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REGULAR ARTICLE

THE EFFECT OF FRUCTOSE ON TURKEY SPERMATOZOA MOTILITY DURING *IN VITRO* CULTIVATION AT 41°C

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ABSTRACT

The aim of our study was to analyze the effect of different fructose concentrations: 20 mg.ml^{-1} – Y20/O20; 15 mg.ml^{-1} – Y15/O15; 10 mg.ml^{-1} – Y10/O10; 5 mg.ml^{-1} – Y5/O5 on the two age groups of turkey spermatozoa motility parameters (group Y – from 28 to 52 weeks and group O – from 53 to 68 weeks) after an *in vitro* cultivation at 41°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 selected spermatozoa motility parameters (MOT, PRO, VCL, ALH, BCF) were found to be balanced or significantly lower in all tested samples. After 1 hours of cultivation at temperature of cultivation (41 °C) significant decrease of all parameters was found. The evaluation of spermatozoa motility parameters between two different age groups turkeys detected higher values in group Y (Young; 28 – 52 weeks). The additions of fructose alone to the extender have not positive effect on turkey spermatozoa motility.

Keywords: fructose, turkey, spermatozoa, motility, CASA

INTRODUCTION

Extensive use of artificial insemination in turkeys has led to development of in vitro storage of semen, either in a liquid form or in a cryopreserved state (**Thurston *et al.*, 1995**). 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, semen must be pre-cooled to 2–8 °C and diluted in an appropriate extender (**Douard *et al.*, 2004**).

Diluents used for AI are buffered salt solutions used to extend semen, maintain the viability of spermatozoa in vitro, and maximize the number of hens that can be inseminated. Semen extension is important since poultry semen is viscous and highly concentrated, containing 6 (roosters) to 12 (toms) billion spermatozoa per ml **(Donoghue and Wishart, 2000)**. Semen diluents are based on the biochemical composition of chicken and turkey semen (**Lake, 1995**). Glutamic acid, the most prominent anionic constituent of avian seminal plasma, became a standard component of diluents (**Lake and Mc Indoe, 1959**). Extenders should 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**). Briefly, most of the extenders provide the requirements for both energy metabolism and buffering capacity (**Akçay *et al.* 2006**).

The response of fowl spermatozoa to exogenous acetate and glucose is lower in avian than mammalian spermatozoa. The overall metabolic activity, per unit cell, is lower than with other species, and the pattern of carbohydrate metabolism is different. Morphologically avian spermatozoa are dissimilar to mammalian spermatozoa, and the enzyme complement could be much less and thus account for a lower metabolic rate (**Scott *et al.*, 1962**). **Amir et al. (1985)** state in their study that fructose is formed by turkey spermatozoa from glucose and accumulated in the medium under aerobic conditions. Fructose originating from glucose is used for fructolysis when the glucose reserve in the medium is almost exhausted.

The aim of this study was to analyze the influence of different fructose concentration on the turkey spermatozoa motility parameter during *in vitro* cultivation at 41°C.

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 28 to 52 weeks (Young) and from 53 to 68 weeks (Old). 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 $mg.ml^{-1}$ – Y20/O20; 15 $mg.ml^{-1}$ – Y15/O15; 10 $mg.ml^{-1}$ – Y10/O10; 5 $mg.ml^{-1}$ – Y5/O5 diluted in the physiological solution (D-Fructose G.R., Lach-ner s.r.o., Neratovice, Czech Republic). The letter corresponding of turkey age group: Y – Young and O – Old (older) turkeys. Samples were cultured at 41°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

The highest spermatozoa motility (MOT) was detected in the control sample YK at the beginning of cultivation. In the groups of young (Y), significantly lower values were recorded in the sample Y20 ($p<0.05$) and samples Y15, Y5 ($p<0.001$). In the groups of old (O) only sample O10 proved significantly lower motility than in control sample at the time 0. However after 1 hours of cultivation significantly lower values ($p<0.001$) of spermatozoa motility were observed in all samples of group O (O20, O15, O10, and O5) in comparison to the control sample (OK). All results of the spermatozoa motility are shown in Figure 1 and Figure 2. Non-significant differences were found in both age groups in other time of *in vitro* cultivation.

Spermatozoa progressive motility (PRO) followed the tendency of spermatozoa motility. Also the highest progressive motility (39.96 %) was found in the sample YK at the time 0 hours (34.62 % in the sample OK). Initially (Time 0) progressive motility was significantly lower in the samples Y20 (27.49%), Y15 (22.00%), Y10 (28.31%), Y5 (22.66%) compared to the sample YK (Figure 3). Nevertheless in all samples of group O non-significant differences were found at the beginning of cultivation. Significantly lower values of progressive motility were detected after 1 hours of culture in samples O20, O15, O10 and O5 than in the control sample OK (Figure 4). Considerable decrease values were observed after 1 hours of cultivation in both groups Y and O. The addition of fructose has not positive effect on this parameter.

Analysis of velocity curved line (VCL) between samples Y15, Y5 and control YK revealed no significant differences (Figure 5). Significantly lower values were observed only at the time 0 hour in the sample Y15 and Y5. In all samples of group O significantly lower values of VCL than in the control OK after 1 hour and in the samples Y20 and Y15 after 4 hour of cultivation (Figure 6) were detected.

Very balanced values of amplitude of lateral head displacement (ALH) were found in all samples (Figure 7 and 8). Significantly ($p<0.05$) lower values were detected only at the time 0 hours in samples Y20, Y5 and at the time 1 hours in sample O5.

Analysing the beat cross frequency (BCF) significant differences were not detected in the samples of group Y (Figure 9). Very equal values were observed in all tested samples. Significantly lower values were detected in samples O15 ($p<0.05$) at the time 0 hours and in sample O5 ($p<0.001$) at the time 1 hour (Figure 10).

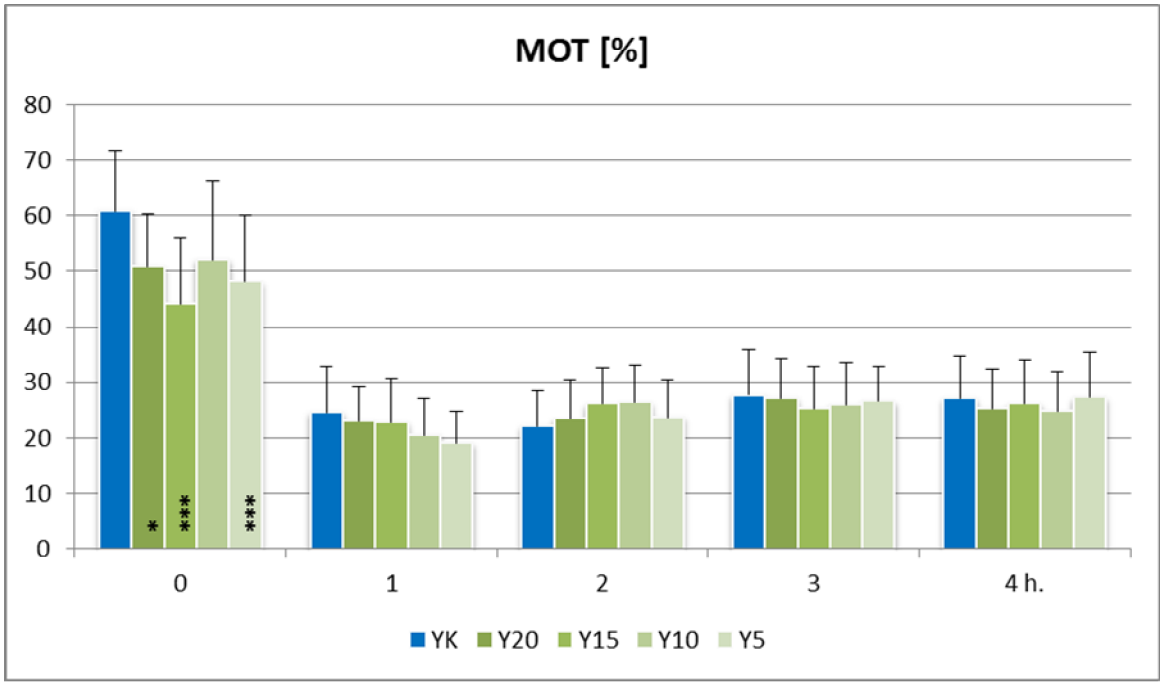

Figure 1 Spermatozoa motility (in %) in group Y and in samples with different concentrations of fructose and time periods [hours]. Significant differences *$p<0.05$; **$p<0.01$; ***$p<0.001$

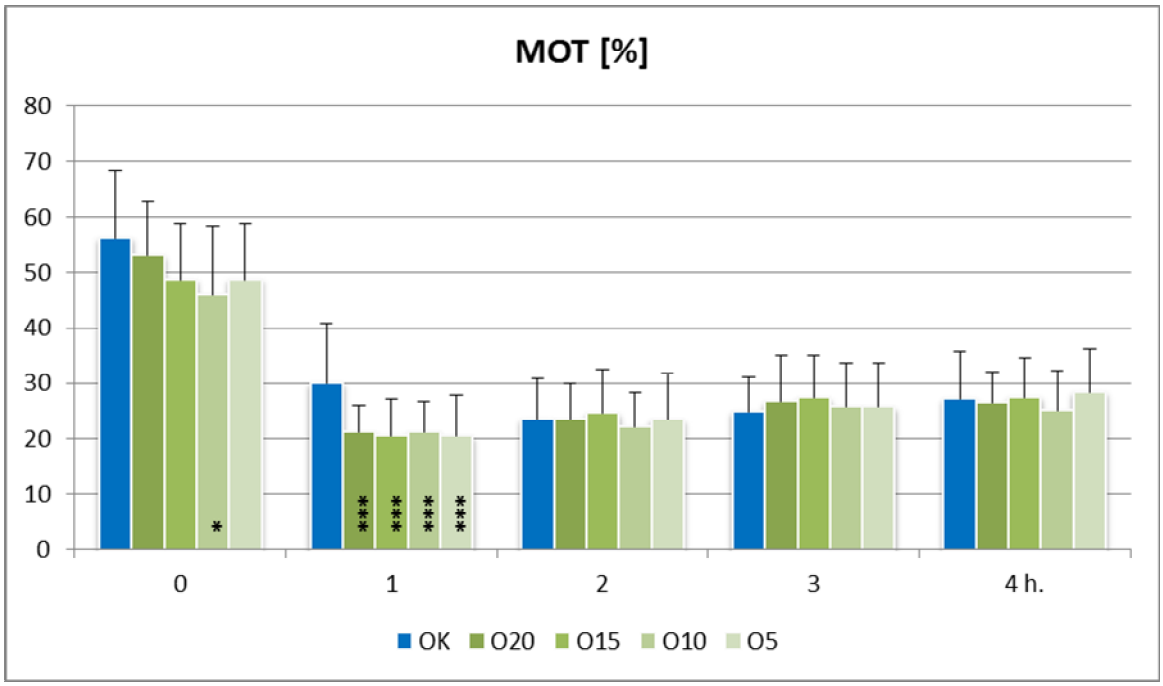

Figure 2 Spermatozoa motility (in %) in group O and in samples with different concentrations of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001

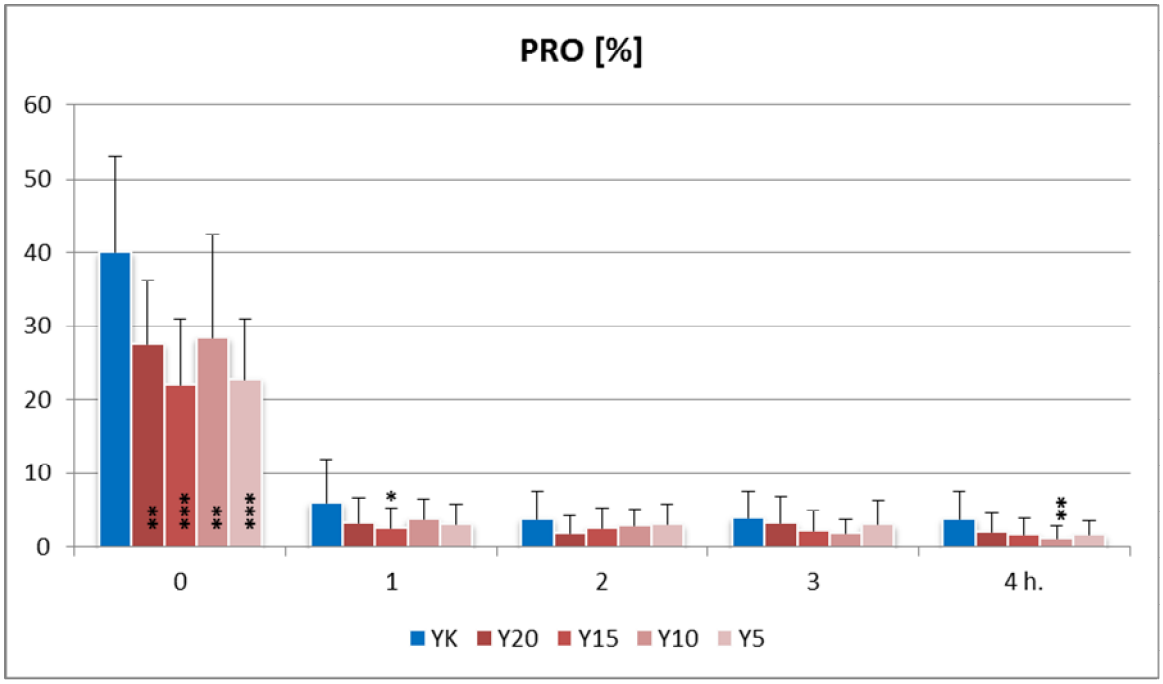

Figure 3 Spermatozoa progressive motility (in %) in group Y and in samples with different concentrations of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001

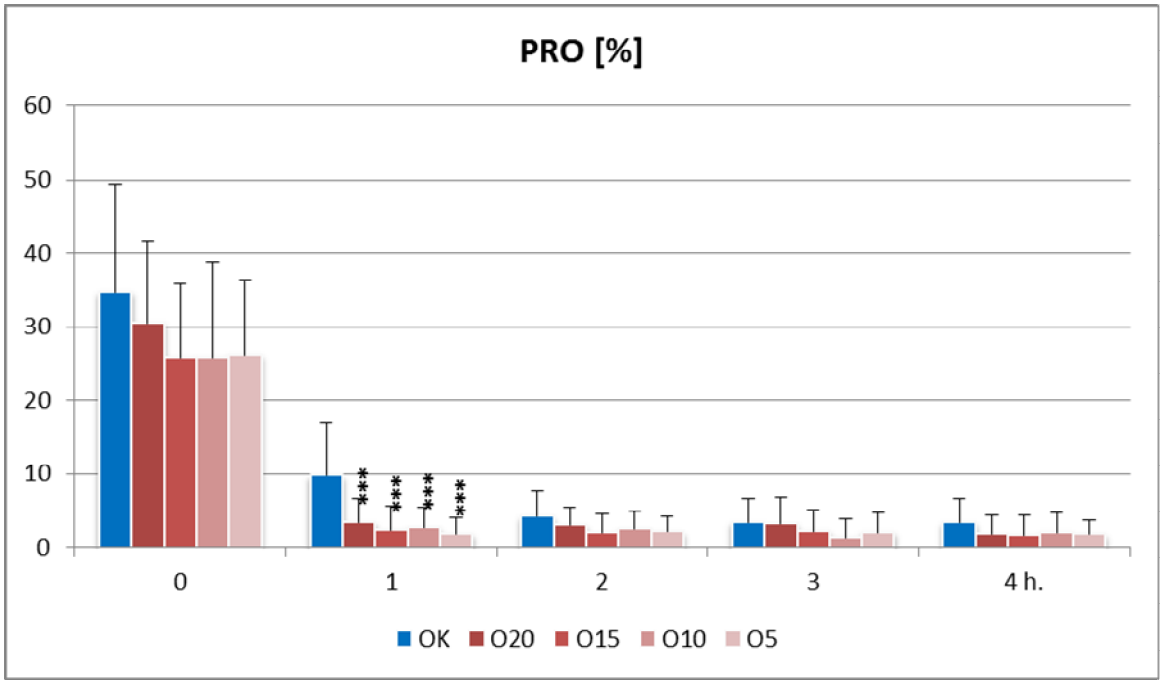

Figure 4 Spermatozoa progressive motility (in %) in group O and in samples with different concentrations of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001

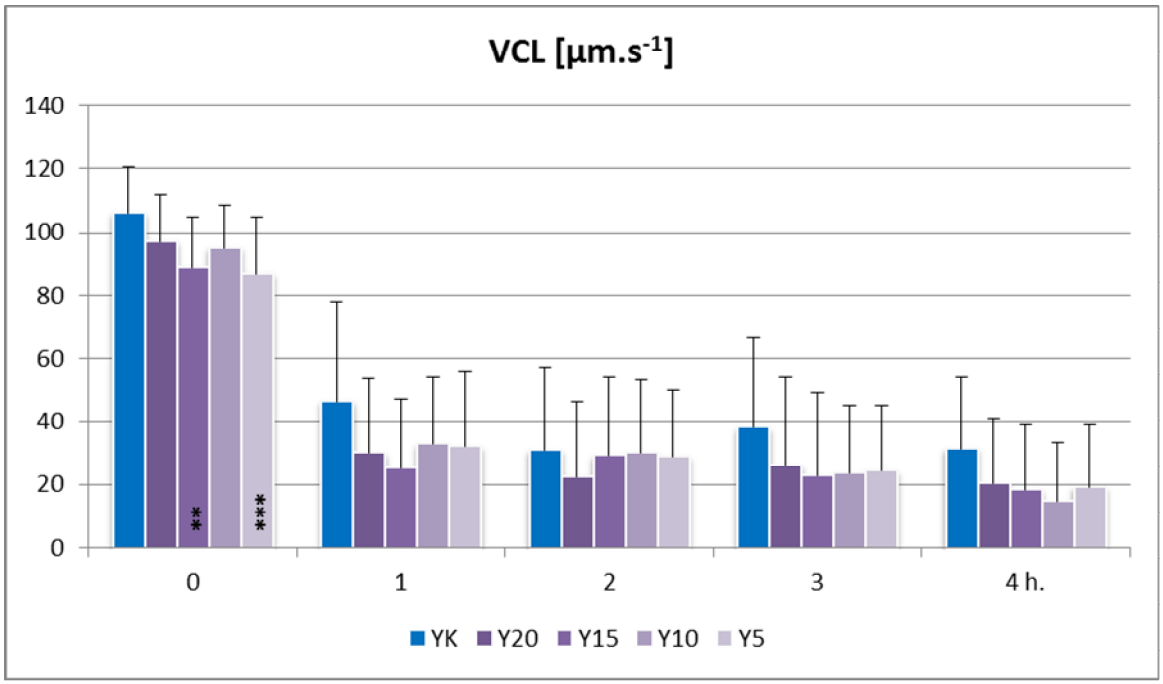

Figure 5 Velocity curved line (in $\mu m.s^{-1}$) in group Y and in samples with different concentrations of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001

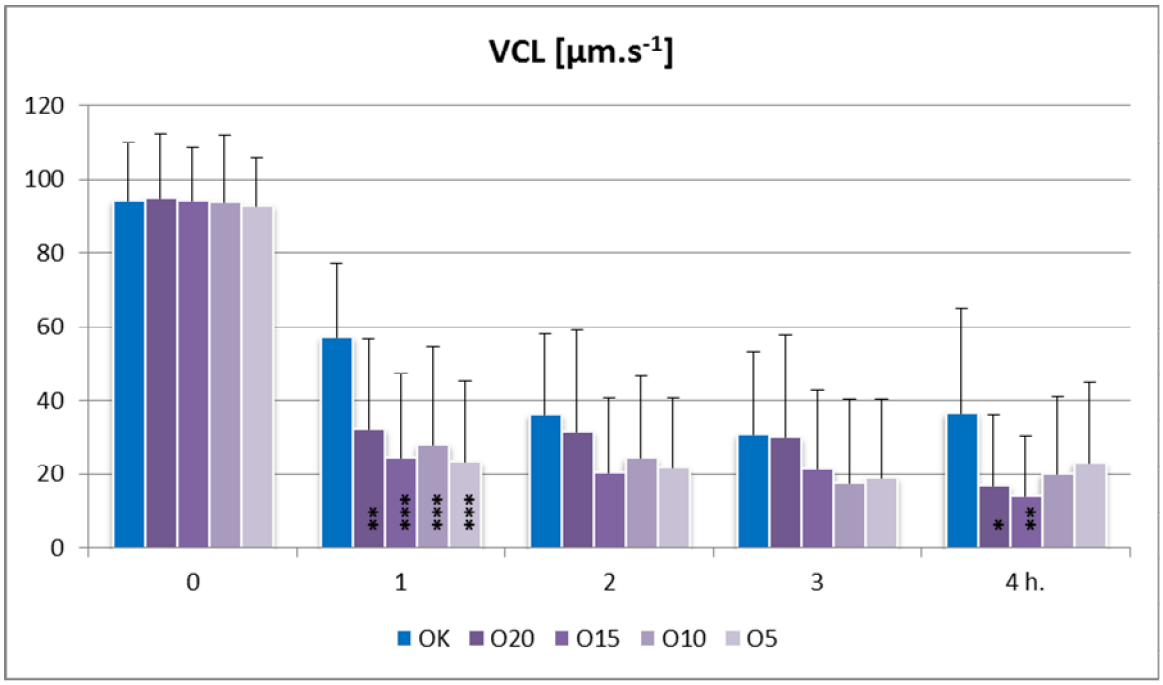

Figure 6 Velocity curved line (in µm.s^{-1}) in group O and in samples with different concentrations of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001

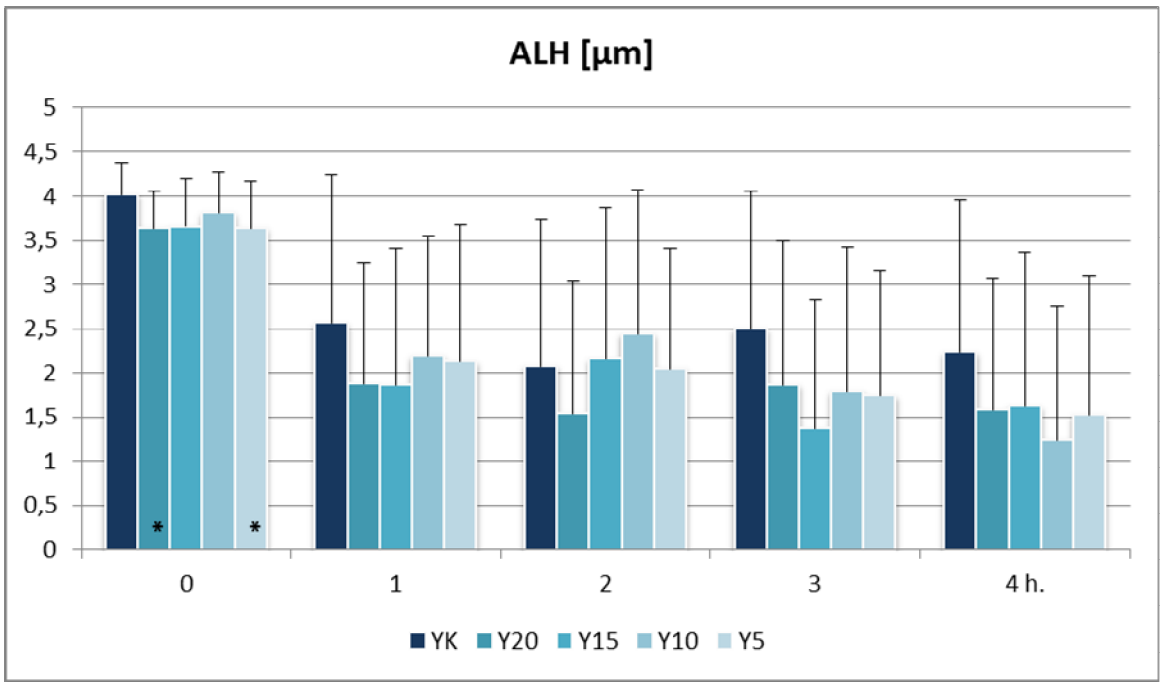

Figure 7 Amplitude of lateral head displacement (in µm) in group Y and in samples with different concentrations of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001

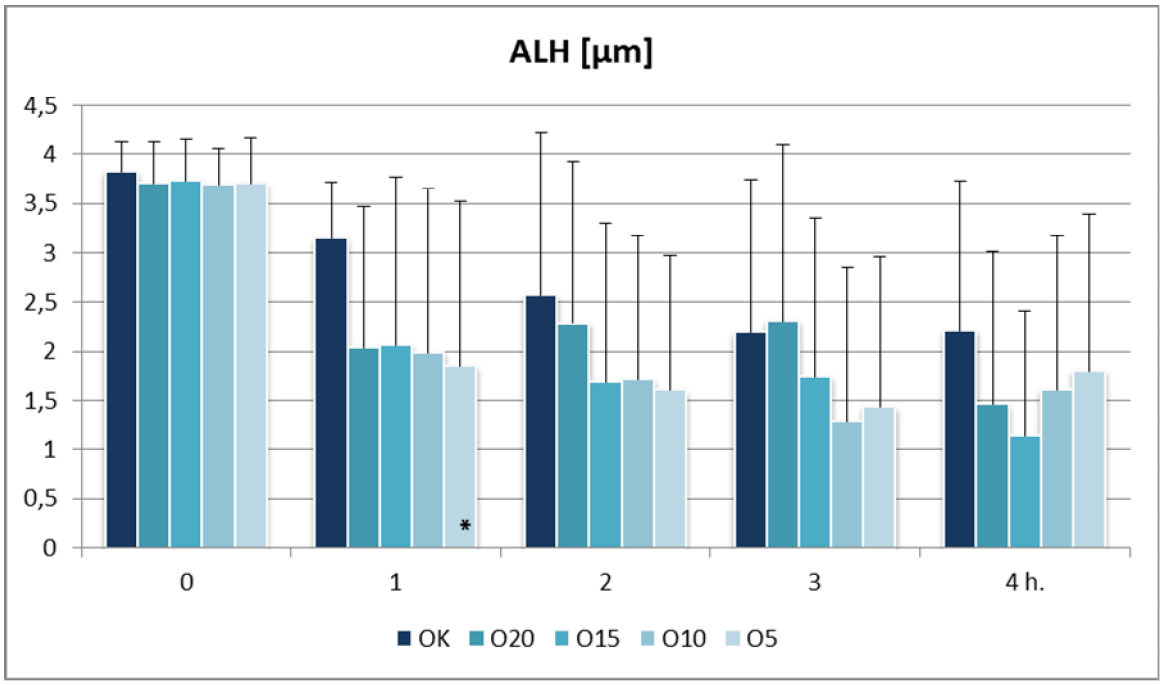

Figure 8 Amplitude of lateral head displacement (in µm) in group O and in samples with different concentrations of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001

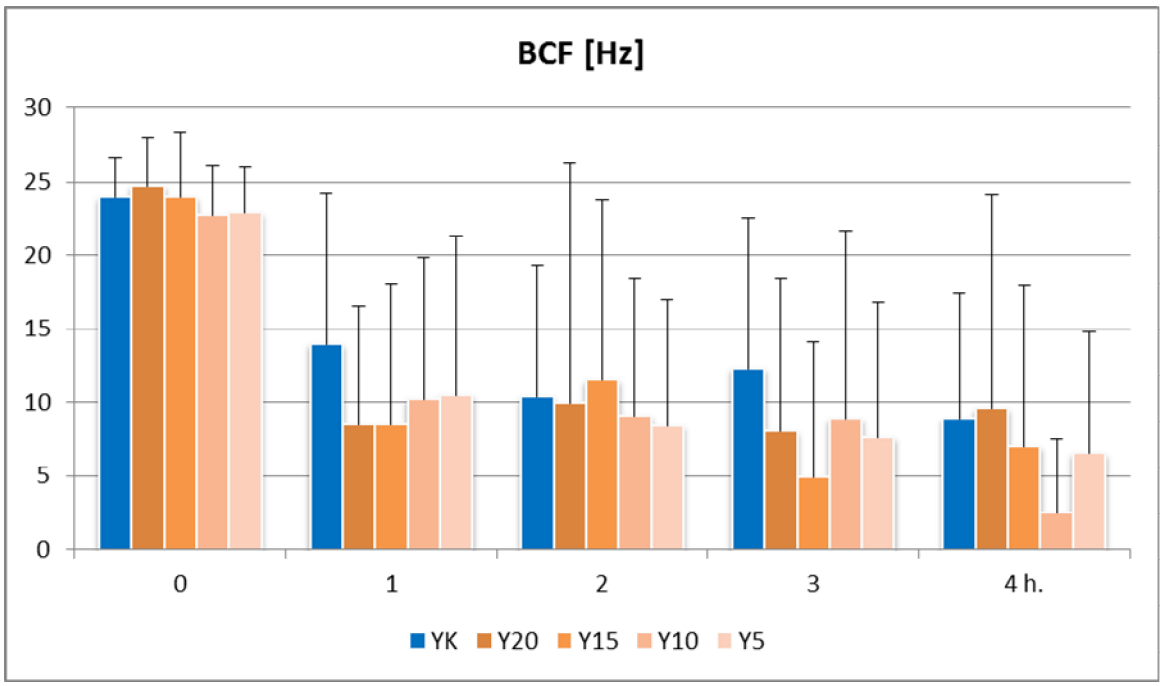

Figure 9 Beat cross frequency (in Hz) in group Y and in samples with different concentration of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001

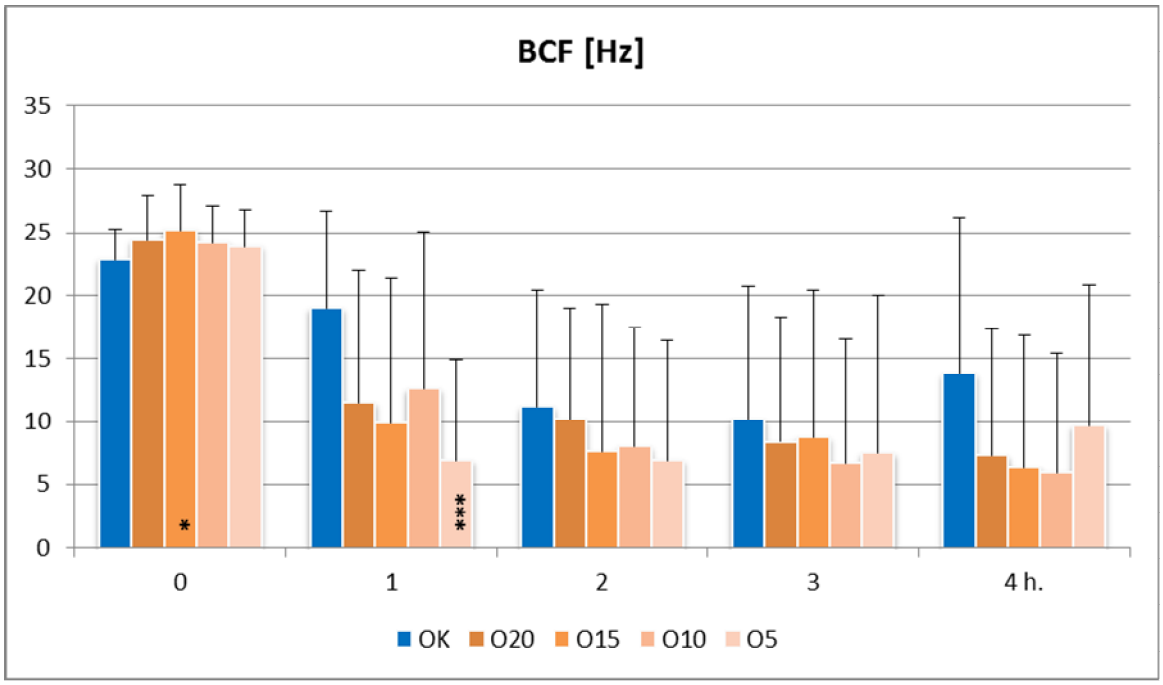

Figure 10 Beat cross frequency (in Hz) in group O and in samples with different concentration of fructose and time periods [hours]. Significant differences *p<0.05; **p<0.01; ***p<0.001

In our previous study **Slanina *et al.* (2012)**, we obtained similar tendencies of spermatozoa motility parameters of turkey cultured at 5°C in the same concentrations of fructose, where the significantly higher values of spermatozoa motility (MOT) and progressive motility (PRO) were observed only in the control sample. Subsequently, similar values in all samples were detected at the next time of cultivation. Very balanced values of velocity curved line (VCL) and amplitude of lateral head displacement (ALH) were detected in all samples. Analyzing the beat cross frequency (BCF) no significant differences were detected. However after 1 hour cultivation considerable decrease values were not observed compared with the current study, what was caused by a different temperature of cultivation.

The effect of fructose and glucose on the turkey spermatozoa was studied by **Amir *et al.* (1985)**. The motility of the spermatozoa at the end of the 1 hour incubation period at 37°C was adversely affected by the presence of fructose in the medium, under aerobic conditions. Under anaerobic conditions, the spermatozoa motility was significantly lower than under aerobic conditions. On the other hand, an adverse effect was obtained when the spermatozoa were suspended in a medium containing fructose. The spermatozoa motility was not affected when the fructose in the medium was formed from glucose. Although 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**).

A similar tendency was detected also in other species. **Rigau et al. (2001)** studied effects of glucose and fructose on motility of canine spermatozoa. Incubation of spermatozoa from fresh ejaculates in a basal medium without sugars for 60 min at 37°C induced a progressive decrease in the percentage of motile spermatozoa and in some mean motility parameters, such as mean velocity (VAP), linear coefficient (LIN) and dance (DNC), and an increase in the mean frequency of head displacement (BCF). This indicates a progressive loss of linearity and an increase in oscillatory movement. Addition of 10 mM fructose prevented these effects. Incubation in a basal medium with 10 mM glucose for 60 min at 37°C provoked a fast and intense decrease of LIN and a slight increase of DNC, inducing a less linear and more oscillatory mean movement. Neither fructose nor glucose modified the percentage of motile spermatozoa. The response to both sugars was dose-dependent, with differences appearing at concentrations as low as 1 mM.

Sarıözkan *et al.* (2012) evaluated the protective effects of supplementation with three different sugars (raffinose, trehalose and fructose) on the motility, morphology and DNA integrity of rat epididymal spermatozoa chilled and stored at 4 °C. 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. They evaluated spermatozoa motility without using CASA system.

Analysis of the influence of different energetic substrates used in culture media on the bovine spermatozoa motility was the aim of **Kňažická *et al.* (2010)** study. 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 motility parameters were found to be balanced or significantly lower in all tested samples. A 1 hours of cultivation at temperature of cultivation 41°C resulted in significant decrease of all motility parameters. Results of our experimental work suggest that fructose has no positive effects on turkey spermatozoa motility parameters when cultured under *in vitro* conditions at 41°C in comparison to the control diluted with physiological solution. The evaluation of spermatozoa mobility parameters between two different age groups turkeys was detected higher values in group Y (Young; 28 – 52 weeks).

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