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**Original Paper**

# High taurine concentrations negatively effect stallion spermatozoa parameters *in vitro*

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Over the past decades natural substances are widely used in the maintaining of spermatozoa viability. The target of present study was to evaluate the effect of various taurine concentrations on stallion spermatozoa during 37 °C cultivation. Fresh semen was collected from 10 breeding stallions. The experimental groups were supplemented with six different concentration of taurine (in mg/ml): A – 2.5, B – 5, C – 7.5, D – 10, E – 15, F – 20 and compared to control (CON – 0). Spermatozoa motility was assessed using the Computer Assisted Semen Analyzer (CASA) system in 6 time periods (0, 1, 2, 3, 4 and 5 hours). The MTT test was used for detection of viability. For measuring antioxidant activity FRAP and TOS methods were used. Significantly negative effect was observed in the samples with the highest concentration of taurine (20 mg/ml). Spermatozoa viability was not significantly affected in analysed concentrations of taurine. Significant higher antioxidant activity was detected in the sample with the highest taurine concentration. Data clearly showed negative effects of high taurine concentrations on stallion spermatozoa.

**Keywords:** taurine, CASA, antioxidant activity, spermatozoa, stallion

## 1 Introduction

The main factors to assess the selection of stallions for breeding should be health, riding ability, performance, pedigree and finally the fertility of stallions. With the increasing trend of modern reproductive technologies and the transport of insemination doses it is necessary to monitor the fertility and quality of stallion ejaculate. Viability of spermatozoa for longer period (more than 24 hours) is one of the significant factors for successful insemination (Halo Jr. et al., 2018). The addition of substances such as taurine which may act as an antioxidant, membrane stabilization factor, capacitation factor and factor of spermatozoa motility is desirable to prolong fertilization ability. Taurine has been recognized for having important role in many physiological processes that include osmoregulation, antioxidation, detoxification and membrane stabilization (O´Flahery et al., 1997).

The aim of the present study was to investigate different concentrations of taurine on the motility, viability parameters of stallion spermatozoa during *in vitro* incubation at 37 °C and its effect on antioxidant capacity.

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## 2 Material and methods

### *2.1 Semen collection and processing*

Spermatozoa samples were collected from 10 breeding stallions (Nitra region, Slovakia) in age range of 5–20 years that consisted of following breeds: Lipizeaner, Holsteiner, Hucul, Oldenburger horses. Semen was diluted in saline solution (NaCl 0.9% Braun, B. Braun Melsungen AG, Germany) in 1 : 3 ratio for the control group (CON). Experimental groups were prepared using same dilution – six concentrations of taurine (Taurine ≥99%, Sigma Aldrich, USA) dissolved in the saline solution (mg/ml): CON – 0, A – 2.5, B – 5, C – 7.5, D – 10, E – 15, F – 20. Semen was divided in two aliquots. Part of ejaculate was used for motility analyses and the second part was centrifuged (15 min, 10,000 × g, 4 °C), seminal plasma was transferred into Eppendorf tubes and kept frozen (-80 °C) for FRAP and TOS analysis. Samples for motility analyses were incubated at 37 °C, over the entire duration of experiment.

### *2.2 Motility analyses*

CASA (Computer Assisted Semen Analyzer) method with SpermVision software (Minitube, Tiefenbach, Germany) and the Olympus BX 51 (Olympus, Japan) were used to analyse spermatozoa motility. Cultivation times were set to 0, 1, 2, 3, 4 and 5 hours. Multiple spermatozoa vitality parameters were observed (Slanina et al., 2018; Halo Jr. et al., 2019).

### *2.3 Assessment of mitochondrial toxicity*

Viability of cells exposed to taurine *in vitro* assessment was executed using the metabolic activity (MTT) assay. This colorimetric assay measures the conversion of 3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, USA) to purple formazan particles. Conversion mediated by mitochondrial succinate dehydrogenase produced by intact mitochondria of viable cells was determined by ELISA reader (Multiscan FC, ThermoFisher Scientific, Vantaa, Finland) at 570 nm against 620 nm wavelengths. Data was expressed as percentage – comparing to metabolic activity of spermatozoa of the control group (Jambor et al., 2017).

### *2.4 Ferric reducing ability of plasma (FRAP) assessment*

Analysis for FRAP was performed according to method by Benzie and Strain (1996). This test determines the total antioxidant power, based on the reduction of a ferric-tripyridyl triazine complex to its ferrous coloured form in the presence of antioxidants. $FeSO_4 \cdot 7\ H_2O$ solutions were used for calibration. Working reagent was added to 96-wells micro plate and a reagent blank reading was at 593 nm with Promega GloMax Multi+ Microplate Reader (Madison, Wisconsin, USA). Then standards and samples were added. Second absorbance was taken after 4 minutes and FRAP was calculated using the standard curve and expressed in $FeSO_4 \cdot 7\ H_2O$.

### *2.5 Total oxidant status (TOS)*

The principle of analysis of the total oxidation status was based on the oxidation of ferrous ion-o-dianisidine complexes by oxidants present in the sample to ferrous ions and the oxidation reaction was supported by glycerol molecules present in the reaction solution. The iron ions then form a coloured complex with xylenol orange in the acidic environment of the reaction solution. Analysis for TOS was performed according to method described by Erel (2005). Sample/standard were added to 96 well microplate with first reaction solution and absorbance (blank) at 560 nm was measured using the Promega GloMax Multi+ Microplate Reader (Madison, Wisconsin, USA). Then second reaction solution was added and after 3 minutes, resulting absorbance was measured at 560 nm and the results were calculated. The test was calibrated with hydrogen and the resulting values were expressed in micromoles of $H_2O_2$ equivalent per liter.

### *2.6 Statistical analysis*

Statistical analysis was done by the GraphPad Prism 5 (GraphPad Software Inc., USA) using one-way ANOVA with Dunnett's post-test. All statistical tests were carried out at levels of significance at $*P < 0.05$; $**P < 0.01$, $***P < 0.001$ and results were interpreted as means and expressed as mean and *SD*.

 

## 3 Results and discussion

In general, a decreasing tendency in motility (MOT) parameter in all experimental groups compared to control is evident. Lowest motility was always in the sample with the highest concentration of taurine (group F) in sample. On other hand, after 5 hours a significant increase in group C compared to control was detected. Results of progressive motility (PRO) have seen similarly tendency as results of motility. After 2 hours of experiment, the sample with 2.5 mg/ml taurine (A) significantly increased (39.71%) compared to control (29.5%) whereas samples with higher concentrations – C, D, E, F significantly decreased compared to control. Interestingly, after 5 hours the concentrations have significantly risen compared to control in group C, but the increasing tendency in groups A, B and D was visible. In most samples an increased value of velocity curved line (VCL) compared to control in all groups and all time periods were found (Figure 1). After 5 hours of incubation, viability of stallion spermatozoa showed an increasing trend in all experimental groups except group F with the highest concentration of taurine which was in the contrary (Figure 2).

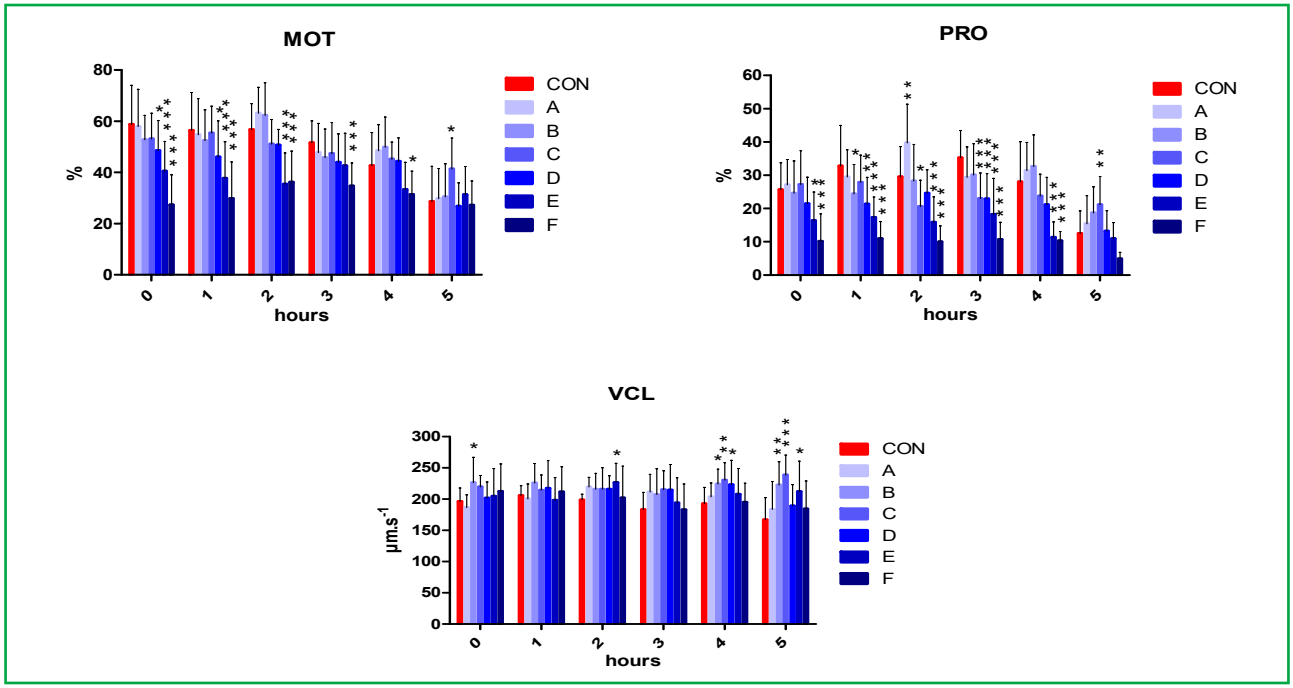


**Figure 1** The effect of various concentrations of taurine on the motility (%), progressive motility (%) and velocity curved line (µm/s) of stallion spermatozoa ($n$ = 10) at 0 h, 1 h, 2 h, 3 h, 4 h and 5 h after incubation at 37 °C. Each bar represents the mean (±*SD*). The level of significance was set at **P* <0.05; ***P* <0.01, ****P* <0.001. CON – 0, A – 2.5, B – 5, C – 7.5, D – 10, E – 15, F – 20 mg/ml of taurine

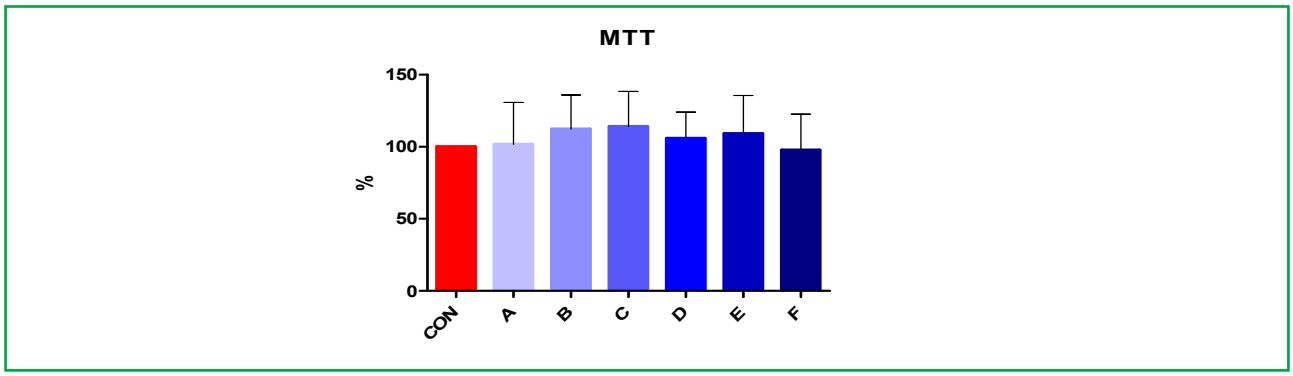


**Figure 2** The effect of various concentration of taurine on the viability (%) of stallion spermatozoa ($n$ = 10) assessed after 5 hours of incubation at 37 °C. Each bar represents the mean (±*SD*) of measured optical density and converted to percent. The control was recalculated to 100%. CON – 0, A – 2.5, B – 5, C – 7.5, D – 10, E – 15, F – 20 mg/ml of taurine

Taurine has been used in several studies to increase and protect spermatozoa motility in horses and other animal species. The concentration of taurine used depended on the species and on cultivation temperature of spermatozoa. Taurine has been used to enhance the motility of frozen stallion semen (Stephens et al., 2013). Addition of taurine (25 mM) to post-thaw stallion spermatozoa did not significantly affect motility (MOT), progressive motility (PRO), velocity parameters (VCL, VAP, VSL). Only linearity (LIN) was significantly increased after taurine treatment. Iljaz and Ducharme (1995) studied effect of taurine (70 mM) in three different semen extenders for 96 hours storage at 5 °C. After 24, 48, 72 and 96 hours motility in extender INRA82 with taurine was higher compared to extender without addition of taurine. Addition of taurine to rabbit spermatozoa in three concentrations (3.125, 6.25 and 12 mM) during *in vitro* incubation at 39 °C showed that the most optimal concentration was 6.25 mM during long-term incubation (Tirpák et., 2017). In study carried by Bucak et al. (2007) on ram semen, taurine at a dose of 25 mM after the cryopreservation/thawing increased spermatozoa motility and showed antioxidant properties. Antioxidant profile parameter FRAP has shown and increasing trend in all groups compared to the control but with only the sample with the highest concentration of taurine being statistically significant at 156.5 µmol/l $Fe^{2+}$ compared to control – 127.0 µmol/l $Fe^{2+}$. Total oxidative status (TOS) results have been fluctuating between 2.055 µmol $H_2O_2$ and 2.214 µmol $H_2O_2$ and have not shown any statistically significant differences (Figure 3).

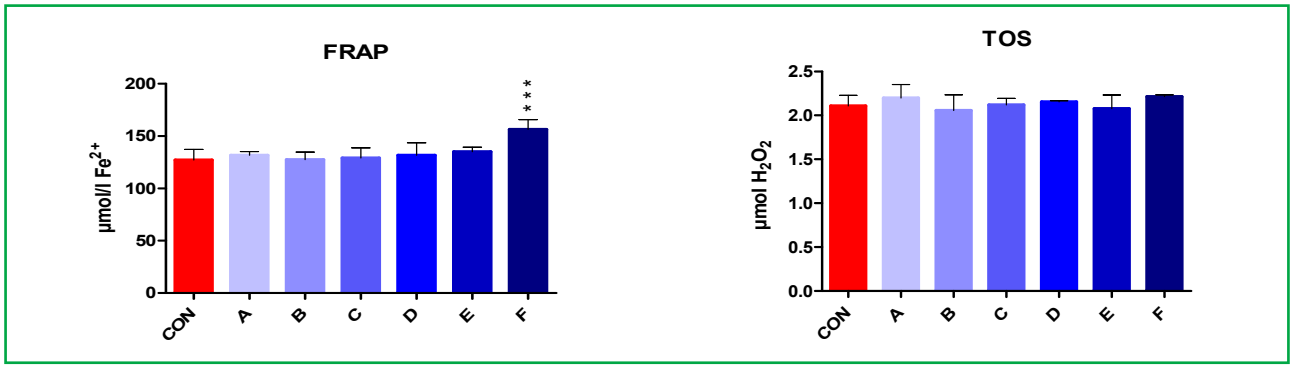


**Figure 3** The effect of various concentration of taurine on the ferric reducing ability (µmol/l $Fe^{2+}$) and total oxidant status (µmol $H_2O_2$) of stallion seminal plasma ($n = 10$). Each bar represents the mean (±*SD*). The level of significance was set at **P* <0.05; ***P* <0.01, ****P* <0.001. CON – 0, A – 2.5, B – 5, C – 7.5, D – 10, E – 15, F – 20 mg/ml of taurine

Reddy et al. (2010) observed the effect of taurine (50 mM) on bull spermatozoa using the FRAP assay and found out a similar trend of increase in FRAP compared to control as we did in our samples collected from stallions.

## 4 Conclusions

Data from the present *in vitro* study demonstrate that taurine in concentration 7.5 mg/ml had protective effects on the stallion spermatozoa in later time intervals. With increasing taurine concentration soared its negative effect. From the achieved results we can state that optimal taurine supplementation may be a suitable component of stallion semen extenders.

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## References

Benzie, I. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, 239(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Bucak, M. N. et al. (2007). The influence of trehalose, taurine, cysteamine and hyaluronan on ram semen: Microscopic and oxidative stress parameters after freeze–thawing process. *Theriogenology*, 67(5), 1060–1067. https://doi.org/10.1016/j.theriogenology.2006.12.004

Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry*, 38(12), 1103–1111. https://doi.org/10.1016/j.clinbiochem.2005.08.008

Halo, M. and Tirpák, F. (2018) Stallion fertility – the basis of successful reproduction. *Svet koní*, 18.

Halo Jr., M. et al. (2019) Time and dose-dependent effects of Viscum album quercus on rabbit spermatozoa motility and viability *in vitro*. *Physiological Research*, 68(6), 955–972. https://doi.org/10.33549/physiolres.934223

Ijaz, A. and Ducharme, R. (1995). Effect of various extenders and taurine on survival of stallion sperm cooled to 5 C. *Theriogenology*, 44(7), 1039–1050. https://doi.org/10.1016/0093-691x(95)00290-o

Jambor, T. et al. (2017) In vitro effect of 4-nonylphenol on human chorionic gonadotropin (hCG) stimulated hormone secretion, cell viability and reactive oxygen species generation in mice Leydig cells. *Environmental Pollution*, 222, 219–225. https://doi.org/10.1016/j.envpol.2016.12.053

O'flaherty, L. et al. (1997) Intestinal taurine transport: a review. *European Journal of Clinical Investigation*, 27(11), 873–880. https://doi.org/10.1046/j.1365–2362.1997.2000747.x

Reddy, N. S. S. et al. (2010). Effects of adding taurine and trehalose to a tris-based egg yolk extender on buffalo (*Bubalus bubalis*) sperm quality following cryopreservation. *Animal Reproduction Science*, 119(3-4), 183–190. https://doi.org/10.1016/j.anireprosci.2010.01.012

Slanina, T. et al. (2018) Effect of taurine on turkey (*Meleagris gallopavo*) spermatozoa viability and motility. *Czech Journal of Animal Science*, 63(4),127–135. https://doi.org/10.17221/79/2017-CJAS

Stephens, T. D. et al. (2013) Effects of pentoxifylline, caffeine, and taurine on post-thaw motility and longevity of equine frozen semen. *Journal of Equine Veterinary Science*, 33(8), 615–621. https://doi.org/10.1016/j.jevs.2012.10.004

Tirpák, F. et al. (2017) Low taurine concentrations possitively affect rabbit spermatozoa properties in later time intervals. *Journal of Microbiology, Biotechnology and Food Sciences*, 7, 128–131. https://doi.org/10.15414/jmbfs.2017.7.2.128-131