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SPERM MOTION OF *IN VITRO* STORED MUSCOVY DRAKE SEMEN

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ABSTRACT

A study on sperm motion characteristics of Muscovy drake semen *in vitro* stored for 6 and 24 hours at 4° C, and of frozen-thawed semen by Computer-assisted sperm analysis was carried out. The semen was divided into three equal samples and diluted with the AU extender in ratio 1:3 (semen:extender). Two of the samples were stored at temperature 4° C for 6 and 24 hours respectively. The third semen sample was frozen by pellet method and kept in liquid nitrogen for two months.

Sperm motion parameters – curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity (LIN), straightness (STR), wobble (WOB) were measured using a Sperm Class Analizer (Micropticum, Spain).

After 6 and 24 hours *in vitro* semen storage, and in frozen-thawed semen VCL was 104.61±10.31 μм/s, 40.4±5.67 μм/s and 39.55±4.50 μм/s, VSL – 27.97±2.93 μм/s, 17.01±1.68 μм/s and 17.99±1.65 μм/s, VAP – 54.52±5.26 μм/s, 26.80±3.23 μм/s and 27.87±2.65 μм/s, LIN – 26.72±0.45 %, 43.15±3.19 % and 45.78±2.89 %, STR – 51.25±0.90 %, 64.23±2.48 % and 64.59±0.80, WOB – 52.14±0.10, 66.85±2.61 and 70.82±3.60 % respectively.

Obtained results indicated significant differences ($P<0.001$) between motion parameters of semen stored for 6 hours on one hand, and semen stored for 24 hours and frozen-thawed semen on other hand. Muscovy spermatozoa after 6 hours *in vitro* storage at 4° C preserved a good characteristic of sperm motion and sperm velocity.

KEYWORDS: Muscovy duck, spermatozoa, semen storage, sperm motility, sperm velocity

INTRODUCTION

Motility is one of the most important features of a fertile spermatozoa. It was the first, and continues to be the most widely used indicator of sperm function. Sperm motility is an important attribute, because it is readily identifiable and reflects several structural, and functional competence, as well as essential aspects of spermatozoa metabolism (*Partyka et al.*, 2012). The main disadvantage of conventional semen evaluation by using of light microscope is the subjectivity and variability of obtained results associated mainly with experience and skill of the observer, and the method of specimen preparation. Computer-assisted sperm analysis is very rapid, objective and sensitive method in detecting subtle motility characteristics (*Klimowicz et al., 2008, Klimowicz-Bodys et al 2012, Partyka et al., 2012*).

The studies on the sperm motion parameters in avian semen using this method of analysis are insufficient, and they are more in rooster spermatozoa

than the others *(McDaniel et al., 1998; Fromann et al. 1998, 2000, 2006; Bowling et al. 2003).*

The purpose of the study was to appreciate motion characteristics of Muscovy drake semen *in vitro* stored for 6 and 24 hours at 4° C, and of frozen-thawed semen by Computer-assisted sperm analysis.

MATERIAL AND METHODS

Birds and semen collection

In the study were used six one-year-old Muscovy drakes (White variety), kept in individual cages with size 0.6/0.8/0.6 m. The ejaculates were collected by placing a Muscovy female (teaser method) in the male's cage using an artificial vagina *(Tan, 1980; Gerzilov, 2000).* The artificial vagina consisted of a rubber muff and a graduated test-tube.

Dilution and store of semen

All ejaculates with the following qualities: colour – pearly-white; purity – free of any contamination with cloacal products; volume – above 0.3 ml were merged. The pooled semen was diluted in ratio 1:3 (semen:extender) with the AU extender and divided into three equal samples.

The AU extender consisted following components: 0.40 g D–glucose, 0.80 g D–fructose, 0.80 g sugar, 0.90 g sodium citrate, 0.84 g sodium glutamate, 0.40 glycin, 0.04 g ethylenediaminetetraacetic acid disodium salt dihydrate, and plus 100 mL double distilled water. The osmolarity was 320 mOsmol/kg and pH 7.00 *(Gerzilov, 2002).*

Two of the samples were stored at temperature 4° C for 6 and for 24 hours respectively.

In the third semen sample was added egg yolk in a concentration of 15 % (v/v). The semen sample was equilibrated in a refrigerator at 4° C for 30 min, thereafter was added cryoprotectant glycerol in 5 % as a final concentration and equilibrated at 4° C for 30 min again. Equilibrated semen sample was dropped directly in concave cavities of dry ice at -79° C for 10 min. The semen pellets were placed in an atmosphere of liquid nitrogen for 5 – 10 min and finally they were put in cryotubes and plunged into liquid nitrogen (LN_2). The pellets were kept frozen in the LN_2 container for two months. The semen samples were thawed with AU extender (1:3 v/v) at 42° C.

Sperm motion assessment

Sperm motion parameters were measured in the Institut of Biology and Immunology of Reproduction "Academician Kiril Bratanov" at Bulgarian Academy of Sciences – Sofia using a Sperm Class Analizer (Micropticum, Spain) and the software Motility&Concentration, which detected motile/immotile spermatozoa automatically. Leja 20 chambers were used in the investigations with 2 µl volume of drops. Each sample was evaluated by following parameters:

- Characteristic of sperm motion in % – static, progressive and non-progresive sperm motility;
- Velocity distribution of the spermatozoa in % – rapid, medium, slow, static;

- Curvilinear velocity (VCL) in µm/s - a measure of the total distance traveled by a given sperm cell divided by the time elapsed (average velocity measured over the actual point-to-point track followed by the sperm cell);
- Straight line velocity (VSL) in µm/s - the straight line distance from beginning to end of a sperm track divided by the time taken;
- Average path velocity (VAP) in µm/s - the average path velocity of sperm;
- Linearity (LIN) in % – the linearity of the curvilinear trajectory, it is the ratio VSL/VCL;
- Straightness (STR) in % – linearity of the spatial average path, by the ratio VSL/VAP;
- Wobble (WOB) in % – measure of oscillation of actual trajectory about its spatial average path

Statistical analysis

Data were subjected one-way analysis of variance (ANOVA) followed by t-test to determine the level of significance among mean values. The results are presented as mean±SD.

RESULTS AND DISCUSSION

The progressive motile spermatozoa and sperm velocity in 6-hour stored semen was significantly higher, while the static spermatozoa was significantly lower compared to 24-hour stored semen and the frozen-thawed semen (table 1 and 2). Probably the higher percentage of non-progressive motile and static spermatozoa was due to their longer *in vitro* storage. It is noteworthy, partly the better results in motion of the thawed spermatozoa versus those stored for 24 hours at temperature 4° C.

Table 1. Sperm motion analysis in % (n=6)

Parameters	After 6 hours storage	After 24 hours storage	After thawing
Static spermatozoa	0.19 ± 0.09	24.19 ± 9.80	10.76 ± 6.11
Non-progresive motile spermatozoa	80.21 ± 1.12	68.99 ± 1.67	81.78 ± 7.20
Progressive motile spermatozoa	19.60 ± 1.20	6.82 ± 1.83	7.46 ± 1.70
Total	100	100	100

Table 2. Velocity distribution of the spermatozoa in % (n=6)

Parameters	After 6 hours storage	After 24 hours storage	After thawing
Rapid	58.48 ± 7.74	4.90 ± 2.64	3.13 ± 1.00
Medium	32.91 ± 4.13	17.40 ± 5.22	22.94 ± 1.58
Slow	8.42 ± 3.86	53.52 ± 6.12	65.95 ± 9.27
Static	0.19 ± 0.09	24.19 ± 9.80	10.76 ± 6.11
Total	100	100	100

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Computer-assisted sperm analysis (CASA system) indicated that Muscovy spermatozoa after 6 hours *in vitro* storage preserved a rapid velocity - 58.48 ± 7.74 % from all sperm cells, while rapid sperm cells after 24 hours of *in vitro* storage and after thawing were remarkably little - 4.90 ± 2.64 % and 3.13 ± 1.00 % respectively.

The curvilinear velocity of sperm cells (VCL) in three storage ways (6 hours, 24 hours and thawed semen) were 104.61±10.31 μм/s, 40.4±5.67 μм/s and 39.55±4.50 μм/s. For the other velocity parameters the mean values were as follows: VSL – 27.97±2.93 μм/s, 17.01±1.68 μм/s and 17.99±1.65 μм/s, VAP – 54.52±5.26 μм/s, 26.80±3.23 μм/s and 27.87±2.65 μм/s, LIN – 26.72±0.45 %, 43.15±3.19 % and 45.78±2.89 %, STR – 51.25±0.90 %, 64.23±2.48 % and 64.59±0.80, WOB – 52.14±0.10, 66.85±2.61 and 70.82±3.60 % respectively (figure 1). Obtained results indicated significant differences (P<0.001) between sperm motion parameters after 6 hours of cool storage of semen on one hand and after 24 hours of semen storage as well as thawed semen on other hand. There were not any significant differences between velocity parameters of sperm cells after 24 hours of storage and after thawing. Blesbois et al. (2008) established that all studied velocity semen parameters in chicken semen with exception of straightness (STR) showed lower values after cryopreservation. This suggesting that cryopreservation slows down the movement of chicken spermatozoa without changing the shape of trajectories. Increasing the duration of *in vitro* storage and cryopreservation damage sperm cells and decrease their quality.

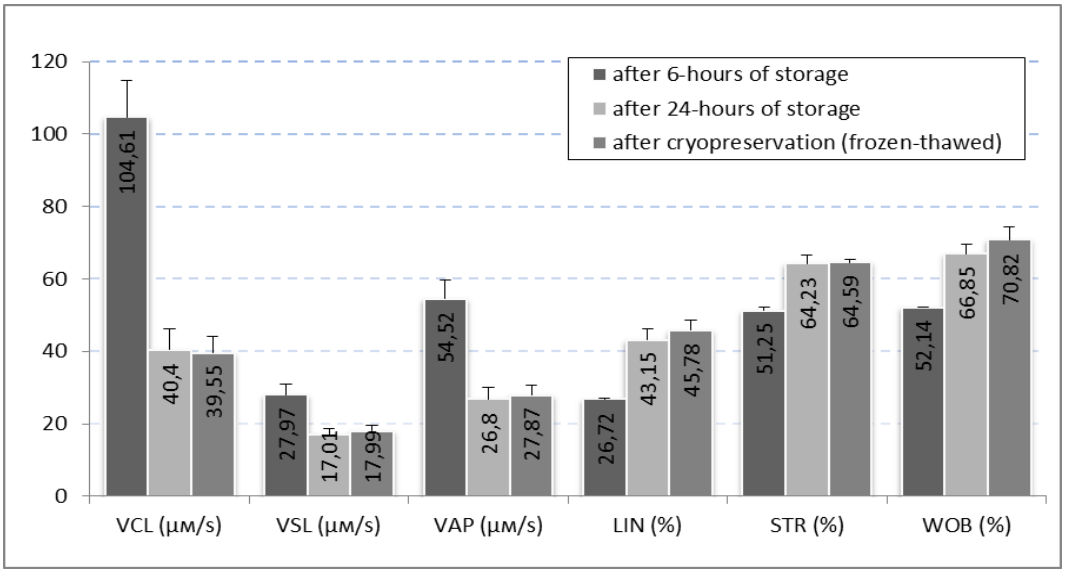

Fig. 1 Velocity of the spermatozoa

CONCLUSION

Computer-assisted sperm analysis ascertained that Muscovy spermatozoa after 6 hours *in vitro* storage at 4° C preserved a good characteristic of sperm motion and sperm velocity, while after 24 hours of *in vitro* storage and after thawing parameters were significantly reduced.

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