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Effect of ejaculation frequency on spermatozoa survival in diluted semen from Pleven Blackhead rams

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Abstract: The effect of ejaculation frequency on spermatozoa survival in diluted Pleven Blackhead ram semen was investigated. Thirty-two ejaculates were examined. From each ram 4 ejaculates were collected at 10-min intervals in the morning and in the afternoon. Semen parameters, namely, color, density, transparency, volume, concentration, motility, and abnormal sperm, for each ejaculate were determined. The sperm motility was assessed from the moment of dilution up to 60 min at 10-min intervals, then again at 90 min and 120 min. The semen characteristics were determined by means of "Motic Image Plus" equipment and the data were processed statistically.

Sperm volume and concentration in semen samples decreased gradually with increase in ejaculation frequency. The values were significantly lower for the 4th ejaculate compared to preceding ones ($P < 0.05$). The highest survival rate ($P < 0.05$) of sperm was found in the 2nd ejaculate, and this trend was preserved up to the second hour. Up to 50 min after dilution, the sperm motility did not change considerably, after which it was found to be reduced ($P < 0.05$).

The frequency of ejaculation and the period of semen collection had an impact on sperm motility in extended and short-time stored semen from Pleven Blackhead rams. The spermatozoa in a 2nd ejaculate showed higher survival rates and could be recommended for additional processing.

Key words: Ram, frequency of ejaculation, semen, motility

Introduction

Artificial insemination is a primary biotechnological approach aiming at increasing the genetic potential in sheep husbandry (1-3). In practice, double artificial insemination with liquid semen at 8- or 12-h intervals is commonly performed. When sheep for insemination are numerous, several ejaculates are successively collected and, after extension, are stored in a liquid state or frozen (4-7).

One of the essential parameters of sperm survival and fertility is the motility of spermatozoa (8-11). According to Phillips and Lardy (12) and Willett and Salisbury (13) the egg yolk-phosphate and egg yolk-citrate sperm extenders maintain the high sperm motility within the first hour after semen collection. Maxwell and Salamon (14) state most authors do not provide sufficient information about temperature of storage, dilution rate, and dose of inseminate. Bonev et al. (15) reported that sperm motility in diluted semen correlated positively to fertility and depended directly on the time of storage and time of insemination.

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The rams with best semen parameters should be used as donors, and successful fertilization is guaranteed by sufficient amount of spermatozoa with a high survival rate, reaching the site of ovum fertilization (16). In the opinion of Gündoğan (17), the season, age, ram breed, and the frequency of ejaculation had a considerable effect upon the biological parameters of semen (volume, concentration, motility, normal and abnormal sperm etc.). Another factor is also the interval between collected ejaculates (18,19). According to Kaya et al. (20), the motility of spermatozoa in the semen of German Mutton Merino × Native Akkaraman rams was reduced with increasing the number of ejaculations.

In contrast, Nel-Themaat et al. (21) observed a higher motility in the 2nd ejaculate compared to the 1st, collected at a 10-min interval in Gulf Coast Native rams. Kistanova et al. (22) reported a negative correlation between sperm volume and sperm concentration and a trend towards increased motility in 3 ejaculates consecutively obtained at 30-min intervals from Ile de France rams

The sheep from the local Pleven Blackhead breed are widely distributed in Bulgaria and are used to obtain crosses with other breeds (23). Nevertheless, the information about the influence of ejaculation frequency on the quality of semen from Pleven Blackhead rams is not well documented.

Furthermore, the investigations performed with other sheep breeds with regard to the relationship of sperm motility and number of consecutive ejaculations are conflicting.

This raises the question of utilization of ram donors with the best semen quality when the semen is intended for insemination of numerous sheep immediately after collection or has to be stored and transported.

The purpose of the present study was to determine the effect of ejaculation frequency upon the survival of spermatozoa in the semen of Pleven Blackhead rams after dilution and short-time storage at a temperature of 35 °C.

Materials and methods

Donor rams (n = 4) from the Pleven Blackhead breed aged between 2 and 4 years, weighing 55-70 kg, were used. The animals were reared in individual boxes under uniform conditions and an immunoprophylaxis regimen.

The region was located at latitude of 42°25′N, and the experiment was carried out in the breeding season (November).

Prior to the experiment, an abstinence period of 30 days was provided and a complete physical examination was performed. The semen samples were collected by the artificial vagina method in the presence of a ewe in estrus. A total of 32 ejaculates were used for this study. From each ram, 4 ejaculates were obtained at 10-min intervals (at 0600-0800) and another 4 observing the same schedule after 8 h (1400-1600), numbered as 1st, 2nd, 3rd, and 4th.

The average air temperature throughout ejaculate collection was 11.2 ± 0.4 °C in the morning and 15.9 ± 0.2 °C in the afternoon.

Immediately after semen collection, the color, density, transparency, sperm volume (mL), sperm concentration (no. × 10^9/mL), sperm motility (%), and abnormal sperm (%) were determined.

The semen was stored in a water bath at 35 °C and diluted with 0.9% saline to achieve 0.2 mL semen containing 80 × 10^6 motile spermatozoa. After that, the sperm motility of each ejaculate was evaluated at 10-min intervals within the period from dilution up to 60 min, and again at 90 min and 120 min.

The concentration of sperm was determined by microscope (200× magnification) in a Thoma counting chamber. The viability of spermatozoa by means of their motility (%) was assessed by 2 independent observers. Abnormal sperm (%) were determined by the microscopic examination of semen by "Motic Image Plus" digital software equipment (Motic China Group Ltd, 2001-2004), including a phase contrast microscope with a hot plate, digital camera, and relevant software (24).

The results were statistically processed with the StatSoft statistical software (Microsoft Corp. 1984 - 2000 Inc.) with ANOVA and nonparametric analysis of means and proportions using the Student's t-criterion.

Results

In this experiment, a total of 32 ejaculates were examined, successfully collected in an attempt to

mount a teaser ewe. During the experiment, there were no deviations in the normal macroscopic parameters of ejaculates. The results from the initial screening of semen are given in the Table.

A decrease in the average volume of ejaculates with increasing frequency of ejaculations was observed for both periods of collection: in the morning and in the afternoon. For morning collections, the average volumes of the 1st and 2nd ejaculates (1.5 ± 0.3 mL, 1.2 ± 0.3 mL) differed statistically significantly ($P < 0.05$) from that of the 4th (0.5 ± 0.1 mL). A similar result was obtained with afternoon ejaculates: the volume of the 1st ejaculate (1.0 ± 0.3 mL) was bigger ($P < 0.05$) than those of the 3rd and the 4th.

The trend towards reduction in sperm concentration in afternoon ejaculates was preserved. Again, there was a decrease in values with every next ejaculation ($P < 0.05$). The highest spermatozoa counts for both periods of collection ($2.0 \pm 0.3 \times 10^9$/mL and $1.4 \pm 0.1 \times 10^9$/mL) were observed for 1st ejaculates, with the lowest ($0.9 \pm 0.1 \times 10^9$/mL and $0.6 \pm 0.1 \times 10^9$/mL) in the 4th ejaculates (Table).

Unlike the first 2 parameters, the determination of motility percentage did not show any significant differences among ejaculates obtained either in the morning or in the afternoon. The highest motility (90.0 ± 1.2% and 90.0 ± 2.2%) for both periods was observed for the 2nd ejaculate.

The 2nd ejaculate differed statistically significantly ($P < 0.05$) from the 1st (80.6 ± 3.3% and 79.6 ± 3.3%), 3rd (83.1 ± 3.3% and 83.0 ± 2.3%), and 4th (76.7 ± 3.2% and 76.5 ± 3.0%) ejaculates.

The percentages of abnormal sperm vary, with values ranging from 4.3 ± 0.9% to 7.3 ± 0.9%. The significant increase in values ($P < 0.05$) was registered in the last 3 ejaculates collected in the afternoon.

The time course of the motility of spermatozoa from each ejaculate showed a reduction with advancing time of storage (Figure 1). From collection to 50 min thereafter, there were no significant deviations from the initial motility for either collection period. After 60 min, however, the sperm motility decreased considerable ($P < 0.05$) in the 1st and 4th ejaculates. The lowest motility was registered in the 4th ejaculate at 120 min, with values of 40 ± 2.2% (morning) and 38.6 ± 1.8% (afternoon). The lowest variation in sperm motility was observed for the 2nd ejaculate.

Discussion

The results of the present study showed that the frequency of ejaculations and the time interval between ejaculations influenced the survival of spermatozoa in Pleven Blackhead ram semen. The effect of frequency of semen collection upon sperm quality in domestic animals is also reported by other investigators (16,18,25).

The sperm volume vs. sperm concentration decreased gradually with every next ejaculation

Table. Parameters of subsequent ejaculates collected in the morning and in the afternoon in Pleven Blackhead rams (mean ± S.E.M.).

	Morning				Afternoon			
Ejaculate number	I	II	III	IV	I	II	III	IV
Parameters								
Volume (mL)	1.5 ± 0.3a	1.2 ± 0.3a	0.8 ± 0.2ab	0.5 ± 0.1bc	1 .0 ± 0.3a	0.8 ± 0.2ab	0.5 ± 0.1bc	0.4 ± 0.1bc
Concentration (no. × 10^9/mL)	2.0 ± 0.3a	1.4 ± 0.2b	1.0 ± 0.1c	0.9 ± 0.1c	1.4 ± 0.1b	1.0 ± 0.1c	0.8 ± 0.2cd	0.6 ± 0.1d
Motility (%)	80.6 ± 3.3a	90.0 ± 1.2b	83.1 ± 3.3a	76.7 ± 3.2a	79.6 ± 3.3a	90.0 ± 2.2b	83.0 ± 2.3a	76.5 ± 3.0a
Abnormal sperm (%)	4.3 ± 0.9a	5.7 ± 0.7a	5.3 ± 0.7a	4.3 ± 0.3a	5.7 ± 1.2a	6.3 ± 0.3b	7.3 ± 0.7c	7.3 ± 0.9c

The means that do not have common letters in the same row are different ($P < 0.05$)

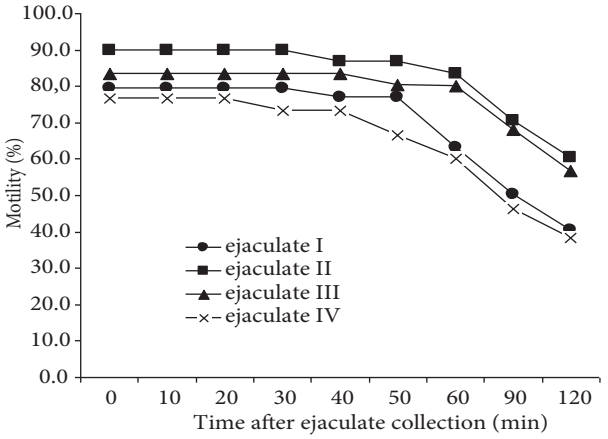

Figure 1. Sperm motility from morning ejaculates, extended and stored at 35 °C for 120 min.

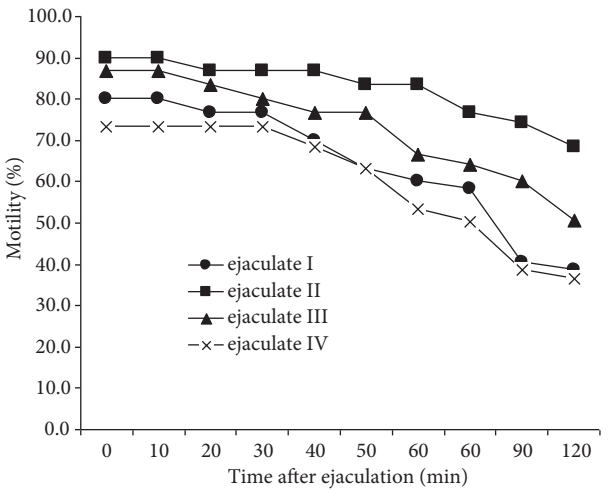

Figure 2. Sperm motility from afternoon ejaculates, extended and stored at 35 °C for 120 min.

as shown in the studies by Kaya et al. (20). Unlike the data reported by Nel-Themaat et al. (21), a statistically significant ($P < 0.05$) reduction in semen volume and concentration was determined between the 1st and 2nd, and 4th ejaculations for both periods of collection.

This could be attributed to the reservoir function of studied rams and supports the hypothesis of Stellflug and Berardinelli (27) about a breed-related effect upon sperm production in rams.

The present study also supported the importance of the interval between successive ejaculations upon sperm quality as reported also by Ollero et al. (18).

The abnormal spermatozoa percentages in the different ejaculates were in agreement with those obtained by Kaya et al. (20) in German Mutton Merino × Native Akkaraman rams. The higher values in the last ejaculates collected in the afternoon could be due to the differing secretions of epididymis and accessory glands in high frequency of ejaculation, detected also in boar semen (26).

According to data from this experiment, the 8-h period was sufficient for partial restoration of semen volume and sperm concentration in Pleven Blackhead rams, when several successive ejaculates are needed. This fact could be utilized to determine the necessary number of male breeders for artificial insemination of numerous sheep.

The data about sperm motility showed clearly that a statistically significantly ($P < 0.05$) higher motility was observed in the 2nd ejaculate compared to all others, as also shown by Nel-Themaat et al. (21) in Gulf Coast Native rams. In contrast to data reported by Kistanova et al. (22) in Ile de France rams, the motility of spermatozoa in the 3rd ejaculate tended to increase compared to the 1st.

The lower motility of the 1st ejaculate could be attributed to the higher number of mature spermatozoa. According to Marengo (28) they are stored in a quiescent state in the tail of the epididymis and in a long abstinence period a portion is passively transferred into the vas deferens and may undergo changes.

Picket and Komarek (29) reported that spermatozoa in the 2nd fraction contained more lipids. This could determine a higher resistance and increased motility.

The initial motility was preserved after extension of ejaculates and their storage at 35 °C.

The time course of motility showed that the survival of spermatozoa in diluted semen collected either in the morning or in the afternoon did not change considerably up to 50 min and afterwards decreased considerably ($P < 0.05$). This could be due to reduction of semen pH and to dilution rate (16,26).

The obtained results could assist in throwing light on the problem with attainment of high-quality ejaculates and thus higher fertilization rates when multiple sheep are to be inseminated.

In Pleven Blackhead rams we regard the semen from 4 consecutive ejaculates collected at 10-min intervals as suitable for artificial insemination immediately after collection and dilution. When storage, transportation, or freezing are required, the 2nd and the 3rd ejaculates could be the most appropriate.

The utilization of semen for artificial insemination within 50 min of collection could provide spermatozoa with a higher survival rate in the insemination dose.

In conclusion, the frequency of ejaculation and the period of semen collection influence the sperm motility in diluted and short-time stored semen from Pleven Blackhead rams, with the highest survival rates in the 2nd ejaculate. Future field trials are probably needed to reveal the effect of ejaculate number and the time of storage on conception.

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