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Effect of Different Processing Temperature on Motility and Viability in Canary Sperm

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ABSTRACT

Background: Canaries have attracted people's attention with their colors, shapes and impressive songs. The fact that canaries are domesticated and easy to obtain has made these birds attractive for songbird studies. In our study, canary semen was examined at different temperature values. This study aims to determine the most efficient spermatozoa examination temperature in canaries.

Methods: In this study, five different temperature values (36, 38, 40, 42 and 44°C) were determined. The study used 6 active male Gloster canaries in each group. After each semen collection application, a 3-day break was given for the birds to rest. Dulbecco's Modified Eagle Medium (DMEM) expander, which is preferred by songbirds, was used in the research. The semen was diluted 1:2 in a water bath at specified temperatures (36, 38, 40, 42, 44°C) and immediately examined for motility and viability.

Result: When the study results were evaluated, it was seen that the viability rate at 38°C was higher than the other groups and 40°C gave the highest results in terms of motility. In addition to the viability rate at 38°C, the swimming speed of spermatozoa was also found to be more stable over a wider area than other groups. As a result, based on the data obtained from the study, it can be said that the most appropriate temperature for motility and viability in canary spermatozoa is 38°C.

Key words: Canary, Diluent temperature, Sperm motility, Sperm viability.

INTRODUCTION

Semen quality decreases significantly as body temperature rises in birds (Karaca *et al*., 2002). It has been reported that the body temperature in the canary is 41.6°C (Brain and Prozesky, 1963). The temperature value determining spermatological parameters in passerine birds varies according to the animal species (Lüpold *et al*., 2009). It has been stated that the motility values examined at 38-40°C are close, but slower movement is observed compared to 40-42°C in sparrows. A study reported that 40°C is optimal for sparrows (Yang *et al*., 2019). In the evaluation of motility in budgerigars at 38°C and 41°C degrees, it was observed that the highest motility value was obtained at 41°C (Madeddu *et al*., 2022). In the study of determining the speed of spermatozoa in the songbird Eurasian bullfinch (*Pyrrhula pyrrhula*), it was determined that although the average body temperature in songbirds was 40°C, temperatures below body temperature (35°C) gave more optimal results in determining the speed of spermatozoa in the preliminary study (Birkhead *et al*., 2006). It is predicted that the semen temperature of the love parrot (*Agapornis roseicollis*) is 35.7-38.2°C. In addition, studies on songbirds have emphasized that 37-38°C temperatures are ideal (Dogliero *et al*., 2015). Although the general body temperature in birds is 40-41°C, spermatogenesis develops a few degrees below body temperature (Beaupre *et al*., 1997). Because of the increase in cloacal temperature due to the increase in ambient temperature, an increase in the rate of abnormal spermatozoa and a decrease in the rate of spermatozoa were observed in male zebra finch (Taeniopygia guttata) birds (Hurley *et al*., 2018). Heat stress can cause infertility in poultry (Karaca *et al*., 2002). It has been stated that optimum temperatures determine motility and viability at 19-24°C temperatures in rock pigeons (*Columba livia*). It was determined that there was a significant decrease in spermatological parameters above the temperature value of 28°C (Cheng *et al*., 2002). In birds, semen can be stored in the female reproductive tract longer than in mammals, possibly at temperatures as high as 40°C (Holt and Lloyd, 2010). In a domestic bird species, spermatozoa in the female reproductive tract can survive up to 15 weeks (Sasanami *et al*., 2013). It has been reported that the properties of semen extender, semen storage temperature and semen storage time affect semen quality in Ross broiler breeder male roosters (Dumpala *et al*., 2006). Although semen can survive for a long time at body temperatures in the reproductive tract of both male and female birds, this period is significantly reduced in the external environment at the same temperatures. In addition, it has been observed that the rate of motility and viability can be more stable at 20-37°C in the external environment. This situation is thought to be due to the decrease in metabolic movements at low temperatures

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(Sarkar, 2020). The aim of this study is to determine the most appropriate processing or waiting temperature to be used in the examination of canary semen.

MATERIALS AND METHODS

Ethics committee permission

Ethics committee approval of this study was received from Ondokuz Mayıs University Animal Experiments Local Ethics Committee (Date: 22.06.2023; Acceptance No: 2023-47).

Animals

Canaries reach sexual maturity at ten months of age (Coutteel, 2003). In the study, six male canaries between the ages of 1 and 2 years were used in each group to examine the motility and viability rate, while five birds were used to calculate the percentage average of the sperm swimming speed. Birds were housed singly in cages measuring 60 cm × 40 cm × 50 cm. Birds were sexually activated with a photoperiod of 16 hours of light and 8 hours of darkness (Trivedi *et al.*, 2006). Semen collection from canaries was performed using the cloacal massage method (Kucera and Heidinger, 2018). In birds, feces are excreted from the cloaca and the residues there can cause contamination during semen retrieval (Santiago-Moreno and Blesbois, 2020). To prevent contamination during the semen collection, birds were defecated with abdominal stimulation before semen collection. However, when defecation behavior was observed during semen collection from canaries, contaminated semen was not used so that it would not affect the study results. Before starting the study, semen was taken from each canary and motility values were determined at 37°C (Schmoll and Kleven, 2016). It was observed that the mean sperm motility was over 50%. Thus, motility and vitality losses that may occur during sperm collection from birds were minimized.

Extenders

High glucose Dulbecco's Modified Eagle's Medium (DMEM) diluent was used in the study (Opatová *et al.*, 2016). DMEM contains 42 500 mg glucose/L, 110 mg/L sodium pyruvate and L-glutamine (Humann-Guilleminot *et al.*, 2019). In studies on semen performance, it is known that extenders such as DMEM extenders are frequently used in addition to suitable temperature conditions for semen (Lüpold *et al.*, 2009). It has been reported that motility values are higher in DMEM diluent than in phosphate buffer saline (PBS) diluent. In addition, it was observed that the ratio of motile spermatozoa to semen diluted with DMEM was more stable than that of the PBS extender (Cramer *et al.*, 2019). It was diluted 1:2 with a DMEM diluent previously heated in a 36°C, 38°C, 40°C, 42°C and 44°C water bath (Hudson *et al.*, 2016). The semen density in 1 mL was calculated by the hemocytometer method by semen density examination in poultry (Bakst and Cecil, 1997). The density for Gloster canary semen collected at different times was 84×10^6 - 96×10^6 spp/mL. It was predicted that the average spermatozoa density in the semen diluted 1:2 and used was 28×10^6 - 32×10^6 spp/mL.

Evaluation of sperm

The rate of motility and viability in diluted semen was examined. Since the mean volume of Gloster canary semen is minimal (2 µl), it was thought that the semen might be adversely affected by external effects during waiting. Separately collected semen from canaries was used in each study. For the semen taken from canaries to be affected by external negativities as little as possible, the semen taken was examined without waiting. After the sperm was diluted at the desired temperature, it was evaluated by observing it under a heated light microscope for approximately 30-45 s. It is known that this time difference does not have any negative effect on motility determination (Hurley *et al.*, 2023). The sample taken from the diluted semen was stained immediately and then dried for approximately 1 min and evaluated. The time used for viability assessment did not exceed 1.5 min. This situation was repeated for all trial temperatures. The results were evaluated by taking the statistical mean.

The evaluation of motility

The study evaluated motility as the rate of progressive spermatozoa and sperm swimming speed (Sætre *et al.*, 2018). Sperm motility was evaluated as slow, moderate and fast-moving forward (Yang *et al.*, 2019). After semen was diluted 1:2 with DMEM extender, which was previously heated at desired temperatures, it was evaluated at 400× magnification with the help of Bresser Researcher Lcd light microscope and Tokai Hit heating plate at determined temperatures.

The evaluation of viability

The eosin staining method determined the viability rate of semen diluted at different temperatures (Jafari Ahangari *et al.*, 2013). After the collected semen was mixed with eosin dye at a ratio of 1:2 on the slide, a smear was taken and dried with dry air as quickly as possible. At least 200 spermatozoa were counted at 400× magnification with the help of a Bresser Researcher Lcd light microscope and Tokai Hit heating plate. Those who did not receive dye were evaluated as alive. Spermatozoa that received dye were considered dead (Fig 1).



Fig 1: Eosin staining of dead spermatozoa.

Statistical analysis

Analysis of variance one-way Analysis of Variance (ANOVA) and comparisons between groups (Duncan's test) were performed using the SPSS 22.0 package programs. The effects (significance) of the groups were evaluated at P<0.05 level (IBM., Corp., 2011). In addition, the number of animals used in the study was determined by G*Power analysis (Version, 3.1.9.7).

RESULTS AND DISCUSSION

Spermatozoa motility and viability

The effects of different temperatures (36, 38, 40, 42 and 44°C) on the motility and viability rates in canary semen are given in Table 1. The effect of different temperature values on the motility and viability rate in canary semen was very significant (P<0.001). The highest motility value (62%) was found in the Trial-3 group (at 40°C). The sperm viability rate was the highest in the Experiment-2 (at 38°C) groups. In addition, according to the polynomial analysis, linear (P<0.001) and quadratic (P<0.001) effects were very significant.

In our study, the viability rate was higher at 38°C. In our study, the difference between 38 and 40°C motility values was statistically insignificant. Although 38°C (56%) and 40°C (62%) were statistically close to each other in terms of motility, it was seen that 40°C gave more positive results numerically. The average body temperature of the canary is 41.6°C (Brain and Prozesky, 1963). When the results of our study were examined, it was seen that temperatures below body temperature were more positive. However, temperatures below a specific temperature negatively affected spermatozoa's viability and motility values. Our study observed a rapid decrease in motility and viability at 42°C after 40°C. After 40°C, viability and sperm motility decreased due to increasing temperature values. It was also observed that sperm motility remained stable for a shorter period above 38°C. Significant was found: a sharp decrease in motility and viability at 42°C and 44°C. The viability of poultry sperm can be preserved for a long time in the female reproductive tract at body temperature. However, it is known that sperm viability is negatively affected in the external environment at the same temperature. However, poultry semen can be preserved longer at low temperatures in the external environment (Sarkar, 2020; Sasanami *et al.*, 2013). It is thought that slowing down the metabolic rate at low temperatures in the external environment prolongs the survival time of spermatozoa (Sarkar, 2020). Spermatogenesis in birds occurs a few degrees below body temperature (Beaupre *et al.*, 1997). According to the results of our study, temperatures below body temperature were more optimistic regarding spermatological parameters in canaries. However, it is interesting that the difference between the body temperature expressed for canaries and the temperature values determined as a result of the study is not high and is at close values. Studies on songbirds have emphasized that temperatures of 37-38°C are ideal (Dogliero *et al.*, 2015). When the results obtained from the studies are evaluated, it is seen that they overlap with our study.

Spermatozoa speed

The effect of different temperature values on spermatozoa velocity in canary semen is given in Table 2. When the spermatozoa swimming speeds of the experimental groups were examined, it was observed that most of the five specimens (60%) in the Trial-1 group moved 'slowly.' In Trial-3, Trial-4 and Trial-5, it was determined that most of the five samples (60%, 80% and 60%, respectively, according to the trial groups) were 'fast.' In one group (Trial-2), a large majority (80%) of the five samples was determined to have a 'moderate' speed.

When sperm speeds were evaluated, it was seen that the average speed of 38°C was more stable. Notably, as the temperature increased to 42°C, the movement speed also increased. However, the viability rate decreased due to increasing temperature values after 40°C. It was also observed that sperm motility could not be maintained for a long time above 38°C. Contrary to general usage, he emphasized the need to use different temperature values according to the animal species to minimize the effect of the semen from the external environment. In a study on

Table 1: Effect of different temperature values on motility and viability in canary sperm.

Groups		Spermatological parameters	
		Motility (%)	Viability (%)
Trial 1		42±2.000^{b}	63.00±1.517^{b}
Trial 2		56±2.450^{a}	73.00±1.871^{a}
Trial 3		62±3.742^{a}	67.70±2.040^{b}
Trial 4		28±2.000^{c}	54.20±1.158^{c}
Trial 5		20±3.162^{c}	41.40±2.159^{d}
Combine		P<0.001	P<0.001
Linear	P	P<0.001	P<0.001
Quadratic		P<0.001	P<0.001

a, b, c, d: The differences between the means shown with different letters in the same column are significant (P<0.05). Trial 1, Trial 2, Trial 3, Trial 4, Trial 5: 36, 38, 40, 42, 44°C (respectively).

Table 2: Effect of different temperature values on spermatozoa velocity in canary sperm.

Velocty	1*		2**		3***	
Groups	N	%	N	%	N	%
Trial 1	3	60	2	40	-	-
Trial 2	-	-	4	80	1	20
Trial 3	1	20	1	20	3	60
Trial 4	-	-	1	20	4	80
Trial 5	1	20	1	20	3	60

*Slow, **Medium, ***Fast; N = Number of samples Trial 1, Trial 2, Trial 3, Trial 4, Trial 5: 36, 38, 40, 42, 44°C (respectively).

sperm speed and morphology, a temperature of 35°C was preferred. However, depending on the animal species, 37-39°C was used (Lüpold *et al*., 2009). A study conducted on budgies stated that the mobility value at 41°C was higher than at 38°C (Madeddu *et al*., 2022). A study reported that spermatozoa speed increases when appropriate temperature values are exceeded (Yang *et al*., 2019). As a result, although there was an increase in spermatozoa speed when the optimum temperature was increased to a certain degree, a decrease in viability and motility rates was observed. Studies conducted on songbirds appear to support our study.

Sperm motility values and viability rates vary in poultry species (Sarkar, 2020). In the study, the effects of temperature values on bird semen were examined and it was observed that the viability of semen kept outside at body temperature decreased quickly. Generally speaking, sperm motility decreases at both low and high levels. It is also known that sperm viability is higher at lower temperatures up to specific temperature values (Sarkar, 2020). When the results of our study were evaluated, it was observed that the highest survival rate was at 38°C. Although the viability rates were similar at 36°C and 40°C, it was observed that 38°C and 40°C were close to each other in terms of motility. The motility value is lower at 36°C than at 38°C. However, after 40°C, the decrease in motility and vitality values increased and the maximum decrease was observed at 44°C. Therefore, our study supports the view expressed in the study that sperm motility decreases at both high and low temperatures. In this regard, it can be said that a temperature of 38°C was successful for Gloster canary semen. As a result, it was observed that when the optimum temperature was increased to a certain degree, although there was an increase in spermatozoa speed, there was a decrease in viability and motility rates. It seems that studies conducted on songbirds support our study.

It has been reported that the motility and viability values of fresh semen in turkeys are over 70% (Kuzlu and Taskın, 2017). In the study, fresh semen was collected with a 1 mL plastic syringe and diluted with physiological saline at a ratio of 1:2. In addition, the density value for turkey semen was found to be 3.5-5×10^9 sp/mL. In our study, fresh semen was collected from a canary. 2 µL of semen was examined by diluting it with DMEM diluent at a ratio of 1:2. It is seen that the density value and semen volume of the semen diluted at equal dilution rates are significantly lower than turkey semen. Considering this situation, it is thought that canary semen is more sensitive to external temperature changes. In our study, using equal time intervals as much as possible in the evaluation of each canary semen and having the same person make the evaluation minimizes individual errors between samples and shows that the appropriate temperature under equal conditions is 38°C.

CONCLUCION

Gloster canary is a small domesticated songbird. Determination of motility and viability rate in Gloster canary semen may contribute to species-specific studies. It was noteworthy in the study that the swimming speed of the semen was more stable at 38°C; There was no statistical difference between the percentage of motility at 38°C and 40°C, but the viability rate at 38°C was significantly higher. As a result, it can be said that 38°C was more successful in the study. However, in order to have a better idea about the appropriate working temperature, it is thought that it may be useful to examine canary semen in terms of motility and viability at temperature values of 37-41°C.

Conflict of interest

The authors declare that they have no conflict of interest.

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