THE EFFECT OF FRUCTOSE ON TURKEY SPERMATOZOA MOTILITY *IN VITRO*

VPLYV FRUKTÓZY NA POHYBLIVOSŤ SPERMIÍ MORIAKOV *IN VITRO*

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ABSTRACT

The aim of our study was to analyse the effect of different fructose concentrations: 20 $mg.ml^{-1}$ – M20; 15 $mg.ml^{-1}$ – M15; 10 $mg.ml^{-1}$ – M10; 5 $mg.ml^{-1}$ – M5 on the turkey spermatozoa motility parameters after an *in vitro* cultivation at 5°C. Semen samples diluted with physiological solution were used as the control. Individual motility parameters were recorded at five time periods: 0, 1, 2, 3, 4 hours. Each sample was evaluated using the Computer Assisted Semen Analyzer (CASA) system. The highest spermatozoa motility of 60.76% was recorded at the time 0 in the control. At the time 0 significantly higher values of motility were observed in the control sample than in the M20 ($p<0.05$) and M15, M5 ($p<0.001$) samples. A significantly lower motility ($p<0.05$) was recorded in the sample M10 in comparison with the control at the time 1 and 4 hours. The samples M15 and M5 showed a significantly lower motility also at the time 2, 3 and 4 hours. A significantly lower progressive motility ($p<0.01$; $p<0.001$) was observed after 0 hours of culture in all samples in comparison with the control. Subsequently, similar values in all samples were detected at the next time of cultivation. . Very balanced values of velocity curved line and amplitude of lateral head displacement were detected in all samples. Analysing the beat cross frequency no significant differences were detected. Results of our experimental work suggest that fructose does not have positive effects on the turkey spermatozoa motility parameters when cultured under *in vitro* conditions.

Key words: fructose, turkey, spermatozoa, motility, CASA

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INTRODUCTION

In vitro liquid storage of semen is of practical interest in the management of male turkeys. However, turkey spermatozoa rapidly lose viability and fertilizing capacity when stored either undiluted or diluted at physiological temperatures.. In order to maintain the fertilizing ability of *in vitro*-stored spermatozoa, sperm must be pre-cooled to 2–8 °C and diluted in an appropriate extender (Douard et al., 2004). Extenders also provide energy substrates. Therefore, extenders used for avian semen are enriched with carbohydrates (glucose or fructose) and other components likely to provide energy (citrate, glutamate, acetate) (Graham et al., 1982; Christensen, 1995; Thurston, 1995).

In addition, turkey spermatozoa differ from fowl spermatozoa in many aspects of their metabolism and resistance to cooled storage. The metabolism of glucose and acetate are lower in avian than mammalian spermatozoa (Scott et al., 1962). Fructose is formed by turkey spermatozoa from glucose and accumulated in the medium under aerobic conditions (Amir et al., 1985). Most of the extenders provide the requirements for both energy metabolism and buffering capacity. The exogenous substrates added to extenders for turkey semen may not be sufficient or appropriate for the energy needs of gametes during *in vitro* storage (Akcay et al., 2006).

The aim of this study was to analyse the influence of different fructose concentrations on the turkey spermatozoa motility parameters during short *in vitro* cultivation.

MATERIAL AND METHODS

Biological material

In this study semen was obtained by penal massaging of the turkeys of the line Big 6 (BUT – British United Turkeys Ltd., Chester, United Kingdom) aged from 35 to 42 weeks. Semen samples were a mixture of several groups of identical individual turkeys.

Sample preparation

Semen was diluted in a ratio of 1 part of semen and 200 parts of physiological solution (Sodium chloride 0,9% Braun, B. Braun Melsungen AG, Melsungen, Germany) – Control sample K. At the same ratio the semen was diluted with four different concentrations of fructose solution: 20 $mg.ml^{-1}$ – M20; 15 $mg.ml^{-1}$ – M15; 10 $mg.ml^{-1}$ – M10; 5 $mg.ml^{-1}$ – M5 diluted in the physiological solution. Samples were cultured at 5°C and recorded at five time periods: 0, 1, 2, 3, 4 hours. The experiment was realized in 6 replicates.

Analytical method

Each of thus prepared samples was evaluated using a Computer Assisted Semen Analyzer (CASA) system – Sperm Vision (Minitub, Tiefenbach, Germany) equipped with a microscope (Olympus BX 51, Japan) to assess the spermatozoa motility. Each sample was placed into Makler Counting Chamber (depth 10 μm, Sefi–Medical Instruments, Germany). Using the turkey specific set up the following parameters were evaluated – total motile spermatozoa (MOT), progressively motile spermatozoa (PRO), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) in different time periods.

Statistical analysis

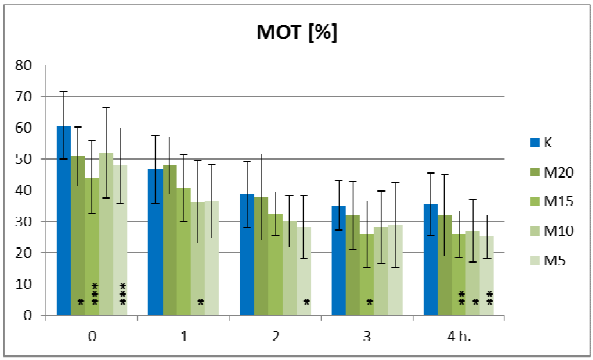
Obtained data were statistically analyzed using PC program Excel and a statistics package SAS 9.1 (SAS Institute Inc., USA) using Student's t–test and Scheffe's test. Statistical significance was indicated by p values of less than 0.05; 0.01 and 0.001.

RESULTS AND DISCUSSION

Results of the spermatozoa motility are shown in *Tab. 1*. The highest motility of 60.76 % was recorded at the time 0 h. in the control sample – K. This value was significantly higher than in the samples M20 ($p<0.05$) and M15, M5 ($p<0.001$). With increasing time of cultivation a gradual decline in all samples was observed. A significantly lower motility ($p<0.05$) was recorded in the sample M10 when compared to the control at the time 1 and 4 h. The samples M15 and M5 showed a significantly lower motility ($p<0.05$; $p<0.01$) also at the time of 2, 3 and 4 hours.

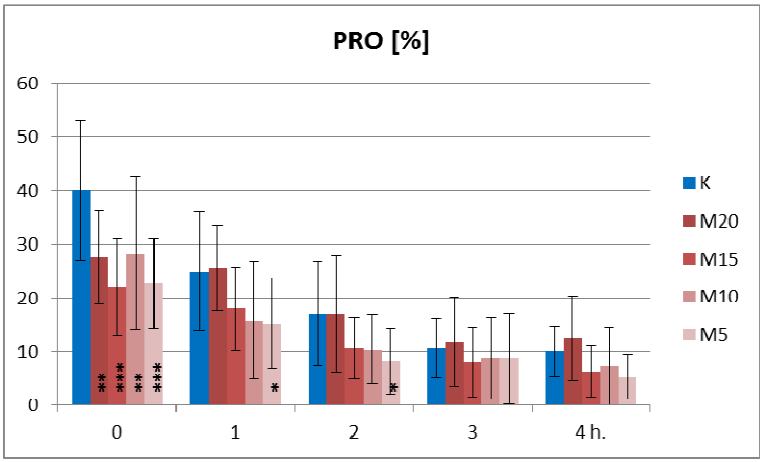
These results are in accordance with results of Amir et al. (1985), who obtained no consistent results as to the effect of fructose on the motility of turkey spermatozoa. . On the other hand, an adverse effect was obtained when the spermatozoa were suspended in a medium containing fructose. The sperm motility was not affected when the fructose in the medium was formed from glucose.

*Tab. 1 Spermatozoa motility (in %) in groups with different concentrations of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001.*



The highest progressive spermatozoa motility (39.96 %) was detected in the sample K at the beginning of cultivation (*Tab. 2*). When compared the experimental groups to the control sample, significantly lower values ($p<0.01$; $p<0.001$) were observed after 0 hours of culture in all samples. Significantly ($p<0.05$) decreased values were observed in the sample M5 when compared to the control sample at the time of 1 and 2 hours.

*Tab. 2 Spermatozoa progressive motility (in %) in groups with different concentrations of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001.*

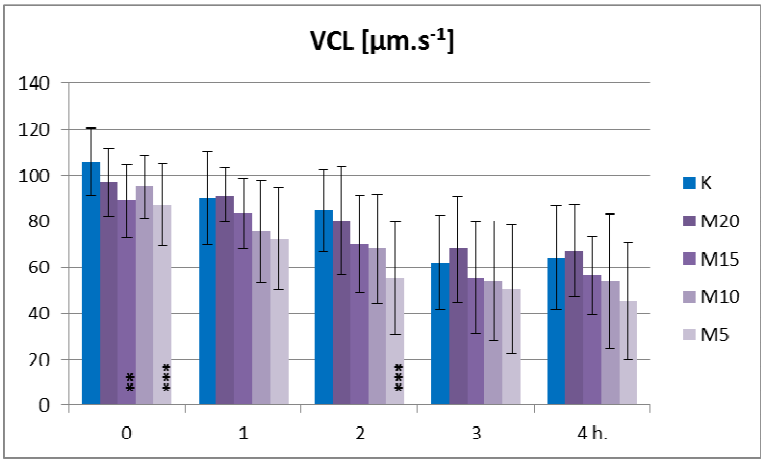


Analysis of velocity curved line (VCL) between samples M20, M10 and control revealed no significant differences (*Tab. 3*). Non-significant differences were found also for other fructose groups after 1, 3 and 4 hours of cultivation. Significantly lower values ($p<0.001$) were observed in the sample M5 when compared to the control at the time of 0 and 1 h. VCL was significantly lower ($p<0.01$) in the M5 sample at the time 2 h.

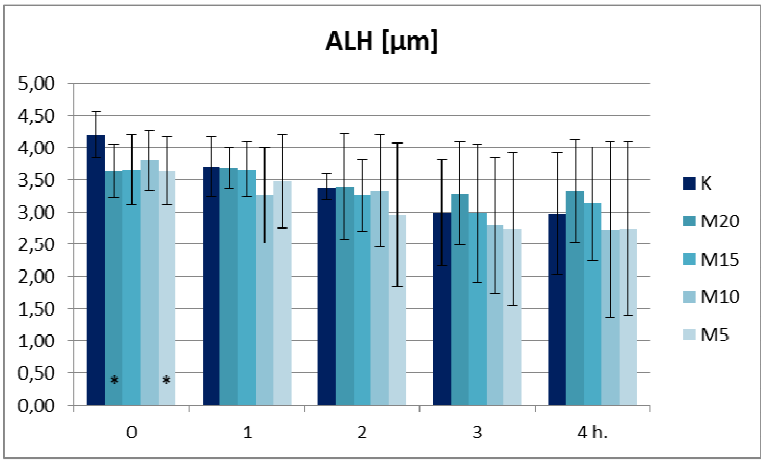
Very balanced values of amplitude of lateral head displacement (ALH) were detected in all samples (*Tab. 4*). Significantly ($p<0.05$) lower values were detected only at the time 0 hours in samples M20 and M5.

Analysing the beat cross frequency (BCF, *Tab. 5*) significant differences were not detected. Very equal values were observed in all tested samples.

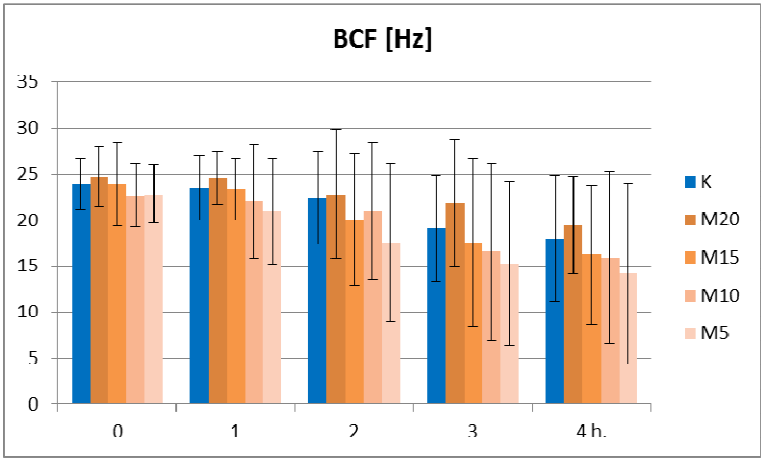
*Tab. 3 Velocity curved line (in μm.s^{-1}) in groups with different concentrations of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001.*



*Tab. 4 Amplitude of lateral head displacement (in μm) in groups with different concentrations of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001.*



*Tab. 5 Beat cross frequency (in Hz) in groups with different concentration of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001.*



A similar tendency was detected also in other species. Sarıözkan et al. (2012) evaluated the effects of different sugar supplementations on the rat spermatozoa motility at 0 and 12 h after chilling. No significant difference was observed in any of the parameters evaluated at 0 h, before storage ($p>0.05$). After 12 h of storage, all sugar additives led to statistically higher motility in comparison to the control group. In conclusion, raffinose, trehalose and fructose provided a better protection of sperm functional parameters against chilling injury, in comparison to the control group.

In the study of Rigau et al., (2001) total motility of canine fresh ejaculates was 70%, and progressively decreased to 35% after incubation for 60 min in a medium without sugars. Similar results were observed when the cells were incubated in the presence of 10 mM glucose or 10 mM fructose, although fructose slightly increased the total motility to about 45% at 60 min. A progressive increase of BCF was observed in spermatozoa incubated without hexoses. The addition of 10 mM fructose, but not 10 mM glucose, prevented this increase for at least 60 min. This difference was maintained when spermatozoa were incubated in increasing concentrations of monosaccharides.

Analysis of the influence of different energetic substrates used in culture media on the bovine spermatozoa motility was the aim of Kňačická et al. (2010). In the medium with the addition of fructose the spermatozoa motility significantly increased ($p<0.001$) after immediate dilution of the sample. With increasing time of cultivation this difference began to be reduced ($p<0.01$). After 24 h cultivation no significant differences were recorded. Progressive motility copied the tendency of spermatozoa motility as the medium with the addition of fructose reached significantly ($p<0.001$) higher values at time 0 and 1h, but after 24 hours of cultivation the progressive motility was significantly ($p<0.05$) higher in the control sample.

CONCLUSION

The selected spermatozoa mobility parameters (MOT, PRO, VCL, ALH, BCF) were found to be balanced or significantly lower in all tested samples. Results of our experimental work suggest that fructose does not have positive effects on turkey spermatozoa motility parameters when cultured under *in vitro* conditions in comparison to the control diluted with physiological solution.

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