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REGULAR ARTICLE

THE EFFECT OF DIFFERENT CONCENTRATION OF TREHALOSE ON TURKEY SPERMATOZOA MOTILITY *IN VITRO*

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ABSTRACT

Target of this study was to analyze the effect of a different trehalose concentration (T10 – 10 mg.ml^{-1}; T5 – 5 mg.ml^{-1}; T2.5 – 2.5 mg.ml^{-1}; T1.25 – 1.25 mg.ml^{-1}) on the turkey spermatozoa motility parameters after a culture *in vitro* at 5°C. For control the semen samples were diluted with physiological solution. Individual parameters were recorded at six time periods: 0, 30, 60, 90, 120, 150, 180 minutes. The highest values of spermatozoa motility were determined in samples containing 10 mg.ml^{-1} of trehalose. The highest motility of 88.16% was recorded after 30 minutes of culture in the sample T10. Significantly ($p<0.01$) higher data were detected in T10 samples compared to control after 90 minutes of culture. After 180 minutes of culture a significantly ($p<0.01$) higher motility was detected in control. In the samples T5; T2.5 and T1.25 similar significantly lower values were recorded compared to control, except for T1.25 at the time of 90 minutes of culture. The highest progressive motility 77.97% was observed in samples T10 after 30 minutes of culture. A significantly lower value in group T10 ($p<0.05$) was detected at time 180. When compared to other control lower values at of all time periods were found.

Keywords: turkey, spermatozoa, motility, CASA, trehalose

INTRODUCTION

Short-term liquid storage of turkey semen is of great interest in the management of turkey reproduction due to the extensive use of artificial insemination. Unfortunately, the length of storage of undiluted or extended semen is very restricted and usually after 24 h of storage significant decreases in spermatozoa viability and fertilizing capacity are observed **(Donoghue a Wishart, 2000).** For this reason, in practical conditions, turkey semen is diluted in an appropriate extender and used within a few hours (typically up to 6 h; **Sexton and Giesen, 1983**) to facilitate artificial insemination **(Kotłowska et al., 2007).**

Although it has yet to be achieved, mimicking the environment for storage of spermatozoa within the hen would profoundly alter current systems for storing semen for extended periods of time *in vitro* **(Donoghue and Wishart, 2000).**

Many factors may contribute to the loss in fertilizing ability of spermatozoa stored *in vitro* **(Zaniboni and Cerolini, 2009).** Spermatozoa metabolism is not completely arrested during liquid storage at reduced temperature; the main changes which occur include an irreversible reduction in motility, morphological integrity and fertility of spermatozoa **(Maxwell and Stojanov, 1996).** In order to maintain the fertilizing ability of *in vitro* stored spermatozoa, spermatozoa must be pre-cooled to 2–8 °C **(Donoghue and Wishart, 2000)** and diluted in an appropriate extender **(Akçay et al., 1997).**

The fundamental principle is that chicken or turkey semen is diluted in a medium based on the ionic environment of the male reproductive tract (notably with the main anion as glutamate rather than chloride), although hypertonic compared with seminal plasma, with an added glycolytic substrate and a buffer maintaining a pH of around 7.1. The suspension of spermatozoa in this medium is then held at low temperature – usually around 5°C – to minimize spermatozoa metabolic rate and any deleterious catabolic processes, as well as proliferation of microorganisms. However, chicken spermatozoa may be stored under anaerobic conditions, in which they can maintain ATP levels and fertilizing ability, but turkey spermatozoa, which have poor glycolytic capacity and require oxidative phosphorylation to maintain energy levels, must be stored under aerobic conditions **(Hocking, 2009).**

Extenders should also contain energy substrates. Therefore, extenders used for poultry semen are enriched in carbohydrates (glucose or fructose) and in other components likely to provide energy (citrate, glutamate, acetate) **(Graham et al., 1982; Christensen, 1995; Thurston, 1995).** The metabolism of glucose and acetate are lower in avian than mammalian

spermatozoa. Briefly, most of the extenders provide the requirements for both energy metabolism and buffering capacity **(Akçay et al., 2006).**

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

MATERIAL AND METHODS

In this study semen obtained by massaging the turkey line of Big 6 (BUT - British United Turkeys Ltd., Chester, United Kingdom) was used. Semen samples are always a mixture of several groups of identical individual turkeys. Semen was diluted in a ratio of 1 part of semen, and 2 parts of physiological saline (Sodium Chloride 0.9% w/v intravenous Infusion Bieffe, Bieffe Midetal S.p.A., Grosotto, Italy). Experiment was realized in 6 replicates. Sample measured at time 0 minutes was considered as 100% (of control) and taken as the basal value for all additional measurements. Subsequently, the sample was diluted with 5 µl of diluted semen with 500 µl of physiological saline and this sample was used as a control. At the same ratio the semen was diluted semen with four different concentrations of trehalose solution – 10 $mg.ml^{-1}$; 5 $mg.ml^{-1}$; 2.5 $mg.ml^{-1}$; 1.25 $mg.ml^{-1}$ diluted in the physiological solution. The final dilution was 1:200. The average pH of saline used was 6.50 (pH 213; Hanna Instruments Inc.).

From each diluted semen sample, two samples which were labeled as T10; T5; T2.5; T1.25 with the number corresponding to the concentration of trehalose and control identified as K were prepared. Samples were cultured at 5 °C

Each of thus prepared samples were 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, progressively motile spermatozoa, curvilinear velocity (VCL), amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) in different time periods. Obtained data were statistically analyzed with the help of the 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 values all of spermatozoa motility parameters were recalculated and expressed in per cents.

Results of spermatozoa motility are shown in Figure 1. The highest motility of 88.06 % was recorded after 30 minutes of cultivation in the sample T10. With increasing time of cultivation a gradual decline in all samples was observed. The sample T10 showed similar values as in the control. Significant higher motility ($p<0.01$) in the T10 sample compared to control was recorded at the time 90. However, after 180 minutes of culture significantly ($p<0.01$) higher value of 61.88 % in control was detected. In the sample T5 at all time periods decreased values than in the control sample were observed: at the time of 30 minutes ($p<0.05$), at the time 90 minutes ($p<0.01$), and in further times ($p<0.001$). In groups T2.5 and T1.25 almost identical values were reported and these were lower compared to the control group.

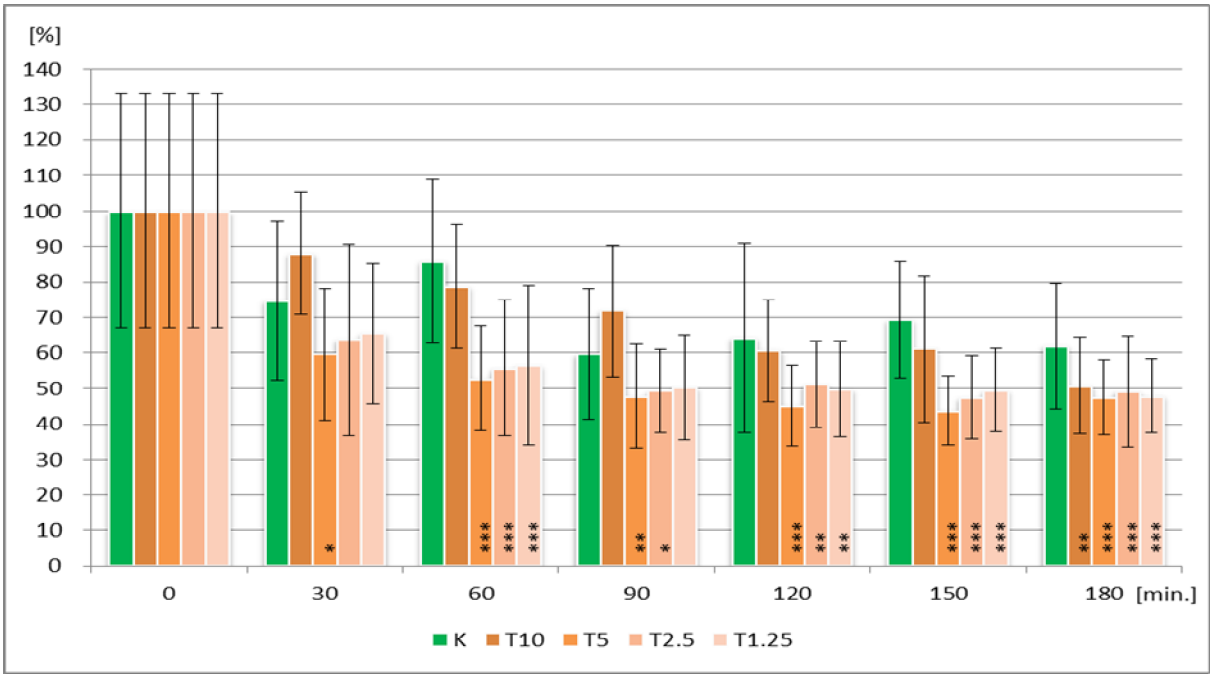

Figure 1 Spermatozoa motility (in %) in groups with different concentration of trehalose and time periods [min.]. Significant differences *p<0.05; **p<0.01; ***p<0.001.

The highest progressive spermatozoa motility (77.97%) in sample T10 was detected after 30 minutes of cultivation (Fig. 2). Statistically significant difference ($p<0.05$) was observed only at the time of 180 minutes, where the highest progressive motility (41.96%) in

control group was recorded. When compared experimental groups to control sample a significant lower values ($p<0.001$) were detected after 60, 120, 150 and 180 minutes of culture. Progressive motility after 90 minutes of cultivation was significantly decreased ($p<0.01$) in groups T2.5 and T5. For group T1.25 after 90 minutes of culture significantly ($p<0.05$) lower values were found compared to the control group.

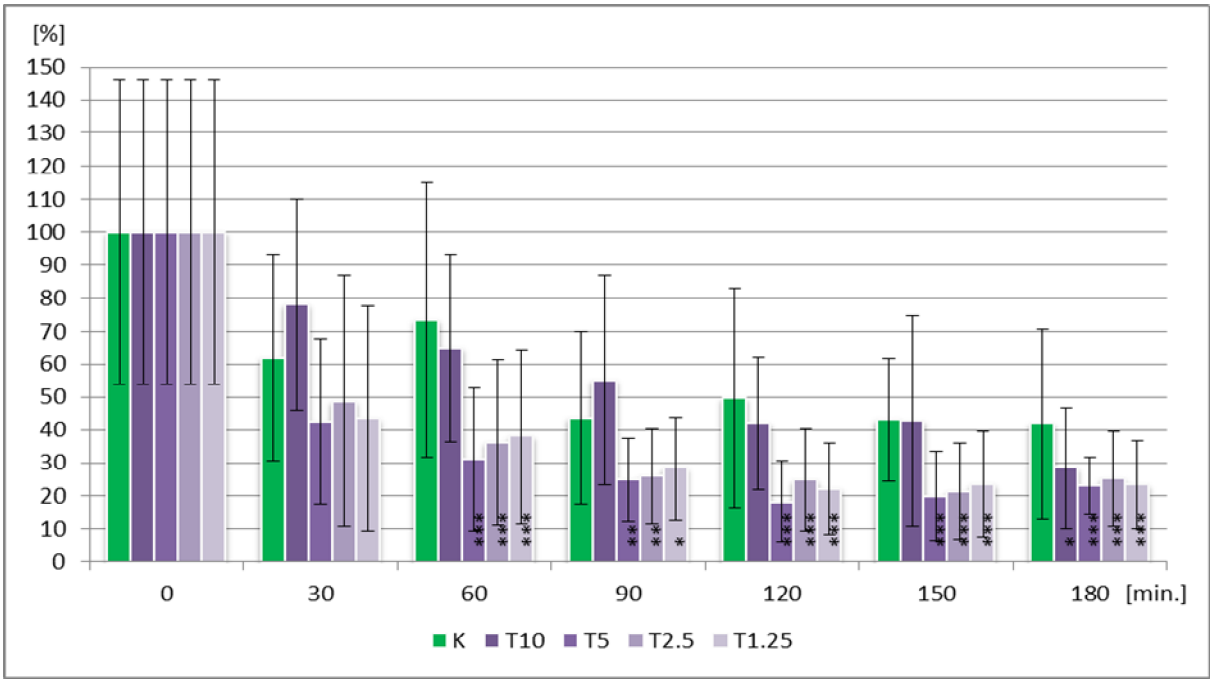

Figure 2 Spermatozoa progressive motility (in %) in groups with different concentration of trehalose and time periods [min.]. Significant differences *p<0.05; **p<0.01; ***p<0.001.

Analysis of selected velocity parameter – velocity curved line (VCL) between group T10 and control found any significant differences (Fig. 3). VCL for these samples ranged from 89.38 % to 62.82%. Non-significant differences were found also for others trehalose groups after 150 minutes of cultivation and for group T2.5 at time 30. Significant decrease of the VCL parameter was detected mainly in groups with lower concentration of trehalose.

Very balanced values of amplitude of lateral head displacement (ALH) in sample T10 in comparison to control sample were observed. The sample T5 showed significantly ($p<0.01$) lower value only at the time 60 minutes of culture. ALH was significantly ($p<0.05$) lower in sample T2.5 after 60 and 90 minutes of *in vitro* culture. For group T1.25 significantly lower values at time of 30, 60, 120 and 180 minutes was found (Fig. 4).

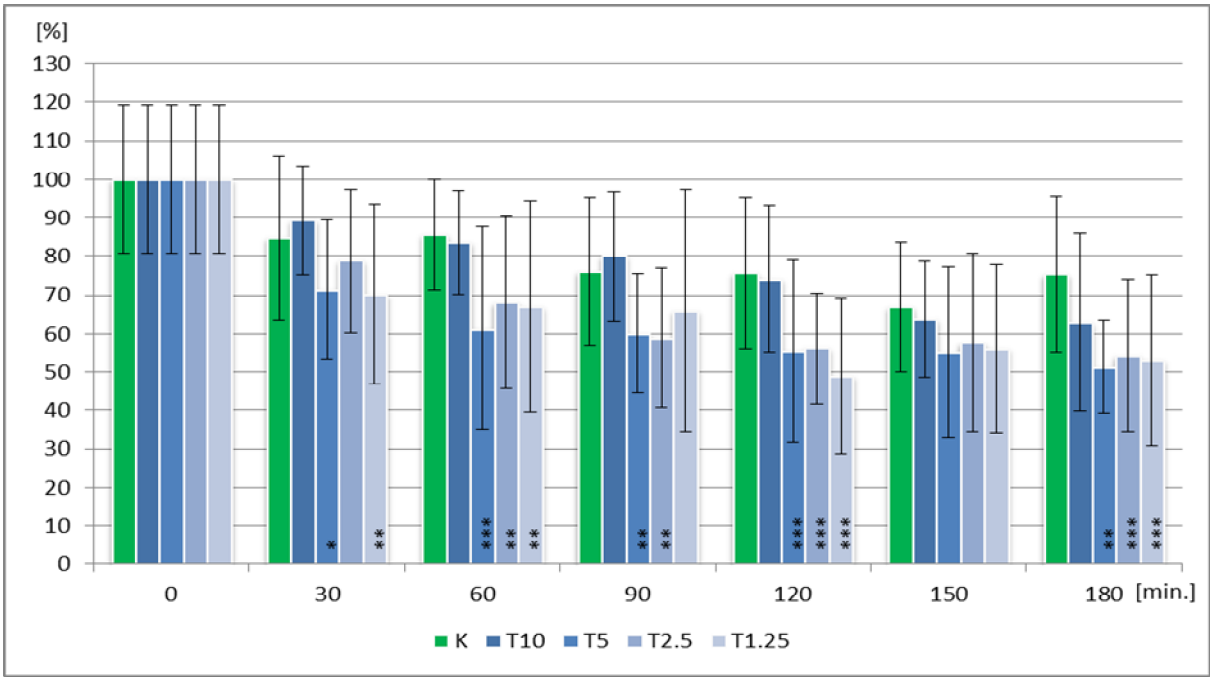

Figure 3 Velocity curved line (in μm.s^{-1}) in groups with different concentration of trehalose and time periods [min.]. Significant differences *p<0.05; **p<0.01; ***p<0.001

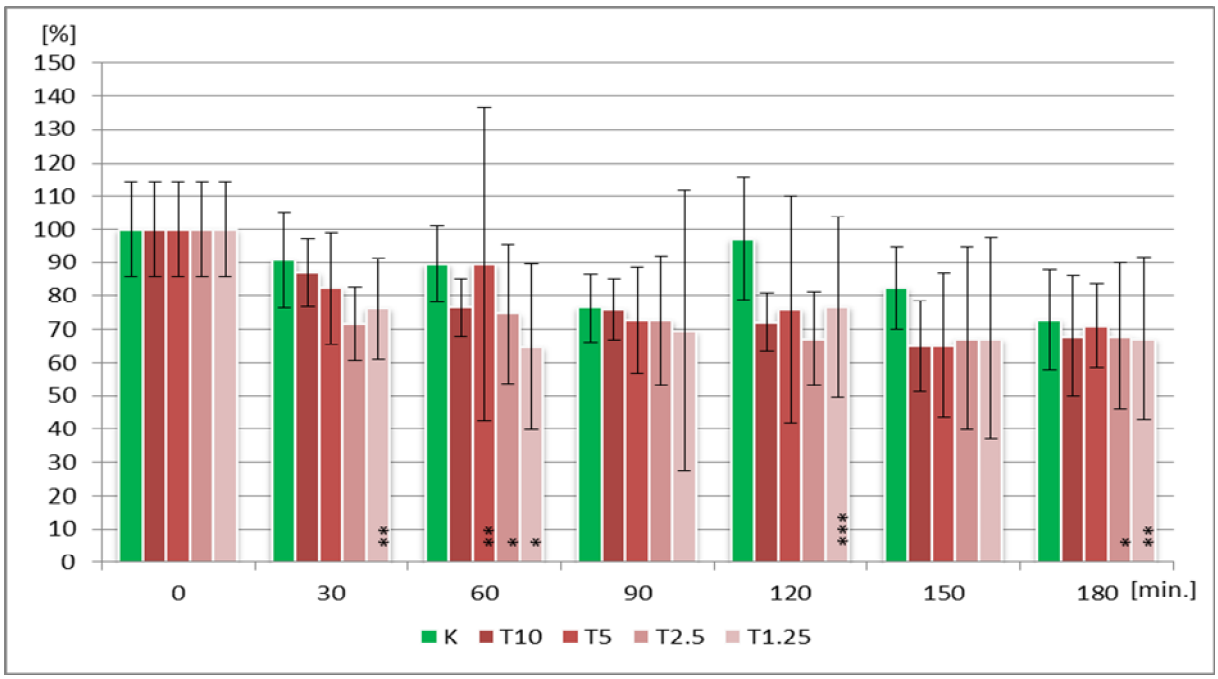

Figure 4 Amplitude of lateral head displacement (in μm) in groups with different concentration of trehalose and time periods [min.]. Significant differences *p<0.05; **p<0.01; ***p<0.001.

Similarly as for the previous two parameters, the beat cross frequency (Fig. 5) in the group T10 was not significantly altered. Statistically significant lower values in samples T5

and T2.5 only after 180 minutes cultivation were found. For sample T1.25 a significantly lower values after 60 and 180 minutes of cultivation were detected.

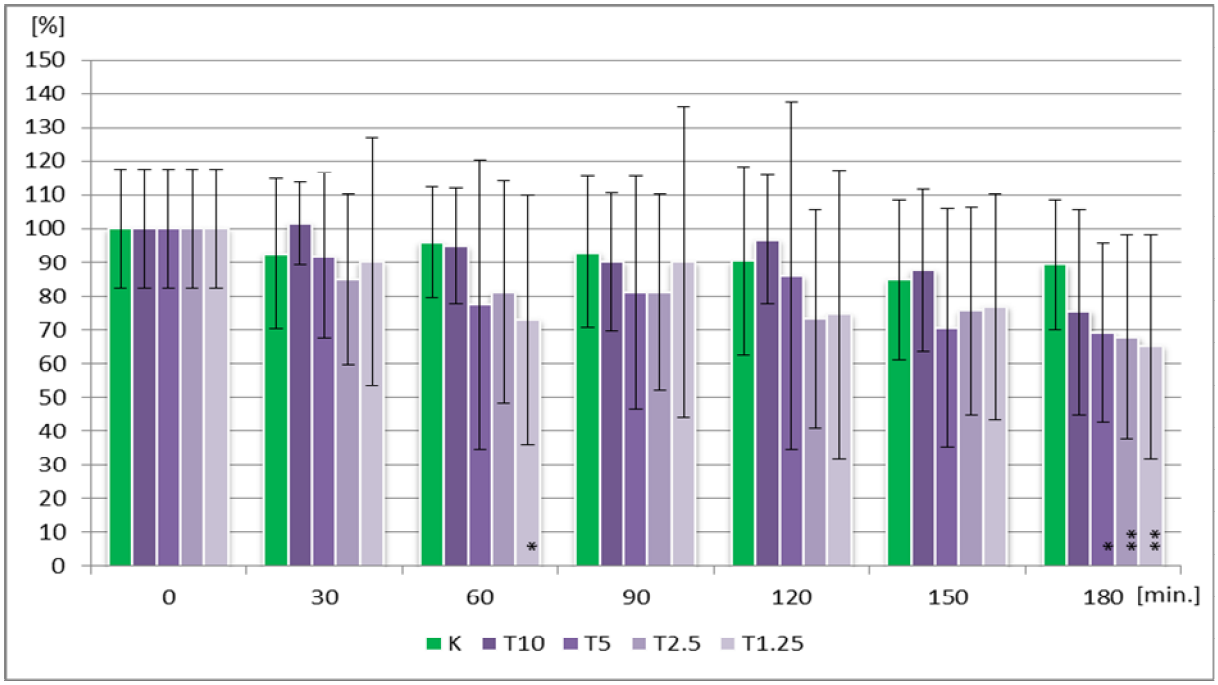

Figure 5 Beat cross frequency (in Hz) in groups with different concentration of trehalose and time periods [min.]. Significant differences *p<0.05; **p<0.01; ***p<0.001.

Positive effect of higher concentration of trehalose on spermatozoa motility described **Hu et al., (2010).** The purpose their study was to determine the effects of various doses of trehalose on *in vitro* semen quality parameters and antioxidant activities of frozen-thawed bovine semen. The semen samples, diluted with an extender containing trehalose (0, 25, 50, 100 and 200 mM), were evaluated. The extender supplemented with 100 mM trehalose exhibited the highest percentages of spermatozoa motility, acrosomal membrane integrity, and plasma membrane integrity, in comparison to the control group ($p<0.05$). No difference was observed for spermatozoa motility between trehalose 50 and 100 mM groups ($p>0.05$).

Trehalose has the antioxidant effect on membrane integrity of spermatozoa and protective function against the thermal shock for frozen insemination doses **(Molinia et al. 1994). Eiman et al. (2003)** analyzed the influence of trehalose cryopreservation of goat spermatozoa. Trehalose solution (0.375 M) was added to TCG solution (Tris-citric acid-glucose) to give the following trehalose extenders: 0% (only TCG), 25%, 50%, 75%, and 100% (v:v) (only trehalose). The spermatozoa motility parameters improved significantly by increasing the concentration of trehalose ($p<0.05$) and significantly high recovery rates for the

motility parameters were also achieved by a high concentration of trehalose ($p<0.05$). Motility of the frozen-thawed spermatozoa after a 3-h incubation improved significantly with increasing concentrations of trehalose in the extender ($p<0.05$).

Hu et al. (2009) investigated the effect of different concentrations of trehalose (0, 25, 50, 100 and 200 mM) on boar semen. Trehalose was added to basic diluent – TCF (Tris-citric acid- fructose). Trehalose 100 mM conferred the highest spermatozoa motility values, with an improvement of a 19% over the control group ($p<0.01$). No significant difference was observed for spermatozoa motility between freezing extenders containing trehalose 50 mM and 100 mM ($p>0.05$). Addition of 25 mM of the trehalose to the cooling extender showed an intermediate effect on motility preservation. Concerning percentage of linear motile spermatozoa when trehalose concentrations higher than 100 mM significantly decreased after thawing ($p<0.01$). These results suggest that 100 mM trehalose afford better protection ability than that of others during the freeze-thaw process in boars.

Another experimental work dealing with the influence of trehalose on spermatozoa motility was realized by **Kozdrovski (2009).** The study describes the effect of a different trehalose concentration on the post-thaw viability and fertility of European brown hare spermatozoa. Immediately after collection, the semen was diluted with an extender of the following composition: Tris 250 mM, citric acid 80 mM, glucose 70 mM, DMSO 1.0 M, egg yolk (17%, v/v), and kanamycin (80 $mg.l^{-1}$) – Protocol I. In Protocols II and III, respectively, 50 mM and 100 mM of trehalose were added to the extender. Immediately after thawing and after 3 hour incubation at 37◦C, motility characteristics of frozen/thawed semen were assessed with computer-assisted semen analysis system After 3 hours of incubation, motility and spermatozoa with progressive motility (PMOT) were the lowest in the semen frozen with supplementation of 100mM of trehalose ($p<0.01$).

CONCLUSION

Results of our experimental work suggest a positive *in vitro* effect of trehalose in higher concentration (10 $mg.ml^{-1}$) in comparison to control diluted with physiological solution. Lower concentrations of trehalose have a negative impact on spermatozoa motility parameters (motility; progressive motility; velocity curved line; amplitude of lateral head displacement and beat cross frequency). At lower concentrations of trehalose (5; 2.5; 1.25 $mg.ml^{-1}$), decreased motility parameters were found. Generally, the results of this study found a dose–dependent effect of trehalose on turkey spermatozoa motility *in vitro*.

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