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EFFECT OF DIFFERENT EXTENDERS ON RAM SPERM TRAITS DURING STORAGE

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Abstract

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The aim of the study was to test commercial extenders used for short-term and long-term sperm preservation. Semen was collected in the reproduction season, i.e. from June to December. The ejaculates were obtained from single services and the routine analysis of the semen was performed immediately after the collection. The examination included semen volume, colour and texture, sperm concentration and motility, ejaculate turbulence and percentage of sperm with abnormal morphology. The semen was diluted with an extender in the ratio of 1:4. The processed semen was transported in an insulated container at 16–18 °C to the laboratory and stored in a stationary thermostat under the same temperature. Sperm motility tests were performed 24, 48, 72 and 96 hours after the placement in to thermostat.

Ejaculates diluted with Ovipro, Optidyl, Triladyl and Andromed CSS gave very good results of viability (81.23 %–83.41 %) after 24 hours of storage. After 48 hours, Ovipro, Andromed, Optidyl and Triladyl gave values above 75 %. The Triladyl extender proved to be a good stabilizing agent, showing consistent results during a long-term storage. It was chosen as a control one for overall assessment. Other preservation media did not show any improving or worsening effects. The extender Ovipro showed a high motility effect in the first 48 hours only, and hence it appears to be the best solution for the short-term preservation.

ram, sperm activity, extender

Preparation of insemination doses (from suitable donors or sperm import) and their subsequent use for insemination may help increase numbers of high merit animals of desired breeds and performance types. The routinely analysed parameters of breeding ram semen include specific reproduction traits that do not predict the ewe fertility but serve as important indicators of ram fertility. These techniques enable to improve the flock quality by using high merit animals for natural service or artificial insemination.

As the short-term and long-term semen preservation and subsequent intracervical insemination have not given satisfactory results so far, all the breeding efforts are based on rearing rams with high breeding value and relying on natural reproduction.

Therefore, in the first phase of this study, the aim was to test the efficacy of commercial extenders available in short-term and long-term semen preservation.

Literature review

In breeding practise, both the breeding methods are still in use: artificial insemination (AI) and natural mating (NS). In spite of a lower pregnancy rate, AI brings substantial breeding benefits (Bucak and Tekin, 2007). Cryopreservation of ram semen significantly decreases the fertilizing ability of intracervical insemination. The intracervical insemination gives higher pregnancy rates, yet it is less suitable for the practical use (Yaniz *et al.*, 2005). The lower fertilizing ability of frozen semen is due to the use of inadequate methods of cryopreservation (Aitken and Clarkson, 1989) or improper handling during transport (Fair *et al.*, 2007).

The sperm motility test is generally used for evaluation and comparison of preservation media

quality (Věžník *et al.*, 2004; Louda, 2001; Gamčík and Kozumplík, 1976).

Štolc *et al.* (2008) tested differences in sperm activity between extenders. They did not find any significant differences between the extenders tested. The smallest reduction in sperm activity was observed with the Triladyl (TRI) extender and the greatest one with Andromed (AND).

Similar results were reported by Afroz *et al.* (2008). They found different sperm activities after semen diluting and thawing, depending on an extender. Triladyl appeared to be better, owing to high sperm activity after the thawing. This is in accordance with our results.

Paulenz (1998) monitored sperm viability, using four different extenders: a skim milk extender, sodium citrate extender, and two egg yolk-based extenders (T1 and T2). Sperm viability was measured after 6, 12, 24 and 30 hours. The motility was influenced by the storage time and type of extender; sperm motility was influenced by the relationship between storage time and extender type. The results showed a higher sperm viability in the egg yolk-based extender (T2).

Fukui Y. *et al.* (2008) studied pregnancy rate in ewes artificially inseminated with frozen semen, using different extenders (Andromed, egg yolk-based extenders). Suffolk ewes were used in the study. The results suggested that it is possible to use extenders free of animal origin products with success.

MATERIAL AND METHODS

The commercial extenders listed below were used for the laboratory testing of short-term and long-term ram semen preservation:

- extenders free of products of animal origin – Andromed, Andromed CSS I. and II., Biociphos, Biladyl, Optidyl,
- extenders containing products of animal origin – Triladyl, Ovipro.

The study included Suffolk (SF), Sumava (S) and Walachian (W) breeding rams (n = 9), 2 to 4 years old, showing no clinical disease signs. Semen was collected in the reproduction season (June–December). The collected semen for each ram was obtained from a single service; the routine sperm analysis was performed right after the collection. The ejaculates were examined for volume, colour, texture, sperm concentration, sperm motility, turbulence, and percentage of spermatozoa with abnormal morphology. Semen was diluted with a relevant extender at the ratio 1:4.

The processed semen was transported in an insulated box at 16–18 ^{0}C in a laboratory where it was stored in a stationary thermostat under the same temperature. Further motility assessment was carried out 24, 48, 72 and 96 hours after the placement in to the thermostat.

The data obtained were subjected to the analysis of variance (ANOVA).

RESULTS

Table I shows mean activity values of breeding ram sperm, obtained in the long-term sperm motility test, by different storage times and extenders.

Ejaculates diluted with Ovipro, Optidyl, Triladyl and Andromed CSS gave very good results of viability (81.23 %–83.41 %) after 24 hours of storage. After 48 hours, Ovipro, Andromed, Optidyl and Triladyl gave values above 75 %. After 72 hours, high viability values were obtained with Triladyl, Andromed, Andromed CSS. After 96 hours, the highest viability was obtained with Triladyl. For Andromed and Andromed CSS, sperm viability exceeded 40 %. Afroz *et al.* (2008) studied motility of sperm after cooling to 5 °C in Triladyl diluents ranged from 65.00% to 66.67.

Statistically significant differences were found between Triladyl-Ovipro and Triladyl-Biladyl ($P < 0.01$), and between Triladyl and Biociphos ($P < 0.05$). Štolc *et al.* (2009) did not find significant differences among the deluters. Triladyl gave consistent results in a long-term and appeared to be the best stabilizing agent. It was chosen as a control for the overall assessment. Other preservation media showed neither improving nor worsening effects. The results suggested that Biociphos and Biladyl

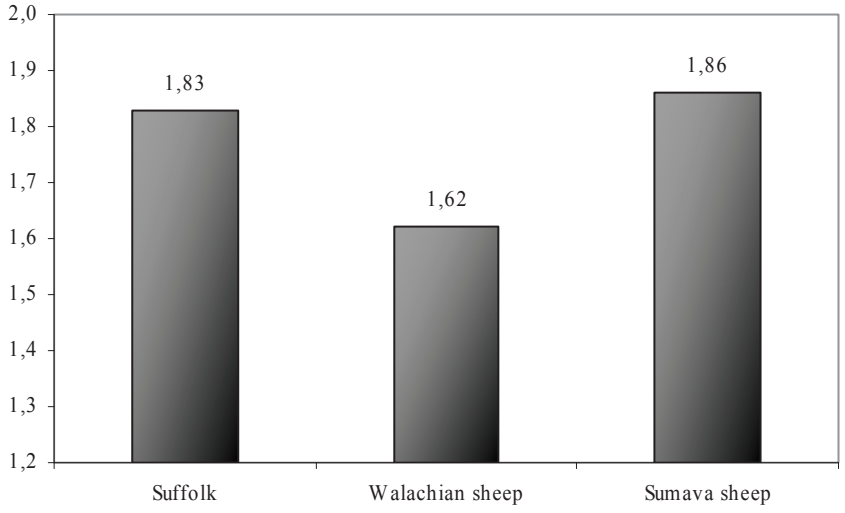
I: *Mean sperm activity in breeding rams in the long-term viability test, listed by storage time and extender used*

Extenders	Dilution ratio	No. of ejaculates	24 h	48 h	72 h	96 h	Total	Viability index
		n	%	%	%	%	%	%
Triladyl	1:4	9	**81.9**	75.6	68.0	55.7	70.3	100.00 [a]
Andromed	1:4	9	78.6	77.1	66.9	43.0	66.4	94.5
Ovipro	1:4	9	**83.4**	79.2	34.7	9.5	51.7	73.5 [b]
Biladyl	1:4	9	77.9	64.9	51.9	28.6	55.8	79.4 [b]
Optidyl	1:4	9	**82.4**	76.0	62.9	39.5	65.2	92.7
Andromed CSS I. and II.	1:4	9	81.2	73.5	65.7	48.4	67.2	95.5
Biociphos	1:4	9	75.6	70.0	62.5	29.7	59.5	84.6 [c]

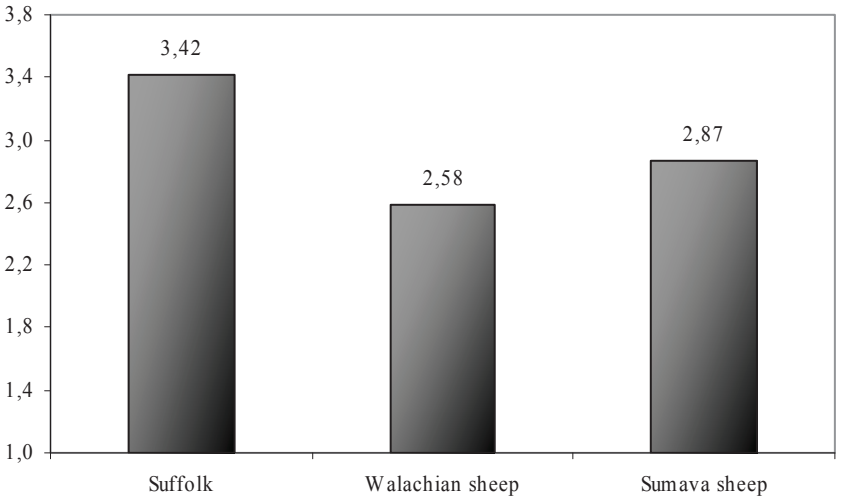
a, b $P < 0.01$
a, c $P < 0.05$

II: *Mean values and standard deviacion of spermatologic parameters of ram ejaculate used for the testing of extenders*

Breed	Number of collections	Ejaculate volume	Sperm activity	Sperm concentration	Turbulence rate	Morphologically abnormal sperm
	n	ml	%	$\times 10^6 \cdot mm^{-3}$	(1–3)	%
Suffolk	9	1.83 ± 0.528	79.9 ± 0.151	3.42 ± 0.632	2.62	12.5 ± 0.264
Walachian sheep	9	1.62 ± 0.611	78.4 ± 0.178	2.58 ± 0.585	2.50	16.9 ± 0.480
Sumava sheep	9	1.86 ± 0.668	78.0 ± 0.145	2.87 ± 0.689	2.68	15.0 ± 0.323



1: *Ejakulate volume (ml)*



2: *Sperm concentration (×106.mm^{-3})*

are almost cost-ineffective. Ovipro showed a high motility first 48 hours only and hence it appears to be the best extender for the short-term sperm preservation. Paulent (1998) tested differences in sperm activity between extenders. He found that all sperm viability parameters were influenced by storage time and extender, while sperm motility was the only evaluated parameter that was influenced by the interaction between extender and temperature.

Furthermore, semen quality was evaluated. Table II shows the semen quality results (ejaculate volume, sperm activity, sperm concentration – Graph 2, turbulence rate and percentage of morphologically abnormal spermatozoa).

The highest ejaculate volume was given by the Suffolk and Sumava rams (Graph 1). The highest sperm activity and sperm concentration (Graph 2) values, and turbulence rates were obtained with the Suffolk rams.

The percentages of spermatozoa with abnormal morphology were within the physiological range. A detailed assessment revealed that immature

spermatozoa (with a protoplasmatic droplet) made up the greatest portion of all the abnormal sperms, followed by spermatozoa with altered head bases and those with an excentrically located base of tail.

Generally, the quality of the breeding ram ejaculates under study was high, and the breeding flocks met high breeding and veterinary standards.

CONCLUSION

The Triladyl extender gave consistent results in a long-term and appeared to be the best stabilizing agent. The results obtained with the extenders Biociphos and Biladyl were on the limit of cost-effectiveness. The Ovipro extender seems to be the best one for short-term preservation, showing a high viability effect in the first 48 hours only.

It can be concluded that the quality of the breeding ram ejaculates under study was very high and the flocks monitored fully complied with high breeding and veterinary standards.

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SUMMARY

As the short-term and long-term semen preservation and subsequent intracervical insemination have not given satisfactory results so far, all the breeding efforts are based on rearing rams with high breeding value and relying on natural reproduction.

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