TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2021, vol. 6, No 2(11): 117–120

THE EFFECT OF THE DEGREE OF DILUTION ON THE MOTILITY AND VELOCITY PARAMETERS OF SPERM IN RAMS

Hristina Blagova[1], Madlena Andreeva[1,2], Rossen Stefanov[1]

[1]Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria

[2]Laboratory of Free Radical Processes, Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia Bulgaria

E-mail: blagovahristina@gmail.com g

ABSTRACT

The aim of the present study was to trace the influence of the degree of dilution on the motility and velocity parameters of ram sperm. For this purpose, 12 ejaculates of 6 rams, Northeast Bulgarian fine- fleece breed. Sperm motility and velocity parameters were analyzed by computer sperm analyzer (CASA). Samples were diluted 1: 3, 1: 6, 1: 9, 1:12; 1:24 with extender 6 A. It was found that the total motility (TM) at dilutions from 1: 3 to 1:12 decreased by 2% ($P \leq 0.001$), while at 1:24 by 25% ($P \leq 0.001$. A significant effect of dilution was found in progressive sperm motility (PM). At dilutions from 1: 3 to 1:12 it decreased by 27.47% ($P \leq 0.001$), while at 1:24 the decrease was by 56.63% ($P \leq 0.001$). The degree of dilution affects some velocity parameters of the sperm, as Rapid, Medium, VCL, VSL, LIN, STR, WOB and AHL.

Key words: dilution, motility, velocity parameters, sperm, rams.

Introduction

An unconditional prerequisite for the widespread use of artificial insemination is the ability to store semen after receipt, evaluation and dilution under "*in vitro*" conditions.

The use of semen extenders with appropriate ingredients and physicochemical parameters optimizes energy metabolism and preserves the fertility of male gametes. This ensures high reproductive results through artificial insemination (Bottwalla and Miles, 1992). Among the many factors that determine the effectiveness of fertilization is sperm motility (Donnelly et al., 1998). Therefore, the assessment of sperm motility is one of the most important methods for fertility of male animals (Bratton et al., 1956). In connection with this, many methods for assessing sperm quality have been introduced, one of which is analysis by computer sperm analyzer (CASA), allowing objective determination of sperm concentration, variety of motility parameters and assessment of morphological abnormalities (Thurston et al., 1999; Verstegen et al., 2002). The computer-assisted sperm analysis (CASA) system allows accurate, repeatable, reliable, and objective assessment of various sperm parameters such as concentration, total and progressive motility, and various velocity parameters (Rijselaere et al., 2012). Various reports announce that CASA not only measures the proportion of motile sperm, but also measures other sperm movement parameters derived from individual sperm, and has greater prognostic power on sperm fertility potential (Mortimer, 1994). The high concentration of ruminant semen usually necessitates its dilution for further analysis with CASA to obtain valid results, however, according to Farrell et al. (1995), the dilution of semen may cause changes in motility parameters.

The aim of the present study was to evaluate the effect of the degree of dilution on the motility and velocity parameters of sperm in Northeast Bulgarian fine- fleece rams.

Materials and methods

Experimental Design

The experiment was conducted with six clinically healthy rams Northeast Bulgarian fine-fleece breed. The rams are bred in the National Center for Agriculture (NCC) – Targovishte. The animals were aged 2–5 years, all with previous sexual experience, placed under the same conditions of rearing, feeding and sexual use, in accordance with the regulatory requirements.

The collection of ejaculates took place in the middle of the breeding season, which for the Northeast Bulgarian fine- fleece breed is June-July. Two consecutive ejaculates were obtained from each ram by the artificial vagina method. A total of 12 ejaculates were examined for the experiment. Each ejaculate was tested for the indicators – motility, concentration and velocity, by analyzing the sperm at a dilution of 1: 3; 1: 6; 1: 9; 1:12 and 1:24. All ejaculates are diluted with extender 6A (sodium citrate, lactose, sucrose, d.H2O), prepared at the Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov" – BAS.

Sperm Analysis

The analysis of sperm was performed in a specialized laboratory of the Institute of Biology and Immunology of Reproduction, Sofia, Bulgaria. The evaluation of sperm quality parameters – concentration, motility and various kinematic parameters of sperm is performed with the CASA system (Sperm Class Analyzer [SCA] 5.0. Microptic, Barcelona, Spain). Sperm concentration was adjusted to SCA assays by dilution, loaded into a Leja 20 chamber (Leja Products B.V., Nieuw-Vennep, The Netherlands) and examined using a warm-stage microscope (Nikon, Tokyo, Japan). In addition to the total number of motile sperm, the SCA software also measures the speed of movement, the width of the sperm head trajectory, the frequency of change in the direction of the sperm head, and the kinematics for each analyzed sperm. The following velocity parameters are recorded:

- Rapid, % – Rapid motility spermatozoa;
- Medium, % – Medium motility spermatozoa;
- Slow, % – Slow motility spermatozoa;
- VCL, μm/s – curvilinear velocity;
- VSL, μm/s – straight line velocity;
- VAP, μm/s – average path velocity;
- LIN,% – linearity (the ratio between VSL and VCL);
- STR,% – straightness (the ratio between VSL and VAP);
- WOB,% – Wobble (the ratio between VCL and VAP);
- ALH, μm – amplitude of lateral head displacement;
- BCF, Hz – beat-cross frequency (the frequency of sperm trajectory crosses the average path trajectory).

Statistical analysis

The analysis of all results was performed with a specialized statistical analysis package IBM SPSS Statistics, 23 (SPSSInc., Chicago, USA). The comparison of sperm characteristics was performed by statistical tests of ANOVA. Significance of group differences was assessed with One Sample T-Test. Results are presented as mean ± standard error (SE).

Results and Discussion

As the dilutions increase from 1: 3 to 1:24, the sperm concentration gradually decreases. In our study, total sperm motility (TM) was weakly affected at dilutions of 1: 3 to 1:12, but at a dilution

of 1:24 the difference was significantly greater (P≤0.001). We found a significantly stronger effect of dilutions in the progressive motility (PM) of sperm. With increasing dilutions PM decreases, as at 1: 3 it is 95.71%, and at 1:24 it is only 39.08%. In non-progressively motile sperm (NPM), the opposite trend is observed. As the dilutions increased, the NPM percentage increased significantly from 3.89% (1: 3) to 41.53% (1:24). The same trend is maintained in the percentage of immotile sperm. At dilutions from 1: 3 to 1:12, immotile spermatozoa increased by only 1.97%, while at dilution 1:24 immotile spermatozoa were 19.38% (Table 1).

Table 1: Influence of the degree of dilution on the motility of sperm.

Parameters	DILUTION				
	1:3	1:6	1:9	1:12	1:24
Concentration, mill/ml	2462.20±42.67[a]	1895.73±80.91[a]	1156.21±25.67[a]	923.67±32.11[a]	885.73±23.73[a]
TM, %	99.63±0.24 [a]	98.81±0.30 [a]	98.31±0.22 [a]	97.65±0.31 [a]	75.03±0.87 [a]
PM, %	95.71±0.54 [a]	92.25±1.99 [b]	70.95±2.90 [a]	68.24±2.06 [a]	39.08±2.32 [a]
NPM, %	3.89±0.63 [a]	6.77±2.07 [b]	27.63±2.76 [a]	29.40±1.93 [a]	41.53±1.99 [a]
Immotile, %	0.37±0.24 [a]	1.18±0.30 [b]	1.68±0.22 [a]	2.34±0.31 [a]	19.38±1.34 [a]

Note: X±SE; [a] P≤0.001; [b] P≤0.01

Kinematic parameters such as progressive motility (PM), straight line velocity (VSL), curvi-linear velocity (VCL), amplitude of lateral head (ALH) and linearity (LIN) are positively correlated with fertility (Perumal et al., 2011). In our studies, we found that the degree of dilution affects the velocity parameters of sperm. With increasing dilutions from 1: 3 to 1:24 the parameters – Rapid, VCL, STR and AHL decrease, and the parameters Medium, Slow, VSL, LIN, WOB increase. The parameters that were not affected by the degree of dilution were VAP and BCF (Table 2). Our results are consistent with that found by Perumal et al. (2014) that velocity parameters correlated with sperm motility. Other authors (Cox et al., 2006; Mostafapor and Ardebili, 2014) investigated how the extenders affects the parameters of sperm motility when evaluating sperm by CASA and found a positive effect.

Table 2: Influence of the degree of dilution on the velocity parameters.

Parameters	DILUTION				
	1:3	1:6	1:9	1:12	1:24
Rapid, %	95.77±0.64 [a]	91.39±2.05 [a]	67.47±.06 [a]	64.02±2.34 [a]	23.44±1.94 [a]
Medium, %	4.02±0.59 [a]	6.35±1.71 [b]	20.25±1.73 [a]	21.10±0.82 [a]	35.18±1.61 [a]
Slow, %	0.20±0.09 [c]	1.06±0.50	10.58±1.44 [a]	12.52±1.52 [a]	21.98±1.32 [a]
VCL, µm/s	118.88±1.44 [a]	120.48±3.05 [a]	92.74±2.71 [a]	91.31±1.80 [a]	79.56±3.08 [a]
VSL,µm/s	30.34±0.62 [a]	31.57±0.49 [a]	33.25±1.12 [a]	34.14±0.63 [a]	40.83±1.74 [a]
VAP, µm/s	59.73±0.68 [a]	62.87±1.21 [a]	56.71±1.68 [a]	56.27±0.76 [a]	59.81±2.31 [a]
LIN, %	25.51±0.34 [a]	26.62±0.38 [a]	35.20±1.05 [a]	35.89±0.76 [a]	47.29±1.58 [a]
STR, %	62.59±1.42 [a]	55.80±0.78 [a]	33.25±1.12 [a]	48.89±0.41 [a]	49.40±0.59 [a]
WOB, %	50.23±0.14 [a]	46.62±0.38 [a]	60.34±0.98 [a]	65.34±0.86 [a]	70.11±1.02 [a]
AHL, µm	5.61±0.04 [a]	5.03±0.37 [a]	4.06±0.12 [a]	3.94±0.10 [a]	3.09±0.17 [a]
BCF, Hz	5.77±0.04 [a]	5.64±0.13 [a]	5.54±0.07 [a]	5.55±0.08 [a]	5.77±11 [a]

Note: X±SE; [a] P≤0.001; [b] P≤0.01; [c] P≤0.05

Conclusion

1. The degree of dilution of ejaculates affects the PM of sperm, with optimal dilutions are from 1: 3 to 1:12.
2. With increasing dilutions from 1: 3 to 1:24 the parameters – Rapid, VCL, STR and AHL decrease, and the parameters Medium, Slow, VSL, LIN, WOB increase.
3. For the VAP and BCF parameters, no regularities were found regarding to the influence of the dilution.

Acknowledgements

The financial support of National Scientific Program for young scientists and postdoctoral fellows, funded by the Bulgarian Ministry of Education and Science (MES) with PMC № 577 (17.08.2018).

References

1. Bottwalla S.M., Miles R.D. (1992). *Development of diluents for domestic fowl semen*. World's Poult. Sci. J. 48, 121–128.
2. Bratton, R. W., Foote R. H., Henderson C. R., Musgrave S. D., Dunbar R. S. Jr., Dunn H. O., Beardsley J. P. (1956). *The relative usefulness of combinations of laboratory tests for predicting the fertility of bovine semen*. J. Dairy Sci. 39, 1542–1549.
3. Cox J.F., Alfaro V., Montenegro H. (2006). *Computer-assisted analysis of sperm motion in goats and its relationship with sperm migration in cervical mucus*. Theriogenology 66 (4), 860–867.
4. Donelly E.T., Scheena E.M., MC Nally J.A., Thompson W. (1998). *In vitro fertilization and pregnancy rates: the influence of sperm motility and morphology on IVF outcome*. Fert. Steril. 70(2), 305–31.
5. Farrell P.B., Trouern-Trend V., Foote R.H., Douglas-Hamilton D. (1995). *Repeatability of measurements on human, rabbit, and bull sperm by computer-assisted sperm analysis when comparing individual fields and means of 12 fields*. Fertil Steril., 64(1):208–210.
6. Mortimer D. (1994). *Practical Laboratory Andrology*. OxfordUniversity Press, New York, US.
7. Mostafapor S., Farrokhi Ardebili F. (2014). *Effects of diluting medium and holding time on sperm motility analysis by CASA in ram*. Vet Res Forum. 2014 Spring; 5(2):101–5.
8. Perumal P., Selvaraju S., Selvakumar S., Barik A.K., Mohanty D.N., Das S., Das R.K., Mishra P.C. (2011). *Effect of pre-freeze addition of cysteine hydrochloride and reduced glutathione in semen of crossbred jersey bulls on sperm parameters and conception rates*. Reproduction in Domestic Animals, 46(4): 636–641.
9. Perumal P., Srivastava S.K., Ghosh, S.K., Baruah K.K. (2014). *Computer-Assisted Sperm Analysis of Freezable and Nonfreezable Mithun (Bos frontalis) Semen*. Journal of Animals, Article ID 675031, 6 pages.
10. Rijselaere T., Van Soom A., Maes D., Ni¿añski W. (2012*). Computer-assisted sperm analysis in dogs and cats: An uptadeafter 20 years*. Reproduction in Domestic Animals 47(6): 204–07
11. Thurston L.M., Watson P.F., Holt W.V. (1999). *Sources of variation in the morphological characteristics of sperm sub-populations assessed objectively by a novel automated sperm morphology analysis system*. J. Reprod. Fertil., 117(2):271–280.
12. Verstegen J., Iguer-Ouada M., Onclin K. (2002). *Computer assisted semen analyzers in andrology research and veterinary practice*. Theriogenology, 57(1):149–179.