



THE EFFECT OF DIFFERENT TEMPERATURE AND MEDIUM ON TURKEY SPERMATOOZOA MOTILITY *IN VITRO*

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ABSTRACT

Target of this study was to analyze the effect of different diluents (saline solution, commercial diluent and yolk diluent) on the functional parameters of turkey spermatozoa after a culture *in vitro* at 5 °C and 20 °C. Samples were subjected to repeated measurements at intervals of 0, 30, 60, 90, 120, 180, 240, 300 minutes. Significantly the highest motility was found in the samples with yolk diluent at 5 °C and at 20 °C. Lower values were measured in samples diluted with saline solution and commercial diluent. Progressive motility copied the finding results of the overall motility with the exception of yolk diluent, which were reported significantly lower values.

Keywords: turkey, spermatozoa, diluents, egg yolk, CASA

INTRODUCTION

Spermatozoa viability and fertilizing activity is determined by metabolism. Metabolic processes that take place in spermatozoa are used to ensure the movement of spermatozoa. The quality of these processes depends on the quantity and quality of available energy resources (**Breuer and Wells, 1977**).

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**).

Preservation of poultry semen fertilization capacity for 24 to 48 hours requires oxygen. The diluent must provide inputs of fructose or glucose as substrates for the production of ATP, a sufficient buffering capacity, pH and maintain the ambient temperature from 4 to 7°C (**Akçay et al., 2006**).

Suitable diluent must provide energy for spermatozoa, maintain pH and osmolarity on the level of seminal plasma with an identical and a natural medium for spermatozoa (**Siudzińska and Łukaszewicz, 2008**). For longer storage of birds semen have developed a number of diluents (**Akçay et al., 2006**).

Most saline solutions are suitable for direct spermatozoa survival because they provide an osmotic pressure (330 to 400 mOsm) and pH (7.0 to 7.4) identical with seminal plasma (**Thurston, 1995**). Solvents should also contain a variety of energy substrates. Therefore, the diluents used for poultry ejaculate are enriched of carbohydrates (glucose or fructose) and other components are likely to provide energy (citrate, glutamate, acetate) (**Thurston, 1995**).

Phospholipids make up almost 60% of the total lipids in turkey semen (**Cerolini et al., 1997**). Avian spermatozoa phospholipids are further characterized by a large proportion of polyunsaturated fatty acids (PUFAs) that are associated with a high index of peroxidase (**Surai et al., 2001**). Physiologically high ratios of PUFA in avian spermatozoa are integral (indivisible) to maintain membrane fluidity and flexibility during the fertilization process. In birds, lipids were considered a possible source of energy during *in vitro* storage under aerobic conditions. A study has demonstrated a direct link between compromised spermatozoa of poultry after *in vitro* storage and lipid peroxidation (**Long and Kramer, 2003**). According **Kotłowska et al. (2007)** addition of solvent acetate and vitamin E is also beneficial for the maintenance of sperm motility during storage.

Important for the development of diluents and storage systems for poultry ejaculate are physiological differences and metabolic requirements of spermatozoa in different species.

Cock spermatozoa are metabolically competent in both aerobic and anaerobic environments *in vitro*. In contrast, turkey spermatozoa require a high level of oxygen to survive (**Wishart, 1982**).

Yolk diluent is a combination of egg yolk and phosphate buffer. This combination is able to reliably preserve life and fertility on the ability of spermatozoa in fresh semen. Egg yolk contains nutritional substances that make around spermatozoa protective colloids, thereby preventing the harmful effects of external factors. Its most important feature is the ability of spermatozoa to increase resistance to thermal shock. This feature is characterized by lecithin, but also the lipid-protein complex, which is contained in the raw yolk. The maximum lifetime was found in spermatozoa diluent with 20 to 25% share of the yolk. Today, the phosphate buffer is replaced by sodium citrate. The optimum composition of yolk diluent is 3 parts of sodium citrate and 1 part yolk. At 5 °C the culture spermatozoa fertility capacity maintains till 2 or 3 days (**Gamčík et al., 1976**).

It is difficult to maintain the quality of turkey spermatozoa for *in vitro* storage for 24 hours. (**Thurston et al., 1994**). 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**).

The aim of this study was to analyze the influence of different extenders 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, UK) was used.

Semen samples were transferred to the laboratory and before analysis diluted as turkey semen contains high concentrations of spermatozoa. As diluents were used – saline solution (Sodium Chloride 0.9%; Birffe Midetal S.p.A., Grosotto, Taliansko), commercial diluent and yolk diluent in a ratio of 10 µL of semen, and 2000 µL of diluent, so the final dilution was 1:200 in the analysis. Half of the samples were maintained at 5 °C and rest at laboratory temperature 20 °C.

The functional parameters of spermatozoa motility were evaluated and compared at 5 °C and at 20 °C at intervals of 0, 30, 60, 90, 120, 150, 180, 240 and 300 minutes. Subsequently 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 (**Slanina et al., 2012; Massányi et al., 2012**). 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 study was aimed to determine the impact of different media types (saline solution, commercial and yolk diluent) on turkey spermatozoa motility parameters. Kinetic parameters were studied *in vitro* in a time frame from 0 to 300 minutes at two incubation temperatures: 5 °C and 20 °C.

In analysis of the percentage of total spermatozoa motility was found that the sample diluted with yolk diluent at 5 °C and time 0 minutes showed the highest values. Similar values were found in the sample also at 20 °C. The effect of this diluent show a higher efficiency compared to a commercial diluent and saline solution at intervals of 0-60 minutes ($p < 0.05$, $p < 0.01$). In the time interval from 60 to 90 minutes differences in the effectiveness of saline solution, which significantly decreased at 5 °C and at 20 °C ($p < 0.05$, $p < 0.001$) were detected. After 90 minutes to 300 minutes in the sample diluted commercial diluent at 5 °C ($p < 0.05$, $p < 0.01$) reduction of motility was observed. At the time of 180 minutes in a sample containing a saline solution at both temperatures ($p < 0.05$, $p < 0.001$) a conclusive decline of the motility was recorded and at the time interval from 180 to 240 minutes the motility was significantly increased at both temperatures ($p < 0.05$, $p < 0.001$) (Figure 1).

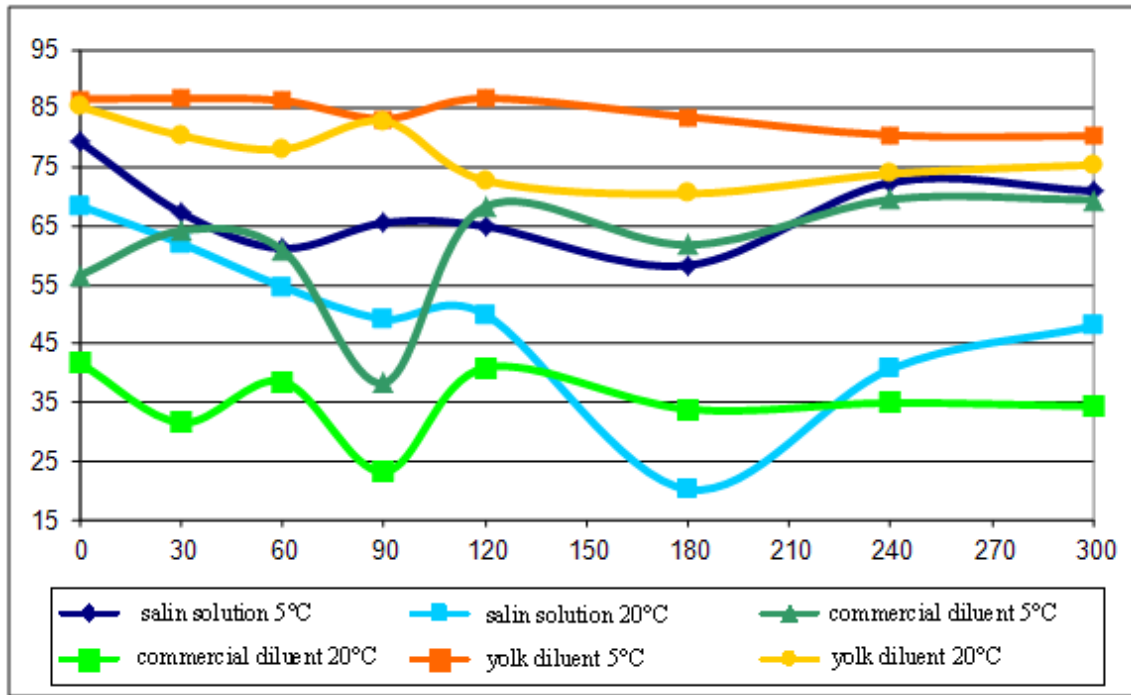


Figure 1 Spermatozoa motility (in%) at temperatures 5 °C and 20 °C in various diluents and the times

The percentage of progressively motile spermatozoa at Time 0 was reported significantly ($p < 0.05$, $p < 0.001$) the highest in sample diluted with saline solution at 5 °C and subsequently also at 20 °C, followed by the yolk diluent at 5 °C and yolk diluent at 20 °C. The significantly low levels of motility ($p < 0.05$, $p < 0.01$) were found in a commercial diluent at 5 °C and significantly lowest values were measured in samples diluted commercial diluent at 20 °C ($p < 0.05$, $p < 0.001$). At the time 30 minutes differences in the effectiveness of diluents were observed. At 5 °C yolk diluent yolk significantly force upward trend ($p < 0.05$), but on the other hand the effect of saline solution significantly decreases ($p < 0.05$, $p < 0.01$). At the time of 60 minutes there is a decrease in efficiency of yolk diluent at 5 °C, a commercial diluent at both temperatures, and conversely increase of the efficacy of saline solution at 5 °C ($p < 0.05$, $p < 0.001$) and at 20 °C ($p < 0.05$, $p < 0.001$), as well as yolk diluent at 20 °C ($p < 0.05$, $p < 0.01$). At the time interval (90-300) minutes significantly increase ($p < 0.05$, $p < 0.001$) of progressive motility in the commercial diluent at 5 °C, in this time significantly decreased ($p < 0.05$, $p < 0.01$) progressive motility in yolk diluent at 20 °C ($p < 0.05$, $p < 0.001$) and in a saline solution at 20 °C ($p < 0.05$, $p < 0.001$) was found (Figure 2).

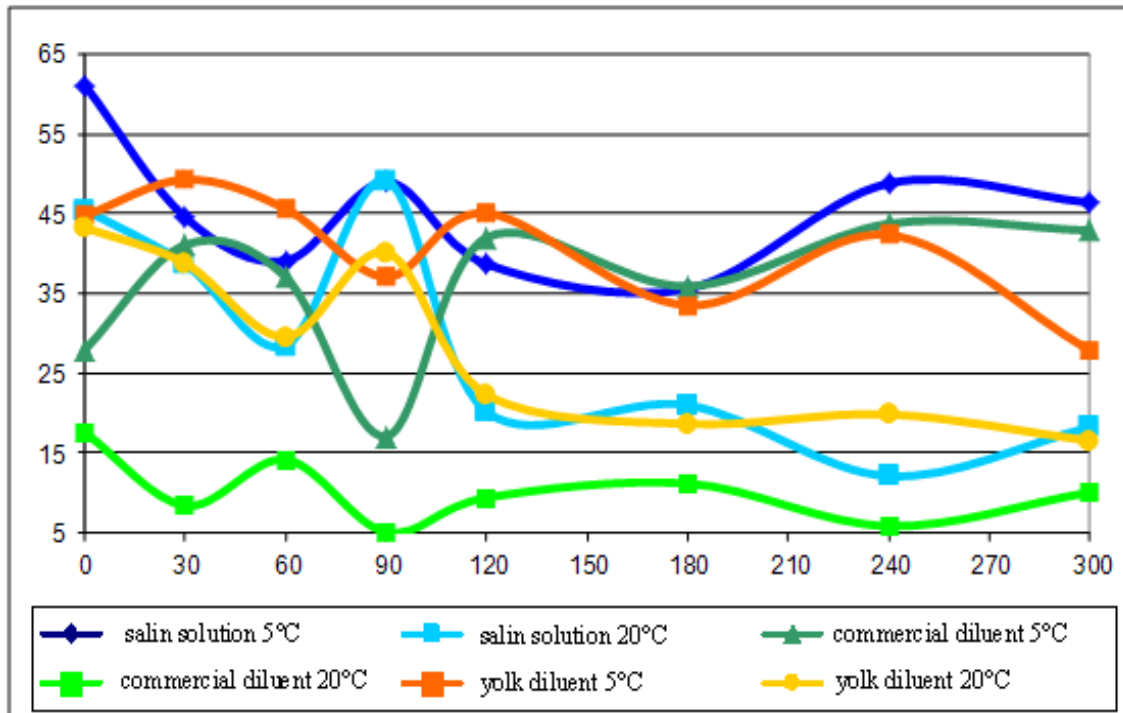


Figure 2 Progressive spermatozoa motility (in%) at temperatures 5 °C and 20 °C in various diluents and the times

From velocity parameters was evaluated parameter VCL - velocity curved line. The highest value of VCL was observed at the beginning of the measurement at time 0 minutes in saline solution at 5 °C ($p < 0.05$, $p < 0.001$). Significant differences were found later at the time of 60 minutes in a commercial diluent at 20 °C ($p < 0.05$, $p < 0.01$) at 5 °C ($p < 0.05$, $p < 0.01$) and in saline solution at 5 °C ($p < 0.05$, $p < 0.001$). At the time of 120 minutes were shown significant differences in yolk diluent at 5 °C ($p < 0.05$, $p < 0.01$) and at 20 °C ($p < 0.05$, $p < 0.001$), in a commercial diluent at 5 °C ($p < 0.05$, $p < 0.001$), in saline solution at 5 °C ($p < 0.05$, $p < 0.001$) and in saline solution at 20 °C ($p < 0.05$, $p < 0.01$). (Figure 3).

Amplitude of lateral head displacement (ALH) in sample diluted with saline solution value at 5 °C varied from 4.51 μm (0 minutes) to 4.03 μm (300 minutes) and at 20 °C from 3.93 μm (0 minutes) to 3.45 μm (300 minutes) and was declined. In sample diluted with commercial diluent at 5 °C values were from 3.70 μm (0 minutes) to 4.36 μm (300 minutes) and at 20 °C from 3.41 μm (0 minutes) to 3.29 μm (300 minutes). Samples diluted with egg yolk reached at 5 °C values of 3.72 μm (0 minutes) to 3.02 μm (300 minutes) and at 20 °C from 3.60 μm (0 minutes) to 3.02 μm (300 minutes) (Figure 4).

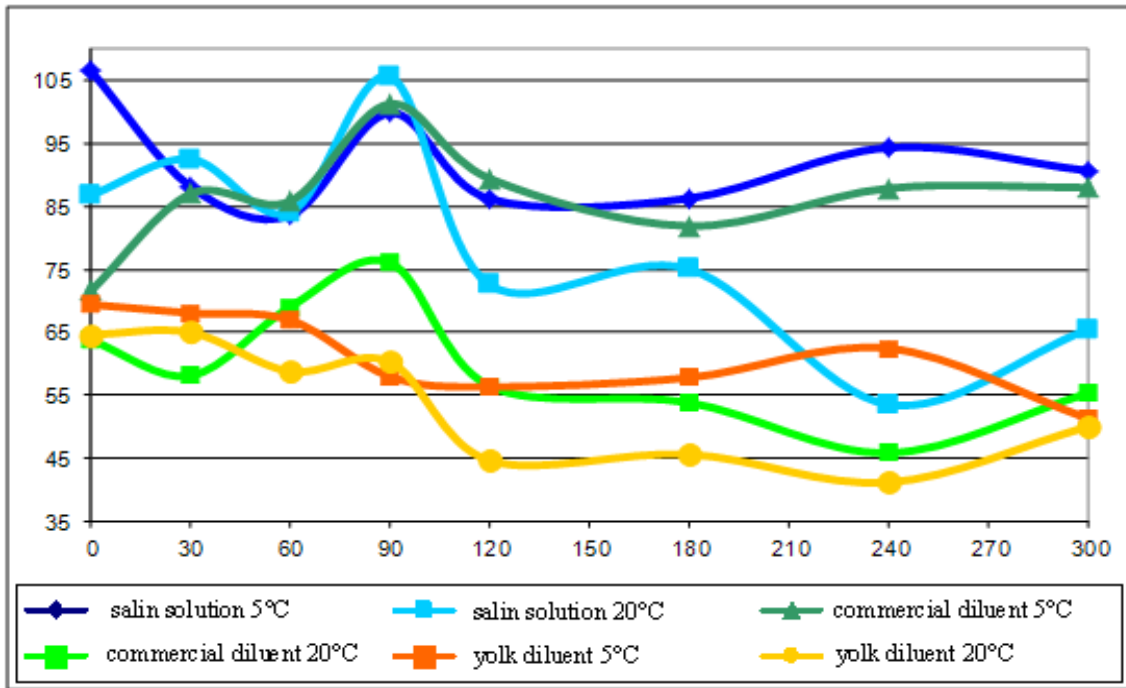


Figure 3 Velocity curved line (in $\mu\text{m}\cdot\text{s}^{-1}$) at 5°C and 20°C in various diluents and the times

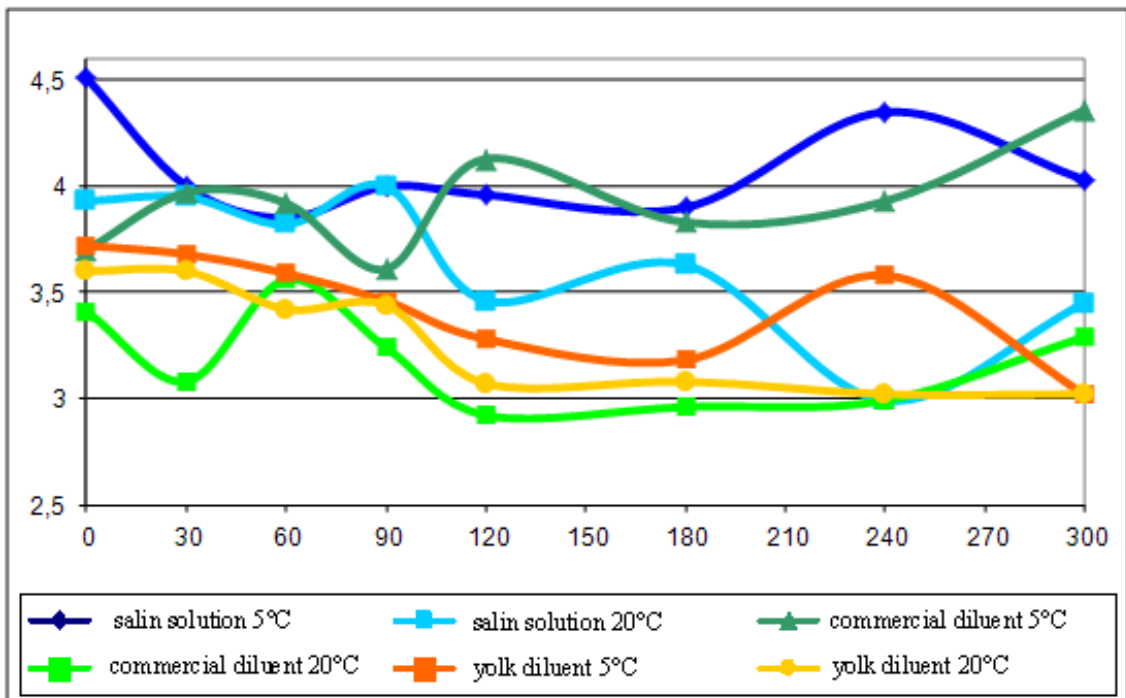


Figure 4 Amplitude of lateral head displacement (in $\mu\text{m}\cdot\text{s}^{-1}$) at 5 °C and 20 °C in various diluents and the times

The lowest BCF (beat cross frequency) at 0 minutes for a sample diluted with commercial diluent and cultured at 20 °C was recorded (19.77 Hz). On the other hand, in samples diluted with saline solution and cultured at 5 °C at time 0 minutes highest value

(22.46 Hz) were measured. In all diluents on both temperature the most significantly decrease was found only the yolk diluent at 5 °C between 90 and 120 minutes. Comparing the samples in various diluents it was found that in sample cultured at 5 °C, the BCF was higher than that in sample cultured at 20 °C. For the samples diluted with saline solution this status only to 180 minutes was recorded.

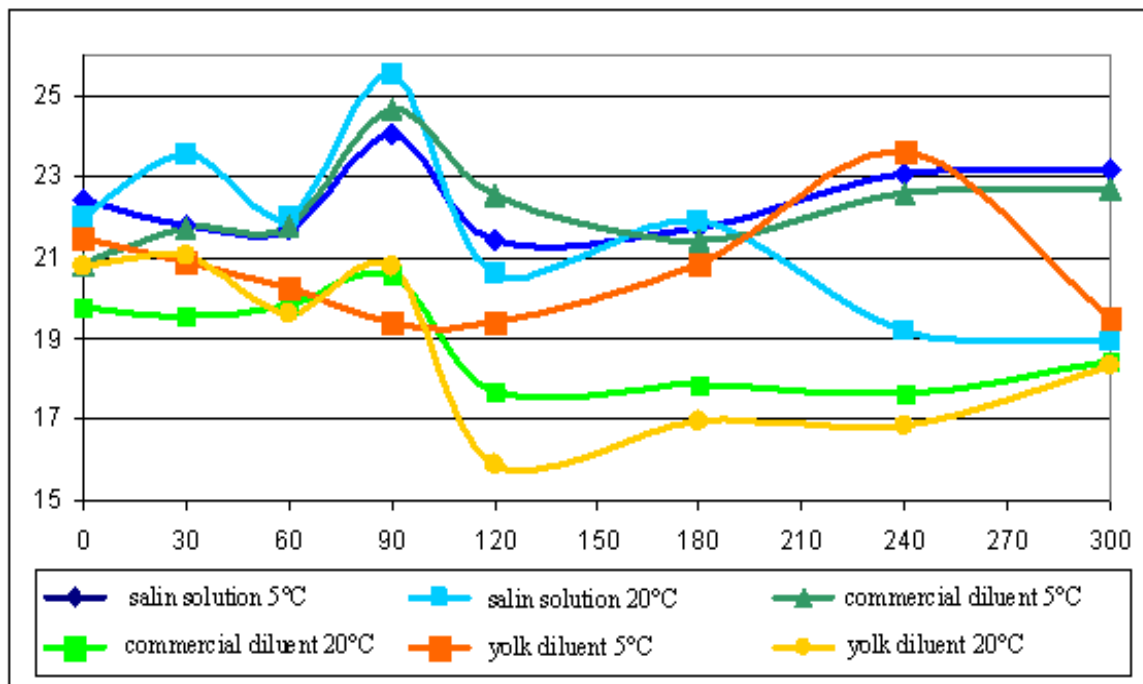


Figure 5 Beat cross frequency (in Hz) at 5 °C and 20 °C in various diluents, and the times

Contribution was aimed to determine the effects of different types of diluent (saline solution, commercial diluent and yolk diluent) the motility characteristics of turkey semen and showing its fertilizing ability.

Turkey semen usually can not be stored longer than 6 hours without loss of fertilizing ability, even if oxygenic and stored in the diluent at reduced temperature. According to **Pakhurst et al. (2000)** stored turkey spermatozoa showed a curvilinear decrease in spermatozoa motility after 48 hours, but 36% loss of initial activity accounted for the first 6 hours of storage. Spermatozoa which were studied between 6 and 24 hours lost 18% of their original activity and after 48 hours decrease by 30%.

Several attempts of **Sexton and Giesen (1983)** were performed by measuring the effect of diluent, pH, size, volume of insemination dose and frequency of insemination on motility and fertilization capacity of turkey spermatozoa. The measurement was carried out at an interval of 6 hours at 15°C. Fertilizing capacity and motility of both samples in storage was

suppressed more if diluted with Beltsville Sperm Poultry Extender at pH 5.5 than at pH 6.5 or 6.0. There were observed differences in fertilization ability of semen in storage and stored.

Hirai et al. (1997) examined three parameters of motility in fresh bull semen with the addition of yolk diluent. The average velocity of progressively motile spermatozoa (VPM), the velocity of linear progressively motile spermatozoa (VLP) and the percentage of linear swimming spermatozoa (LIN) were evaluated. The addition of 10, 20 or 30% egg yolk to Tris buffer (pH 6.5) resulted in a linear decrease of VPM and a decrease in the percentage of progressively motile spermatozoa, but it increased the relative rate of LIN in fresh diluted semen. Increasing the levels of egg yolk in the diluent resulted in higher viscosity. The VLP was significantly higher than the VPM.

Gündoğan et al. (2003) examined the effect of five different diluents and the glucose-phosphate (GP), cream-yolk diluent (MY), diluent containing egg yolk and citrate (YC), blood serum (S) and seawater (SW) to spermatozoa motility, while semen was stored at 5 °C. The percentage of moving spermatozoa was evaluated every 24 hours until 10 days after the dilution. Sea water had no effect on primary motility. Spermatozoa motility in this diluent was 0. Diluent glucose-phosphate of the highest motility values after 24 h of incubation ($80.5 \pm 0.88\%$) than blood serum ($69.5 \pm 1.70\%$) and creamy yolk diluent ($78.0 \pm 1.17\%$) while diluent containing egg yolk and citrate exhibited motility values ($79.0 \pm 1.24\%$) were reported. Diluents glucose-phosphate and egg yolk-citrate maintained the more than 50% of the spermatozoa motility to 4 day of cultivation, while the cream-yolk diluent only the third day of cultivation and blood serum in only 2 days.

In our work it was found that the dilution of the semen and subsequent storage at 5 °C generally show better values than samples that were cultured at 20 °C. Using analyzes have shown that dilution with financially undemanding yolk diluent, in comparison with saline solution and the commercial diluent has the best effect on semen quality, and therefore has a positive impact on the functional parameters of spermatozoa motility.

CONCLUSION

Our results show that diluent with egg yolk have positive effect on turkey spermatozoa motility parameters. In comparison with saline solution and commercial solution, yolk diluent causes the best motility at time 0 and after 5 hours at both temperatures. The progressive motility results indicate that the sample diluted with yolk diluent has only the third best value (27.80%), after saline solution (46.37%) and commercial diluent (42.87%) at time 300

minutes and at 5 °C. In general the positive effect on the functional parameters of spermatozoa motility was found for spermatozoa treated at 5 °C in comparison with 20 °C.

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