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Semen production of boars from the East Balkan swine breed

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Abstract

For the first time in our country for the purposes of artificial insemination, semen was regularly obtained from trained boars of the East Balkan Swine breed. The aim of the study was to determine ejaculate volume, concentration, number of doses for insemination. The study covered the period from October 2023 to May 2024, and seventy-seven ejaculates from ten boars were examined. Analysis revealed an average volume of the ejaculate of 161.417 ± 5.281 ml, with the minimum and maximum values being within very wide range – $40 \div 300$ ml. Sperm concentration and total concentration were, respectively, $0.575 \times 10^9/\text{ml} \pm 0.012$, and $90.89 \times 10^9/\text{ml} \pm 2.675$. The reported mean number of doses received from one ejaculate was 10.618 ± 0.43 . The boar as a fixed factor had a significant effect on the studied parameters of the sperm ($p < 0.001$). The month of semen collection appeared to be a significant source of variation in the total concentration and number of insemination doses. The boar, as a factor within the year, significantly influenced ejaculate volume and concentration ($p < 0.001$), as well as total concentration ($p < 0.05$). In the analysis of monthly dynamics of the main semen traits, lower values were observed for most traits during the first two months of the experiment. Despite the relatively aggressive temperament of animals from primitive breeds like the East Balkan Swine breed, obtaining semen manually was entirely feasible and applicable with appropriate training and adaptation of animals.

Keywords: East Balkan Swine, boars, sperm concentration, ejaculate volume

INTRODUCTION

The East Balkan Swine (EBS) is the only one autochthonous breed preserved in a pure state in Bulgaria, and one of the few native pig breeds in Europe (Palova & Ivanova, 2019). An alarming reduction in the number of animals has been observed over the past 20 years. Zapryanova *et al.* (2013) reported that between 2005 and 2011, the population decreased from 55 000 to 9 684 animals. According to the Ministry of Agriculture and Food, Executive Agency for Selection and Reproduction of Animals, in the Republic of Bulgaria (2023) at the end of 2023, due to deteriorating epidemiological situation in the country caused by African Swine Fever (ASF) the number of animals accounted for one hundred and six sows and only two boars, raised in two farms.

East Balkan Swine is endangered breed due to significant change in the farming technology comprised of enclosed rearing areas, restrictions imposed by state institutions to limit the spread of ASF. This is contrary to the free-range farming of animals that has been a tradition for centuries, leading to a loss of profitability due to the feeding of animals entirely with compound feed. Regional restrictions (Regulation No. 6, 2007) on raising EBS also contribute to the elimination of breed as a national genetic resource. These circumstances necessitate immediate actions of all non-governmental organizations and governmental institutions to preserve genetic diversity in pig farming and to protect the EBS (National Biodiversity Conservation Strategy, 2024).

Preserving genetic resources requires a clear and consistent strategy which have to ensure protection of the breed under any circumstances. In this regard, the FAO recommends both *in vitro* and *in vivo* approaches for preservation. It is expected that by 2050, the demand for meat products in developing countries will increase by 76% (FAO, 2015).

In our country, studies on the breeding dynamics, meat productivity, sexual behavior, and partially on the quality of semen in EBS have been conducted by various Bulgarian and foreign authors (Ivanova-Peneva, 2010; Marchev & Szostak, 2013; Popova *et al.*, 2015; Yordanov, 2017). Currently, targeted work on preserving the East Balkan Swine breed and conducting experiments to obtain sperm from boars for the purpose of genetic material preservation for artificial insemination, is being carried out in the nucleus of the Golyamo Vranovo industrial pig farm, Ruse region. The scientific team's efforts aimed a sustainable and rational conservation of the East Balkan Swine as a national gene pool avoiding inbreeding. Additionally, it aims to create an economic impact for EBS raised farms through popularization of breed's qualities, conducting tests, and creating hybrid lines. Determining the specific parameters and characteristics of the ejaculate is crucial for subsequent breeding policies to preserve the breed and accordingly, a scientifically based and reasoned selection of boars.

This study aimed to determine sperm ejaculate volume, sperm concentration and total sperm concentration, number of doses for insemination and to evaluate effects of a month, year, and boar on these parameters for East Balkan Swine breed.

MATERIALS AND METHODS

The study investigated the sperm production of boars from the autochthonous East Balkan Swine breed of the same age. The

covered period is from October 2023 to May 2024, during which 77 ejaculates were obtained from 10 boars. The ejaculates were collected at equal time intervals. The animals were raised on a pig farm in individual pens and were fed a specialized balanced diet formulated for breeding animals. The feed was the same as that used at the Golyamo Vranovo – (industrial pig farm) Invest corp. The compound feed for the boars consisted of wheat (24%), barley (15%), corn (34%), sunflower expeller (12%), soybean meal (10%), and other ingredients. The nutritional composition was precisely balanced to provide the necessary elements for complete and balanced nutrition: dry matter (85%), crude protein (12.8%), crude fiber (5.5%), metabolizable energy (2500 Kcal/kg), crude fat (4.53%), and crude ash (5.25%). Additionally, twelve amino acids, essential micro- and macro-elements, and vitamins were also provided.

The semen was collected manually in a specially isolated room, separated from other animals. The facility was equipped with an enclosure area, providing a quick and easy exit for the technician in case of boar aggression. A fixed dummy was installed in the room. After collection, the semen was transferred to the laboratory, which was directly connected to the collection room. In the laboratory, the ejaculate was evaluated based on several main parameters: volume of the ejaculate (ml), concentration of spermatozoa ($\times 10^9$ sperm/ml), measured with a sperm densitometer, and total concentration of spermatozoa in the ejaculate ($\times 10^9$), calculated by multiplying the concentration of spermatozoa per milliliter by the volume of the ejaculate. The dilution was performed using a semen extender for boar semen at temperature of 37°C. After dilution, the semen was divided into doses in polyethylene vials and cooled to 17°C in a thermal chamber. The semen was transported in cooling bags to the industrial farm in Golyamo Vranovo.

The data analysis was done by SPSS (IBM, v.24). The utilized multivariate linear model had the following statistical form:

$$Y_{ijkl} = \mu + M_i + Y_j + B_k + YB_{jk} + e_{ijkl}$$

where: Y_{ijkl} – observation vector, μ – overall average constant, M_i and Y_j are fixed effects of the month (i =October to May) and year (j =2023-2024), B_k is fixed effect of the boar (n =10), YB_{jk} is random effect of interaction between year of obtaining*boar, e_{ijkl} – residual variance.

RESULTS AND DISCUSSION

In analyzing the semen from boars of the East Balkan Swine breed (Table 1), we determined an average ejaculate volume of 161.4 ± 5.281 ml, with minimum and maximum values ranging widely from 40 to 300 ml. Despite the lower volume, the sperm concentration per milliliter was $0.575 \pm 0.012 \times 10^9$ /ml. The recorded average number of doses obtained from a single ejaculate was 10.62 ± 0.33 . Kondracki (2003) reported similar results for ejaculate volume—153.5 ml and sperm concentration— 574.1×10^6 /ml in Duroc boars, indicating a significantly lower volume and higher concentration compared to other studied in his study breeds. The total concentration in Duroc, due to the lower volume, is significantly lower (71.7 ± 0.39 ml) than that in the East Balkan Swine— $90.89 \pm 2.675 \times 10^9$. The Mangalitsa breed, although also of East European origin similar to the East Balkan Swine, exhibited significantly different

ejaculate parameters as follows: an average volume of 202.8 ml, a concentration of 213×10^6 spermatozoa/ml (43.9×10^9 /ejaculate) (Ciorei et al., 2013).

From the factors studied, the boar significantly influenced all examined sperm parameters ($p < 0.001$). Tsakmakidis *et al.* (2010) also reports a significant impact of the individual on ejaculate quality, with a strong positive correlation between ejaculate quality and farrowing rate.

The month of semen collection appeared to be a significant source of variation regarding total concentration and the number of doses for insemination, while the year of collection did not influence any of the examined traits. However, the combination of year and boar significantly affected ejaculate volume ($p < 0.001$) and total concentration ($p < 0.05$).

During the analysis of the monthly variation in ejaculate volume over the study period, two peaks in average values were observed in January and April (Fig.1). The highest average volume of ejaculate, 181.0 ml, was recorded in April, with relatively high volumes maintained throughout the spring months (from March to May). The seasonal changes likely contributed to the increase in volume during this period. The effect of the season will be the subject of subsequent study.

On the other hand, sperm concentration (Fig. 2) initially showed an inverse relationship with volume, but relatively consistent and similar values were observed during the spring months.

Table 1. Seminal characteristics of Eastern Balkan boars

Traits	LS	± SE	Minimum	Maximum
Volume, ml	161.4	5.281	40.00	300.0
Concentration, $\times 10^9$ /ml	0.575	0.012	0.420	0.780
Total concentration, $\times 10^9$	90.89	2.675	28.00	173.0
Number of insemination doses	10.62	0.330	3.000	26.00

the year, and the boar on the quality of sperm of EBS

Model	F-criterion and level of significance			
	Semen parameters			
Factor	Volume, ml	Concentration, $\times 10^9/\text{ml}$	Total concentration, $\times 10^9$	Number of insemination doses
Month	1.302	1.038	2.405*	2.107*
Year	365	0.053	0.638	0.471
Boar	7.658***	2.659**	7.832***	4.28***
Year*Boar	3.940***	1.161	2.208*	0.556

Legend: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

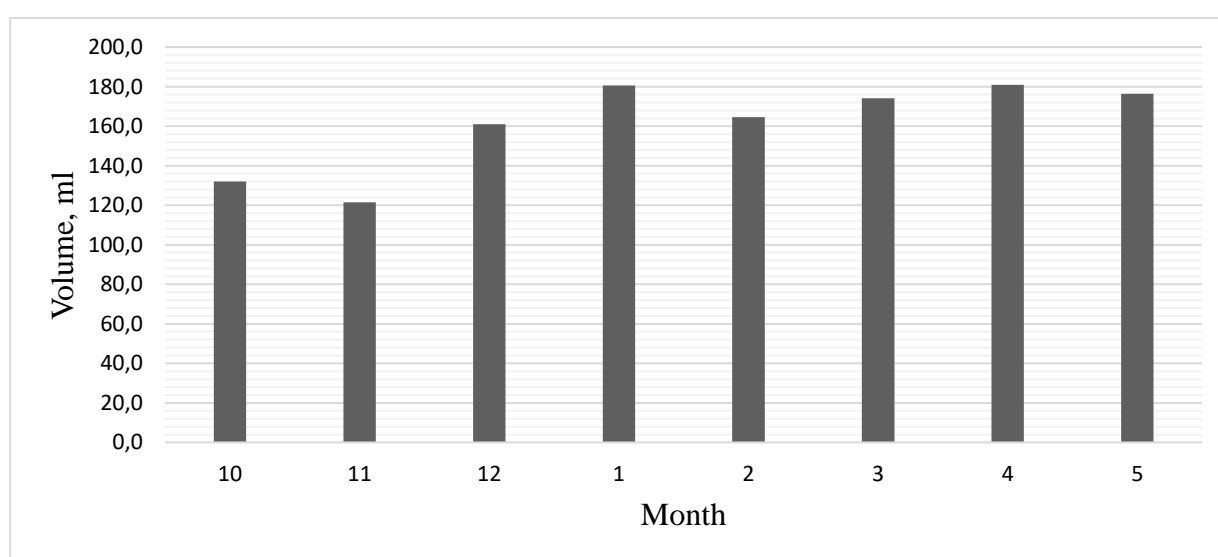


Figure 1. Monthly variation of semen volume in EBS boars

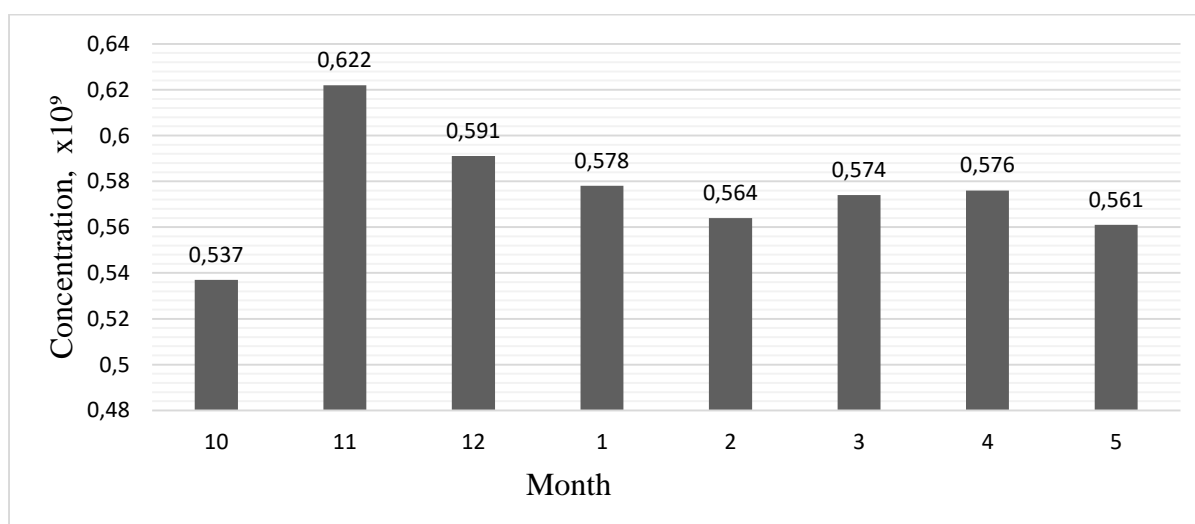


Figure 2. Monthly variation of concentration of total spermatozoa in ejaculate ($1 \times 10^9/\text{ml}$) of EBS boars

Consistent with the current results, Kunowska-Słószarz & Makowska (2011) reported that boars of Polish Landrace, Polish Large White, and Duroc × Pietrain breeds exhibited the highest ejaculate volumes during the autumn and winter months, whereas during the same period, sperm concentration was at its lowest. Similarly, in a large-scale study involving 230 750 ejaculates from 2 712 boars, Smítal (2009) found the lowest values for ejaculate parameters during summer and the highest during autumn and winter. Knecht *et al.* (2014), who studied Duroc × Pietrain boars, observed the highest number of insemination doses per ejaculate during winter (26.0 ± 0.51 doses).

CONCLUSIONS

Despite the relatively aggressive temperament of animals from primitive breeds like the East Balkan Swine (EBS), obtaining semen manually was entirely feasible and applicable with appropriate training and adaptation of the animals. The average ejaculate volume for EBS was $161.4 \text{ ml} \pm 5.281$, with minimum and maximum values in a wide range from 40 to 300 ml. In terms of sperm concentration per milliliter, the EBS breed surpassed many commercial breeds with a concentration of $0.575 \times 10^9/\text{ml} \pm 0.012$. The total sperm concentration in EBS was relatively high at $90.89 \times 10^9/\text{ml} \pm 2.675$, which supported the production of an adequate number of insemination doses per ejaculate, averaging 10.62 ± 0.33 doses. The boar had a significant effect on all examined sperm parameters ($p < 0.001$). The month of semen collection was a significant source of variation, with high volumes observed throughout the spring months.

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