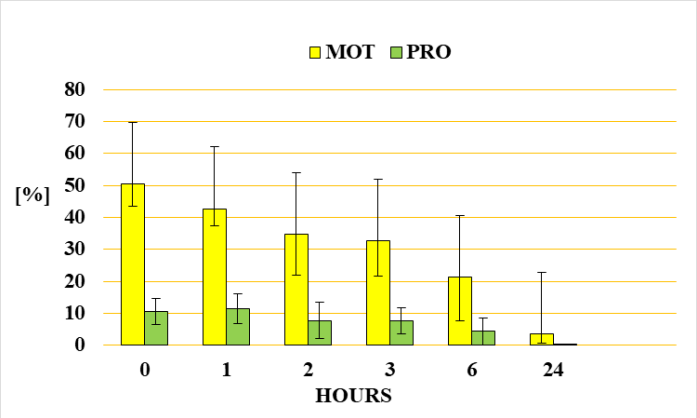

Figure 1 Motility (MOT) and progressive motility (PRO) of stallion spermatozoa (n=12) in six time periods (0, 1, 2, 3, 6, 24 hours).

As described in Figure 2 the analysis of velocity curved line detected approximately equal level in the first five measured time intervals. Velocity in these time intervals was in range from 140 to 150 $\mu m.s^{-1}$. After 24 hours, rapid decrease of spermatozoa quality was observed, reflecting the motility and progressive motility. Results of present study suggest that spermatozoa without conservative medium after 6 hours of storage have lower quality of spermatozoa.

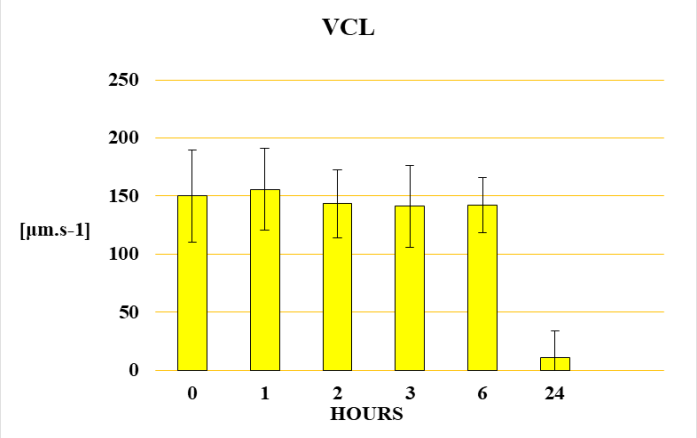

Figure 2 Velocity curved line (VCL) of stallion spermatozoa (n=12) in six time periods (0, 1, 2, 3, 6, 24 hours).

Other aspect of present study was aimed on content of biochemical parameters in stallion seminal plasma. Concentration of biochemical parameters in stallion seminal plasma are presented in Table 1.

Table 1 Concentration of biochemical parameters in stallion seminal plasma (n=12).

Parameter	Mean	S.D.	CV %
Ca (mM/L)	1.332	1.241	93.174
P (mM/L)	1.397	1.094	78.374
Mg (mM/L)	2.482	1.137	45.826
TP (g/L)	11.398	6.519	57.191
UREA (mM/L)	4.112	0.443	10.771
ALT (µkat/L)	0.151	0.055	36.408
AST (µkat/L)	3.359	1.210	36.032
TG (mM/L)	0.481	0.469	97.655
CHOL (mM/L)	0.135	0.118	87.207

Legend: S.D. – standard deviation, CV – coefficient of variation, Ca – calcium, P – phosphorus, Mg – magnesium, TP – total proteins, ALT – alanine aminotransferase, AST – aspartate aminotransferase, TG – triglycerides, CHOL – cholesterol.

Main aspect of present study was to describe effect of biochemical parameters on stallion spermatozoa properties. Evaluation of the biochemical parameters showed a positive significant correlation with motility and progressive motility (Table 2). Nevertheless, only two elements correlated with motility parameters. The correlation analysis showed a statistically significant (P<0.05) effect of Mg (0.850) and Ca (0.848) on the spermatozoa motility. Further, positive significant (P<0.05) correlation was found between Mg (0.841) and progressive motility. No correlation was found between biochemical parameters of stallion seminal plasma and velocity curved line (VCL).

Table 2 Correlations between motility parameters and biochemical parameters of stallion spermatozoa (n=12).

Parameter	MOT (%)	PRO (%)	VCL ($\mu m.s^{-1}$)
Ca (mM/L)	0.848*	0.58	0.42
P (mM/L)	0.24	0.48	0.54
Mg (mM/L)	0.850*	0.841*	0.74
TP (g/L)	-0.60	-0.16	0.08
UREA (mM/L)	-0.19	-0.03	-0.07
ALT (µkat/L)	-0.49	-0.15	0.06
AST (µkat/L)	-0.29	0.35	0.55
TG (mM/L)	-0.07	-0.03	0.04
CHOL (mM/L)	-0.06	0.34	0.48

Legend: Ca – calcium, P – phosphorus, Mg – magnesium, TP – total proteins, ALT – alanine aminotransferase, AST – aspartate aminotransferase, TG – triglycerides, CHOL – cholesterol, the level of significance was set at *P<0.05; **P<0.01; ***P<0.001.

According to **Kareskovski and Katila (2008)** number of researches described negative effect of seminal plasma on storage of equine spermatozoa. Also individual differences between stallions have impact on seminal plasma.
Detailed study of stallion semen was conducted by many researchers **(Křížková *et al.*, 2017; Giaretta *et al.*, 2017)**. Motility was controlled in different time periods as in the study of other authors: **Valsa *et al.*, (2016)** in human spermatozoa and **Slanina *et al.*, (2015)** in turkey spermatozoa. In comparison with results of **Križková *et al.*, (2017),** values of total motility in the initially measured time (88%) were higher than in present study.
According to **Valsa *et al.*, (2016)**, prostate gland was main contributor of magnesium and calcium in semen. Similar findings in concentration of calcium to present results were claimed by **Valsa *et al.*, (2015, 2016).** For comparison, concentrations of calcium and magnesium in human seminal plasma were higher in study of **Wong *et al.*, (2001)** than in present study. Same findings in concentration of calcium had **Hamad *et al.*, (2014)**. Further, magnesium in stallion seminal plasma in results of **Usuga *et al.*, (2017)** had higher concentration compared to submitted results.
Excess exposure of magnesium and calcium especially ionised calcium in seminal plasma correlate with infertility and may damage male reproductive system **(Pesch *et al.*, 2006)**. Same findings to present study had **Marzec-Wróblewska *et at.*, (2012)**, that calcium and magnesium have positive effect on motility and concentration of spermatozoa. Calcium in seminal plasma is necessary for stimulation of steriodogenesis in Leyding cells of the testis **(Asadpour, 2012)**. Magnesium is a very important element in cell physiology, which plays a role in spermatogenesis **(Wong *et al.*, 2001)**. According to **Valsa *et al.*, (2016)** magnesium engages in several functions of spermatozoa motility and further stimulates ATPase. ATPase catalyse the decomposition of ATP and this reaction releases energy.

CONCLUSION

In conclusion, findings in the present study suggest that biochemical parameters of stallion seminal plasma have effect on stallion spermatozoa. However, a significant correlation was observed between calcium and motility further between magnesium and motility and progressive motility. Results indicate that optimal concentration of calcium and magnesium in seminal plasma have beneficial effect on motility parameters of stallion spermatozoa. This phenomenon might be taken into consideration in order to improve the percentage of pregnant mares. Breeders of stallions should assecurate well-balanced feed with vitamins and minerals. Studies on effects of biochemical parameters of stallion seminal plasma to motility parameters of stallion spermatozoa should be continued to ascertain impact of calcium and magnesium.

Acknowledgments: The research was financially supported by the VEGA 1/0760/15, VEGA 1/0857/14, APVV-16-0289, APVV-15-0544, KEGA 006/SPU-4/2015 and AgroBioTech Research Centre built in accordance with the project Building „AgroBioTech" Research Centre ITMS 26220220180.

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