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# RELATIONSHIPS AMONG HERD, RAM BREEDS, AGE OF RAMS, SPERM DENSITY BEFORE DILUTING AND SPERM MOTILITY DURING THERMAL SURVIVAL TEST

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## Abstract

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The objective of this study was to determine the effect of herd, breed, the age of rams, their semen density, and the type of diluter used on sperm motility during a survival test. The total level of sperm activity of 49 rams was evaluated. A statistically significant effect of the herd, breed, the age of the rams, and the density of their semen on sperm motility after 30, 60, and 90 minutes was determined. Significant differences among herds were detected in the level of sperm activity after 90 minutes of the survival test only, $P < 0.05$. A significant difference ($P < 0.05$) between breeds was determined only in the case of sperm activity after 60 minutes of the test. Higher sperm motility during the entire survival test was detected in Bohemia Forest rams. A non-significant difference ($P > 0.05$) was found in the sperm activity in relation to the age of the rams. Sperm survival during the test significantly differed in relation to sperm density before diluting ($P < 0.05$). No significant differences among diluters used were confirmed. Relationships among sperm activity before and after diluting and during the entire survival test were confirmed by significant Pearson´s correlation coefficients ($P < 0.001$).

ram, herd, breed, sperm motility, thermal survival test, sperm density, diluter

The economy of farm animal breeding is associated with levels of production and secondary traits. Reproduction belongs to the economically important traits of farm animal breeding. Breeders use artificial insemination or natural service to ensure females' pregnancy (Stádník et al., 2008). Efficient animal reproduction involves accurate estimations of fertilization capability (Grasa et al., 2005). Artificial insemination (AI) can be a important and intensive method of sheep breeding (Bucak, Tekin, 2007). AI in sheep is currently limited by the poor fertility achieved after cervical insemination with frozen semen (Yániz et al., 2005), probably due to problems with semen preservation (Aitken and Clarkson, 1989) or failure of normal sperm transport in the female and/or early embryo development (Fair et al., 2007).

The objective of this study was to determine the effect of herd, breed, the age of rams, their semen density, and the type of diluter used on sperm motility during a survival test.

## MATERIAL A METHODS

The experiment was conducted in eight herds of sheep (Bohemia Forest sheep, n = 4, and Wallachia sheep, n = 4) during the mating seasons from 2003 to 2006. A total of 49 rams (Bohemia Forest breed, n = 32, and Wallachia breed, n = 17) in these herds were observed and monitored. The rams were divided into three groups according to their age at the time of observation. The first group consisted of rams younger than 36 months; the second group included rams from 36 to 60 months of age; and the third group was comprised of rams over 60 months of age. Sperm were collected from each ram repeatedly during mating seasons in evalution period, in selected rams during subsequent days or weekends, in other

rams repeatedly during subsequent years. The results of individual semen collection were used for evaluation. The sperm density was evaluated by estimation immediately after each collection and was determined by microscopic estimation in relation to spatial distribution of spermatozoas in visual field of microscope. Individual levels of sperm density were estimated on the commonly used levels in relation + = dense – D with more detailed division to D1, D2, and D3; ++ = medium dense – MD with more detailed division to MD1, MD2, and MD3; and +++ = very dense – VD. The levels of diluting of collected sperm was made in relation to constant quality of AI doses produced and differed from 3 to 8 portions of added diluter. Sperm activity was estimated immediately after each collection, after dilution and repeatedly after 30, 60, and 90 minutes of a thermal survival test. Sperm activity was determined by the same high power field microscope as that for the percentage of live spermatozoa, and the percentage that was progressively motile was estimated from this. Two types of diluters mainly used for diluting of bull semen were applied, Optidyl and Triladyl. Optidyl differed from Triladyl in content of ionized egg yolk and glycerol, while Triladyl contained in addition sodium citrate, milk citrate, and fructose. Optidyl is commercial preparation for the direct usage (Cryotech, s.r.o.) and Triladyl is also commercial preparation (Minitüb, Tiefenbach, Germany), but in concentrated form and subsequent diluting before usage is necessary.

Sperm motility of the rams´ sperm during the survival test (30, 60, and 90 minutes at temperature 39 ± 1°C) was evaluated with relation to the herd, breed, age group, density of sperm before diluting, and type of diluter used.

The dataset was analysed by ANOVA (Rasch and Mašata, 2006) using the statistical program SAS STAT 8.0 – GLM (SAS, 2001). The following equation was used:

$$Y_{ijklmn} = \mu + HERD_i + BREED_j + AGE_k + DEN_l + DIL_m + e_{ijklmn}$$

where:

$Y_{ijklmn}$..... observed value of the dependent variable (sperm motility after 30, 60, and 90 minutes of the survival test),

μ..... average value of dependent variable,

$HERD_i$ .. fixed effect of i-class of HERD (i = 1st herd, n = 9; 2nd herd, n = 5; 3rd herd, n = 12; 4th herd, n = 3; 5th herd, n = 6; 6th herd, n = 7; 7th herd, n = 5; 8th herd, n = 2),

$BREED_j$ fixed effect of j-class of breed (j = Bohemia Forest sheep (BFS), n = 32; Wallachia sheep (WS), n = 17),

$AGE_k$ ..... fixed effect of k-class of ram's age (k = to 36 months of age, n = 27(AG1); from 37 to 60 months of age, n = 10 (AG2); more than 60 months of age, n = 12 (AG3)),

$DEN_l$ ..... fixed effect of l-class of sperm density (l = D - dense, n = 4; D1, n = 2; D2, n = 8; D3, n = 16; MD – medium dense, n = 7; MD1, n = 3; MD2, n = 3; MD3, n = 3; VD3 – very dense, n = 3),

$DIL_m$..... fixed effect of m-class of diluter (m = Optidyl (OPT), n = 17; Triladyl (TRI), n = 32),

$e_{ijklmnop}$ ... residual effects.

The differences between the estimated variables were tested at the levels of significance $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

## RESULTS AND DISCUSSION

Sperm motility of the rams´ ejaculates was evaluated during observations performed in 8 selected herds of Bohemia Forest sheep (n = 32) and Wallachia sheep (n = 17) during the mating seasons from 2003 to 2006. Sperm motility of 49 rams after 30, 60, and 90 minutes of the thermal survival test was evaluated with relation to the factors included in the model. Table I presents the basic statistical characteristics of the linear model evaluated. Coefficient of repeatability ranged from $r^2 = 0.7937$ to $r^2 = 0.8607$ during evaluation of the observed traits, similar lower values being determined with relationship to sperm motility after 30 and 60 minutes of the survival test, and the highest values in relationship to the level of sperm activity after 90 minutes of the test. The statistical significance of all the models used for evaluation of the sperm activity during the survival test was $P < 0.01$ in the case of sperm motility after 30 and 60 minutes and $P < 0.001$ in evaluation of sperm motility after 90 minutes.

A significant effect of the herd was found only with relation to sperm motility after 60 ($P < 0.05$) and 90 ($P < 0.001$) minutes of the test. The effect of the breed was significant only with relation to sperm motility after 60 minutes of the survival test ($P < 0.05$). Makawi et al. (2007) found significant differences in sperm motility among the evaluated rams in relation to the individual season. Fair et al. (2007) recorded interbreed differences in reproduction results especially in the area of the sheep reproductive capabilities, and David et al. (2008) confirmed their findings with a significantly higher level of heritability and repeatability for traits of sheep fertility. A significant effect of the age group was detected in relation to sperm motility after 30 and 60 minutes ($P < 0.05$). The level of sperm density was significantly related to sperm motility after 60 minutes ($P < 0.01$) and after 30 and 90 minutes ($P < 0.001$). A non-significant effect of the diluter was found in relation to sperm motility during the survival test ($P > 0.05$).

A total of 8 herds were evaluated. Table II describes the results of sperm quantitative and qualitative characteristics with relation to the effect of the herd. Significant differences were detected in the level of sperm activity after 90 minutes of the survival test only, $P < 0.05$. However, we could determine no trend with relation to these results. The effect of herd can be associated with different levels of nutrition, climate conditions, quality of management, health status of the animals, etc., and all these factors can

I: *Statistical significance of basic factors included in the linear model*

| | $r^2$ | MODEL | | HERD | | BREED | | AGE | | DENSITY | | DILUTER | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F-test | P | F-test | P | F-test | P | F-test | P | F-test | P | F-test | P |
| **A30** | 0.7991 | 3.98 | 0.0034 | 1.90 | 0.1325 | 1.06 | 0.3127 | 3.75 | 0.0449 | 7.14 | 0.0004 | 1.60 | 0.2283 |
| **A60** | 0.7937 | 3.85 | 0.0041 | 2.56 | 0.0498 | 4.35 | 0.0460 | 5.07 | 0.0187 | 5.71 | 0.0013 | 1.17 | 0.3308 |
| **A90** | 0.8607 | 6.18 | 0.0002 | 7.66 | 0.0003 | 2.19 | 0.1496 | 2.70 | 0.0961 | 7.27 | 0.0003 | 0.01 | 0.9950 |

A30, A60, A90 – activity of sperm after 30, 60 and 90 minutes of the survival test

cause differences among herds (Yoder et al., 1990). Megahed and Etman (2006) demonstrated a higher pregnancy rate from rams of herds with feeding rations of better quality.

Sperm motility during the survival test of Bohemia Forest and Wallachia rams´ semen was compared. Table III documents these results. A significant difference ($P < 0.05$) between breeds was determined only in the case of sperm activity after 60 minutes of the test, other differences being non-significant. However, higher sperm motility during the entire test was detected in Bohemia Forest rams, the differences ranging from 3.89% after 30 minutes of the test to 14.16% after 60 minutes of the test. Makawi et al. (2007) found non-significant inter-breed differences in sperm quality after thawing, but with a tendency similar to that in our observation.

Table IV presents the results of the rams´ sperm motility traits with relation to their ages. The rams were divided into 3 groups – to 36 months, from 37 to 60 months and more than 60 months of age. A non-significant differences ($P > 0.05$) were found in sperm activity. The youngest rams presented the non-significantly highest sperm motility after 30 and 60 minutes of the test. However, the oldest rams had the highest sperm activity after 90 minutes of the survival test. In relation to these results, the lowest decline in sperm motility from 60 to 90 minutes of the test, 4.1%, was detected in the group of the oldest rams, while the decline in sperm activity ranged from 16.07% in rams to 36 months of age to 23.56% in rams from 36 to 60 months of age. On the other hand, Rodriguez-Almeida et al. (2008) found interaction between age and type of semen preservation, when better acrosomal reaction of spermatozoa 24 hours after refrigerating was detected in younger rams. We can assume that younger rams have better qualitative characteristics of semen before diluting and after 30 and 60 minutes of the survival test. Štolc et al. (2009) detected a lower decline of sperm activity of younger rams in relation to diluting of their semen. Their results confirm our findings. However, young rams in our observation demonstrated a lower ability to maintain a high level of sperm motility during the survival test.

II: *Effect of observed herd on activity of sperm after 30, 60 and 90 minutes of the survival test*

| | A30 | | A60 | | A90 | |
|---|---|---|---|---|---|---|
| | $\mu + \alpha$ | SE | $\mu + \alpha$ | SE | $\mu + \alpha$ | SE |
| **HD1 (n = 9)** | 74.48 | 3.73 | 70.00 | 6.70 | 55.48 | 7.85 |
| **HD2 (n = 5)** | 68.04 | 10.47 | 53.01 | 18.82 | 47.04 | 12.04 |
| **HD3 (n = 12)** | 71.22 | 4.60 | 61.20 | 8.26 | 45.82 | 9.68 |
| **HD4 (n = 3)** | 74.11 | 7.36 | 53.49 | 13.22 | 41.32 | 15.50 |
| **HD5 (n = 6)** | 73.51 | 4.04 | 68.41 | 7.26 | 60.71 | 8.50 |
| **HD6 (n = 7)** | 70.04 | 5.33 | 53.50 | 9.58 | 55.15 | 11.22 |
| **HD7 (n = 5)** | 68.11 | 5.21 | 55.49 | 9.37 | 31.32 | 10.98 |
| **HD8 (n = 2)** | 79.11 | 7.36 | 78.50 | 13.23 | 78.82 | 15.49 |
| **P < 0.05** | | | | | 1-7; 2-5,8; 5-7; 7-8 | |

HD1 – HD8 – 1st – 8th herd included in the observation; A30, A60, A90 – activity of sperm after 30, 60 and 90 minutes of the survival test

III: *Effect of breed on activity of sperm after 30, 60 and 90 minutes of the survival test*

| | A30 | | A60 | | A90 | |
|---|---|---|---|---|---|---|
| | $\mu + \alpha$ | SE | $\mu + \alpha$ | SE | $\mu + \alpha$ | SE |
| **BFS (n = 32)** | 72.89 | 1.98 | 66.00 | 3.54 | 50.98 | 4.68 |
| **WS (n = 17)** | 69.00 | 3.48 | 51.84 | 6.28 | 37.70 | 8.26 |
| **P < 0.05** | | | * | | | |

BFS – Bohemia Forest sheep, WS – Wallachia sheep; A30, A60, A90 – activity of sperm after 30, 60 and 90 minutes of the survival test

IV: *Effect of ram´s age on activity of sperm after 30, 60 and 90 minutes of the survival test*

| | A30 | | A60 | | A90 | |
|---|---|---|---|---|---|---|
| | μ + α | SE | μ + α | SE | μ + α | SE |
| **AG1 (n = 27)** | 72.73 | 2.06 | 63.00 | 3.70 | 46.93 | 4.86 |
| **AG2 (n = 10)** | 71.06 | 4.44 | 60.92 | 7.96 | 37.36 | 10.52 |
| **AG3 (n = 12)** | 69.04 | 3.33 | 52.83 | 5.98 | 48.73 | 7.90 |
| **P < 0.05** | | | | | | |

AG1 – group of rams to 36 months of age, AG2 – group of rams from 36 to 60 months of age, AG3 – group of rams over 60 months of age; A30, A60, A90 – activity of sperm after 30, 60 and 90 minutes of the survival test

V: *Effect of sperm density on activity of sperm after 30, 60 and 90 minutes of the survival test*

| | A30 | | A60 | | A90 | |
|---|---|---|---|---|---|---|
| | μ + α | SE | μ + α | SE | μ + α | SE |
| **D (n = 4)** | 77.84 | 5.18 | 85.90 | 10.12 | 73.85 | 10.82 |
| **D1 (n = 2)** | 49.91 | 4.86 | 37.38 | 9.49 | 9.03 | 10.15 |
| **D2 (n = 8)** | 72.34 | 3.16 | 61.19 | 6.17 | 42.46 | 6.60 |
| **D3 (n = 16)** | 75.53 | 1.84 | 69.30 | 3.59 | 53.10 | 3.84 |
| **MD (n = 7)** | 83.03 | 6.09 | 74.23 | 11.90 | 69.45 | 12.74 |
| **MD1 (n = 3)** | 48.05 | 6.53 | 19.81 | 12.76 | 6.86 | 13.65 |
| **MD2 (n = 3)** | 68.35 | 4.86 | 33.23 | 9.49 | 33.10 | 10.15 |
| **MD3 (n = 3)** | 67.36 | 4.50 | 58.48 | 8.79 | 32.36 | 9.40 |
| **VD3 (n = 3)** | 80.11 | 4.,34 | 62.50 | 8.48 | 61.13 | 9.07 |
| **P < 0.05** | 1-2,6; 2-3,4,5,6,7,8,9; 3,4,5-6; 5-8; 6-7,8,9 | | 1-2,3,4,5,6,7,8; 2-3,4,5; 3,4,5-6,7; 6-8,9; 7-9 | | 1-2,3,6,7,8; 2-3,4,5,9; 3,4,5-6; 5,6-7,8; 6,7,8-9 | |

D, D1, D2, D3 – dense levels of sperm; MD, MD1, MD2, MD3 – medium dense levels of sperm; VD3 – very dense level of sperm; A30, A60, A90 – activity of sperm after 30, 60 and 90 minutes of the survival test

VI: *Effect of different type of diluter on activity of sperm after 30, 60 and 90 minutes of the survival test*

| | A30 | | A60 | | A90 | |
|---|---|---|---|---|---|---|
| | μ + α | SE | μ + α | SE | μ + α | SE |
| **OPT (n = 17)** | 72.50 | 3.90 | 63.78 | 7.95 | 36.64 | 12.66 |
| **TRI (n = 32)** | 65.89 | 2.99 | 47.30 | 6.11 | 38.44 | 9.73 |
| **P < 0.05** | | | | | | |

OPT – diluter Optidyl, TRI – diluter Triladyl; A30, A60, A90 – activity of sperm after 30, 60 and 90 minutes of the survival test

VII: *Residual Pearson´s correlation coefficients among evaluated traits of the rams' sperm quality*

| | A30 | A60 | A90 |
|---|---|---|---|
| **ACT** | 0.9191*** | 0.7659*** | 0.7580*** |
| **ACT2** | 0.8729*** | 0.7704*** | 0.6989*** |
| **A30** | | 0.8075*** | 0.7637*** |
| **A60** | | | 0.7780*** |

ACT – activity of sperm before diluting, ACT2 – activity of sperm after diluting; A30, A60, A90 – activity of sperm after 30, 60 and 90 minutes of the survival test, P < 0.001 (***)

Table V shows the level of evaluated traits with relation to rams´ sperm density after its collection during the sheep´ mating season. Significant differences were documented in sperm activity after 30, 60, and 90 minutes of the survival test. The highest level of sperm motility after 30, 60, and 90 minutes was found in the groups of rams with a density level – dense (D), medium dense (MD), and very dense

(VD), and the lowest level with relation to density D1 and medium sperm density – MD1 ($P < 0.05$). Štolc et al. (2009) observed similar results in sperm activity before and after diluting. However, O'Meara et al. (2008) discussed whether the fertility of a given semen sample can be easily quantified using available in vitro tests.

The results of the diluter effect on sperm motility during the survival test are described in Table VI. Two types of diluter were used and evaluated; however, no significant differences among these diluters were confirmed. The lowest decrease in sperm activity was detected in semen diluted with Triladyl (TRI), and the highest decrease in this trait was found with the use of Optidyl (OPT). The differences in sperm activity were non-significant. Afroz et al. (2008) observed a different range of sperm motility after dilution and thawing of sperm with relation to the type of diluter. They considered Triladyl a better diluter due to higher sperm motility after thawing. Their findings, together with those of Mara et al. (2007) and Fukui et al. (2008), confirm our results.

Table VII contains selected statistically significant residual Pearson's correlation coefficients among the evaluated traits. The highest coefficients $r = 0.9191$ ($P < 0.001$), respectively $r = 0.8729$ ($P < 0.001$) were detected between sperm activity before dilution and sperm activity after 30 minutes of the test, respectively between sperm activity after diluting and sperm activity after 30 minutes. Significant coefficients from $r = 0.6989$ to $r = 0.7704$ ($P < 0.001$) were found with relation to sperm activity after 60 and 90 minutes of the survival test. Correlation coefficients among sperm motility after 30, 60 and 90 minutes of the test ranged from $r = 0.7637$ to $r = 0.8075$ ($P < 0.001$). Snowder et al. (2004) estimated genetic correlations between the sexual performance score of the ram and the number of lambs weaned, and they detected significant variations in relation to the breed of the rams. We can try to find opportunities for the use of qualitative sperm characteristics before and after diluting, and during the survival test for selection of rams in relation to their reproduction results achieved in individual herds.


## SUMMARY

A statistically significant effect of the herd, breed, age of rams, and density of their sperm on sperm activity after 30, 60, and 90 minutes of the survival test was determined. Different breeding conditions in individual herds resulted in significant differences in sperm activity after 90 minutes of the test. Bohemia Forest rams demonstrated a higher level of sperm motility during the entire test, but significantly only after 60 minutes. Younger rams achieved non-significantly higher sperm activity after 30 and 60 minutes of the test; however, their sperm activity declined markedly within 90 minutes in comparison with the oldest rams. A significant relationship between the level of density before diluting and sperm activity during the survival test was detected. A non-significant effect of the diluter on sperm activity was determined. Relationships among sperm activity before and after diluting and during the entire survival test were confirmed by significant Pearson´s correlation coefficients. Our findings raise the question of the usefulness of these relationships for the selection of rams before their introduction into the reproduction process in the herd.



## FULL SUMMARY

The experiment was conducted in eight herds of sheep and 49 rams (4 herds of Bohemia Forest sheep – 32 rams, and 4 herds of Wallachia sheep – 17 rams) during the mating seasons from 2003 to 2006. The rams were divided into three groups, the first group consisted of rams younger than 36 months; the second group included rams from 36 to 60 months of age; and the third group was comprised of rams over 60 months of age. The sperm density was evaluated immediately after each collection from each ram and was determined by microscopic estimation. Sperm activity was estimated repeatedly after 30, 60, and 90 minutes of a thermal survival test (39 $\pm$ 1°C). Sperm activity was determined by the same high power field microscope as the percentage of progressively motile spermatozoa.

Sperm motility of the rams´ sperm during the survival test was evaluated with relation to the herd, breed, age group, density of sperm before diluting, and type of diluter used. The dataset was analysed by ANOVA using the statistical program SAS STAT 8.0 – GLM.

Coefficient of repeatability of the basic statistical characteristics of the linear model ranged from $r^2 = 0.7937$ to $r^2 = 0.8607$ during evaluation of the observed traits. The statistical significance of all the models used for evaluation of the sperm activity during the survival test was $P < 0.01$ in the case of sperm motility after 30 and 60 minutes and $P < 0.001$ in evaluation of sperm motility after 90 minutes.

A significant effect of the herd was found only with relation to sperm motility after 60 ($P < 0.05$) and 90 ($P < 0.001$) minutes of the test. The effect of the breed was significant only with relation to sperm motility after 60 minutes of the survival test ($P < 0.05$). A significant effect of the age group was detected in relation to sperm motility after 30 and 60 minutes ($P < 0.05$). The level of sperm density was significantly related to sperm motility after 60 minutes ($P < 0.01$) and after 30 and 90 minutes ($P < 0.001$).

A non-significant effect of the diluter was found in relation to sperm motility during the survival test ($P > 0.05$).
Significant differences of the effect of the herd were detected in the level of sperm activity after 90 minutes of the survival test only, $P < 0.05$. The effect of herd can be associated with different levels of nutrition, climate conditions, quality of management, health status of the animals, etc.
Sperm motility during the survival test of Bohemia Forest and Wallachia rams´ semen was compared. A significant difference ($P < 0.05$) between breeds was determined only in the case of sperm activity after 60 minutes of the test, other differences being non-significant. However, higher sperm motility during the entire test was detected in Bohemia Forest rams, the differences ranging from 3.89% after 30 minutes of the test to 14.16% after 60 minutes of the test.
A non-significant differences ($P > 0.05$) of sperm motility with relation to age of rams were found in sperm activity. The youngest rams presented the non-significantly highest sperm motility after 30 and 60 minutes of the test. However, the oldest rams had the highest sperm activity after 90 minutes of the survival test. In relation to these results, the lowest decline in sperm motility from 60 to 90 minutes of the test, 4.1%, was detected in the group of the oldest rams, while the decline in sperm activity ranged from 16.07% in rams to 36 months of age to 23.56% in rams from 36 to 60 months of age. We can assume that younger rams have better qualitative characteristics of semen before diluting and after 30 and 60 minutes of the survival test. However, young rams in our observation demonstrated a lower ability to maintain a high level of sperm motility during the survival test.
Significant differences of the level of evaluated traits with relation to rams´ sperm density after its collection were documented in sperm activity after 30, 60, and 90 minutes of the survival test. The highest level of sperm motility was found in the groups of rams with a density level – dense (D), medium dense (MD), and very dense (VD), and the lowest level with relation to density D1 and medium sperm density – MD1 ($P < 0.05$). The results of two types of the diluter effect on sperm motility during the survival test are described. The lowest decrease in sperm activity was detected in semen diluted with Triladyl (TRI), and the highest decrease in this trait was found with the use of Optidyl (OPT). The differences in sperm activity were non-significant.
The highest residual Pearson´s correlation coefficients among the evaluated traits $r = 0.9191$ ($P < 0.001$), respectively $r = 0.8729$ ($P < 0.001$) were detected between sperm activity before dilution and sperm activity after 30 minutes of the test, respectively between sperm activity after diluting and sperm activity after 30 minutes. Significant coefficients from $r = 0.6989$ to $r = 0.7704$ ($P < 0.001$) were found with relation to sperm activity after 60 and 90 minutes of the survival test. Correlation coefficients among sperm motility after 30, 60 and 90 minutes of the test ranged from $r = 0.7637$ to $r = 0.8075$ ($P < 0.001$). We found possibility for the use of qualitative sperm characteristics before and after diluting, and during the survival test for selection of rams in relation to their reproduction results achieved in individual herds.

## SOUHRN

Vztahy mezi stádem, plemenem, věkem beranů, hustotou jejich spermatu a motilitou spermií během testu přežitelnosti

Hodnoceny byly charakteristiky spermatu u 49 beranů dvou plemen v osmi chovech (čtyři chovy plemene šumavská ovce – 32 beranů, čtyři chovy plemene valašská ovce – 17 beranů) během připouštěcího období v letech 2003–2006. Berani byli rozděleni do skupin podle věku (do 36 měsíců, 36–60 a starší než 60 měsíců). Po každém odběru berana byla hodnocena hustota spermatu odhadem pod mikroskopem (podle prostorového uspořádání spermií v zorném poli mikroskopu). Aktivita spermií byla určována mikroskopicky (zvětšení 200x) při teplotě 38–39 °C pomocí vyhřívací destičky na stolku mikroskopu. Aktivita byla určována jako procentický podíl spermií s progresivním pohybem, a to po odebrání spermatu a dále po jeho naředění a opakovaně po 30, 60 a 90 minutách termického testu přežitelnosti ($39 \pm 1$ °C).
Data byla analyzována metodou ANOVA statistickým programem SAS STAT 8.0 – GLM.
Koeficient opakovatelnosti sledovaných ukazatelů kolísal od $r^2 = 0{,}7937$ do $r^2 = 0{,}8607$. Statistická významnost modelu použitého pro hodnocení vybraných ukazatelů plodnosti byla ve většině případů $P < 0{,}01$ a v případě hodnocení aktivity spermií během testu přežitelnosti $P < 0{,}01$ po 30 a 60 minutách a $P < 0{,}001$ po 90 minutách.
Byl zjištěn signifikantní vliv chovu na motilitu spermií po 60 ($P < 0{,}05$) a 90 minutách testu ($P < 0{,}001$). Vliv plemene na aktivitu spermií byl průkazný pouze po 60 minutách testu přežitelnosti ($P < 0{,}05$). Věk beranů měl statisticky průkazný vliv na aktivitu spermií zjišťovanou v čase 30 a 60 minut testu přežitelnosti ($P < 0{,}05$). Stupeň hustoty spermatu po odběru ovlivnil průkazně aktivitu spermií během testu přežitelnosti (po 60 minutách $P < 0{,}01$ a po 30 a 90 minutách $P < 0{,}001$). Byl vypočten neprůkazný vliv použitého ředidla na aktivitu spermií v průběhu testu ($P > 0{,}05$).

Byl detekován průkazný vliv chovu (farmy) na aktivitu spermatu po 90 minutách testu ($P < 0,05$). Vliv chovu souvisí s rozdílnou kvalitou krmiva, klimatických podmínek, managementu, zdravotního stavu zvířat atd.
Dále byla porovnávána motilita spermatu v testu přežitelnosti mezi plemeny. Signifikantní vliv plemene ($P < 0,05$) na aktivitu spermií byl zjištěn pouze po 60 minutách testu. Vyšší aktivita spermií byla v průběhu testování změřena u beranů šumavské ovce, rozdíly činily od 3,89 % po 30 minutách, do 14,16 % po 60 minutách testu.
Nejmladší berani měli neprůkazně vyšší aktivitu spermií během testu (30 a 60 minut), ale nejstarší berani měli nejvyšší aktivitu spermií na konci testu (90 minut). Aktivita spermií klesla u nejstarších beranů mezi 60 a 90 minutami testu o 4,1 %, zatímco u beranů do 36 měsíců věku to bylo o 16,07 % a ve skupině beranů ve věku mezi 36 a 60 měsíci činil pokles aktivity 23,56 %. U nejmladších beranů byly zjištěny lepší kvalitativní charakteristiky spermatu, ale přežitelnost spermií v průběhu testu je nižší než u beranů starších.
Statisticky průkazný rozdíl byl zjištěn při porovnání hustoty spermatu po odběru a jeho aktivity v průběhu testu přežitelnosti. Nejvyšší aktivita byla vypočtena u skupiny beranů s hustým (D), středně hustým (MD) a velmi hustým (VD) ejakulátem. Byl hodnocen také vliv použitého ředidla na motilitu spermatu beranů. Nejnižší pokles aktivity spermií po naředění byl zjištěn při použití ředidla Triladyl (TRI) a nejvyšší pokles při použití ředidla Optidyl (OPT). Tyto rozdíly byly statisticky neprůkazné.
Nejvyšší reziduální Pearsonovy korelační koeficienty mezi hodnocenými ukazateli $r = 0,9191$ ($P < 0,001$), resp. $r = 0,8729$ ($P < 0,001$) byly vypočteny mezi aktivitou spermií před naředěním a ve 30 minutách testu, resp. aktivitou spermií po naředění a po 30 minutách testu. Další signifikantní koeficienty ($r = 0,6989$ až $r = 0,7704$, $P < 0,001$) byly vypočteny ve vztahu mezi aktivitou spermií v 60 a 90 minutách testu přežitelnosti.
Korelační koeficienty mezi aktivitou spermií po 30, 60, a 90 minutách testu přežitelnosti kolísaly od $r = 0,7637$ do $r = 0,8075$ ($P < 0,001$).
Kvalitativní charakteristiky spermatu před a po naředění a během tepelného testu přežitelnosti by bylo možno využít pro výběr beranů podle výsledků reprodukce dosahovaných v jednotlivých chovech.

beran, stádo, plemeno, aktivita spermií, test přežitelnosti, hustota spermatu, ředidlo

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