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REGULAR ARTICLE

EFFECT OF STORAGE TEMPERATURE ON THE MOTILITY CHARACTERISTICS OF ROOSTER SPERMATOZOA

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ABSTRACT

The objective of our study was to evaluate the influence of different storage temperature on rooster sperm motility. Semen was collected once a week from Lohmann Light breeder males into prepared sterile tube. The heterospermic pool was diluted at the ratio of 1:100 in a commercial avian extender, divided into two aliquots and incubated at 8°C or 37°C. The quality of semen samples were evaluated using CASA system (Sperm Vision™) after 30, 60 and 120 min of incubation. We observed significantly ($P<0.05$) the highest progressive motility of rooster sperm after 60 min of spermatozoa incubation at 8°C, that was also significantly ($P<0.05$) higher in comparison to spermatozoa incubated for 60 min at 37°C. Basing on the observed results, we hypothesized that law temperatures (about 8°C) would be better for long-term storage of rooster spermatozoa. Further experiments with different semen diluents and storage temperatures and intervals are required in order to prove our hypothesis.

Keywords: rooster, spermatozoa, temperature, storage interval, CASA

INTRODUCTION

The increasing use of artificial insemination (AI) in the poultry industry emphasizes the need for the distribution of good quality sperm. In order for the poultry industry to take advantage of modern AI techniques, proper storage of poultry semen is necessary. As chicken semen is highly concentrated and is of low volume, the extension of neat semen with a proper diluent is required prior to AI and storage. Several factors play a role in maintaining the quality of semen over storage. For example, the diluents used for semen extension and storage conditions such as time, aeration, and holding temperature play a major role (**Dumpala *et al.*, 2006**).

It is known that sperm motility and the fertilizing ability of undiluted neat fowl semen stored *in vitro* usually decreases within 1 h of collection (**Carter *et al.*, 1957**). Therefore, to store fowl semen, the type of diluent and storage temperature are very important to avoid a reduction in sperm quality (**Dumpala *et al.*, 2006**). Sperm 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 fact, semen storage is always accompanied by deterioration in spermatozoa quality and fertility (**Thurston, 1995**).

Sperm motility is a critical factor in the maintenance of fertility. Historically, it has been difficult to use sperm motility assessment as a predictor of fertility potential in poultry, possibly because of the subjectiveness of the methods used (**King *et al.*, 2000**). However, in the recent twenty years computer-aided sperm analysis (CASA) technology has been used to investigate sperm motility parameters from several species: rats (**Moore and Akhondi, 1996**), rabbits (**Farrell *et al.*, 1993**), boars (**Holt *et al.*, 1997**), bulls (**Amann, 1989**), turkeys (**Bakst and Cecil, 1992**), and humans (**MacLeod and Irvine, 1995**). Significant correlations of CASA motility measurements with fertility have been found in most of the mentioned studies.

The objective of our study was to evaluate the influence of different storage temperature on rooster sperm motility.

MATERIAL AND METHODS

Animals

Sexually mature (61 – 67 weeks old) and clinically health Lohmann Light breeder males (n = 15) reared in a private breeding facility (Liaharenský podnik Nitra Ltd., Slovak Republic) were used in experiments. The roosters were housed in individual cages, under a constant photoperiod of 14 h of light day and were fed a commercial standard diet with water given *ad libitum*.

Semen collection and handling

Semen was routinely collected from each rooster once a week by dorso-abdominal massage into prepared sterile tube. The heterospermic pool was transported to the laboratory for CASA analysis. Pooled semen was diluted at the ratio of 1:100 in a commercial extender (Avian diluent; IMV Technologies, France) and divided into two aliquots. One aliquot of diluted liquid rooster semen was stored at 8°C, whereas the other was stored at 37°C. The motility characteristics of diluted rooster spermatozoa stored under different storage temperature were analyzed using CASA system (Sperm Vision™; MiniTüb, Tiefenbach, Germany) after 30, 60 and 120 min. Briefly, the semen samples were placed into Standard Count Analysis Chamber Leja 20 micron (MiniTüb, Tiefenbach, Germany) and evaluated using the CASA system under a Zeiss Axio Scope A1 microscope. In each sample the following parameters were evaluated: the concentration (10^9 per ml), percentage of motile spermatozoa (motility > 5 μm/s) and percentage of progressively motile spermatozoa (motility > 20 μm/s).

Statistical analysis

The experiment was replicated 3 times. Observed results were evaluated statistically using one-way ANOVA (Duncan's method) using SigmaPlot software (Systat Software Inc., Germany) and expressed as the means ± SEM. P-values at $P<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The assessment of semen quality characteristics of poultry birds gives an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs (**Peters *et al.*, 2004**). Fertility and hatchability on the other hand are the major determinant of profitability in the hatchery enterprise, therefore it is necessary to find suitable harmless protocol for the rooster spermatozoa handling.

In our study computer-aided sperm analysis (CASA) did not reveal any differences in the concentration of spermatozoa incubated at 8°C or 37°C (Tab 1). No differences were also observed in sperm concentration after different storage intervals (30, 60 or 120 min). We found low percentage of motile and progressively motile spermatozoa (Tab 1) that would be expected. For example **Peters *et al.* (2008)** noticed that spermatozoa motility in seven different strains of chickens varied from 60 to 90%. That surprising low sperm motility values in our experiments could be due to the specific composition of the commercial avian diluent. The diluent could decrease the sperm motility in order to preserve enough energy for the moment when spermatozoa will reach the female reproductive system.

The rooster spermatozoa stored at 37°C retained similar motility during the whole period of incubation (120 min), whereas the motility of spermatozoa stored at 8°C insignificantly increased after 60 min of incubation (Tab 1). Moreover we observed significantly ($P<0.05$) the highest progressive motility of rooster sperm after 60 min of spermatozoa incubation at 8°C, that was also significantly ($P<0.05$) higher in comparison to spermatozoa incubated for 60 min at 37°C (Tab 1).

Table 1 CASA parameters of rooster spermatozoa incubated under different storage temperature and time period

Parameter	Storage temperature	Time period		
		30 min	60 min	120 min
Concentration (10^9 per ml)	8°C	2.36±0.97	3.03±1.19	2.89±1.01
	37°C	3.03±1.09	2.62±1.01	2.80±1.20
Motility (%)	8°C	26.35±9.44	35.45±1.37	20.20±1.94
	37°C	23.43±3.66	20.78±3.18	23.17±5.46
Progressive motility (%)	8°C	13.68±1.57[b]	27.10±0.97[a]	13.85±0.39[b]
	37°C	14.73±5.00	11.43±2.32[b]	13.83±2.69

Results are expressed as means ± SEM; [a] vs [b] were statistically significant at $P<0.05$

Exposing sperm to subphysiological temperatures, especially in the range of 0 to 20°C may cause damage, or "chilling injury" (**Watson and Morris, 1987**). This may occur immediately upon cooling (direct chilling injury), or as an indirect chilling injury inflicted over an extended storage interval (**Arav *et al.*, 1996**). Nevertheless, in our study no negative effects of the low temperature and the length of storage on the motility values of rooster spermatozoa were observed. But on the contrary the progressive motility of spermatozoa was significantly ($P<0.05$) higher after 60 min of incubation than those incubated at higher temperature (37°C; Tab 1). Similarly, **Slanina *et al.* (2011)** reported that use of the low temperature (4 – 8°C) for long-term storage of turkey spermatozoa could maintain better motility than higher storage temperature. This could be due to several factors. One of them may be a low metabolic rate, since it is known that every 10°C reduction in storage temperature halves the metabolic rate of any cell type (**Hammerstedt and Andrews, 1997**). At low metabolic rates, consumption and exhaustion of the limited sperm bioenergetic resources (ATP) is reduced. As demonstrated in chicken and turkey sperm, ATP consumption is reduced by 75 to 80% when semen is stored at 5°C compared to 40°C (**Wishart, 1984**). This would be associated with lower oxygen consumption at low temperatures, as reported in chicken and turkey sperm stored at 5°C compared to 15, 25, or 41°C for 3 or 6 h (**Clarke *et al.*, 1982**). Better sperm quality after storage at low temperature could be also due to lower lipid peroxidative damage at 5 and 10°C compared to 20°C, as reported for turkey semen (**Cecil, 1993**).

CONCLUSION

Basing on the observed results, we hypothesized that law temperatures (about 8°C) would be better for long-term storage of rooster spermatozoa. However, according to the low motility of spermatozoa diluted in commercial avian extender, we suggested to use other extender for semen dilution. Therefore further experiments with different semen diluents and storage temperatures and intervals are required in order to prove our hypothesis.

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