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REGULAR ARTICLE

THE EFFECT OF *IN VITRO* SEMEN STORAGE TEMPERATURE AND AGE OF MALES ON SPERMATOZOA MOTILITY PARAMETERS OF TURKEYS SEMEN

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ABSTRACT

This work was to evaluate the effect of *in vitro* storage temperature and age of males on turkey spermatozoa motility. For this purpose samples were collected from British United Turkeys (BUT) Big 6 line and semen quality was assessed by using Computer Assisted Semen Analyzer (CASA) system. After 60 minutes of storage spermatozoa motility, progressive motility and amplitude of lateral head displacement decreased significantly both in 4° and 41°C regardless of birds age. However the lowest values of all parameters were noted after storage in thermostat. Spermatozoa motility after 0 and 60 minutes in 4°C was higher in samples collected from turkeys of 35 – 42 weeks of age (60.94% and 53.33% respectively). Whereas the value of that parameter in semen stored in 41°C was lower in that age group. The same tendency was found in progressive motility. The results showed that higher temperature of *in vitro* storage (even that similar to animal body temperature, in this case 41ºC) has more negative effect on spermatozoa motility parameters than lower temperature.

Keywords: spermatozoa, motility, turkey, CASA

INTRODUCTION

In poultry breeding one of the most important issue is the identification of males with high fertilizing ability. Beside genetic conditionings there are many factors affecting this ability. These include age, season, amount of light, state of health and nutrition, number of copulations or ejaculations per day and sperm competition.

Semen quality and quantity changes during the year. Semen volume and sperm concentration increase from December to the end of April and decline in summer **(Krzymowski, 1981)**. It is connected with day length. Appropriate long day length induces gonadotropin secretion and gonadal growth **(Bentley et al., 2007)**, thereby semen volume.

The ejaculate size also decreases with number of copulation or ejaculation per day. Significant differences occur even after 3 - 4 successive ejaculations **(Sturke, 1970)**.

The sperm competition results from the males necessity to defend their paternity. Because of that even facing the rival increases the ejaculate volume **(Briskie and Montgomerie, 2007)**.

Even though the ejaculate size influences on semen quality the more significant correlation is between fertilizing ability and semen quality than quantity. That is the reason why the assessment of sperm quality is of great interest. For this purpose sperm morphology, motility, integrity of organelles, metabolism and ability to interact with the egg investments is evaluated.

The assessment of spermatozoa morphology concentrates on determination of proportion of normal and abnormal types using a light microscope. It is very time - consuming and subjective method.

Many dyes enable evaluation of membrane integrity. Some of them such as eosin stain only organelles with damaged membrane while fluorescent dyes also penetrate nuclear membranes. For assessment of metabolism the oxidoreduction dyes are used. They are coloured in their reduced or oxidized form so when they enter spermatozoa they may be reduced by sperm metabolism, thereby the coloured or uncoloured product is made.

The evaluation of spermatozoa ability to interact with egg investments is possible using one of two methods. The first focuses on assessing the number of spermatozoa bound to perivitelline proteins and the second tests ability of spermatozoa to produce points of hydrolysis in the inner perivitelline layer **(Wishart, 2009)**.

The most distinctive feature for spermatozoa is their motility. There are many methods for its evaluation. The most subjective is microscopic observation **(Tabatabei, 2010)**. Some

of the objective methods based on light scaterring of sperm movement within the light path of a spectrophotometer or the movement of a population of spermatozoa from a low - viscosity medium into a medium of high viscosity containing the polymer Accudenz **(Wishart, 2009)**. Moreover for motility assessment Computer Assisted Semen Analyzer (CASA) system is employed. Beside motility and progressive motility this system enables measurement of average path distance (DAP, μm), curved line distance (DCL, μm), straight line distance (DSL, μm), average path velocity (VAP, μm.s^{-1}), curvilinear velocity (VCL, μm.s^{-1}),straight line velocity (VSL, μm.s^{-1}), straightness index (STR), linearity index (LIN), wobble (WOB), amplitude of lateral head displacement (ALH, μm) and beat cross frequency (BCF, Hz) **(Roychoudhury, 2010)**.

The aim of this study was to analyze the influence of age of males and *in vitro* storage temperature on the turkey spermatozoa motility parameters.

MATERIAL AND METHODS

Semen collection

14 semen samples were collected from British United Turkeys (BUT) Big 6 line from husbandry in Podhorany (Slovakia). 7 samples came from toms of 35 – 42 week of age (group A) and next 7 from males in 63 – 73 week of age (group B). Each sample was a mix of ejaculated semen from 8 males.

All samples were diluted with physiological solution (1:200). Then from every sample were prepared two (one was placed in 4 °C and one in 41°C).

Semen evaluation

Motility parameters were assessed using CASA system (Computer Assisted semen Analyzer) with a microscope (Olympus BX 51, Japan) after 0 and 60 minutes of storage. 10 µl of each sample was loaded onto Makler Counting Chamber (Sefi–Medical Instruments, SRN) slide which was then placed on a microscope stage. The system analyzed 7 different fields from one slide.

Motility parameters which were measured included: motility (%), progressive motility (%), VCL (curvilinear velocity, μm.s^{-1}), ALH (amplitude of lateral head displacement, μm) and BCF (beat cross frequency, Hz).

Statistical analysis

For statistical comparison of the data from different storage temperature and from two age groups was used Student's t-test. Statistical significance was indicated by *P* values of less than 0.05, 0.01, 0.001.

RESULTS AND DISCUSSION

After 60 minutes of storage spermatozoa motility decreased significantly both in 4° and 41°C regardless of age of birds ($p<0.001$) (Figure 1). However, the lowest values in both age groups were observed after 60 minutes in higher temperature (26.20% in samples collected from younger males, 30.42% in samples from older).

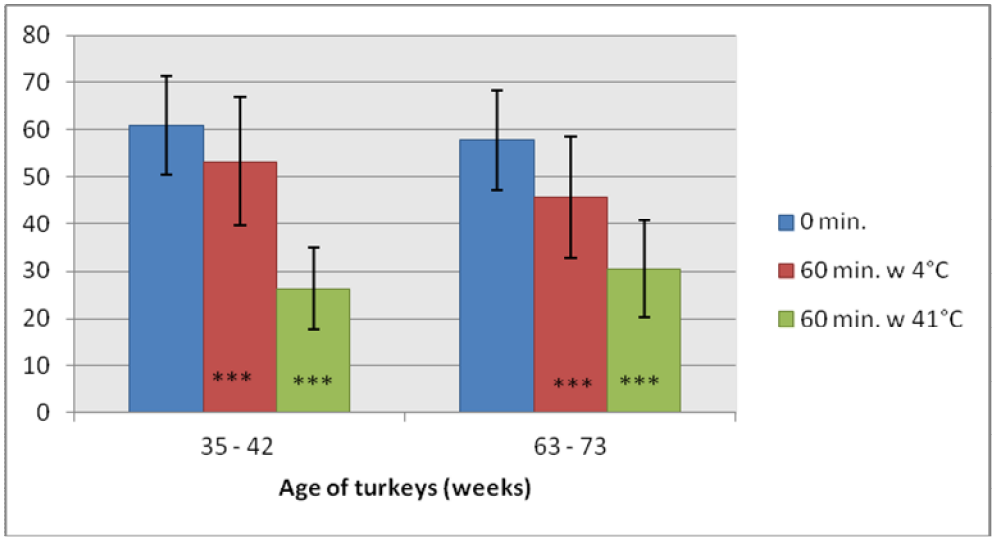

Figure 1 Spermatozoa motility (%) in semen of turkeys from two age groups storage at different temperature (°C). Significant differences between storage temperature: *p<0.05, **p<0.01, ***p<0.001

Likewise progressive motility values decreased significantly after 60 minutes of storage both in semen samples from younger and older toms ($p<0.001$) (Figure 2). The higher values of that parameter were detected in semen from males of 35 – 42 weeks of age. Except of samples stored in thermostat where higher values had older males.

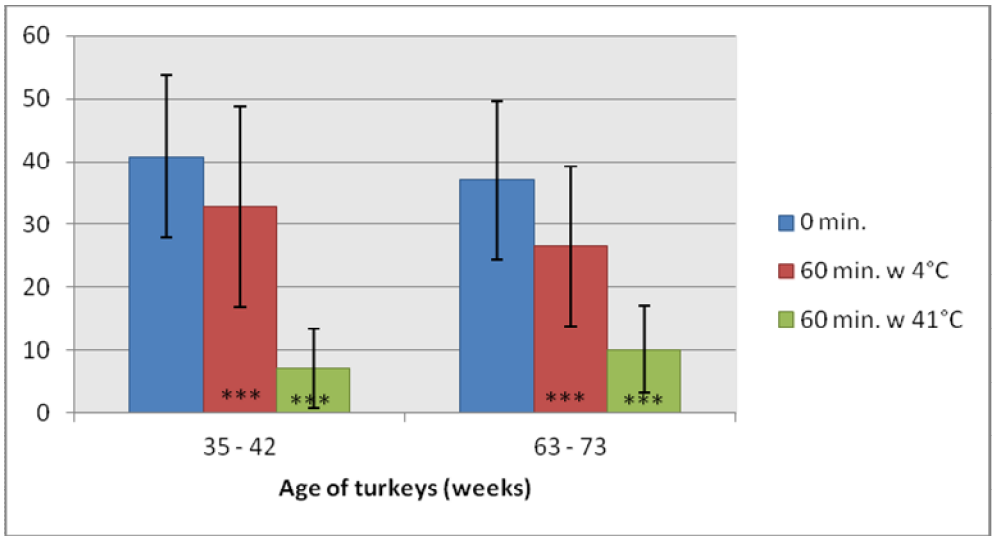

Figure 2 Progressive spermatozoa motility (%) in semen of turkeys from two age groups storage at different temperature (°C)

The curvilinear velocity decreased significantly after storage in higher temperature in both age groups (Figure 3). Whereas significantly lower value in samples stored in 4°C was recorded only in younger group of birds ($p<0.001$).

The similar tendency was observed in amplitude of lateral head displacement (Figure 4). The highest values were noted after 0 minutes of storage in both age groups.

The beat cross frequency declines after 60 minutes of storage but significant differences were noted only after storage in thermostat ($p<0.001$) (Figure 5).

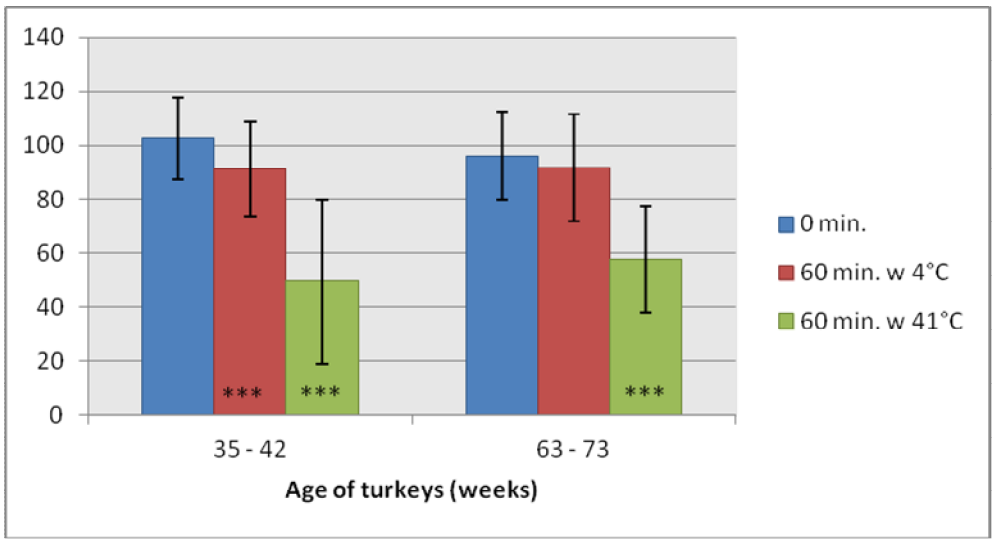

Figure 3 Curvilinear velocity ($\mu m.s^{-1}$) in semen of turkeys from two age groups storage at different temperature (°C)

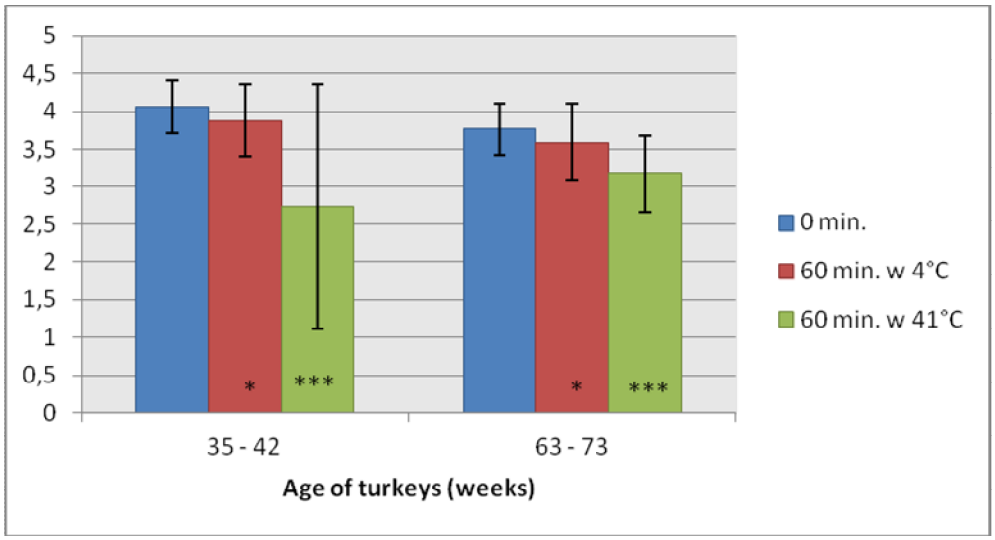

Figure 4 Amplitude of lateral head displacement (µm) in semen of turkeys from two age groups storage at different temperature (°C)

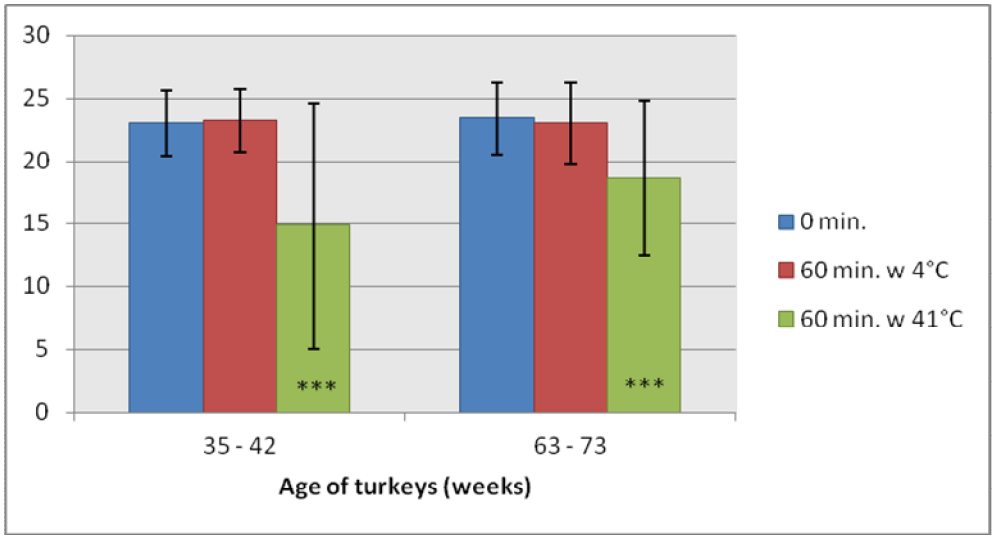

Figure 5 Beat cross frequency (Hz) in semen of turkeys from two age groups storage at different temperature (°C)

Gained results showed that after 60 minutes of storage motility parameters decreased both in 4 and 41°C. The same results were described by **Kotłowska *et al.* (2007)**. In their experiment the turkey semen from males of 34 – 43 week of age was stored in 4 - 7°C for 48 hours. Measurements were made after 2.5, 24 and 48 hours, respectively. For sperm quality assessment motility, curvilinear velocity, average path velocity, straight line velocity, linearity and beat cross frequency were noted. Values of these parameters declined during storage time but significant difference was observed after 48 hours.

Similar results were detected by **Douard *et al.* (2000)**. They also used semen collected from British United Turkeys (BUT) Big 6 line which was stored in 4ºC for 48 hours. Results were recorded after 1, 24 and 48 hours and showed that percentage of motile spermatozoa decreased during time of storage.

Dumpala *et al.* (2006) analyzed effect of semen storage temperature and diluent type on the sperm quality of Broiler Breeder. They stored samples in 4, 21 and 41 ºC for 8 hours. The gained data showed that percentage of dead spermatozoa was increased during time of storage and was the highest in samples stored in 41 ºC.

Iaffaldano *et al.* (2008) analyzed effect of turkeys age on semen quality. They collected semen samples from British United Turkeys (BUT) Big 6 line classified in two groups according their ages: 32 – 40 and 44 – 52 weeks of age. Ejaculate samples were diluted with BPSE (Beltsville poultry semen extender) and placed in 5 ºC. Motility was measured after 3, 24 and 48 hours using Sperm Motility Test (SMT). As it was expected higher values were noted in samples of younger toms. The semen quality declined after storage in both groups.

Similar observation described **Kelso *et al.* (1996)**. They compared ejaculates collected from 25 and 60 weeks old broiler fowls. For this purpose they measured sperm concentration, percentage of live spermatozoa and their metabolic activity. It turned out that better quality represents sperm from younger males.

CONCLUSION

The gained data showed that semen quality declines after storage. What is more higher temperature (even that similar to animal body temperature, in this case 41ºC) has more negative effect on spermatozoa motility parameters than lower temperature. Regardless of age of birds semen quality changes significantly.

The further researches need to investigate type of diluents which improve semen quality during storage.

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