Bulgarian Journal of Agricultural Science, 20 (No 4) 2014, 967-972
Agricultural Academy

EFFECT OF BREEDING SEASON ON THE KINEMATIC PARAMETERS AND MORPHOLOGY OF RAM' SPERM FROM SYNTHETIC POPULATION BULGARIAN MILK SHEEP BREED

D. ABADJIEVA[1*], M. CHERVENKOV[1], R. STEFANOV[1], N. METODIEV[2], E. KISTANOVA[1], D. KACHEVA[1] and E. RAYCHEVA[2]
[1] Bulgarian Academy of Sciences, Institute of Biology and Immunology of Reproduction, BG - 1113 Sofia, Bulgaria
[2] Institute of Animal Science, BG - 2232 Kostinbrod, Bulgaria

Abstract

ABADJIEVA, D., M. CHERVENKOV, R. STEFANOV, N. METODIEV, E. KISTANOVA, D. KACHEVA and E. RAYCHEVA, 2014. Effect of breeding season on the kinematic parameters and morphology of ram' sperm from synthetic population Bulgarian milk sheep breed. *Bulg. J. Agric. Sci.,* 20: 967-972

The aim of this study was the investigation of the breeding season effect on the kinematic and main spermatological parameters of the rams from Synthetic Population Bulgarian Milk sheep breed (SPBM), new Bulgarian breed certificated in 2005. The experiment was carried out with seven **rams.** Two consecutive ejaculates from each ram were obtained by artificial vagina before and during the breeding campaign (n=28). Overall sperm motility and the individual kinematic parameters of motile spermatozoa were assessed by the computer-aided sperm analysis system Sperm Class Analyzer (SCA). The sperm morphology was estimated after sperm blue stain and calculated as a percent of abnormal cells among 100 sperm cells from several fields on the slide.

It was found that the ejaculates obtained from SPBM rams during the breeding season had better features of sperm motion kinetics. The values of the velocity parameters ($P< 0.05$), motility ($P< 0.05$), and percentages of spermatozoa with rapid ($P< 0.01$) and medium ($P< 0.001$) speed were higher than those from the ejaculates collected before the breeding season. Minor and not significant changes in the kinematic parameters of motile spermatozoa in consecutive ejaculates were observed. No significant differences were established in morphological status of spermatozoa in nonbreeding and breeding season. It seems that the better sperm motility kinematic parameters during the breeding season ensure the higher sperm fertility and success on the future insemination.

Key words: rams, spermatozoa, velocity, motility, morphology

Abbreviations: SPBM: Synthetic Population Bulgarian Milk sheep breed; SCA: Sperm Class Analyzer; VCL: curvilinear velocity; VSL: straight-line velocity; VAP: average path velocity; LIN: linearity; STR: straightness; ALH: amplitude of lateral head displacement; BCF: beat cross frequency; WOB: wobble

Introduction

The studies on sperm motility are of great importance for determining the quality of semen and for deepening the knowledge of cell biology. These analyzes have been performed traditionally by tracing the kinetic motion of the head or the tail of spermatozoa. They provide insight into the cell biological mechanisms, responsible for the control of the movement.

The accuracy and precision of the kinematic measurements are limited by a number of technical factors. However these parameters are valuable because they reflect the ability of sperm to migrate through the female genital tract and to interact with oocyte at fertilization.

Motility and morphology of spermatozoa are accepted as markers of fertility for a long time. It has been shown that these parameters of semen are strongly associated with successful conception in vivo (Davis and Siemers, 1995).

E-mail: dessi_l@abv.bg

Additionally, the authors indicated a high correlation coefficient between the morphology and fertilization capacity of the spermatozoa (Zhang et al., 1998; Bohlooli1 et al., 2012). Also poor morphology has been associated with deviant kinematic and inefficient penetration of both cervical mucus and the zona pellucida (Morales and Overstreet, 1998). Garrett and Clarke, (2003) underlined that the kinematic parameters, concentration, tail properties and morphology of the spermatozoa had positive correlation with the successful fertilization.

The variations in the basic semen parameters during breeding and non-breeding season is one of the limiting factors for reproduction in sheep (Karagiannidis et al., 2000; Azawi et al., 2012b). There are scientific reports for reduction of the quantity and quality of semen production and sperm fertility in rams during the non-breeding season (Colas, 1980; Makawi et al., 2007; Azawi and Ismaeel, 2012a). Data about seasonal changes in main sperm parameters of rams from the Synthetic Population Bulgarian Milk sheep breed (SPBM) were not found in the available scientific literature. It is actually to study the male reproductive properties of this widespread and new Bulgarian breed certificated in year 2005 (Nikolov et al., 2008).

The aim of this work was to study the effect of breeding season on the kinematic and main spermatological parameters of the rams from SPBM breed.

Materials and Methods

Experimental Design

The experiment was carried out in the animal facility of the Institute of Animal Science - Kostinbrod with seven sexually matured, clinically healthy **rams** from Synthetic Population Bulgarian Milk sheep breed. The **average live weight of rams was 95 kg.** They were under uniform nutritional conditions. The water was provided ad libitum.

The ejaculates (n = 28) were collected by using artificial vagina. The experiment has been done in two replications. The samples were divided in 4 groups according to the period of collection of sperm and the sequence number of the ejaculate as follows: 1stgroup- the first ejaculates, obtained from rams 1 month before the breeding campaign (n_1=7), 2nd- the second ejaculates obtained from rams 1 month before the breeding campaign (n_2=7), 3th- the first ejaculates, obtained from rams during the breeding campaign (n_3=7), 4th- the second ejaculates obtained from rams, during the breeding campaign (n_4=7). The ejaculates were initially diluted (1:3, vol/vol) in 6A ram semen extender and shipped to the laboratory of IBIR-BAS within 1 hour.

Sperm Analysis

The assessment of semen quality parameters and various kinematic parameters of motile spermatozoa were carried out by Sperm Class Analyzer (SCA, Microptic, Spain). After delivering to the laboratory, the ejaculates were diluted additionally with 6A extender to reach appropriate concentration for performing the analysis. The extended semen was loaded into a Leja 20 chambers (Leja Products B.V., Nieuw-Vennep, The Netherlands) and examined using a microscope with warmed stage (Nikon, Tokyo, Japan). The analyses of sperm motility patterns were performed with SCA operating system. In addition to the overall percentage of motile spermatozoa, the software of SCA also measured the concentration, velocity of movement, the width of the sperm head's trajectory, the frequency of the change in direction of the sperm head and the kinematic values for each analyzed spermatozoon.

The following velocity values were recorded:

- the curvilinear velocity (VCL, mm/s - the average path velocity of the sperm head along its actual trajectory);
- the straight-line velocity (VSL, mm/s - the average path velocity of the sperm head along a straight line from its first to its last position);
- the average path velocity (VAP, mm/s - the average velocity of the sperm head along its average trajectory);
- the percentage of linearity (LIN, % - the ratio between VSL and VCL);
- the percentage of straightness (STR, % - the ratio between VSL and VAP).

The width of the sperm head's trajectory was recorded as the mean amplitude of lateral head displacement (ALH, mm) reflected the average value of the extreme side-to-side movement of the sperm head in each beat cycle. Finally, the frequency of the change in direction of the sperm head was recorded by means of the beat cross frequency (BCF, Hz), and the wobble (WOB, %), which reflects the measure of oscillation of the actual path about the average path.

For analyzing of sperm morphology were prepared several smears from each ejaculate. The smears were air-dried and stained with Sperm Blue (Microptic, Barcelona, Spain), according to the description of the manufacturer, and observed using the light microscope. The presence of abnormal cells out of at least 100 sperm cells from several fields on the slide was counted and their total percentage calculated.

Statistical Analysis

The data were calculated by statistical program SPSS 13.0 for Windows. The significance of the differences between the groups was evaluated by t-criterion of Student. Findings are considered statistically significant if $P<0.05$.

Results

Determination of movement characteristics is important for a prediction of sperm fertilization capacity. The kinematic parameters of the SPBM ram spermatozoa from two consecutive ejaculates before and during the breeding campaign are presented in Table 1 (mean ± SE).

The main velocity parameters of spermatozoa have higher value in ejaculates (I^{st} and II^{nd}), obtained during the breeding season. Especially, for the second ejaculates was established the statistically significant difference in all kinematic parameters in comparison with the non-breeding season ($P< 0.01$; $P< 0.05$; $P< 0.01$; $P< 0.01$; $P< 0.05$; $P< 0.05$; $P< 0.05$; $P< 0.05$). This data indicates a high sperm motility. It has to be noticed that the values of VCL are much higher than those of VAP and correspond to lower LIN percentage. These results show a high degree of lateral deviation of the spermatozoa head from the direction of movement.

Significant differences in sperm parameters between first and second ejaculates in both seasons were not established. However, the spermatozoa in the second ejaculate during breeding campaign show a tendency to be more active than spermatozoa in the first ejaculates (Table 1). The higher means of VCL, ALH, and BCF in second ejaculates show about higher energetic value of sperms.

In the present study we found higher levels of total motility in the ejaculates obtained during the breeding season (Figure 1). The difference between first ejaculates in the seasons is significant ($P< 0.05$). Also the effect of breeding season on the sperm concentration was observed. The both groups of ejaculates (I^{st} and II^{nd}) from the breeding season have higher ($P< 0.01$) concentration of spermatozoa.

The analysis with SCA is characterized by high sensitivity to the motility of the sperm cells and effectively distinguishes between the velocity of their motion, dividing them into fast, medium, slow-moving and static (Table 2). In accordance with our results, during the breeding season increas-

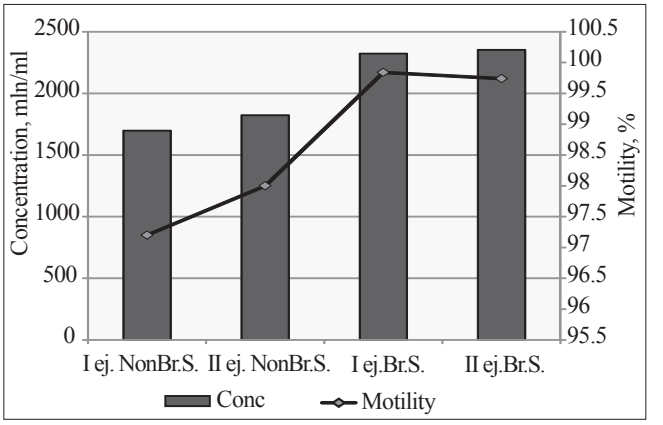

Fig. 1. Dynamic of concentration and motility parameters of spermatozoa in non-breeding and breeding seasons

Table 1
Velocity parameters of ram spermatozoa from SPBM breed in nonbreeding and breeding seasons

Samples	Non-breeding season		Breeding season	
Paramet.	I^{st}ejacul. (n_1=7)	II^{nd}ejacul. (n_2=7)	I^{st}ejacul. (n_3=7)	II^{nd} ejacul. (n_4=7)
VAP, µm/s	39.59 ± 3.95 b	46.90 ± 5.09 b_1	58.1 ± 3.46 b	66.08 ± 0.98 b_1
VSL, µm/s	25.43 ± 2.03	26.5 ± 3.29 a	30.68 ± 1.61	34.14 ± 0.42 a
VCL, µm/s	65.16 ± 8.25 b_2	64.04 ± 11.60 b_3	106.22 ± 9.06 b_2	122.54 ± 2.94 b_3
ALH, µm	3.1 ± 0.37 a_1	3.2 ± 0.34 b_4	4.38 ± 0.38 a_1	4.8 ± 0.19 b_4
BCF, Hz	6.06 ± 0.37	6.04 ± 0.20	6.20 ± 0.09 a_2	6.54 ± 0.08 a_2
STR, %	65.93 ± 4.44 a_3	64.26 ± 4.22 a_4	52.92 ± 1.02 a_3	51.68 ± 0.35 a_4
LIN, %	45.16 ± 6.32	42.90 ± 5.26	29.32 ± 1.44 a_5	27.92 ± 0.45 a_5
WOB, %	66.59 ± 4.15	65.66 ± 3.45	55.24 ± 1.70 a_6	54.0 ± 0.55 a_6

Significance: $a_{1...n}$= P<0.05, $b_{1...n}$= P<0.01;

Table 2
Distribution of the ram spermatozoa from SPBM breed by motility speed in nonbreeding and breeding seasons

Samples	Non-breeding season		Breeding season	
Parameters	I^{st}ejacul. (n_1=7)	II^{nd}ejacul. (n_2=7)	I^{st}ejacul. (n_3=7)	II^{nd} ejacul. (n_4=7)
Rapid, %	30.54 ±10.00 b	42.62 ± 11.60 b_1	77.88 ± 6.23 a_1, b	94.0 ± 1.15 a_1,b_1
Medium,%	27.49 ±3.38	31.70 ± 3.90 c	17.24 ± 4.52 a_2	5.1 ± 0.95 a_2, c
Slow, %	37.07 ±7.47 b_2	23.48 ± 8.34 a_3	4.72 ± 1.71 b_2	0.8 ±0.26 a_3
Static, %	4.9 ±1.96	2.20 ±1.44	0.16 ± 0.05	0.1 ± 0

Significance: $a_{1...n}$= P< 0.05, $b_{1...n}$= P< 0.01, c =P< 0.001

es the percentage of the fast motile sperms in both groups ejaculates ($P< 0.01$) and decreases the percentage of medium and slow moving spermatozoa ($P < 0.05$; $P < 0.01$) while the static spermatozoa are approaching the zero value.

The values of the sperms with normal morphology and those with defects in the head, the midpiece, the tail or with cytoplasmic droplets are presented in Table 3. There were no significant differences between samples in non- and breeding seasons. It was observed a tendency ($P > 0.05$) to lightly increase of the spermatozoa with the tail and head defects in the second ejaculates in both seasons that lead to the decrease of the total percent of normal spermatozoa.

Morphological status of the ram sperms from the SPBM breed are visually presented on Figure 2.

Discussion

Motility is considered as one of the most important features for assessment of fertilizing ability of the sperms A close relationship between fertilizing capacity and the kinematic parameters, describing the path of the gametes, has been established by Farrell et al. (1998). The measurement of sperm velocity is accepted as an indirect indicator of mitochondrial function in spermatozoa (Peris et al., 2004). The lower level of the sperm total motility in the ejaculates before the breeding season should be explained by changes in mitochondrial structure of the still immature spermatozoa. This suggestion is confirmed by the results of Piomboni et al. (2012) in human sperm. Also the lower values of VCL in these ejaculates have an impact on the sperm's overall curvilinear motion. This determines the decreasing of the motility, as it is confirmed by our results.

It is known that the quality of the ejaculate depend on the sequence of obtaining. Some authors have observed higher motility in the second ejaculates compared to the first one (Kistanova et al., 2007). The same trend toward increasing sperm motility from first to second ejaculate was found in this study during the non-breeding, but not in breeding season. Yotov et al. (2011) reported that sperm volume and concentration in semen samples decreased gradually with increase of ejaculation frequency, but the survival rate of sperm was the highest in the second ejaculate. Our results show the effect of breeding season on the sperm concentration but not on the sequence of the ejaculates obtaining.

High rates of rapid and morphologically normal spermatozoa correspond with high BCF value in the second ejaculates obtained in breeding season without a dramatic change in ALH. These parameters determine faster and more frequent movements of the tail, and thus ensure the ability of these cells to travel far to reach the oocyte (Murray, 2007).

Table 3
Morphological characteristics of spermatozoa from rams of SPBM breed in nonbreeding and breeding seasons

Samples	Non-breeding season		Breeding season	
Parameters	Istejacul. (n_1=7)	IIndejacul. (n_2=7)	Istejacul. (n_3=7)	IInd ejacul. (n_4=7)
Normal, %	90.00 ± 2.31	71.80 ± 14.17	90.80 ± 2.93	88.60 ± 2.14
Head, %	2.29 ± 0.56	3.20 ± 1.37	1.60 ± 0.46	1.4 ± 0.22
Midpiece, %	0.43 ± 0.19	1.40 ± 1.04	0.80 ± 0.52	0.60 ± 0.36
Tail, %	7.14 ± 2.00	7.20 ± 1.37	6.60 ± 3.13	8.60 ± 1.93
Citoplasmic droplets, %	0.29 ± 0.17	0.20 ± 0.18	0.20 ± 0.18	0.80 ± 0.52

Significance: all differences are non-significant

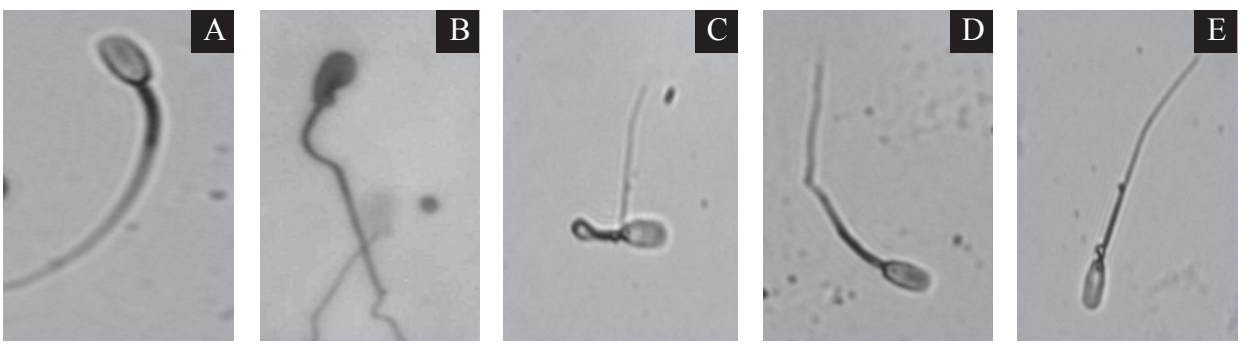

Fig. 2. Spermatozoa with various morphology: A – normal morphology; B – abnormal head shape; C – defects in the midpiece and tail; D – defect in the tail; E – cytoplasmic droplet

Evaluation of spermatozoa morphology is one of the methods for estimating semen quality. Positive correlation was found between the fertilization rate and the percentage of spermatozoa with normal morphology in humans. Sperm midpiece and tail abnormalities also can be indicators of possible infertility (El-Ghobashy and West, 2003). Our results are correspondnt with the data of D'alessandro et al. (2001), who found the decreased number of abnormal sperms in semen collected during the breeding campaign in other sheep breeds. It is described that small defects such as abnormal shape of the head, bending in the midpiece, curved or broken tails, presence of cytoplasmic droplets are caused by environmental factors and higher percent of defects in semen are received outside the breeding season (Ahangari and Hamedani, 2010), which coincides with our results. In the ejaculates obtained before breeding campaign, the lightly higher percentage of spermatozoa with abnormal morphologie were accompanied with decline of sperm motility. Hamidi et al. (2012) reported that the semen volume, sperm motility, percent of live sperm, the percentage of abnormal sperm and sperm concentration determined in breeding and non breeding seasons had significant differences. We declare the same findings for the sperm of rams from SPBM breed.

Based on the literature summaries and the obtained results, it should be underlined that the ejaculates obtained during the breeding season from rams of SPBM breed have better reproductive performance compared to those collected in nonbreeding season. The changes in the studied parameters mainly refer to the seasonality, but not to the sequence of ejaculates obtaining.

Conclusion

In the present work for first time have been studied the dynamic of the kinematic and main spermatological parameters of the rams from Synthetic Population Bulgarian Milk sheep breed in dependence on ejaculate sequence as well as on seasonality of breeding. The results of the performed analyses have great importance because they allow to define the ranges for these parameters in the target breed.

It was found that the ejaculates obtained from SPBM rams during the breeding season had better features of sperm motion kinetics. The values of the velocity parameters ($P< 0.05$), motility ($P< 0.05$), and percentages of spermatozoa with rapid ($P< 0.01$) and medium ($P< 0.001$) speed were higher than those from the ejaculates collected before the breeding season Minor and not significant changes in the kinematic parameters of motile spermatozoa in consecutive ejaculates were observed. No significant difference was established in morphological status of spermatozoa in non-breeding and breeding season. It seems that the better sperm motility kinematic parameters during the breeding season ensure the higher sperm fertility and success on the future insemination.

References

Ahangari, Y. J. and M. Ah. Hamedani, 2010. Monthly Changes in Morphology of Spermatozoa in Zel Rams. *Proc. Aust. Soc. Anim. Prod*., **28**: 83.

Azawi, O. I. and M. A. Ismaeel, 2012a. Effects of seasons on some semen parameters and bacterial contamination of Awassi ram semen. *Reprod. Domest. Anim.,* **47** (3): 403-6.

Azawi, O. I., A. N. T. M. Al-Khashab and N. N. A. Al-Kado, 2012b. Effect of Gonadotropin Releasing Hormone Treatment on Semen Characteristics and Enzymatic Activities of Awassi Rams in Breeding and Non Breeding Seasons. Iranian Journal of Applied Animal, **2** (1): 13-19.

Benoff, S., K. Auborn, J. L. Marmar and I. R. Hurley, 2008. Link between low-dose environmentally relevant cadmium exposures and asthenozoospermia in a rat model. *Fertil. Steril*., **89**: e73–e79.

Bohlooli1, Sh., F. Cedden1, Ş. Bozoğlu1, S. Razzaghzadeh1 and J. Pishjang, 2012. Correlation between conventional sperm assay parameters in cryopreserved Ram Semen. *Annals of Biological Research*, **3** (2): 884-889.

Colas, G., 1980. Seasonal variations of the quality of sperm in the Ile-de-France ram. I. Study of the cellular morphology and ''massal''motility. Reprod. Nutr. Dev., **20**: 1789–99.

Davis, R. O. and R. J. Siemers, 1995. Derivation and reliability of kinematic measures of sperm motion. *Reprod Fertil Dev*., **7**: 857–869.

D'alessandro, A. G., M. A. Collona, A. Bellitti, and G.Martemucci, 2001. Variations during the year in quantitative and qualitative semen characteristics in Leccese rams. *Zootecnia e Nutrizione Animale*, Bologna, **27**: 221–230.

El-Ghobashy, A. A. and C. R. West, 2003. The human sperm head: a key for successful fertilization. *J. Androl*., **24**: 232-238.

Farrell, P. B., G. A. Presicce, C. Brockett and R. H. Foote, 1998. Quantification of bull sperm characteristics measured by computer-assisted spermanalysis and the relationship to fertility. *Theriogenology*, **49** (4): 871–879.

Garrett, C. L. and D. Y. Clarke, 2003. Automated semen analysis: '*zona pellucida* preferred' sperm morphometry and straight-line velocity are related to pregnancy rate in subfertile couples. *Hum. Reprod*., **18**: 1643-1649.

Hamidi, A., M. Mamoei, Kh. Mirzadeh, S. Tabatabaei and H. Roshanfekr, 2012. Seasonal Variations in Semen Characteristics in Arabic Rams. *Pakistan Veterinary Journal,* **32** (1): 41.

Hammerstedt, R. H., 1993. Maintenance of bioenergetic balance in sperm and prevention of lipid peroxidation: a review of the effect on design of storage preservation systems. *Reprod. Fertil. Dev*., **5**: 675–690.

Karagiannidis, A., S. Varsakeli, C. Alexopoulos and I. Amarantidid, 2000. Seasonal variation in semen characteristics of Chios and Friesian rams in Greece. *Small Rumin. Res.*, **37:** 125-130.

Kistanova, E., D. Vasileva, B. Grigorov and N. Metodiev, 2007. The morphological and biochemical characteristics of the consecutive ejaculates from Il de France rams at various ages. *Biotech. Anim. Hunsb*., **23**: 301-310.

Makawi, S. A., B. A. Elsharif and E. A. Babiker, 2007. Effect of Season on Freezability of Semen from Two Breed-Types of Desert Sheep in the Sudan. *Journal of Animal and Veterinary Advances*, **6** (7): 846-849.

Meizel, S., 2004. The sperm, a neuron with a tail: 'neuronal' receptors in mammalian sperm. *Biol. Rev*., **79**: 713–732.

Morales, P. K. and D. R. Overstreet, 1998. The relationship between the motility and morphology of spermatozoa in semen. *J. Androl*., **9**: 241-247.

Murray, G. M., 2007. Acrosome size and kinematics of human spermatozoa. PhD thesis, Stellenbosch University, pp. 43-50.

Nikolov, V. et al., 2008. Livestock breeds in the republic of Bulgaria. Catalogue, 2nd edition, Sofia, Bulgaria (ISBN 978-954-92216-3-3): **90**.

Peris, S. I., A. Morrier , M. Dufour and J. L. Bailey, 2004. Cryopreservation of ram semen facilitates sperm DNA damage: relationship between sperm andrological parameters and the sperm chromatin structure assay. *J. Androl*., **25**: 224-233.

Piomboni, P., R. Focarelli, A. Stendardi, A. Ferramosca and V. Zara, 2012. The role of mitochondria in energy production for human sperm motility. *Int. J. Androl.,* **35**: 109-124.

Yotov, St., I. Fasulkov and N. Vassilev, 2011. Eff ect of ejaculation frequency on spermatozoa survival in diluted semen from Pleven Blackhead rams. *Turk. J. Vet. Anim. Sci.,* **35** (2): 117-122.

Zhang, B. R., B. Larsson, N. Lundeheim and H. Rodriguez-Martinez, 1998. Sperm characteristics and zona, pellucida binding in relation to field fertility of frozen, thawed semen from dairy AI bulls. *Int. J. Androl*., **21**: 207-216.