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The influence of environmental change and seasons on the sperm quality of breeding boars after relocation

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ABSTRACT

This study aimed to determine the impact of relocation as a potential stress factor on the reproductive properties of breeding boars, as well as the impact of the new environment on the quantity and quality of the semen obtained during different seasons. At the beginning of spring, 16 breeding boars (Large White (n=3), German Landrace (n=4), Swedish Landrace (n=6) and Pietrain (n=3)) were relocated from old to new housing conditions and exposed to loading, transport, unloading, and rehousing in a new environment. They had the same conditions of feeding, care, sexual exploitation and keeping, before and after relocation. The health status of all breeding boars was carefully monitored and controlled. No significant differences were observed in the total and average number of doses produced between seasons. There was a significantly higher value of total ejaculate volume and density during the spring compared to the values obtained in other seasons ($P<0.01$). Differences in the average percentage of mobility between autumn and winter were not significant ($P=0.983$), as in spring and summer ($P=0.737$). Ventilation rate, gas concentrations of ammonia, nitrous oxide and carbon dioxide in the boar enclosure were similar before and after relocation. Two days after relocation, one boar (ID 172) showed signs of illness and was treated, but his sperm was rejected due to sperm agglutination and reduced motility, so he was culled. Individual analysis of the production indicators showed that there was no significant change in the spermatogenesis of healthy boars, even though they were relocated.

Key words: boars; environmental conditions; relocation; seasons; sperm quality

Introduction

The environmental factors influencing the sexual behavior of boars include genetics, season, management procedures, housing conditions, and sexual, psychological and social interactions (HEMSWORTH et al., 1977.; ROHRMANN and HOY, 2005.; HEMSWORTH and TILBROOK, 2007.). The most pronounced deviation in the sexual behavior of boars was observed due to poor

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management practices and housing conditions, which can be manifested through decreased sexual motivation and libido (HODEL et al., 2021). Pig housing is important not only because it is closely related to other factors that affect production, such as feeding, breeding and production technology, but also because breeding boars have a number of specificities, with special requirements (MORRISON et al., 2003; ROHRMANN and HOY, 2005.; PETAK, et al., 2010). The issue of housing pigs deserves special attention due to different climatic conditions (SZOSTAK and PRZYKAZA, 2016). Housing conditions very often play a decisive role in ensuring maintenance of basic hygiene measures, which are the first prerequisite for good animal health and welfare (PETAK, et al., 2010). Harmonizing microclimatic factors, the so-called climate of the facility, as one of the natural environmental factors in production, can affect above all the health of the animals, and thus also the production results and reproductive properties of breeding boars. The microclimate of the stable includes the physical and chemical state of the air, comprising the temperature, humidity, particle content, and the saturation of CO_2, H_2S, NH_3, and others (BALENOVIĆ, 1997). The most widely used assisted reproduction technology in pig production is the artificial insemination (AI) (RUNGRUANGSAK et al., 2022). In addition to reducing the risk of disease transmission, AI enables the use of superior genes in pig farming and leads to better profitability (MAES et al., 2016). Standard operating procedures and quality assurance systems in AI centers are crucial (PAVUNA et al., 1983; LOPEZ RODRIGUEZ et al., 2017). Comfortable accommodation, appropriate bedding material, cooling systems, good feeding strategy, and frequency of sperm collection significantly affect sperm quality (SAVIĆ and PETROVIĆ, 2015).

The quantity and quality of ejaculates are affected by both animal (breed, age, health status and feeding) and environmental factors, such as the air temperature, humidity, season, etc. (CHEON et al., 2002; SZOSTAK and PRZYKAZA, 2016). The reproductive activity of boars shows certain seasonal changes. Seasonal variations in boar sperm quality are associated with changes in photoperiod and heat stress during the summer (YESTE et al., 2010). Boars' sexual behavior is coordinated by the central nervous system related to the presence of sex hormones secreted by the gonads. A low level of androgens in the summer months can have a negative effect on the male libido. It is often accompanied by an increased number of rejected ejaculates, characterized by reduced motility and an increased proportion of morphologically changed sperm due to oxidative stress. Among other things, sperm motility is one of the most important indicators for evaluation of semen quality (KARAGEORGIOU et al., 2016; SUWIMONTEERABUTR et al., 2021). Differences in seminal plasma and spermatozoa antioxidative systems in different boar breeds and hybrids were observed (ŽURA ŽAJA et al., 2016a; 2016b). Providing additional cooling and air circulation is a difficult challenge, as boars must be kept within their comfort zone.

On the basis of the fact that the breeding boars in this study were exposed to stress by the act of relocation, there was a justified suspicion that the process of spermatogenesis could be impaired, and thus the boars' sperm quality and production. The study aimed to determine the impact of relocation as a potential stress factor on the reproductive properties of breeding boars, as well as the impact of the new environment on the quantity and quality of the sperm obtained during different seasons.

Materials and methods

Animals. Sixteen breeding boars that were moved from old to new housing conditions and exposed to loading, transport, unloading and rehoused in a new environment, were included in the research. The transfer from the location coordinates N 46.028852, and E 16.548166 to the location N 45.978170, and E 16.540731, lasted a total of 30 minutes. The breeding boars were of different breeds: Large White (n=3), German Landrace (n=4), Swedish Landrace (n=6) and Pietrain (n=3). All breeding boars, aged 2-5 years, had the same conditions of keeping, feeding, care

and sexual exploitation before relocation, and in the new conditions after relocation. As part of their care, the boars were regularly showered and washed, with the difference that instead of once a week, in the summer, with high outside temperatures, showering and washing was done 3-4 times a week.

The health status of all the breeding boars was carefully monitored and controlled immediately before and after relocation, in order to observe any visible changes. Any change in the state of their health during the time of relocation and immediately after could be associated with assumed stress. The boars were relocated immediately after the winter, at the beginning of spring.

Sperm collection and evaluation. The ejaculates of the boars were obtained by a standard procedure of manual fixation of the penis with a sterile disposable glove, while mounting on a phantom, placed in a special collection pen. They were collected during ejaculation in sterile, heated and tissue-wrapped glass bottles with a volume of 350 ml. The first fraction of the ejaculate was not taken (due to possible contamination with urine residues, disintegrated epithelium, and numerous microorganisms). Other fractions were collected and immediately delivered to the laboratory that was part of the Center, where the validity of the ejaculate was assessed (among other things for minimal spermatozoa concentration of 200 million in 1 mL of ejaculate). After evaluation according to the standard protocols, the sperm was diluted with a specific diluent. Different diluents were used, considering the individuality of the boars in relation to specific diluents. All ejaculates that did not meet the minimum passing criteria during the evaluation were rejected. Total ejaculate volume (ml), average ejaculate volume (ml), average ejaculate density (millions), average motility percentage, and average and total doses produced were determined for each ejaculate and each boar. As the doses of liquid semen of breeding boars intended for artificial insemination of sows are the ultimate goal of production, the average number of doses produced per ejaculate of boars was taken as a criterion.

Housing microclimate. The housing pen was equipped with a mechanical negative pressure ventilation system, which was controlled by the temperature. The room was ventilated by two fans. For measurement of gas concentrations (ammonia, nitrous oxide, and carbon dioxide) a photo-acoustic spectrometer, Multi Gas Monitor Model 1312, was used. In addition to housing, the social behavior of the boars was evaluated. In the natural environment, boars are less social animals and naturally live solitary lives. The housing pen was built to specific standards designed to reduce the stress associated with physical contact, by ensuring safe human-animal interaction to avoid injury of boar handlers.

Season. The autumn season refers to September to November of the previous year, winter is from December of the previous year to January and February of the year in question, spring is March to May, and summer June to August.

Meteorological data. The measurements of air temperature, rainfall precipitation, sunshine duration and relative humidity were obtained from the nearest meteorological recording station, located within 0.5 km of the boars' housing system.

Statistical analysis. Data were statistically analysed using the STATA 13.1 program (StataCorp. 2016. Stata Statistical Software: Release 13.1 College Station, TX: StataCorp LP). The distribution of the data obtained for total volume, average volume, density, mobility, number of doses and average number of doses was tested by the Shapiro-Wilks test. The data obtained were normally distributed, so they were statistically analyzed by the one-way ANOVA test, Sidak, Boneferroni and Scheffe post-hoc tests, and regression analysis.

Results

In the study, the minimum qualities of ejaculates for boars used in natural mating or for artificial insemination were applied: ejaculate volume 100 ml, sperm motility at least 70%, at least 150 billion sperm cells in one ml of ejaculate, while the percentage of immature and pathological forms of spermatozoa was 16% and 20%, respectively.

Table 1. Presentation of sperm production indicators of breeding boars (breed of boars, number of sperm collections, total and average doses produced) during different seasons before (autumn, winter) and after (spring and summer) relocation

Season	Breed of boars*	Number of sperm collections	Total doses produced	Average doses produced (M±S.E.M.)
Autumn	LW	23	462	20.09±2.95[a]
	GL	59	1107	18.76±3.14[ab]
	SL	81	1512	18.67±2.87[ab]
	P	60	811	13.52±3.45[b]
Winter	LW	31	539	17.39±2.99[ab]
	GL	66	1233	18.68±2.82[ab]
	SL	89	1804	20.27±3.72[a]
	P	64	916	14.31±4.83[b]
Spring	LW	65	1082	16.65±3.05[ab]
	GL	63	1326	21.05±3.77[a]
	SL	130	2186	16.82±3.35[ab]
	P	84	1197	14.25±2.05[b]
Summer	LW	49	772	15.76±6.19[b]
	GL	43	894	20.79±4.05[a]
	SL	106	2055	19.39±4.11[a]
	P	51	883	17.31±5.19[ab]

[a,b] Significantly different values ($P<0.05$) in the same row are marked with different superscript letters
*LW-Large White, GL-German Landrace, SL-Swedish Landrace, and P-Pietrain

No significant differences were observed in the total and average number of doses produced between seasons ($P>0.05$), as presented in Table 1. There was a significantly higher value of total ejaculate volume during the spring compared to the values obtained in other seasons ($P<0.01$). Significantly higher values of average individual ejaculate volumes were observed during spring and summer than in autumn and winter ($P<0.01$). The differences in the average individual ejaculate volume between autumn and winter were not significant ($P=0.969$), as well as the differences between spring and summer ($P=0.345$). There was a significant difference in the total density of ejaculate between seasons ($P<0.01$), so higher values were observed during spring than in winter ($P=0.014$) and in summer compared to autumn and winter ($P<0.01$).

Significantly higher values were observed in autumn and winter than in spring and summer ($P<0.01$). Differences in the average percentage of motility between autumn and winter were not significant ($P=0.983$), or in spring and summer ($P=0.737$), as presented in Table 2.

Table 2. Average total volume (ml), average individual ejaculate volume (ml), average density (billions), and average percentage of sperm motility during different seasons (M± S.E.M.)

Season	Average total volume (ml)	Average individual ejaculate volume (ml)	Average density (billions)	Average percentage of sperm motility
	(M± S.E.M.)			
Autumn	3466.76±148.11[a]	165.75±2.25[a]	366.08±1.77[a]	83.52±0.32[a]
Winter	3193.57±142.04[a]	167.19±2.74[a]	363.72±1.96[a]	83.17±0.28[a]
Spring	3824.20±70.13[b]	186.38±1.72[b]	371.55±1.87[b]	79.41±0.69[b]
Summer	3472.04±106.69[a]	181.47±1.77[b]	377.25±1.99[c]	80.31±0.94[b]

[a,b,c] significantly different values (P<0.05) in the same column are marked with different superscript letters

Table 3. Ventilation rate, gas concentrations of ammonia (NH_3), nitrous oxide (N_2O) and carbon dioxide (CO_2) in the boar enclosure of the Center for Artificial Insemination, Croatia

Housing conditions	Number of measurement	Ventilation rate	mg/m3		
			NH_3	N_2O	CO_2
Before relocation	1.	0.012	16.64	1.88	5024
	2.	0.027	23.94	1.09	2195
	3.	0.024	12.27	0.86	2501
Average		0.021±0.006[a]	17.62±4.22[b]	1.28±0.40[c]	3240±1189[d]
After relocation	4.	0.028	23.62	1.79	4243
	5.	0.012	19.94	1.06	2598
	6.	0.018	14.68	0.9	4725
	Average	0.019±0.005[a]	19.41±3.16[a]	1.25±0.36[c]	3855±838[d]

[a,b,c,d] Significantly different values (P<0.05) in the same row are marked with different superscript letters

A few days before the relocation, no visible changes in the health status of the breeding boars were observed. Two days after the move, the health condition of the one boar (ID 172) changed and appropriate therapy was performed. After overcoming the disease, his sperm did not meet the minimum evaluation criteria, and was rejected due to sperm agglutination and reduced motility. Sperm was collected on 25 occasions and rejected each time for the same reasons. During the study, the average air temperature, amount of precipitation, duration of sunshine and relative humidity differed by month (Fig. 1.).

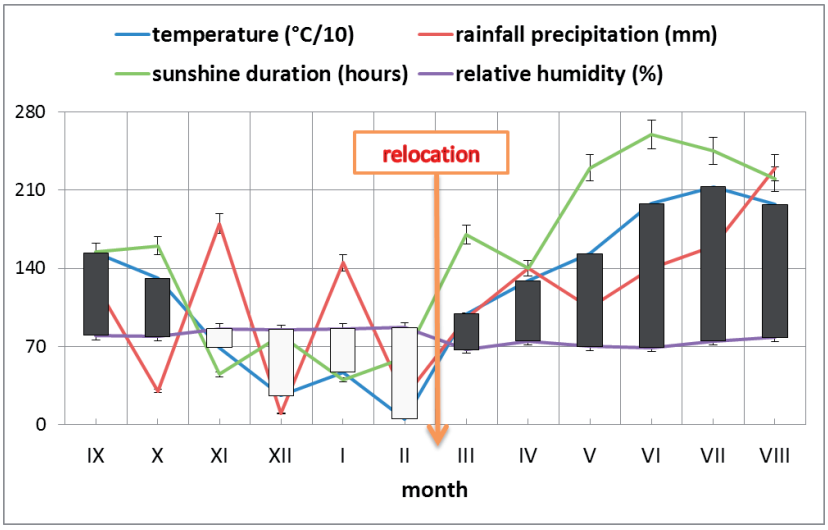

Fig.1. Monthly average air temperature (°C/10), rainfall precipitation (mm), sunshine duration (h), and relative humidity (%) outside the boar housing, six months before and after the relocation of boars

Discussion

It is well known that heat stress significantly affects the quality of boar sperm (FREKING et al., 2012). Boars exposed to higher temperatures (>30°C) for a longer time have lower sperm motility and sperm morphology, but also reduced fertility (WETTEMANN et al, 1976; KUNAVONGKRITET et al., 2005). Since in our study the indicators of the quantity and quality of sperm during the summer did not differ significantly from those in the remaining seasons, we can conclude that the boars were not under stress due to increased environmental temperature or they were not exposed to heat stress. Nevertheless, in order to guarantee an adequate number of insemination doses, there should be a larger number of donor boars in the summer for better organization of insemination centers (KNECHT et al., 2014; KNOX, 2015), as in our study, where there were more sperm collections in the summer than in the winter or autumn.

Although good air quality is required for the comfort and well-being of boars, it has not been proven that air quality, ventilation rate, ammonia or other gas concentrations affect the semen quality of boars (LOPEZ RODRIGUEZ et al., 2017). In our study, no significant changes in air quality, ventilation rate, or emission of harmful concentrations of gases (ammonia, nitrous oxide, and carbon dioxide) were recorded in the previous or the new housing pens.

All boars of the Swedish Landrace breed showed a slight increase in production after relocation. Therefore, even though they were relocated, there was no disturbance in spermatogenesis. Since it has been proven that there are differences in the antioxidant system of seminal plasma and spermatozoa between boars of different breeds and hybrids, it can be assumed that this breeds has good protection against higher environmental temperatures. In addition, Swedish Landraces boars had the highest level of intracellular antioxidant protection of sperm cells (ŽURA ŽAJA et al., 2016b). In boars of the Large White breed, a decrease in the total doses produced per boar was visible after relocation in the spring, and continued in the summer.

Transport and relocation can be stressful for all animals, especially breeding animals (RIOJA-LANG et al., 2019). Stress in breeding boars can impair the development of physiological processes

in the body and weaken their immune system, which can lead to the appearance of organic or conditional infectious diseases. After recovery, there may be a temporary or permanent disruption of the spermatogenesis process, which can be determined by examining the ejaculate. Negative consequences may disappear after a certain time, or may remain permanent, and such boars must be excluded from breeding. The sperm of boars with impaired spermatogenesis has a lower ability to fertilize, and its use for artificial insemination is excluded by strict control measures and procedures.

We can conclude that no significant differences were observed in the total and average number of doses produced between seasons, while in spring significantly higher total volume and ejaculate density values were determined compared to other seasons. Individual analysis of production indicators showed that there was no significant change in the spermatogenesis of healthy boars, in spite of relocation.

Ethics approval

The research protocol and animal management were in accordance with Directive 2010/63/EU (European Union 2010) on the protection of animals used for scientific purposes.

Declaration of Competing interest

The authors declared no potential conflicts of interest with respect to the research, authorship, or publication of this article.

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ĐURIČIĆ, D., F. MARKOVIĆ, R. HORVAT MARKOVIĆ, P. DŽAJA, M. SAMARDŽIJA: Utjecaj promjene okoliša i godišnjih doba na kvalitetu sperme rasplodnih nerasta nakon preseljenja. Vet. arhiv 94, 195-204, 2024.

SAŽETAK

Istraživanje je imalo za cilj utvrditi utjecaj preseljenja kao potencijalnog čimbenika stresa na reproduktivna svojstva rasplodnih nerasta, kao i utjecaj novog okoliša na količinu i kvalitetu dobivenog sjemena tijekom različitih godišnjih doba. Početkom proljeća 16 rasplodnih nerasta (veliki jorkšir n=3, njemački landras n=4, švedski landras n=6 i pietren n=3) preseljeno je iz starih u nove uvjete držanja te su bili izloženi utovaru, transportu, istovaru i dolasku u novo okruženje. Nerasti su imali iste uvjete hranidbe, njege, iskorištavanja i držanja, prije i nakon preseljenja. Zdravstveno stanje svih rasplodnih nerasta pažljivo je praćeno i kontrolirano. Nisu uočene znakovite razlike u ukupnom i prosječnom broju proizvedenih doza sperme između sezona. Tijekom proljeća utvrđena je znakovito viša vrijednost ukupnog volumena i gustoće ejakulata u odnosu na vrijednosti dobivene u ostalim godišnjim dobima ($P<0,01$). Razlike u prosječnom postotku pokretljivosti između jeseni i zime nisu bile znakovite ($P=0,983$), kao ni u proljeće i ljeto ($P=0,737$). Stopa ventilacije, koncentracije plinova amonijaka, dušikovog oksida i ugljikova dioksida u nastambi za svinje bile su slične prije i nakon preseljenja. Dva dana nakon preseljenja nerast (ID 172) je pokazivao znakove bolesti i liječen je, ali mu je sperma odbačena zbog aglutinacije spermija i smanjene pokretljivosti, zbog čega je izlučen iz uzgoja. Pojedinačna analiza proizvodnih pokazatelja pokazuje da nije došlo do značajne promjene u spermatogenezi zdravih nerasta, iako su premješteni.

Ključne riječi: nerasti; okolišni uvjeti; preseljenje; godišnja doba; kvaliteta sperme