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EFFECT OF SEMEN EXTENDERS ON SPERM MOTION OF *IN VITRO* STORED MUSCOVY DRAKE SPERMATOZOA

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Abstract: A study for the influence of semen extenders IMV-buffer, HIA-1 and AU on sperm motion characteristics of Muscovy drake spermatozoa was carried out. The semen from each male (n=6) was divided into three equal parts and diluted in ratio 1:3 (semen:extender) with the IMV-buffer, HIA-1 and AU respectively, and then was stored at temperature 0-4C° for 6 hours. Sperm motion parameters - velocity of spermatozoa (rapid, medium, slow and statistic), VCL, VSL, VAP, LIN, STR, WOB were measured using a Sperm Class Analizer (Micropticum, Spain). Computer-assisted sperm motion analysis indicated that Muscovy spermatozoa preserved a rapid and medium sperm velocity after 6 hours in vitro storage in three examination extenders. The total VCL, VSL and VAP of spermatozoa in the semen diluted with AU extender were 110.47±7.44 μm/s, 29.42±2.02 μm/s and 57.39±3.73 μm/s, in the semen diluted with IMV-buffer were 94.93±11.10 μm/s, 27.57±2.45 μm/s and 51.35±4.98 μm/s, and in the semen diluted with HIA-1 were 68.48±12.74 μm/s, 20.08±4.18 μm/s and 37.75±7.65 μm/s, as the differences were significant between AU and HIA-1 - ($P<0.05$). About LIN, STR, WOB there were no significant differences for the influence of the extenders.

Keywords: Muscovy duck, spermatozoa, extender, CASA system, sperm motility, sperm velocity

Introduction

Semen preservation and artificial insemination (AI) offer many advantages to the poultry industry, particularly in conjunction with production of broiler chicks, turkeys and mule ducks, as well as with genetic evaluation of male breeders and selection programs.

The dilution and the storage of semen at low temperatures is an important element in AI poultry programs, and it aims to increase semen volume, the opportunity to transport of semen samples as well as maintaining the high fertile ability of spermatozoa for several hours *(Cristensen, 1996; Lake, 1996)*.

Three sperm parameters that are most often evaluated when determining a poultry breeder's fertilizing potential include the following: sperm concentration, viability and motility *(McDaniel et al., 1998).* These attributes can be evaluated by several different methods *(Bakst and Cecil, 1997).* Most often the sperm motility estimates visually under light microscope - on a scale ranging in percentages (0-100%) or in balls (0-10). For determination of sperm motility is used hanging drop method *(Wishart and Wilson, 1997).* Important deficiency of visual assessment of sperm motility is a subjective assumption. Modern and very accurate methods are the use of computerized video micrographic (CVM), demonstrated in determining human sperm motility *(Jecht and Rusco, 1973)* and spectrophotometric analysis *(Wishart and Ross, 1985).* By the method of CVM, *Ducci et al. (1992)* determinated sperm motility of six Muscovy drakes from 66 to 87%. According to *Person et al. (2007)* the computer-assisted sperm analysis (CASA) is a useful tool for identifying differences in sperm parameters, related to motility and morphology, and characterization of semen, based on these parameters, and could improve methods for assessing the fertility.The purpose of the study was to appreciate the influence of extenders IMV-buffer, HIA-1 and AU on sperm motion characteristics of Muscovy drake semen diluted and stored for 6 hours at 4°C.

Materials and Methods

Birds. In this experiment were used six one-year-old Muscovy drakes (White variety), kept in individual cages with size 0.6/0.8/0.6 m. The birds were bred at the Poultry farm of the Department of Animal Science, Agricultural University – Plovdiv.

Semen collection The ejaculates were collected by placing a Muscovy female (teaser method) in the cage of the drake using an artificial vagina *(Tan, 1980; Gerzilov, 2000).* The artificial vagina consisted of a rubber muff and a graduated test-tube. In our study were used ejaculates with the following quality: colour – pearly-white; purity – free of any contamination with cloacal products; volume – above 0.3 ml. Thereafter the semen from each male was divided into three equal parts in 1.5 ml Eppendorf conical polypropylene tubes with snap caps and diluted respectively in ratio 1:3 (semen:extender) with the IMV-buffer (patented by IMV – Technologies – France) and created from *Gerzilov (2002)* extenders HIA-1 and AU with the following composition

- HIA-1 – components: 0.25 g D–glucose, 0.25 g D–fructose, 0.07 g sugar, 0.50 g sodium citrate, 0.90 g sodium chloride, and 100 ml double distilled water. The osmolarity was 290 mOsmol/kg and pH 7.00.
- AU – 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 glycocol, 0.04 g ethylene diamine tetra acetic acid disodium salt dihydrate, and 100 mL double distilled water. The osmolarity was 320 mOsmol/kg and pH 7.00.

Diluted semen samples were stored at temperature 4°C for 6 hours in cooler.

Sperm motion assessment. Sperm motion parameters were measured in the Institut of Biology and Immunology of Reproduction – 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 appreciated 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 divided by the time elapsed;
- 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, VSL/VCL;
- Straightness (STR) in %– linearity of the spatial average path, 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

The percentage of progressive motile spermatozoa in two extenders AU and IMV-buffer was better compared to HIA extender (Table 1). The percentage of static spermatozoa was lower (0.19±0.1) in AU extender compared with those in IVM-buffer and HIA-1. Eventually semen diluted with AU extender and with

IVM-buffer had better quality characteristics than semen diluted with HIA-1 extender about sperm motility.

Computer-assisted sperm motion analysis (CASA system) indicated that Muscovy spermatozoa preserved a rapid and medium sperm velocity after 6 hours in vitro storage in three examination extenders (Table 2). The AU extender was with the best influence on the sperm velocity. The percentage of rapid spermatozoa in this extender is highest 58.8 ±7.74% with varied from 32.7% to73.4%, and in the same time the percentage of slow spermatozoa is the lowest 8.42±3.86% with varied from 1.7% to 22.9 %. About these two parameters there was significant difference between AU and HIA-1 extenders ($P<0.05$). The percentage of the spermatozoa with medium velocity remained almost equally for three buffers.

The obtained average values about VCL, VSL, VAP, LIN, STR and WOB are expressed in Table 3. The total average VCL of spermatozoa in semen diluted with AU extender was 110.47±7.44 μm/s with varied from 84.0 μm/s to 124.9 μm/s. The using HIA-1 extender reduced the other velocity parameters VSL and VAP in comparison with another two extenders, as difference between AU and HIA-1 was significant ($P<0.05$). In generally, there were no significant differences of the total values of VCL, VSL, and VAP etc. between the extenders IMV-buffer and AU.

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

Parameters	Semen diluted with extender		
	IMV-buffer	HIA-1	AU
Static spermatozoa	1.52±1.23	5.37±3.20	0.19±0.01
Non-progresive motile spermatozoa	79.02±1.57	81.18±1.67	80.21±1.17
Progressive motile spermatozoa	19.46±1.61	13.46±3.59	19.60±1.22
Total	100	100	100

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

Parameters	Semen diluted with extender		
	IMV-buffer	HIA-1	AU
Rapid	43.55±9.97	21.83±9.25 ^{A}	58.48±7.74 ^{B}
Medium	35.11±3.11	34.88±4.90	32.91±4.13
Slow	19.82±7.58	37.93±11.08 ^{A}	8.42±3.86 ^{B}
Static	1.52±1.23	5.37±3.20	0.19±0.01
Total	100	100	100

Note: Values with different superscript (A-B) within rows differ significantly at $P<0.05$

Table 3. Average values and minimum and maximum levels of velocity parameters (n=6)

Parameters	Unit	Extender	Total	Slow	Medium	Rapid
Curvilinear velocity (VCL)	µm/s	IMV	94.93±11.10 (57.3 – 127.3)	35.19±2.07 (28.8 – 43.1)	75.56±1.63 (70.2 – 79.4)	138.69±1.67 (134.9 – 145.3)
		HIA-1	68.48±12.74 ^{A} (40.7 – 95.4)	31.39±2.18 (27.0 – 37.6)	72.25±2.21 ^{A} (66.3 – 77.0)	128.56±3.54 ^{A} (118.7 – 136.6)
		AU	110.47±7.44 ^{B} (99.4 – 127.9)	36.68±0.90 (34.6 – 40.1)	78.94±1.69 ^{B} (73.2 – 82.4)	138.85±1.41 ^{B} (135.4 – 142.0)
Straight line velocity (VSL)	µm/s	IMV	27.57±2.45 (22.3 – 32.8)	9.81±0.81 (7.7 – 13.1)	23.45±0.76 (21.4 – 26)	41.93±4.50 (35.9 – 62.3)
		HIA-1	20.08±4.18^{A} (11.1 – 30.2)	9.33±0.88 (7.5 – 11.8)	22.04±1.44 (19.5 – 25.6)	33.40±3.12 (24.2 – 40.0)
		AU	29.42±2.02^{B} (23.2 – 34.9)	9.92±0.82 (8.5 – 13.5)	21.89±0.73 (20.3 – 21.4)	36.70±0.51 (35.1 – 38.6)
Average path velocity (VAP)	µm/s	IMV	51.35±4.98 (36.5 – 65.8)	19.32±1.05 (16.5 – 23.4)	42.87±0.80 (40.2 – 45)	75.46±3.28 (71.1 – 90.2)
		HIA-1	37.75±7.65 ^{A} (21.1 – 54.8)	17.53±1.73 (14.1 – 22.5)	40.83±2.21 (35.3 – 46.0)	66.35±4.46 (61.4 – 73.6)
		AU	57.39±3.73 ^{B} (44.4 – 66.40)	19.64±0.95 (17.8 – 23.7)	42.17±1.02 (39.1 – 45.2)	71.60±0.48 (70.5 – 73.7)
Linearity (LIN)	%	IMV	29.73±202 (27.2 – 38.2)	27.96±1.81 (22.8 – 34.0)	31.10±1.33 (29.3 – 37.0)	30.33±3.49 (25.8 – 46.2)
		HIA-1	28.94±0.82 (27.3 – 31.7)	29.64±1.33 (26.4 – 31.9)	30.42±1.15 (27.8 – 33.7)	25.85±1.90 (20.4 – 30.7)
		AU	26.63±0.36 (25.6 – 27.3)	26.97±1.71 (23.3 – 33.8)	27.72±0.59 (26.1 – 29.2)	26.44±0.37 (25.2 – 27.2)
Straightness (STR)	%	IMV	54.00±1.66 (50.5 – 61.1)	50.63±2.15 (43.9 – 56.0)	54.65±1.08 (51.7 – 59.1)	55.02±3.15 (50.1 – 69.0)
		HIA-1	53.06±0.67 (51.8 – 55.2)	53.40±1.69 (49.2 – 58.6)	53.90±0.83 (51.2 – 55.7)	50.05±1.72 (45.6 – 55.2)
		AU	51.23±0.55 (49.0 – 52.5)	50.30±2.05 (45.7 – 54.8)	51.87±0.75 (49.1 – 53.5)	51.25±0.54 (49.1 – 52.5)
Wobble (WOB)	%	IMV	54.84±1.97 (51.7 – 63.6)	55.09±1.87 (48.7 – 61.6)	56.82±1.36 (53.9 – 62.7)	54.48±2.74 (51.0 – 66.9)
		HIA-1	54.52±1.10 (51.7 – 57.5)	55.50±1.74 (51.3 – 59.8)	56.38±1.41 (53.3 – 60.4)	51.44±2.21 (44.6 – 55.5)
		AU	51.99±0.36 (50.5 – 52.9)	53.48±1.56 (50.0 – 59.1)	53.42±0.62 (51.0 – 54.9)	51.58±0.33 (50.3 – 52.5)

Note: Values with different superscript (A-B) in the column within the velocity parameter differ significantly at $P<0.05$

Discussion

CASA system is very rapid, objective and sensitive method in detecting subtle motility characteristics *(Klimowicz et al., 2008)*. According to *McDaniel et al. (1998)*, the Sperm Quality Analyzer provides a very fast and comlete measure of overall avian sperm quality. Various avian sperm extenders were evaluated for

their ability to support spermatozoa mobility. It is established that extender for one species may not be suitable for another species *(Blanco et al., 2000).*

The studies on the sperm motion parameters in avian semen using this method of analysis are insufficient, its are more in rooster spermatozoa *(McDaniel et al., 1998; Fromann et al. 1998, 2000, 2006; Bowling et al. 2003).* The spermatozoa in freshly diluted and in vitro storage at 4°C for 6 hours Muscovy drake semen were extremely active progressively motion and exhibited linear trajectories. CASA system indicated that the percentages of motile spermatozoa varied from 94 to 99 % for different extenders and the progressive sperm motions in IMV and AU extenders were better (above 19 %) than HIA – 1 extender (13.46 %). It indicates that different extenders have different influence on the sperm motions. Probably a higher percentage of non-progressive motile spermatozoa was due to their long in vitro storage in these three buffers.

The same observation was reported for different extenders used for ram semen dilution *(Gil et al., 2000)*.

Further, it was established that the dilution ratio has significant influence on the percentage of motile spermatozoa in the pigeon semen *(Sontakke et al., 2004).*

Conclusion

CASA system indicated that Muscovy spermatozoa after 6 hours in vitro storage in extenders AU, IMV-buffer and HIA-1 at 4°C preserved a good characteristic of sperm motion and sperm velocity. With the best influence on these parameters was extender AU.

Uticaj ekstendera semena na pokretljivost sperme *in vitro* skladištenih spermatozoida mošusne patke

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Rezime

Sprovedeno je istraživanje o uticaju ekstendera sperme IMV-pufera, HIA-1 and AU na osobine pokretljivosti spermatozoida Muscovy pataka. Seme svake životinje (n=6) podeljeno je u tri jednaka dela i razređeno u odnosu 1:3 (seme : ekstender) sa IMV-puferom, HIA-1 and AU respektivno, a zatim držano na temperaturi 0-4C° tokom 6 časova. Izmereni su parametri pokretljivosti spermatozoida- brzina (rapidna, srednja, spora i statična), VCL, VSL, VAP, LIN, STR, WOB korišćenjem Sperm Class Analizer-a (Micropticum, Spain). Kompjuterski asistirana analiza pokretljivosti spermatozoida pokazala je da

spermatozoidi mošusne patke mogu da sačuvaju rapidnu i srednju brzinu pokretljivosti nakon 6 sati *in vitro* skladištenja kod tri ispitivana ekstendera. Ukupni VCL, VSL i VAP spermatozoida u semenu razređenih sa AU ekstenderom bili su 110,47±7.44 μm/s, 29,42±2,02 μm/s i 57,39±3,73 μm/s, u semenu razblaženom sa IMV-puferom bili su 94.93±11.10 μm/s, 27.57±2.45 μm/s i 51,35±4,98 μm/s, u semenu razređenom sa HIA-1 bili su 68,48±12,74 μm/s, 20,08±4,18 μm/s i 37,75±7,65 μm/s, dok su razlike bile signifikantne između AU i HIA-1 - ($P<0.05$). U pogledu LIN, STR, WOB nije bilo statistički značajnih razlika za uticaj ekstendera.

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