

IMPACT OF CLIMATE FACTORS, BREED, AND BOAR UTILIZATION FREQUENCY ON SEMEN QUALITY AND SPERM MORPHOLOGY

Aleksandra Petrović¹, Vladan Bogdanović², Čedomir Radović¹,
Branislav Stanković², Vladimir Živković¹, Nenad Stojiljković¹,
Marija Gogić¹

¹Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Republic of Serbia

²University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia

Corresponding author: Aleksandra Petrović, apetrovic@istocar.bg.ac.

Original scientific paper

Abstract: The primary objective of this research was to evaluate boar ejaculate variability and the occurrence of anomalies in spermatozoa, considering climatic factors during spermatogenesis breed, and utilization frequency. This study involved 17 boars (n=129 ejaculates) and fertility testing was conducted during the most critical period of the year, from August to October. The observed sperm characteristics included: ejaculate volume (VOL), sperm concentration (CON, spermatozoa/ml), total number and number of functional spermatozoa (NT, NF), percentage of sperm motility in the native ejaculate and after dilution (MOTN, MOTD), number of produced doses (NPD), percentage of dead and live spermatozoa (PM, PZ), and sperm anomalies. The assessment of the effect was performed using a General Linear Model procedure. The breed did not influence sperm variability, while the frequency of boar utilization impacted on the occurrence of secondary anomalies. The determined regression coefficient indicated that extending the interval by one day increased PPPK by 0.340-0.348%. The maximum daily temperature during semen collection (model 1) and the value of the TH index during semen collection (model 3) influenced ejaculate volume. An increase of one °C in temperature, or one unit in THI value, led to a (p<0.05) increase in VOL by 3.540 ml and 2.798 ml, respectively. Furthermore, the maximum daily temperature (model 2) and the TH index value (model 4) at the beginning of the epididymal phase of spermatogenesis had an impact on semen motility, as well as the percentage of live and dead spermatozoa.

Key words: boar, spermatogenesis, sperm anomalies, temperature, THI

Introduction

Modern pig farming is based on highly productive breeds with the application of modern technical and technological solutions and biotechnological methods in rearing and reproduction (Savić et al., 2015). The most common biotechnological method used in reproduction is artificial insemination (AI), which is the primary method of reproduction in intensive pig production (Lopez Rodriguez et al., 2017).

Based on the fact that the application of AI enables obtaining of a large number of offspring from one boar, the aim is to include boars with the best possible reproductive and production performance in this process (Apić et al., 2016; Gadea et al., 2005). This can be achieved by permanent monitoring of the fertility of boars and timely culling of animals that are below the average of the studied population (Savić, 2014). Therefore, it is necessary to know the characteristics of the most important ejaculate properties, as well as the factors that influence their variability. The quality and quantity of semen used for artificial insemination are determined by its main parameters: volume, concentration of spermatozoa, live spermatozoa count and number of doses for insemination obtained from one ejaculate after dilution (Smital et al., 2004). In addition, the most important qualitative and quantitative properties of the ejaculate include: sperm motility, percentage of pathological spermatozoa, percentage of dead spermatozoa, total number of spermatozoa in the ejaculate and number of functional spermatozoa (Savić and Petrović, 2019).

There are various spermatozoa defects: spermatozoa with a proximal droplet, with a distal droplet, with an incomplete abnormal head, pear-shaped, short and wide, with a large head, with an acrosomal defect, etc. (Savić, 2014). According to their origin, spermatozoa malformations are divided into two groups: primary malformations, if they arise in the testis during spermatogenesis, and secondary if their genesis takes place in the epididymis during the sperm maturation process (Briz et al., 1996).

A number of genetic and non-genetic factors influence the variability of ejaculate properties. The breed of the boar is certainly one of the most important genetic factors that affect the variability of sperm properties (Cierzko et al., 2000; Stančić et al., 2003; Okere et al., 2005; Wolf and Smital, 2009; Wolf, 2009; Savić et al., 2013; Caisin and Snitco, 2016). In addition to the breed of the boar, the intensity of the boar use has a significant influence on the variation of the properties of the ejaculate. Frequent use of the boar semen negatively affects the quality of the sperm (Lopez Rodriguez et al., 2017). High temperatures also have a negative effect on the development of the spermatogenesis process (Kunavongkritt et al., 2005; Gogić, 2020). As a result of the elevated temperature of the environment, morphological defects occur more frequently, sperm motility is reduced, and overall fertility is lower (Flowers, 2015; Shahat et al., 2020).

However, some studies indicate that not all ejaculate properties vary under the influence of high temperature, such as ejaculate volume and total sperm count (Watteman et al., 1976; Larsson and Einarsson, 1984; Wettemann et al., 1985). The differences in the results obtained in the mentioned researches indicate that it may be necessary to conduct additional research of the influence of temperature and air humidity on the variability of the most important sperm properties.

The aim of this research was to determine whether climatic factors, boar breed and intensity of exploitation influence the variability of ejaculate properties and the occurrence of spermatozoa anomalies.

Materials and Methods

The research was conducted on a pig farm consisting of a reproductive and commercial farm. The boars were placed in a special facility with microclimatic conditions under the control of a semi-automatic ventilation system, using vertical and horizontal ventilation. The animals were fed balanced feed mixtures, and had unlimited access to fresh water.

The research included 17 boars of two different breeds (4 Landrace and 13 Large White). A total of 129 ejaculates were analyzed, but the number of examined ejaculates varied based on observed traits. The fertility of those boars was examined in the time interval from August to October, which represents the most critical period of the year. Each boar had to have at least three successful jumps during the trial period to be included in the analysis. The interval between two sperm collections was average 9 days. The examination included the following parameters: ejaculate volume (VOL, ml), sperm concentration (CON, $\times 10^6$ spermatozoa/ml), total spermatozoa count in the ejaculate (NT, $\times 10^9$ spermatozoa; $NT = VOL \times CON$), total functional spermatozoa count in the ejaculate (NF, $\times 10^9$ spermatozoa; $NF = NT \times MOTN$), percentage of motility of spermatozoa mass in native state (MOTN, %), percentage of motility of spermatozoa mass after dilution (MOTD, %), number of doses produced (NPD), percentage of dead spermatozoa (PM, %), percentage of live spermatozoa (PZ, %), percentage of spermatozoa with protoplasmic droplets (PPK, %), percentage of spermatozoa with a pathological shape on the tail (PPLJR, %), percentage of spermatozoa with pathological changes on the head (POTKG, %) and percentage spermatozoa with pathological changes on the acrosomal part (PAKR, %). The volume of the ejaculate was measured with a graduated cylinder, and was expressed in milliliters with an accuracy of ± 2 ml. The concentration of native sperm was assessed using a photo-colorimeter (Magacell, Magapor, Spain). The native sperm was diluted using a commercial diluent Vitasem (Magapor, Spain). The assessment of the motility of the mass of spermatozoa in the native ejaculate and after dilution was performed by subjective assessment, and observed under a microscope (BA410, Motic®, America, under a

×100 objective). The total number of sperm in the ejaculate was obtained by multiplying the volume of the ejaculate with the concentration. The number of functional spermatozoa was calculated by multiplying the total number of spermatozoa by the percentage of motility of the mass of spermatozoa in the native state. The percentage of dead and live spermatozoa in the semen was determined by observing a permanent preparation under a microscope (BA410, Motic®, America, under a ×400 objective) following staining with eosin-nigrosin (Savić and Petrović, 2019). During staining, dead spermatozoa are partially or completely stained, while live spermatozoa are visible on a dark background without staining.

Evaluation of the presence of pathological forms was performed by microscopic examination on the same permanent preparation, under an adequate immersion lens, and the total number of examined spermatozoa was 100. The number of spermatozoa with a certain anomaly was presented as a relative share of the total number.

For the entire process of spermatogenesis, i.e., the formation of mature spermatozoa from spermatogonia, according to Parrish et al. (2017), a 45 day value was taken, whereby the epididymal phase represented the last ten days of spermatogenesis (36-45 days).

Climatic data (temperature and humidity) were obtained from the weather station in Veliko Gradište near the farm and were available online (<https://www.infoclimat.fr/climatologie-mensuelle/13285/juillet/2013/veliko-gradiste.html>). TH index was calculated according to the formula given by Lallo et al. (2018):

$$THI = T_{\max} - (0.55 - (0.0055 RH)(T_{\max} - 14.5))$$

Where:

T_{\max} = max temperature (°C), RH = relative air humidity (%).

The assessment of the influence of the factors on the variation of the examined properties was performed using the General Linear Model in the statistical package SAS 9.3 (SAS Inst. Inc., 2002-2010), where the following models were used:

1. $y_{ij} = \mu + B_i + b_1(x_{ij} - \bar{x}) + b_2(x_{ij} - \bar{x}) + e_{ij}$
2. $y_{ij} = \mu + B_i + b_1(x_{ij} - \bar{x}) + b_3(x_{ij} - \bar{x}) + e_{ij}$
3. $y_{ij} = \mu + B_i + b_1(x_{ij} - \bar{x}) + b_4(x_{ij} - \bar{x}) + e_{ij}$
4. $y_{ij} = \mu + B_i + b_1(x_{ij} - \bar{x}) + b_5(x_{ij} - \bar{x}) + e_{ij}$

Where:

μ = average value; B_i = fixed effect of boar breed; $b_1(x_{ij} - \bar{x})$ = linear regression effect of the interval between two jumps; $b_2(x_{ij} - \bar{x})$ = linear regression effect of maximum daily air temperature during sperm collection; $b_3(x_{ij} - \bar{x})$ = linear regression effect of the maximum daily air temperature at the beginning of the

epididymal phase of spermatogenesis (36th day); $b_4(x_{ij}-\bar{x})$ = linear regression effect of THI values when collecting sperm; $b_5(x_{ij}-\bar{x})$ = linear regression influence of THI values at the beginning of the epididymal phase of spermatogenesis (36th day); e_{ij} = random error.

Results and Discussion

The differences in all observed properties between the ejaculates included in the research were recorded, as indicated by the absolute and relative indicators of variability (Table 1).

Table 1. Basic statistical parameters of examined sperm properties

Properties	\bar{x}	SD	CV (%)	Minimum	Maximum
VOL (ml)	292.18	95.45	32.67	50.00	520.00
CON (x10⁶/ml)	366.65	98.60	26.89	190.00	707.00
NT (x10⁹)	107.49	43.72	46,68	17.50	225.00
NF (x10⁹)	90.75	36.47	40.18	13.12	191.83
MOTN (%)	83.96	7.16	8.52	65.00	100.00
MOTD (%)	78.18	9.57	12.24	20.00	95.00
NPD	15.26	4.67	30.62	4.00	30.00
PM (%)	23.80	12.38	52.01	2.00	79.09
PZ (%)	76.19	12.38	16.24	20.91	98.00
PPK (%)	13.57	10.20	75.15	0	40.00
PPLJR (%)	2.47	3.06	123.79	0	17.00
POTKG (%)	1.32	1.84	138.99	0	8.00
PAKR (%)	0.19	0.71	368.58	0	6.00

VOL - ejaculate volume, CON - sperm concentration, NT - total spermatozoa count, NF - functional spermatozoa count, MOTN - native sperm motility, MOTD - sperm motility after dilution, NPD - number of produced doses, PM - percentage of dead spermatozoa, PZ - percentage of live spermatozoa, PPK - percentage of spermatozoa with a protoplasmic drop, PPLJR - percentage of spermatozoa with a pathological shape on the tail, POTKG - percentage of spermatozoa with pathological changes on the head, PAKR - percentage of spermatozoa with pathological changes on the acrosomal part; \bar{x} - average value, SD - standard deviation; CV - coefficient of variation

Properties representing the occurrence of anomalies on spermatozoa (PPK, PPLJR, POTKG, PAKR) had very high coefficients of variation. The majority of all the examined anomalies were secondary (PPK; \bar{x} =13.57%), which occur during the maturation of spermatozoa in the epididymal phase of spermatogenesis.

In this research, PPK, unites spermatozoa with proximal and distal protoplasmic droplets, as a secondary form of anomaly. The position of the protoplasmic droplet may indicate the level of "maturity" of the spermatozoa. Namely, spermatozoa with a proximal droplet originate from the testis, with the movement of the spermatozoa toward the epididymis the drop also moves toward the distal parts of the spermatozoa tail. With the arrival of such spermatozoa into the tail of the epididymis, the distal protoplasmic droplet disappears and they take on the appearance of mature spermatozoa. Based on this, it can be concluded that the ratio of spermatozoa to proximal and distal protoplasmic drops can indicate the extent to which the epididymal sperm has matured.

The incidence of a higher proportion of abnormal forms of the secondary type and immature spermatozoa is related to the unfavorable influence of stressogenic factors. According to Savić and Petrović (2019), exposure of the animal to temperatures above 27°C for several days can cause thermal stress that leads to an increase in abnormal forms of spermatozoa as reported by Wetteman et al. (1979), Larsson and Einarsson (1984), Flowers (1997), Flowers (2015), Gogić (2020), Shahat et al. (2020).

Table 2. Statistical significance of the effects included in models 1 and 3

Sperm property	MODEL 1			MODEL 3		
	Linear regression effect IUS	Linear regression effect Max T Uz	R ²	Linear regression effect IUS	Linear regression effect THI Uz	R ²
VOL (ml)	0.176 ^{NS}	3.540*	0.042	0.164 ^{NS}	2.798*	0.044
CON (x10⁶/ml)	1.905 ^{NS}	-1.258 ^{NS}	0.039	1.964 ^{NS}	-0.736 ^{NS}	0.038
NT (x10⁹)	0.613 ^{NS}	0.589 ^{NS}	0.024	0.625 ^{NS}	0.537 ^{NS}	0.026
NF (x10⁹)	0.408 ^{NS}	0.101 ^{NS}	0.025	0.430 ^{NS}	0.187 ^{NS}	0.026
MOTN (%)	-0.061 ^{NS}	-0.206 ^{NS}	0.045	-0.053 ^{NS}	-0.126 ^{NS}	0.038
MOTD (%)	-0.079 ^{NS}	-0.444*	0.056	-0.064 ^{NS}	-0.282 ^{NS}	0.040
NPD	-0.013 ^{NS}	0.067 ^{NS}	0.020	-0.011 ^{NS}	0.062 ^{NS}	0.023
PM (%)	-0.214 ^{NS}	0.296 ^{NS}	0.036	-0.220 ^{NS}	0.198 ^{NS}	0.033
PZ (%)	0.214 ^{NS}	-0.296 ^{NS}	0.036	0.220 ^{NS}	-0.198 ^{NS}	0.154
PPK (%)	0.348*	0.197 ^{NS}	0.052	0.340*	-0.013 ^{NS}	0.052
PPLJR (%)	0.044 ^{NS}	-0.019 ^{NS}	0.023	0.046 ^{NS}	-0.008 ^{NS}	0.022
POTKG (%)	0.026 ^{NS}	0.036 ^{NS}	0.016	0.027 ^{NS}	0.029 ^{NS}	0.018
PAKR (%)	-0.013 ^{NS}	0.009 ^{NS}	0.038	-0.001 ^{NS}	0.007 ^{NS}	0.038

VOL - ejaculate volume, CON - sperm concentration, NT - total spermatozoa count, NF - functional spermatozoa count, MOTN - native sperm motility, MOTD - sperm motility after dilution, NPD - number of produced doses, PM - percentage of dead spermatozoa, PZ - percentage of live spermatozoa, PPK - percentage of spermatozoa with a protoplasmic drop, PPLJR - percentage of spermatozoa with a pathological shape on the tail, POTKG - percentage of spermatozoa with pathological changes on the head, PAKR - percentage of spermatozoa with pathological changes on the acrosomal part; IUS - the interval between two semen collection, Max T Uz - maximum daily air

temperature during sperm collection, THI Uz - THI value during sperm collection; R^2 - coefficient of determination, Statistical significance: ^{NS} - not significant, * - $p < 0.05$

The interval between two sperm collections in both models influenced only the incidence of secondary anomalies - PPK (Table 2). The value of the determined regression coefficient indicates that by extending the interval by one day, the percentage of spermatozoa with a protoplasmic drop increases by 0.340-0.348%. This implies that non-rational boar exploitation can lead to the appearance of a greater proportion of spermatozoa with pathological changes. This is exactly what Savić and Petrović (2019) point out, who state that stressful situations or non-rational use of boars can lead to a decrease in the flow of testosterone at the level of the testes, which leads to the appearance of abnormal forms of spermatozoa or their insufficient maturation. According to Henning (2021) the incomplete maturation of epididymal sperm impairs the ability of *in vitro* capacitation and promotes the destabilization of sperm in stored boar semen.

The maximum daily temperature during semen collection (Model 1) and the TH index value during semen collection (Model 3) influenced the ejaculate volume. Increasing the temperature by 1°C, i.e. THI value by one unit, increased ($p < 0.05$) VOL by 3.540 ml, and by 2.798 ml, respectively. On the other hand, an increase in temperature by one unit leads to a decrease in the mass of sperm motility (MOTD) by 0.444%. The low values of the coefficients of determination (< 5.2 – Model 1; < 15.4 – Model 3), determined by the applied models, indicate that the examined influences explain the variability of the examined sperm properties to a lesser extent. These results are partially similar to those reported by Larsson and Einarsson (1984). Namely, in their research, the ejaculate volume did not vary under the influence of elevated ambient temperature, which is the case in our research, however, there is agreement between the results related to reduced mobility due to elevated temperatures. Wettemann et al. (1985) also report no effect of breed on ejaculate variability. According to Suriyasomboon et al. (2004) the difference in the obtained results may be due to different duration of exposure to elevated temperature, or individual differences in the susceptibility of boars to stress caused by high temperatures.

The maximum daily temperature (Model 2) and the value of the TH index (Model 4) at the beginning of the epididymal phase of spermatogenesis had an impact on the motility properties of native ejaculate and semen after dilution, as well as on the percentage of live and dead spermatozoa (Table 3).

Table 3. Statistical significance of the effects included in models 2 and 4

Sperm property	MODEL 2			MODEL 4		
	Linear regression effect	Linear regression effect	R ²	Linear regression effect	Linear regression effect	R ²
	IUS	Max T Ep		IUS	THI Ep	
VOL (ml)	0.109 ^{NS}	1.641 ^{NS}	0.016	0.022 ^{NS}	1.135 ^{NS}	0.012
CON (x10⁶/ml)	1.700 ^{NS}	-1.407 ^{NS}	0.045	1.713 ^{NS}	-1.152 ^{NS}	0.045
NT (x10⁹)	0.597 ^{NS}	0.283 ^{NS}	0.022	0.578 ^{NS}	0.184 ^{NS}	0.021
NF (x10⁹)	0.349 ^{NS}	-0.144 ^{NS}	0.026	0.345 ^{NS}	-0.135 ^{NS}	0.026
MOTN (%)	-0.012 ^{NS}	-0.336 ^{**}	0.112	-0.115 ^{NS}	-0.256 ^{NS}	0.103
MOTD (%)	-0.166 ^{NS}	-0.538 ^{***}	0.135	-0.153 ^{NS}	-0.418 ^{**}	0.127
NPD	-0.029 ^{NS}	-0.025 ^{NS}	0.015	-0.033 ^{NS}	0.030 ^{NS}	0.017
PM (%)	-0.049 ^{NS}	0.693 ^{***}	0.154	-0.059 ^{NS}	0.540 ^{**}	0.149
PZ (%)	0.049 ^{NS}	-0.693 ^{***}	0.154	0.059 ^{NS}	-0.540 ^{**}	0.149
PPK (%)	0.340 ^{NS}	-0.011 ^{NS}	0.052	0.337 ^{NS}	-0.017 ^{NS}	0.052
PPLJR (%)	0.066 ^{NS}	0.063 ^{NS}	0.040	0.065 ^{NS}	0.057 ^{NS}	0.038
POTKG (%)	0.019 ^{NS}	-0.007 ^{NS}	0.007	0.019 ^{NS}	0.005 ^{NS}	0.007
PAKR (%)	-0.013 ^{NS}	0.002 ^{NS}	0.034	-0.001 ^{NS}	0.006 ^{NS}	0.035

VOL - ejaculate volume, CON - sperm concentration, NT - total spermatozoa count, NF - functional spermatozoa count, MOTN - native sperm motility, MOTD - sperm motility after dilution, NPD - number of produced doses, PM - percentage of dead spermatozoa, PZ - percentage of live spermatozoa, PPK - percentage of spermatozoa with a protoplasmic drop, PPLJR - percentage of spermatozoa with a pathological shape on the tail, POTKG - percentage of spermatozoa with pathological changes on the head, PAKR - percentage of spermatozoa with pathological changes on the acrosomal part; IUS - the interval between two semen collection, Max T Ep - maximum daily air temperature during the epididymal phase, THI Ep - THI value during the epididymal phase; R² - coefficient of determination, Statistical significance: ^{NS} - not significant, ** - p<0.01, ***-p<0.001

In model 2, based on the estimated regression coefficient, increasing the maximum daily temperature by 1°C, decreases (p<0.01; p<0.001) motility (MOTN by 0.336%, MOTD by 0.538%) as well as PZ by 0.693%, which indicates the negative influence of high temperatures during the epididymal phase of spermatogenesis on spermatozoa. A similar regularity is observed when examining the influence of the TH index at the beginning of the epididymal phase of spermatogenesis (reduction in the motility of the mass of spermatozoa after dilution and reduction in the percentage of live spermatozoa). The low values of the determination coefficients obtained by these applied models also indicate that the studied influences explain to a lesser extent the variability of the studied sperm properties.

The interval between two consecutive sperm collections did not affect the variation of the examined sperm properties, which is in contrast to numerous previous studies, showing that the intensity of boar exploitation had an effect on: volume as reported by Smital (2010), Savić and Petrović (2015), Savić et al.

(2015), concentration according to Wolf and Smital (2009), the total spermatozoa count in the ejaculate as reported by Wolf and Smital (2009), Savić et al. (2015), the functional spermatozoa count in the ejaculate according by Smital (2010).

Regardless of which model was applied, the breed of the boar did not affect the average expression and variability of the tested traits (Table 4). The results obtained are contrary to numerous studies such as Stančić et al. (2003), Okere et al. (2005), Smital (2010), Savić and Petrović (2015) which indicate the variation of ejaculate volume under the influence of boar breed. It is similar to sperm concentration ($p=0.354$), which is contrary to research by Chinchilla-Vargas et al. (2018) and Savić et al. (2020).

This research is in agreement with the research of Savić (2014), who concluded that the mobility of native semen and semen after dilution did not vary under the influence of boar breed. In contrast, in the research of Okera et al. (2005), breed has a significant effect on the variation of native semen motility. Also, Savić et al. (2020) state that the breed exerted an influence on the variability of semen mobility after dilution. Knecht et al. (2014) find in their research that the percentage of dead and live spermatozoa varies under the influence of the boar breed, so the results of this research differ from the aforementioned.

Table 4. The average expression of sperm properties under the influence of the boar breed evaluated by the applied models

Sperm property	MODEL 1			MODEL 2			MODEL 3			MODEL 4		
	Boar breed LSMean		p	Boar breed LSMean		p	Boar breed LSMean		p	Boar breed LSMean		p
	VJ	L		VJ	L		VJ	L		VJ	L	
VOL (ml)	304.76	305.19	0.980	304.66	305.40	0.967	304.74	305.23	0.978	304.69	305.33	0.971
CON (x10⁶/ml)	372.72	350.80	0.354	372.68	350.87	0.354	372.78	350.67	0.354	372.65	350.95	0.357
NT (x10⁹)	116.71	107.11	0.343	116.67	107.18	0.349	116.70	107.11	0.343	116.67	107.18	0.349
NF (x10⁹)	98.36	88.12	0.241	98.32	88.18	0.245	98.37	88.09	0.239	98.32	88.20	0.246
MOTN (%)	84.18	81.68	0.106	84.19	81.66	0.090	84.18	81.68	0.107	84.19	81.68	0.095
MOTD (%)	78.55	76.74	0.416	78.59	76.63	0.357	78.55	76.74	0.423	78.58	76.65	0.368
NPD	15.13	16.00	0.335	15.13	15.99	0.339	15.13	16.00	0.334	15.13	16.00	0.339
PM (%)	25.44	22.86	0.401	25.51	22.70	0.327	25.42	22.90	0.411	25.51	22.71	0.331
PZ (%)	74.56	77.13	0.401	74.48	77.29	0.327	74.58	77.09	0.411	74.49	77.28	0.331
PPK (%)	13.24	13.38	0.951	13.23	13.40	0.939	13.24	13.38	0.946	13.22	13.40	0.936
PPLJR (%)	2.29	3.12	0.273	2.31	3.08	0.304	2.29	3.12	0.277	2.31	3.08	0.303
POTKG (%)	1.33	1.52	0.688	1.32	1.54	0.636	1.33	1.52	0.688	1.32	1.54	0.636
PAKR (%)	0.28	0.02	0.155	0.28	0.03	0.165	0.28	0.02	0.157	0.29	0.03	0.164

VOL - ejaculate volume, CON - sperm concentration, NT - total spermatozoa count, NF - functional spermatozoa count, MOTN - motility of native semen, MOTD - motility of semen after dilution, NPD - number of produced doses, PM - percentage of dead spermatozoa, PZ - percentage of live spermatozoa, PPK - percentage of spermatozoa with a protoplasmic droplet, PPLJR - percentage of spermatozoa with a pathological shape on the tail, POTKG - percentage of spermatozoa with pathological changes on the head, PAKR - percentage of spermatozoa with pathological changes on the acrosomal part, VJ –Large white, L-Landrace , p – statistical significance

Conclusions

Of all the investigated factors, only the boar breed showed no significant effect on the properties of the ejaculate in any of the analyzed models. However, it was established that there is a correlation between the interval between two sperm collections and the incidence of secondary anomalies, especially in relation to the percentage of spermatozoa showing certain pathological changes. These results suggest that improper collection of sperm samples can increase the presence of abnormal spermatozoa. Also, the maximum daily temperature and the value of the TH index during the collection of sperm samples affect the ejaculate volume, where higher values of these parameters lead to a larger ejaculate volume. On the other hand, the temperature and the value of the TH index at the beginning of the epididymal phase of spermatogenesis affect the decrease in the mobility of spermatozoa in native and semen after dilution, as well as the decrease in the percentage of live spermatozoa. This indicates that high temperatures during the epididymal phase of spermatogenesis have a negative effect on ejaculate characteristics and sperm vitality.

It is important to note that the analyzed factors explained to a lesser extent the variability of the examined sperm properties, which is shown by the low values of the coefficients of determination in the applied models.

Uticaj klimatskih faktora, rase i intenziteta korišćenja nerasta na kvalitet semena i morfologiju spermatozoida

Aleksandra Petrović, Vladan Bogdanović, Čedomir Radović, Branislav Stanković, Vladimir Živković, Nenad Stojiljković, Marija Gogić

Rezime

Osnovni cilj ovog istraživanja bio je da se oceni varijabilnost osobina ejakulata nerasta i pojava anomalija na spermatozoidima, uzimajući u obzir klimatske faktore tokom spermatogeneze, rasu i učestalost korišćenja nerasta. U istraživanje je uključeno 17 nerasta (n=129 ejakulata), a testiranje plodnosti sprovedeno je tokom najkritičnijeg perioda godine, od avgusta do oktobra. Posmatrane karakteristike sperme obuhvatale su: zapreminu ejakulata (VOL), koncentraciju spermatozoida (CON, spermatozoida/ml), ukupan broj i broj funkcionalnih spermatozoida (NT, NF), procenat pokretljivosti spermatozoida u nativnom ejakulatu i nakon razređenja (MOTN, MOTD), broj proizvedenih doza (NPD), procenat mrtvih i živih spermatozoida (PM, PZ) i anomalije spermatozoida. Procena uticaja izvršena je primenom General Linear Modela. Osobine ejakulata nisu varirale pod uticajem rase nerasta, dok je učestalost korišćenja nerasta uticala na pojavu sekundarnih anomalija. Utvrđeni regresioni koeficijent pokazao je da produženje intervala za jedan dan povećava PPK za 0,340-0,348%. Maksimalna dnevna temperatura tokom prikupljanja semena (model 1) i vrednost TH indeksa tokom prikupljanja semena (model 3) uticali su na zapreminu ejakulata. Povećanje temperature za jedan °C ili vrednosti THI za jednu jedinicu, dovelo je ($p < 0,05$) do povećanja VOL za 3,540 ml, odnosno 2,798 ml. Takođe, maksimalna dnevna temperatura (model 2) i vrednost TH indeksa (model 4) na početku epididimalne faze spermatogeneze uticali su na pokretljivost sperme, kao i na procenat živih i mrtvih spermatozoida.

Ključne reči: nerast, spermatogeneza, anomalije spermatozoida, temperatura, THI

Acknowledgement

This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, on the basis of the Agreement on the realization and financing of scientific research work of SRO No. 451-03-66/2024-03/200022. We would like to thank professor dr Radomir Savić for his assistance in the preparation of this paper.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Apić J., Stančić I., Vakanjac S., Radović I., Milovanović A., Barna T., Maletić M. 2016. Influence of the protein content of boar seminal plasma on spermatozoa viability, motility and acrosome integrity in diluted semen stored for 3 days. *Animal Reproduction*, 13, 1, 36-41.
- Briz M.D., Bonet S., Pinart B., Camps R. 1996. Sperm malformations throughout the boar epididymal duct. *Animal Reproduction Science* 43, 221-239.
- Caisin L., Snitco T. 2016. The influence of the seasonality on boars sperm production - breeder of different species. *Bulgarian Journal of Agriculture Science*, 22, 1, 118 - 122.
- Chinchilla-Vargas J., Kerns K., Rothschild F. M. 2018. Lunar and climatic effect on boar ejaculate traits. *Animal Reproduction Science*, 193, 117-125.
- Ciereszko A., Ottobre J.S., Glogowski J. 2000. Effects of season and breed on sperm acrosin activity and semen quality of boars. *Animal Reproduction Science* 64, 89-96.
- Flowers W. L. 2015. Factors affecting the efficient production of boar sperm. *Reproduction in Domestic Animals*, 50, 52, 25-30.
- Flowers W.L. 1997. Management of boars for efficient semen production. *Journal of Reproduction and Fertility Supplement* 52, 67 – 78.
- Gadea J. 2005. Sperm factors related to in vitro and in vivo porcine fertility. *Theriogenology*, 63:431-444
- Gogić M. 2020. Osobine porasta i razvoja polnih žlezda muških grla (nekastriranih i imunokastriranih) autohtonih rasa svinja. Doktorska disertacija. Univerzitet u Beogradu, Poljoprivredni fakultet.
- Henning H., Luter A. M., Weberski D. 2021. A high incidence of sperm with cytoplasmic droplets affect the response to bicarbonate in preserved boar semen. *Animals*, 11, 2570.
- Knecht D., Środoń S., Duziński K. 2014. The influence of boar breed and season on semen parameters. *South African Journal of Animal Science*, 44, 1, 1-9.
- Kunavongkrit A., Suriyasomboon A., Lundeheim N., Heard W.T., Einarsson S. 2005. Management and sperm production of boars under differing environmental conditions. *Theriogenology* 63, 657-667.
- Lallo C.H.O., Cohen J., Rankine D., Taylor M., Cambell J., Stephenson T. 2018. Characterizing heat stress on livestock using the temperature humidity index (THI) - prospects for a warmer Caribbean. *Regional Environmental Change* 18, 2329-2340.
- Larsson K., Einarsson S. 1984. Seminal changes in boars after heat stress. *Acta Veterinaria Scandinavica* 25, 1, 57-66.

- Lopez Rodriguez A., Van Soom A., Arsenakis I., Maes D. 2017 Boar management and semen handling factors affect the quality of boar extended semen. *Porcine Health Management*, 3, 1, 1-12.
- Okere C., Joseph A., Ezekwe M. 2005. Seasonal and genotype variations in libido, semen production and quality in artificial insemination boars. *Journal of Animal and Veterinary Advances*, 4, 10, 885-888.
- Parrish J. J., Willenburg K. L., Gibbs K. M., et al. 2017. Scrotal insulation and sperm production in the boar. *Molecular Reproduction and Development*, 84, 969-978.
- SAS INST. INC. 2002-2010. The SAS System for Windows. Cary. NC.
- Savić R. 2014. Fenotipska i genotipska varijabilnost plodnosi nerasta. Doktorska disertacija. Univerzitet u Beogradu, Poljoprivredni fakultet.
- Savić R., Petrović M. 2019. *Praktikum iz svinjarstva*. Univerzitet u Beogradu, Poljoprivredni fakultet, str. 182.
- Savić R., Petrović M., Radojković D., Radović Č., Parunović N., Pušić M., Radišić R. 2013. Variability of ejaculate volume and sperm motility depending on the age and intensity of utilization of boars. *Biotechnology in Animal Husbandry* 29, 4, 641-650.
- Savić R., Petrović M., Radojković D., Radović Č., Parunović N., Popovac M., Gogić M. 2015. Ejaculate properties and reproductive efficiency of large white boars during exploitation. *Biotechnology in Animal Husbandry* 31, 3, 397-405.
- Savić R., Radojković D., Stojiljković N., Parunović N., Gogić M., Radović Č. 2020. Effect of breed of performance tested boars on ejaculate traits. *Biotechnology in Animal Husbandry* 36, 3, 309-316.
- Shahat A.M., Rizzoto G., Kastelic J.P. 2020. Amelioration of heat stress-induced damage to testes and sperm quality. *Theriogenology* 158, 84-96.
- Smital J., De Sousa L. L., Mohsen A. 2004. Differences among breeds and manifestation of heterosis in AI boar sperm output. *Animal Reproduction Science* 80, 1-2, 121-30.
- Stančić B., Gagrčin M., Radović I. 2003. Uticaj godišnje sezone, rase i starosti nerastova na kvalitet sperme (1. Nativna sperma.). *Biotechnology in Animal Husbandry*, 19, 1-2, 1-92.
- Wettemann R. P., Wells M. E., Johnson R. K. 1979. Reproductive characteristics of boars during and after exposure to increased ambient temperature. *Journal of Animal Science*, 49, 6, 1501-1505.
- Wolf J. 2009. Genetic correlations between production and semen traits in pig. *Animals*, 3, 8, 1094-1099.
- Wolf J., Smital J. 2009. Effect in genetic evaluation for semen traits in Czech Large White and Czech Landrace boars. *Czech Journal of Animal Science*, 54, 8, 349-358.