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CORRELATION BETWEEN EJACULATE CHARACTERISTICS AND SPERM ABNORMALITIES IN BOARS EXPOSED TO SUMMER HEAT STRESS

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Abstract: Seasonal heat stress is a well-known factor that negatively affects boar fertility, primarily by reducing semen quality and increasing the incidence of abnormal spermatozoa. This study aimed to investigate the relationship between ejaculate characteristics and sperm morphological abnormalities in boars during the most sensitive period of the year. Between August and October, a total of 129 ejaculates were collected from 17 boars. The analyzed semen traits included ejaculate volume, sperm concentration, total and functional sperm counts, motility (in native and diluted semen), viability, and the presence of morphological abnormalities, specifically head, tail, acrosomal defects, and protoplasmic droplets. The results showed that only sperm motility and the number of produced doses significantly correlated with abnormal sperm forms. A weak but consistent positive correlation (r_f>0.200; $p<0.05$ or $p<0.01$) was observed between certain categories of abnormalities, particularly between secondary forms (such as protoplasmic droplets) and primary defects in the tail and acrosomal region. Correlations among primary abnormalities were generally weak. These findings highlight the importance of monitoring sperm motility and morphology as early indicators of heat stress effects in boars.

Key words: boar, semen quality, sperm abnormalities, ejaculate traits, heat stress

Introduction

Given that artificial insemination allows for the production of a large number of offspring from a single boar (Knecht et al., 2014; Apić et al., 2016),

evaluating sperm morphology is a key component of animal breeding programs, as it supports the selection of genetically and reproductively superior males (Gadea, 2025). Routine semen assessments are primarily effective in detecting clear cases of infertility, and in some instances, may also suggest early signs of reduced fertility (Barna et al., 2021). High-quality boar semen plays a crucial role in the success of artificial insemination (Bonet et al, 1993; Li et al. 2023).

The success of AI largely depends on the quantitative and qualitative traits of the ejaculate (Savić et al., 2015). Therefore, the evaluation of boar ejaculate quality represents the first and essential step in semen processing, to ensure that only ejaculates capable of producing quality insemination doses are selected for further use (Rozeboom, 2000). To get the best reproductive performance from each boar, it's important to consider genetic traits and external factors influencing semen quality and production (Lopez Rodriguez et al., 2017).

Spermatogenesis is influenced by numerous endogenous and exogenous factors, among which the most important are exposure to high ambient temperatures, photoperiod, nutrition, ejaculation frequency, and housing conditions (Flowers, 2015). Each of these factors may induce specific alterations, which often allows for the identification of a correlation between the type of sperm malformation observed and the underlying factor contributing to reduced fertility (Bonet et al., 1993). The relationship between different parameters varies depending on the breed and individual boar (Knecht et al., 2014). Heat stress influences the movement characteristics and metabolic profile of sperm, but boars can differ in how strongly they respond to elevated temperatures (Sui et al., 2022). The main consequence of heat stress on the testes is the destruction of germ cells through apoptosis, with spermatocytes, spermatids, and spermatozoa in the epididymis being the most sensitive to this effect (Shahat et al., 2020). Although the negative impact of elevated temperature on semen quality during in vitro exposure has been well documented, the specific molecular mechanisms triggered by heat in mature spermatozoa remain insufficiently understood and require further investigation (Li et al., 2023). Ultrastructural anomalies of boar sperm are divided into head and tail malformations. Observed changes include enlarged or vacuolated acrosomes, nuclear irregularities, swollen or excessive mitochondria, and various tail deformities such as coiling, folding, vesicle formation, and fusion of two tails (Bonet et al., 1993). A shorter flagellum and a higher head-to-flagellum length ratio are characteristic of spermatozoa from low volume ejaculates (Górski et al., 2021).

According to Savić (2014), there are also malformations such as spermatozoa with proximal and distal droplets, which are classified as secondary abnormalities based on their origin. Depending on their origin, sperm abnormalities are generally classified into two groups: primary malformations, which develop in the testes during spermatogenesis, and secondary malformations, which occur later, in the epididymis, during sperm maturation (Briz et al., 1996).

The aim of this study was to investigate the relationship between ejaculate traits and the occurrence of abnormal spermatozoa in boars exposed to summer heat stress.

Materials and Methods

This paper represents a continuation of the research Petrović et al. (2024), and therefore the methodology remains the same. The study involved seventeen boars from two breeds: four Landrace and thirteen Large White. Boars aged 2 to 3 years. The analysis included 129 ejaculates in total; however, the sample size differed across traits. The evaluation of boar fertility was conducted during the August - October period, which is regarded as the most challenging time of the reproductive season. The analysis included several key indicators of semen quality, including ejaculate volume (VOL, ml), sperm concentration (CON, $\times 10^6$/ml), and total spermatozoa count (NT, $\times 10^9$; calculated as VOL × CON). Functional spermatozoa count (NF, $\times 10^9$) was estimated by multiplying NT with the percentage of motility in native semen (MOTN, %). Additionally, motility after dilution (MOTD, %), number of insemination doses produced (NPD), and sperm viability expressed as percentages of live (PL, %) and dead (PD, %) spermatozoa were assessed. Morphological evaluation included the proportion of spermatozoa with protoplasmic droplets (PPK, %), as well as abnormalities of the tail (PPLJR, %), head (POTKG, %), and acrosomal region (PAKR, %).

VOL was measured using a graduated cylinder and expressed in milliliters (±2 ml accuracy). Sperm concentration in native semen was determined by photo-colorimetry using a Magacell device (Magapor, Spain). Semen samples were diluted with a commercial extender (Vitasem, Magapor, Spain). MOTN and MOTD were assessed subjectively under a light microscope (BA410, Motic®, USA) using a ×100 objective. NT was calculated as the product of ejaculate volume and sperm concentration, while the NF was obtained by multiplying NT by MOTN. Sperm viability was evaluated by eosin-nigrosin staining, following the protocol described by Savić and Petrović (2019). Using the applied staining technique, viable spermatozoa appeared unstained against a dark background, while non-viable cells were partially or completely stained. The evaluation of pathological sperm forms was conducted microscopically on the same permanent slide using an appropriate immersion objective. A total of 100 spermatozoa were examined, and the number of spermatozoa exhibiting a particular abnormality was expressed as a percentage of the total count. Prepared slides were examined under the same microscope (BA410, Motic®, USA) at ×400 magnification. Morphological abnormalities were evaluated on permanent stained slides using an immersion objective. For each sample, 100 spermatozoa were examined, and the

frequency of specific abnormalities (protoplasmic droplets, head, tail, and acrosomal defects) was expressed as a percentage of the total count.

Statistical analysis was performed using the SAS software package, version 9.3 (SAS Institute Inc., 2002–2010). Relationships between the examined traits were evaluated using Pearson's correlation coefficient. The strength of the correlations was interpreted based on the classification proposed by Petz (2004), as follows:

0.00-0.20 - negligible,
0.20-0.40 - weak,
0.40-0.70 - moderate, and
0.70-1.00 - strong correlation.

Results and Discussion

Descriptive analysis of boar semen traits revealed considerable individual variation. The average ejaculate volume was approximately 292 ml, while sperm concentration reached around 367 $\times 10^6$/ml. The total and functional sperm counts were 107.5 $\times 10^9$ and 90.8 $\times 10^9$, respectively. Native motility averaged 84%, with post dilution motility slightly lower at 78%. On average, 15 insemination doses were obtained per ejaculate. Regarding sperm viability from native ejaculates, live spermatozoa made up approximately 76% of the sample, while 24% were non-viable. The most frequent morphological defect was the presence of a protoplasmic droplet (13.6%), followed by tail (2.5%), head (1.3%), and acrosomal (0.2%) abnormalities, all showing high variability.

A correlation of varying strength, direction, and statistical significance was established between the examined semen traits (Table 1). A strong positive correlation ($r_f = 0.770$; $p<0.001$) was found between ejaculate volume and both the total sperm count and the number of functional spermatozoa. Similar findings were reported by Smital et al. (2005). A positive, but moderate, correlation ($r_f = 0.423$; $p<0.001$) was also observed between ejaculate volume and the number of insemination doses produced per ejaculate. These results suggest that ejaculates with larger volumes contain higher total and functional sperm counts, enabling the production of a greater number of insemination doses. This is consistent with the findings of Knecht et al. (2014) and Górski et al. (2017).

There is a weak negative correlation between ejaculate volume and sperm motility, which is statistically significant ($p<0.05$) only in the case of the motility percentage of native semen. Górski et al. (2017) also reported a weak negative relationship between these traits, although it was not statistically significant in their study. According to Savić (2014), a higher ejaculate volume does not necessarily imply greater fertility, as ejaculates with larger volumes may contain a lower proportion of progressively motile or functionally competent spermatozoa.

Table 1. Correlations between the examined semen traits

Trait	Parameter	CON	NT	NF	MOTN	MOTD	NPD	PD	PL
VOL	r_f	-0.132	0.770	0.714	-0.190	-0.186	0.423	0.169	-0.169
	p	0.188	0.000	0.000	0.037	0.052	0.000	0.122	0.122
CON	r_f		0.492	0.537	0.247	0.282	0.183	-0.269	0.269
	p		0.000	0.000	0.013	0.056	0.075	0.017	0.017
NT	r_f			0.983	-0.088	-0.098	0.519	0.002	-0.002
	p			0.000	0.383	0.344	0.000	0.988	0.988
NF	r_f				0.085	0.021	0.508	-0.030	0.030
	p				0.401	0.839	0.000	0.794	0.794
MOTN	r_f					0.685	-0.085	-0.242	0.242
	p					0.000	0.374	0.028	0.028
MOTD	r_f						-0.098	-0.454	0.454
	p						0.310	0.000	0.000
NPD	r_f							0.014	-0.014
	p							0.897	0.897
PD	r_f								-1.000
	p								0.000

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, PD - percentage of dead spermatozoa, PL - percentage of live spermatozoa.

On the other hand, a weak positive correlation ($r_f = 0.247$; $p = 0.013$) was observed between sperm concentration and the motility of native semen. These coefficients suggest that more concentrated ejaculates exhibited better mass motility. However, it should be noted that sperm motility was subjectively evaluated.

A weak positive correlation ($r_f = 0.269$; $p<0.05$) was also found between sperm concentration and the proportion of live spermatozoa, while a weak negative correlation ($r_f=0.269$; $p<0.05$) was found with the proportion of dead spermatozoa, indicating that ejaculates with higher concentration had a greater number of viable sperm. Furthermore, sperm concentration showed a moderate positive correlation ($r_f>0.400$; $p<0.001$) with both the total number and number of functional spermatozoa. However, it should be emphasized that these two traits (total number – NT and functional number – NF) are calculated values. Therefore, ejaculates with higher sperm concentrations were found to contain a greater total and functional sperm count. In contrast, Knecht et al. (2014) reported that the correlation between concentration and total sperm count ranged from negligible to weak, depending on the breed. A strong positive correlation ($r_f = 0.983$; $p<0.001$) was established between the total and functional sperm count, which is expected, as NF is derived

from NT. Moderate correlations (r_f = 0.519; r_f = 0.508; p<0.001) also suggest that ejaculates with a higher total and functional sperm count yielded a greater number of insemination doses. Similar results were reported by Knecht et al. (2014), who found an almost perfect correlation ($r_f \approx 1$; p<0.01) between total sperm count and the number of doses produced. A moderately strong positive correlation (r_f = 0.685; p<0.001) was observed between the motility of native semen and motility after dilution. Weak correlations between native sperm motility and the percentages of dead and live sperm indicate that ejaculates with better motility in the native state tend to contain a lower proportion of dead and a higher proportion of live sperm. Additionally, a moderate correlation (r_f = 0.454; p<0.001) between motility after dilution and the proportion of live sperm implies that better post-dilution motility is associated with greater sperm viability.

Correlations between various types of sperm abnormalities are presented in Table 2. Weak positive correlations (r_f > 0.200) of varying statistical significance (p<0.05; p<0.01) were found, particularly between secondary abnormalities (excessive proximal cytoplasmic droplets) and primary abnormalities involving the tail and acrosome. Weak positive correlations (r_f > 0.200) of varying statistical significance (p<0.05; p<0.01) were found, particularly between secondary abnormalities (excessive proximal cytoplasmic droplets) and primary abnormalities involving the tail and acrosome. Weak correlations were also observed among different primary anomalies. These results contradict the findings of Kamanova et al. (2017), who reported a strong negative correlation between spermatozoa with proximal cytoplasmic droplets and those with acrosomal defects. Moreover, the authors concluded that there were moderate to strong negative correlations between primary head and tail abnormalities, with the exception of bent-tail spermatozoa, which were strongly positively correlated with head defects.

Table 2. Correlation between pathological forms of spermatozoa

Trait	Parameter	PPLJR	POTKG	PAKR
PPK	r_f	0.270	0.165	0.213
	p	0.005	0.090	0.028
PPLJR	r_f		0.273	0.060
	p		0.004	0.536
POTKG	r_f			0.226
	p			0.019

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

Among the semen traits, only the motility of native semen and semen after dilution, as well as the number of insemination doses produced, were found to be correlated with pathological forms of spermatozoa (Table 3). Based on the obtained weak negative correlation coefficients, it can be concluded that ejaculates containing a lower proportion of spermatozoa with secondary (excess cytoplasmic droplets) and primary tail defects are expected to exhibit better motility and result in a higher number of insemination doses. These findings are consistent with the earlier study by Barna et al. (2021), who reported that ejaculates with better motility had a lower proportion of spermatozoa exhibiting cytoplasmic droplets and acrosomal damage.

Table 3. Correlation between semen traits and pathological forms of spermatozoa

Trait	Parameter	PPK	PPLJR	POTKG	PAKR
VOL	r_f	0.103	0.026	-0.133	-0.078
	p	0.349	0.815	0.225	0.477
CON	r_f	-0.040	-0.169	-0.103	-0.170
	p	0.725	0.136	0.365	0.134
NT	r_f	-0.106	-0.094	-0.152	-0.188
	p	0.988	0.408	0.181	0.096
NF	r_f	-0.145	-0.145	-0.185	-0.172
	p	0.206	0.205	0.104	0.132
MOTN	r_f	-0.232	-0.319	-0.151	-0.078
	p	0.035	0.003	0.173	0.487
MOTD	r_f	-0.239	-0.220	-0.007	-0.023
	p	0.030	0.047	0.952	0.840
NPD	r_f	-0.243	-0.113	-0.132	-0.024
	p	0.025	0.304	0.231	0.826
PD	r_f	0.069	0.104	0.133	0.086
	p	0.478	0.287	0.171	0.376
PL	r_f	-0.069	-0.104	0.133	-0.086
	p	0.478	0.287	0.171	0.376

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, PD - percentage of dead spermatozoa, PL - 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

Ejaculate volume was not correlated with pathological forms of spermatozoa, which contrasts with the findings of Górski et al. (2021), who reported a slight dependence of anomaly frequency on ejaculate volume.

According to their study, spermatozoa from low-volume ejaculates had shorter tails and a higher head-to-tail length ratio compared to those from medium- and high-volume ejaculates. Kondracki et al. (2020) investigated the impact of sperm concentration on sperm morphology and concluded that in approximately 50% of the ejaculates, spermatozoa with proximal and distal cytoplasmic droplets were observed, and their frequency increased with rising sperm concentration. In contrast, no correlation was found in our study between sperm concentration and pathological sperm forms. Understanding the interaction between sperm morphology particularly cytoplasmic droplets and sperm quality is essential to avoid reduced fertilization efficiency (Henning et al., 2021).

Conclusion

The analysis of the examined semen traits showed correlations of different strength, direction, and significance. Ejaculate volume was strongly positively related to the total and functional sperm count, and moderately related to the number of insemination doses. This suggests that larger ejaculates with more sperm cells can produce a greater number of doses. On the other hand, there was a weak negative correlation between volume and sperm motility, which was statistically significant only for the motility of native semen. Sperm concentration had a moderate positive correlation with both the total and functional sperm count, and a weak positive link with the percentage of live sperm. Native and diluted semen motility were moderately correlated with each other, while their relationship with the percentage of live or dead sperm cells was weaker. When it comes to abnormal sperm forms, weak positive correlations were found between secondary defects and some primary defects, especially those affecting the tail and acrosome. Interestingly, only native and diluted semen motility, along with the number of produced doses, showed a negative correlation with these abnormalities. This means that ejaculates with fewer defective sperm cells are likely to have better motility and yield more doses, making them more useful for artificial insemination.

Korelacija između osobina ejakulata i pojave patoloških oblika spermatozoida kod nerasta tokom perioda letnjeg toplotnog stresa

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Rezime

Sezonski toplotni stres poznat je kao faktor koji negativno utiče na plodnost nerasta, pre svega kroz smanjenje kvaliteta semena i povećanje učestalosti patoloških oblika spermatozoida. Cilj ovog istraživanja bio je da se ispita povezanost između osobina ejakulata i morfoloških anomalija spermatozoida kod nerasta tokom najosetljivijeg perioda u godini. U periodu od avgusta do oktobra prikupljeno je ukupno 129 ejakulata od 17 nerasta, u uslovima povišene spoljašnje temperature. Analizirani su zapremina ejakulata, koncentracija, ukupan i funkcionalni broj spermatozoida, pokretljivost (u nativnom i razređenom semenu), vitalnost, kao i prisustvo morfoloških anomalija - defekata glave, repa, akrozoma i protoplazmatskih kapljica. Rezultati su pokazali da su samo pokretljivost semena i broj proizvedenih doza bili u značajnoj korelaciji sa pojavom patoloških oblika spermatozoida. Utvrđena je slaba, ali dosledna pozitivna korelacija (r_f> 0,200; $p<0,05$ ili $p<0,01$) između određenih kategorija anomalija, naročito između sekundarnih oblika (protoplazmatska kapljica) i primarnih defekata na repu i akrosomalnoj regiji. Među primarnim anomalijama zabeležene su uglavnom slabe povezanosti. Ovi rezultati ukazuju na značaj praćenja pokretljivosti i morfologije spermatozoida kao ranih pokazatelja toplotnog stresa kod nerasta.

Ključne reči: nerast, kvalitet semena, patološke forme spermatozoida, osobine ejakulata, toplotni stress

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Conflict of interest
The authors declare that they have no conflict of interest.

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