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Effects of season on semen parameters and relationships between selected semen characteristics in Hypor boars

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Abstract: The aim of this study was to evaluate the effect of the season of the year on the quality of Hypor boar semen and to determine the correlations between selected semen characteristics in particular seasons. Ejaculates from Hypor boars were analyzed for volume, sperm concentration, percentage of spermatozoa with normal motility, total number of spermatozoa, and the number of insemination doses per ejaculate. Microscopic slides were prepared from semen samples. Each sample was evaluated for the morphology of spermatozoa. Spermatozoa with normal morphology were counted, as well as those with both major and minor abnormalities. Spermatozoa were measured for morphometric traits. Ejaculates were grouped by the season of collection: winter, spring, summer, and autumn. Correlation coefficients were calculated between sperm morphometric traits and the concentration and total count of spermatozoa in ejaculates. In spring and winter, sperm concentrations are higher compared to ejaculates collected in summer and autumn. In spring and summer, ejaculates contain spermatozoa of greatest motility. In spring, sperm cells have largest head dimensions. In autumn, spermatozoa are smallest in terms of dimensions. Significant correlations were observed between sperm concentration and total number of sperm in ejaculates vs. morphometric dimensions of sperm heads in Hypor boars' semen in the autumn, winter, and summer.

Key words: Boar, seasonal changes, ejaculate, sperm morphometry

1. Introduction

Porcine ejaculate performance and semen quality depend on factors such as age, breed, feeding and nutrition, housing conditions, animal care, the way the semen is collected, and many others (1,2). Libido also affects ejaculate traits (3). Reproductive performance of artificial insemination (AI) in boars stems largely from semen quality, which is a function of the incidence of sperm cell morphological abnormalities (4). Besides the semen quality, the AI value of semen is additionally influenced by sperm morphometric features, their sizes, and shapes (5). One of the methods used to assess the biological value of semen and its suitability for inseminating females is examination of sperm morphology (6). In AI practice cases of reduced male fertility are observed. This may be due to seasonal factors such as temperature, humidity, and light intensity (1). The occurrence of seasonal variation in the frequency of morphological sperm anomalies may depend on the breed of pig (2). This is because of varying degrees of sensitivity among breeds to the effects of seasonal factors (7).

The aim of this study was to determine the effects of seasonal factors on ejaculate physical traits and sperm morphology in Hypor boars and to establish correlations between some semen traits and year seasons.

2. Materials and methods

The analysis involved 114 ejaculates collected from 12 Hypor boars utilized in three AI centers located in central Poland. Geographically the area ranges between latitudes 51°60′N and 52°50′N and between 19°10′E and 20°30′E. The average altitude of the area is 109 m above sea level. Four calendar-based seasons of the year were considered: winter (22 December 2014 to 20 March 2015), spring (21 March 2015 to 20 June 2015), summer (21 June 2015 to 22 September 2015), and autumn (23 September 2015 to 21 December 2015). The mean ambient temperature and humidity per season were 1.95 °C and 82.07% (winter), 11.31 °C and 66.71% (spring), 18.76 °C and 65.93% (summer), and 6.26 °C and 84.05% (autumn), respectively. Data on temperatures and humidity of the area in which the boars were located came from the Institute of Meteorology and Water Management-National Research Institute. All the boars from which the semen was collected had been managed under unified conditions,

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conforming with the current animal welfare regulations. Ethics committee approval for this study was given by decision of the District Veterinary Officer (14262001). Food intake was individualized for each boar according to swine nutrition requirements, with ad libitum access to water. The animals were free from contagious and reproduction-related diseases, covered by a routine disease prevention program and receiving permanent veterinary care. Young boars, aged 7–9 months and at their initial stage of reproductive activity, were selected for the study. Ejaculates were collected manually (8). Fresh collected ejaculates were evaluated for physical traits, such as volume, sperm concentration (colorimetry, spectrometer from IMV Technologies, France), and the percentage of sperm motility (based on microscopic examination of a small sample of fresh semen). Sperm motility evaluation was conducted using a Nikon Eclipse-50i light microscope equipped with a phase-contrast condenser. The total number of sperm cells per ejaculate and number of AI doses were estimated using the SYSTEM SUL (v. 6.3.5) package.

The ejaculates were collected every 4 days. Ten ejaculates from each boar collected at 1-month intervals were evaluated. Microscopic slides were prepared from semen samples. Slides were fixed and stained according to the methods of microscopic slide preparation described by Kondracki et al. (9). The microscopic examinations were carried out at a magnification of 100× using immersion objectives and the Nikon Eclipse-50i light microscope. For each slide, morphology of 500 sperm cells was evaluated, noting the number spermatozoa with morphological abnormalities, divided into major and minor changes according to the classification by Blom (10). Morphometric measurements were carried out for 15 randomly selected sperm cells with normal morphology in each slide. The measured values were obtained using an image analysis software package (Screen Measurement v. 4.1). The following morphometric sperm cell measurements were performed: head length, head width, head perimeter, head area, flagellum length, and total sperm length. Measurements were carried out according to Kondracki et al. (11).

Descriptive statistics of the data included the mean and standard deviation (SD). The data were analyzed statistically according to the following model:

$Y_{ij} = m + a_i + e_{ij}$

where:

Y_{ij} - trait level,

m - population mean,

a_i - effect of season,

e_{ij} - error.

Significance of differences between groups were inferred based on Tukey's test. Correlation coefficients were calculated between the dimensions of spermatozoa and their ejaculate concentration and total number. Pearson's correlation coefficients were computed. Computations were carried out using the STATISTICA 10 PL package (StatSoft, Tulsa, OK, USA).

3. Results

Table 1 presents data on physical traits of ejaculates collected from Hypor boars in each season.

The data show that the lowest ejaculate volume occurred in spring (276.66 mL). In summer and autumn, ejaculate volume increased to about 313 mL, and decreased again to about 287 mL in winter. Samples collected in winter exhibited the highest sperm concentration (on average 448.93×10^6), which was followed by a gradual decrease to about 365.54×10^6 in autumn. The difference in sperm cells concentrations between winter and autumn was more than 83×10^6 ($P \leq 0.05$). The number of AI doses obtainable from a single ejaculate depends on sperm concentration. The lowest number of sperm cells, 87.05×10^9 on average, was found in autumn. In the remaining seasons of the year, total sperm cell number ranged from 94.48 to 101.29×10^9. The lowest percentage of spermatozoa with progressive

Table 1. Effect of season on physical ejaculate parameters in Hypor boars.

Specification	Season			
	Winter	Spring	Summer	Autumn
Number of ejaculates (n)	32	30	21	31
Ejaculate volume (mL)	286.87 ± 69.99[a]	276.66 ± 58.15[a]	313.80 ± 68.80[a]	313.22 ± 75.02[a]
Sperm concentration ($\times 10^6$/mL)	448.93 ± 126.39[b]	445.33 ± 97.79[ab]	384.76 ± 110.20[ab]	365.54 ± 123.96[a]
Total number of sperm ($\times 10^9$)	94.48 ± 30.68[a]	101.29 ± 31.95[a]	97.41 ± 29.09[a]	87.05 ± 34.81[a]
Percentage of spermatozoa with normal motility (%)	75.31 ± 5.07[ab]	78.33 ± 3.79[b]	78.09 ± 4.02[b]	74.19 ± 5.01[a]
Number of insemination doses (n)	33.43 ± 11.15[a]	31.70 ± 8.15[a]	31.04 ± 9.83[a]	28.35 ± 10.45[a]

Values are expressed as means ± SD. Means with different letters in a row are significantly different ([a, b]: $P \leq 0.05$).

motility was found in autumn (74.19%), whereas it was highest in spring (78.33%) and summer (78.09%). Differences between the spring-summer period and the autumn season are significant ($P \leq 0.05$). Data in Table 1 also reveal that the most insemination doses (about 33) may be obtained from a single ejaculate of a Hypor boar in winter. The lowest number of insemination doses were prepared from semen collected in autumn. Seasonal changes in the number of insemination doses obtained from ejaculates of the analyzed boars have not been confirmed as statistically significant.

Table 2 presents data pertaining to morphological measurements of sperm cells in relation to season of collection.

Spermatozoa in ejaculates collected in spring had the largest dimensions of their heads and tails. In their heads, the largest dimensions were length and width, as well as area and perimeter. The lowest width and length of the head were found in spermatozoa collected in autumn. The sperm cells of the semen collected in autumn also had heads of the lowest perimeter and surface area. These differences were significant at $P \leq 0.05$. The effect of the season on flagella dimensions was small, and any differences between groups in this respect were nonsignificant.

Table 3 presents data on the frequency of sperm morphological abnormalities in ejaculates collected in particular seasons.

The data allow the conclusion that the percentage of major morphological changes did not change considerably between seasons. The highest rate of major abnormalities was found in spring (1.58%), whereas the lowest was found in autumn (0.80%). The differences, however, were nonsignificant. Differences were noted, on the other hand, in the percentage of minor abnormalities. The highest percentage of sperm with minor abnormalities was found in summer (3.89%), by 1.37% more compared to ejaculates collected in the autumn season ($P \leq 0.05$).

The Figure presents data on the frequency of spermatozoa with abnormalities of morphology depending on the season of the year.

Among the major morphological abnormalities, proximal droplets were most frequent. The percentage of spermatozoa with this defect was small, however, and did not exceed 0.6%. Minor morphological abnormalities in the spermatozoa mostly referred to spermatozoa with simple bent tails. The mean percentage of spermatozoa with this defect was low, only slightly above 3%.

Data in Table 4 pertain to the dependence between sperm morphometrics and the concentration and total number of spermatozoa in Hypor boar semen ejaculates collected in various seasons.

Strong correlations were found between head dimensions vs. concentration and total number of sperm per ejaculate collected in autumn ($r = 0.36–0.69$) and winter ($r = 0.29–0.58$). High correlation coefficients ($r = 0.45–0.58$) were found in the summer season between head length and head area vs. concentration and total number of sperm per ejaculate. These correlations were statistically significant. Correlation coefficients between sperm dimensions and concentration and total number of sperm per ejaculate in spring were low and nonsignificant.

4. Discussion

Based on these data, one may conclude that the physical characteristics of ejaculates and sperm cell morphology in Hypor boars are significantly varied depending on the season. Some authors suggest that the effect of season on semen traits should be considered in the context of such factors as day length and air temperature and humidity (1,12). This effect may vary depending on the breed or the sire, geographic location, and the degree of adaptation of the animal to different climate conditions. Studies on mammals revealed that with the shortening daylight in the autumn season, physiological changes appear that

Table 2. Effect of season on morphometric characteristics of spermatozoa in Hypor boars.

Specification	Season			
	Winter	Spring	Summer	Autumn
Number of ejaculates (n)	32	30	21	31
Head length (μm)	9.03 ± 0.57^{ab}	9.29 ± 0.35^{b}	9.12 ± 0.43^{ab}	8.97 ± 0.61^{a}
Head width (μm)	4.86 ± 0.40^{ab}	5.02 ± 0.28^{b}	4.86 ± 0.32^{ab}	4.77 ± 0.30^{a}
Perimeter of the head (μm)	23.45 ± 1.24^{ab}	24.02 ± 1.15^{b}	23.44 ± 1.09^{ab}	23.26 ± 1.15^{a}
Head area (μm^2)	39.19 ± 4.73^{ab}	41.52 ± 3.28^{b}	38.97 ± 4.27^{ab}	37.57 ± 4.73^{a}
Flagellum length (μm)	43.49 ± 1.22^{a}	43.67 ± 1.62^{a}	43.42 ± 1.38^{a}	43.61 ± 0.70^{a}
Total length (μm)	52.67 ± 1.25^{a}	52.96 ± 1.72^{a}	52.59 ± 1.43^{a}	52.58 ± 0.93^{a}

Values are expressed as means ± SD. Means with different letters in a row are significantly different ($^{a, b}$: $P \leq 0.05$).

Table 3. Effect of season on the frequency of normal and abnormal spermatozoa in Hypor boars.

Specification	Season			
	Winter	Spring	Summer	Autumn
Number of ejaculates (n)	32	30	21	31
Sperm with major abnormalities (%)	1.55 ± 2.20[a]	1.58 ± 2.38[a]	1.08 ± 0.77[a]	0.80 ± 0.60[a]
Sperm with minor abnormalities (%)	2.87 ± 2.86[ab]	2.72 ± 2.16[ab]	3.89 ± 2.93[b]	2.52 ± 2.35[a]
Percentage of normal spermatozoa (%)	95.58 ± 3.95[a]	95.76 ± 3.58[a]	95.09 ± 3.18[a]	96.65 ± 2.40[a]

Values are expressed as means ± SD. Means with different letters in a row are significantly different ([a, b]: $P \leq 0.05$).

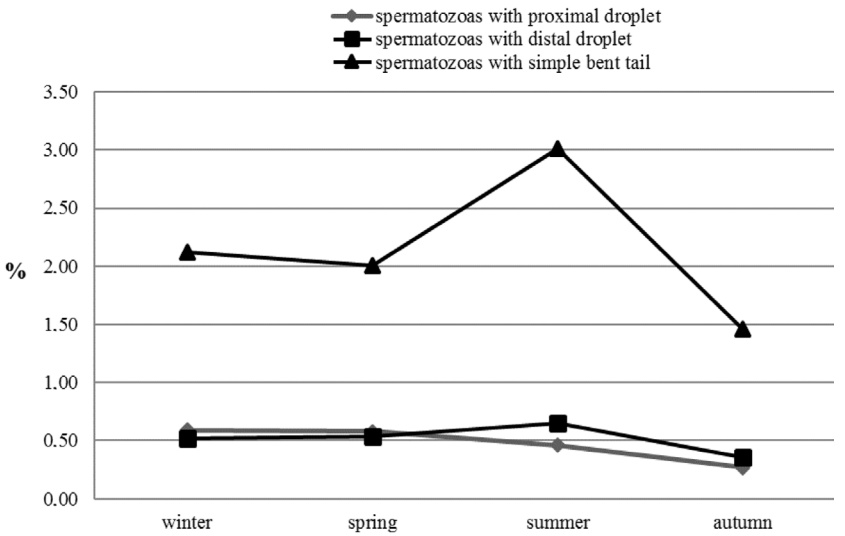

Figure. Frequency of spermatozoa with selected abnormalities of morphology depending on the season of the year.

stimulate reproductive functions in males and promote sperm production (13). Auvigne at al. (14) presented the view that reproductive seasonality in the boar is a feature that has been inherited from its ancestor, the European wild boar (*Sus scrofa ferus*).

Domestic pigs exhibit intensified sexual activity during the natural heat period in wild boars and conversely show reduced sexual activity if the period is unfavorable for wild pig reproduction. Application of lighting programs in spring results in enhanced testosterone production in the serum of domestic boars, up to the level usually observed in autumn and winter, when the best semen is mostly produced (15). Changes in daylight duration affect the secretory activity of the pineal gland and the amount of melatonin produced, which in mammals determines the seasonal changes in the reproductive processes (16). Under a declining day photoperiod, porcine spermatozoa exhibit poorer motility (17). At that time, the percentage of sperm cells with morphological abnormalities also increases. The results demonstrated that the photoperiod in different seasons affected sperm concentration and sperm motility. The study showed that as the day lengthened the sperm concentration in the ejaculate decreased. The best ejaculates were obtained in the winter and spring, and thus when the day was relatively short. These ejaculates had the highest sperm concentration and sperm motility and yielded the most insemination doses. A study by Sancho et al. (17) showed that as the day lengthens the percentage of morphologically normal sperm increases. In our study the highest percentage of sperm with minor abnormalities was noted in summer, and thus during the period when the day was longest. Thus, the data obtained in the present study do not confirm the observations of Sancho et al. (17). Small ejaculate volumes of semen collected in spring were found in crossbred pigs (2).

Elevated temperature may have an adverse effect on spermatogenesis. This results in degeneration of the seminiferous epithelium in the testes (18) and a

Table 4. Effect of season on Pearson's correlation coefficients between ejaculate parameters and morphometry of spermatozoa.

Correlation coefficients	Season of year			
	Winter	Spring	Summer	Autumn
Sperm concentration/head length	0.52*	0.10	0.58*	0.69*
Sperm concentration/head width	0.29*	–0.24	0.27	0.36*
Sperm concentration/perimeter of the head	0.37*	0.01	0.41	0.59*
Sperm concentration/head area	0.56*	0.12	0.48*	0.55*
Sperm concentration/flagellum length	–0.17	0.13	–0.24	–0.14
Sperm concentration/total length	0.09	0.15	–0.05	0.35
Total number of spermatozoa/head length	0.58*	0.07	0.48*	0.63*
Total number of spermatozoa/head width	0.42*	0.15	0.35	0.53*
Total number of spermatozoa/perimeter of the head	0.44*	–0.01	0.31	0.47*
Total number of spermatozoa/head area	0.48*	–0.26	0.45*	0.63*
Total number of spermatozoa/flagellum length	0.00	0.07	0.00	–0.15
Total number of spermatozoa/total length	0.29	0.08	0.11	0.29

*: $P \leq 0.05$.

reduced concentration of sperm cells in the discharged ejaculate. In this study we demonstrated that ejaculates collected in summer and early autumn, and so at higher air temperatures, were significantly lower in terms of the concentration of sperm. Increasing sperm concentrations were observed from the beginning of the winter season, when the temperature dropped considerably, which had a positive effect on the process of spermatogenesis. Air temperature may also affect sperm motility. In this study, an increase in sperm progressive motility was observed during spring and summer, followed by a substantial ($P \leq 0.05$) decline during autumn and winter. Similar observations were made by Sarder et al. (19) and Tomar and Gupta (20). Jasko et al. (21) also reported the lowest motility during winter. It emerged in the studies by Cheon et al. (22) that sperm motility was higher during summer in comparison to other seasons. Wettemann et al. (23) detected that sperm motility dropped from 79.5% down to 46.4% under increased air temperature. Stone (24) noted that a rise of boar housing facility air temperature from 20 °C to 40 °C caused reduction in motility from 93% to 19%. Nevertheless, seasons of the year had no compelling effect on sperm motility as reported by Mathevon et al. (25). The increase in temperature in summer is not conducive to reproduction in pigs. During that time, reduced male sexual activity is observed (26). Flowers (27) observed a lower number of spermatozoa in semen collected in summer months compared to other parts of the year. In this study the relatively high semen quality observed during spring and summer suggested that Hypor boars can tolerate the high temperature during this seasons.

This study demonstrated that season of the year affects sperm cell morphometrics in Hypor boars. Sperm cells of semen collected in spring had longer and wider heads compared to those collected in autumn. Significant differences were also found in the perimeter and surface area of the head between sperm cells in spring and autumn. Hypor boars' sperm cells collected in spring were slightly longer compared to those in other seasons. These differences were, however, nonsignificant. This would confirm the hypothesis, as the sperm cells from the spring had larger dimensions (28). According to Gomendio and Roldan (29) and Mossman et al. (30), longer mammalian spermatozoa are more competitive in terms of fertilization. These authors proved this length to be positively correlated with the maximum velocity of the sperm. A relationship was also found between head dimensions and fertility (31). According to Hirai et al. (32), boars attaining higher fertility produce sperm cells with smaller and shorter heads compared to boars whose fertilizing efficiency is lower. The results of the present study indicate that spermatozoa from the ejaculates collected in the autumn had the smallest and shortest heads. Autumn is the period of the highest fertility in boars and the most efficient reproduction in pigs (15). Thus, the data obtained indicate a link between sperm head dimensions and male fertility.

This study showed that the frequency of sperm morphological abnormalities in the analyzed semen

ranged from about 1% to approximately 4%. We also found that most major malformations occurred in spring, whereas the highest percentage of minor changes was found in summer (3.89%), when ambient temperatures are definitely higher compared to other seasons. Soderquist et al. (33) observed most sperm cells showing head and flagellum abnormalities in spring and summer. Sancho et al. (17), who compared porcine ejaculates collected in spring and in autumn, observed that as the daylight period extends, the percentage of morphologically normal spermatozoa increases. Semen collected in summer, when temperatures are high, contain more spermatozoa with morphological abnormalities (34). An increase in ambient temperatures reduces testicular thermoregulatory capacity and leads to disturbances in the process of spermato- and spermiogenesis. Levels of testosterone and androsterone also decrease in summer, which disturbs sperm production processes. The highest level of androgenic hormones, which promote sperm quality, is observed in autumn (35). This study demonstrated that the least major and minor malformations occurred in autumn. Sancho et al. (17) reported that the percentage of spermatozoa with proximal droplets was higher in autumn than in spring, while the frequency of spermatozoa with distal droplets was higher during spring in comparison to autumn.

In conclusion, the effect of year season on ejaculate traits and the sperm morphological picture in Hypor boars is clear and significant. In the spring and winter months, discharged ejaculates have higher sperm cell concentrations as compared with autumn and summer. Ejaculates collected in spring contained the highest number of sperm cells. Spermatozoa with the highest motility occur in the spring and summer months. Ejaculates collected in winter produce the highest number of insemination doses. Sperm cells in spring have the largest heads. The spermatozoa from ejaculates collected in the autumn had the smallest and shortest heads, which may be linked to the high fertility of boars during this period. The quality of the analyzed semen was very high, and the percentage of sperm cells with morphological abnormalities in summer did not exceed 3.89%. Significant correlations were observed between sperm concentration and total number of sperm in ejaculates vs. morphometric dimensions of sperm heads in Hypor boars' semen in the autumn, winter, and summer seasons.

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