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Changes of sperm morphology in Duroc, Landrace and Large White boars depending on the ambient temperature during the year

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Abstract: Utilisation of artificial insemination has led to the increased role of male animals. They significantly contribute to the efficiency of productivity; therefore, sperm quality has been emphasised. The aim of this study was to analyse changes in the boar sperm morphology during the year in forty-two Duroc, Landrace and Large White boars in the Czech Republic. For each boar, a spermiogram was evaluated every month in 2018. The number of morphologically abnormal spermatozoa was recorded every month to evaluate the morphology. The abnormalities were categorised as abnormalities of the head, abnormalities of the acrosome, proximal cytoplasmic droplets and abnormalities of the tail. Throughout the study, the temperature in the stable was monitored. Changes in the sperm morphology were recorded in all breeds during the year, the changes were different for each breed. In the Landrace boars, the highest concentrations of morphologically abnormal spermatozoa were observed in July and August ($P < 0.05$). In this period, the concentration of the tail defects increased ($P < 0.05$), while, in October, a higher concentration ($P < 0.05$) of spermatozoa with defects of the acrosome was recorded in the Landrace boars. In the Duroc and Large White boars, the highest morphologically abnormal spermatozoa values were observed in September and October ($P < 0.05$). In the Duroc boars, the increase in the morphologically abnormal spermatozoa in this period was mainly caused by an increased occurrence of proximal cytoplasmic droplets ($P < 0.05$). On the contrary, in the Large White boars, the increase was related to a higher incidence of tail defects ($P < 0.05$). These results show that changes in the sperm morphology are influenced by the ambient temperature and the breed.

Keywords: ambient temperature; boar; breed; morphological anomalies; sperm

Modern technologies enable a thorough analysis of semen in order to evaluate its suitability for further utilisation. Only semen of an appropriate quality can be used for the production of insemination doses. Intracervical insemination is still the most frequently used insemination method. Nevertheless, other methods are mentioned more and more often, such as the post-cervical insemination method (Watson and Behan 2002), which allows a reduction in the sperm dose volume without compromising the fertilisation efficiency, or the method of insemination at a fixed time with only one dose applied at the correct time, instead of the original two or three doses per sow (Bortolozzo

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et al. 2015). These trends suggest that the artificial insemination will be characterised by lower sperm doses with a higher emphasis on their quality.

Pigs, just like other homeothermic animals, keep a constant body temperature in changing ambient conditions by regulating the heat loss and heat production (Renaudeau et al. 2006). Animals feel the most comfortable when the ambient temperature is in their thermal comfort zone, when the animals feel neither cold nor hot. The higher the ambient temperature exceeds their comfort zone, the more difficult it is for the pigs to keep cool. Due to a limited ability to perspire, the pigs ventilate to increase the heat loss by respiration. As the temperature exceeds the evaporating critical temperature, a point called the upper critical temperature is reached. At this point, the heat loss by respiration and perspiration is the highest and, if it is not sufficient, the pig is no longer able to regulate its increasing body temperature (Lorschy 2005). Controlling the microclimate in a stable enables one to ensure the optimal temperature for the animals, however, at extremely high outdoor temperatures, which have been more and more common lately, even cooling systems in stables do not prevent an increase in the indoor temperature above the required levels, which causes stress for the animals. Heat stress can affect the primary reproductive function impacting the boar's ejaculate quality or causing morphological changes in the spermatozoa. The occurrence of morphologically abnormal spermatozoa is a problem of insemination stations all over the world. The concentration of morphologically abnormal spermatozoa in semen correlates with its fertilisation ability. According to Collodel and Moretti (2006), a strict evaluation of the sperm morphology has an important prognostic value in assisted reproduction. Spermatozoa with abnormal morphology are negatively reflected in conception rates (Wysokinska and Kondracki 2014), in the lower quality of the embryos (Pena et al. 2017), embryonic deaths and abortions in the early stages of pregnancy (Chenoweth 2005) and, consequently, also, in lower litter sizes (Nagy et al. 2018).

Starting in puberty, the production of sperm cells is a continuous process. During maturation of the spermatozoa in Sertoli cells, which lasts approximately 34–36 days, the sperm cells change their shape from a round head to an oval-shaped head and gain a tail. After the sperm cells leave the testicles, they enter the epididymis for final maturation lasting 9–14 days. The overall process from beginning of the sperm development to their presence in the ejaculate takes approximately 45 days. The occurrence of the morphologically abnormal spermatozoa, thus, suggests alterations during the sperm development or maturation or an improper manipulation with the semen (Okere et al. 2005), so the effect of adverse factors may not be detected until 6–8 weeks from the onset of its action.

The aim of this study was to evaluate the morphological changes in boar spermatozoa depending on the ambient temperature during one year in the breeds used most frequently in the Czech Republic.

MATERIAL AND METHODS

Methodology of the experiment

The observation of changes in the boar sperm morphology was performed at an insemination station in the Czech Republic located at an altitude of 465 m above sea level. A total of forty-two boars were evaluated, of which fourteen boars of the Duroc breed (D), fourteen boars of the Landrace breed (L) and fourteen boars of the Large White breed (LW). The age of the boars at the beginning of observation was 18–24 months to eliminate the effect of age.

The temperature in the stable was measured and recorded with a Voltcraft DL-121TH (Hirsham, Germany) device throughout 2018. The thermometer was placed one meter above the floor. The temperature was recorded every hour, the average temperature was calculated and the highest temperature for each day was recorded.

Housing conditions of the experimental animals

The breeding boars were kept in equal conditions in terms of housing, feeding and caretaking in compliance with the Council Directive EU 2008/120/EC. The boars were stabled in individual pens of 8 m^2 area with a concrete floor and they were fed with a commercial feed mixture KA for breeding boars in a dose of 3.3 kg/boar/day. The metabolisable energy content was 12.6 MJ/kg. The water for drinking was available *ad libitum*. The animals were covered by a routine disease prevention programme and

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regular veterinary care. The stable was not equipped with an automatic microclimate control. The relative humidity was close to 75% (± 10%).

Analysis of sperm morphology

Semen was collected once per week in a collection room by the manual collecting method. Once per month (always in the middle of month), between January and December 2018, native semen of each boar was used to prepare a spermiogram that was dyed according to the method of Cerovsky (1976) and evaluated microscopically at × 1 500 magnification using oil immersion. The morphological analysis was conducted by one trained person only. All the boar semen samples met the minimum standards of 80% motility, a minimum of a 100 ml volume of the sperm-rich fraction and 150 000 sperm cells/mm^3. A total of 200 sperm cells were evaluated for each spermiogram. Individual defects were recorded using the DeSMA software v1.0.5.0 (VRI 2015). The specification of the defects was an important parameter, each defect was therefore calculated separately, even if more defects were present on one sperm. The sperm cells were classified as follows: normal (without morphological defects) and abnormal (with a morphological defect). The morphologically abnormal sperm cells (MAS) were then categorised according to the place and character of the defect to: spermatozoa with a defect of the head (detached heads, head shape and size defects), spermatozoa with defects of the acrosome (condensed, released, no acrosome), proximal cytoplasmic droplets (PCDs) in the neck area and tail defects (bent, contorted, coiled tail). The resulting values were converted to percentages.

Statistical analysis

The resulting data were statistically evaluated using STATISTICA software, v12.0. The results were expressed as an arithmetic mean ± standard error. A mixed model with a repeated measure statement was used with the month as a permanent effect and the breed was used as a random effect. The significance of the differences between the breeds and months was calculated based on Tukey's multiple range test. The level of statistical significance was set at $P < 0.05$.

RESULTS

Figure 1 presents the average and maximum temperatures in the stable during the observation in 2018. It is evident that, from January to April, the temperature ranged between 10 °C and 15 °C, from May, it started to approach 20 °C. From June to August, the range of the average temperature was 20–25 °C. The maximum temperature measured in June was 27 °C, 29 °C in July and 31 °C in August. Between mid-September and mid-October, the temperature dropped to 15–20 °C, from the end of October to December, the temperature ranged between 11 °C and 15 °C.

The statistical analysis confirmed a significant impact of the month for all the evaluated parameters. A significant impact of the breed was recorded for the MAS, acrosome defects, PCD and tail defects. No interaction was observed between the evaluated factors (Table 1).

Detailed results of the effects of the observed factors on the MAS concentration are shown in Table 2. Significant differences in the MAS concentration in the semen during the year were ob-

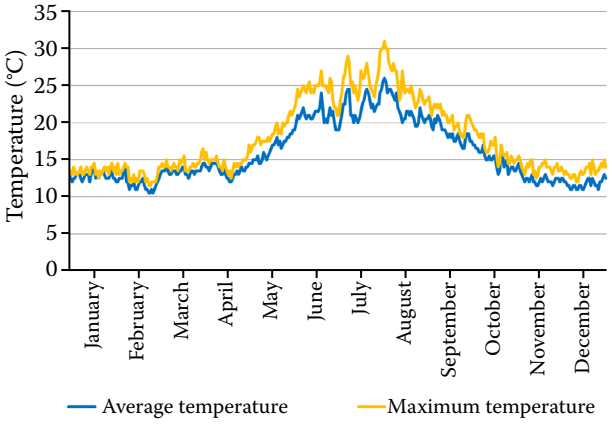

Figure 1. Measured average and maximum daily temperatures in the stable during observation

Table 1. Influence of the month and breed on the individual monitored parameters (*P*-value)

Factor	Parameter				
	MAS	acrosome defects	head defects	PCD	tail defects
Month	0.040	0.026	0.035	0.003	0.035
Breed	< 0.001	0.048	0.253	0.004	0.039
Month × breed	0.852	0.658	0.816	0.755	0.627

MAS = morphologically abnormal spermatozoa; PCD = proximal cytoplasmic droplet

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Table 2. The effect of the month and breed on the percentage of morphologically abnormal spermatozoa

Factor	MAS (%)		
Month	Duroc	Landrace	Large White
January	6.6 ± 1.28^{ab}	6.8 ± 0.94^{ab}	7.4 ± 1.03^{ab}
February	6.8 ± 0.94^{ab}	6.5 ± 1.53^{ab}	7.0 ± 0.89^{a}
March	6.4 ± 1.25^{ab}	6.9 ± 1.57^{ab}	7.3 ± 1.26^{ab}
April	6.8 ± 1.30^{ab}	7.3 ± 0.61^{ab}	7.9 ± 0.78^{ab}
May	6.2 ± 1.08^{a}	7.3 ± 1.31^{ab}	8.0 ± 1.28^{ab}
June	7.1 ± 1.03^{ab}	7.2 ± 1.37^{ab}	8.0 ± 1.21^{ab}
July	8.8 ± 1.04^{ab,x}	12.2 ± 1.14^{b,y}	9.3 ± 1.15^{ab,xy}
August	9.3 ± 1.19^{bc}	10.1 ± 1.20^{b}	11.5 ± 1.36^{ab}
September	10.4 ± 2.44^{bc}	9.6 ± 2.07^{ab}	12.6 ± 1.54^{b}
October	11.9 ± 1.26^{c,y}	9.3 ± 1.46^{ab,x}	11.4 ± 2.06^{ab,xy}
November	9.0 ± 1.32^{b}	8.9 ± 1.27^{ab}	9.4 ± 1.27^{ab}
December	8.0 ± 1.48^{ab}	6.5 ± 0.75^{a}	9.2 ± 1.12^{ab}

MAS = morphologically abnormal spermatozoa
$^{a–c}$Statistically significant difference between the months (values in the columns) for the specific breed with $P < 0.05$; ^{x,y}Statistically significant difference between the breeds (values in the rows) for the specific month with $P < 0.05$

served for all the breeds. For each breed, the lowest and the highest values were recorded in different months. For the L and LW boars, the lowest MAS concentrations were found in February ($P < 0.05$), for the D boars, it was in May ($P < 0.05$).

The highest MAS concentrations for the L boars were recorded in July and August ($P < 0.05$), and, on the contrary, for D and LW boars, the concentration did not increase until the autumn months ($P < 0.05$).

The results of the effect of the breed and month on the occurrence of head defects are shown in Table 3. A significant difference between the values during the year was found only for the D boars with a significant increase in the sperm cells with a morphologically abnormal head between June and July ($P < 0.05$). For the L and LW boars, there were no significant differences in the occurrence of these defects ($P < 0.05$).

The concentrations of the spermatozoa with acrosome defects in the observed animals in the individual months are presented in Table 4. For the D boars, there were no significant fluctuations ($P < 0.05$) during the year. The highest occurrence of abnormal acrosome was found in the LW boars in August in comparison with the other breeds and the other months of the year ($P < 0.05$).

Table 3. The effect of the month and breed on the percentage of spermatozoa with head defects

Factor	Head defects		
Month	Duroc	Landrace	Large White
January	2.7 ± 0.66^{ab}	1.8 ± 0.36	2.4 ± 0.44
February	2.5 ± 0.44^{ab}	1.6 ± 0.58	2.0 ± 0.45
March	2.0 ± 0.56^{ab}	1.6 ± 0.35	2.0 ± 0.50
April	2.0 ± 0.42^{ab}	2.0 ± 0.41	1.8 ± 0.42
May	1.3 ± 0.36^{ab}	1.8 ± 0.48	2.4 ± 0.71
June	1.2 ± 0.36^{a}	2.3 ± 0.59	1.9 ± 0.57
July	2.8 ± 0.34^{b}	2.2 ± 0.56	2.0 ± 0.36
August	2.0 ± 0.63^{ab}	2.0 ± 0.52	3.0 ± 1.15
September	1.7 ± 0.49^{ab}	2.5 ± 0.56	2.4 ± 0.60
October	2.4 ± 0.50^{ab}	2.4 ± 0.37	1.9 ± 0.48
November	2.3 ± 0.86^{ab}	2.9 ± 0.53	2.1 ± 0.36
December	2.2 ± 0.52^{ab}	2.5 ± 0.41	2.6 ± 0.60

^{a,b}Statistically significant difference between the months (values in the columns) for the specific breed with $P < 0.05$

The changes in the occurrence of spermatozoa with proximal cytoplasmic droplets are presented in Table 5. Significant differences between the months were observed in the D boars. For this breed, the highest values were recorded in September and October ($P < 0.05$). A significant difference between the breeds was found in October. The semen of the

Table 4. The effect of the month and breed on the percentage of spermatozoa with acrosome defects

Factor	Acrosome defects		
Month	Duroc	Landrace	Large White
January	0.6 ± 0.20	0.4 ± 0.23^{ab}	0.6 ± 0.25^{ab}
February	0.9 ± 0.49	0.2 ± 0.17^{ab}	0.3 ± 0.18^{a}
March	0.4 ± 0.22	0.0 ± 0.00^{a}	0.5 ± 0.25^{ab}
April	0.6 ± 0.18	0.3 ± 0.16^{ab}	0.4 ± 0.25^{ab}
May	0.1 ± 0.07	0.1 ± 0.06^{ab}	0.7 ± 0.23^{ab}
June	0.2 ± 0.09	0.5 ± 0.28^{ab}	0.3 ± 0.18^{a}
July	0.5 ± 0.22	0.3 ± 0.20^{ab}	0.4 ± 0.18^{ab}
August	0.1 ± 0.06^{x}	0.4 ± 0.15^{ab,xy}	1.5 ± 1.04^{b,y}
September	0.5 ± 0.25	1.2 ± 0.46^{ab}	0.8 ± 0.41^{ab}
October	0.3 ± 0.13	1.3 ± 0.56^{b}	0.5 ± 0.45^{ab}
November	0.5 ± 0.25	0.5 ± 0.26^{ab}	0.5 ± 0.26^{ab}
December	0.6 ± 0.34	0.3 ± 0.18^{ab}	0.5 ± 0.34^{ab}

^{a,b}Statistically significant difference between the months (values in the columns) for the specific breed with $P < 0.05$; ^{x,y}Statistically significant difference between the breeds (values in the rows) for the specific month with $P < 0.05$

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Table 5. The effect of the month and breed on the percentage of spermatozoa with proximal cytoplasmic droplets

Factor	Proximal cytoplasmic droplet		
Month	Duroc	Landrace	Large White
January	2.4 ± 0.65^{a}	2.2 ± 0.50	3.0 ± 0.84
February	2.4 ± 0.62^{a}	1.6 ± 0.56	2.8 ± 0.66
March	2.3 ± 0.69^{a}	1.9 ± 0.83	2.8 ± 0.94
April	2.8 ± 1.18^{ab}	2.4 ± 0.35	3.1 ± 0.69
May	2.8 ± 0.69^{ab}	1.8 ± 0.44	2.9 ± 0.71
June	2.5 ± 0.62^{ab}	1.8 ± 0.69	3.3 ± 0.81
July	2.8 ± 0.69^{ab}	3.3 ± 1.17	4.7 ± 1.00
August	3.9 ± 0.85^{ab}	3.8 ± 1.35	5.1 ± 1.38
September	5.5 ± 1.09^{b}	2.8 ± 1.02	4.0 ± 0.84
October	5.6 ± 0.99^{b,y}	2.9 ± 0.60^{xy}	3.6 ± 0.76^{x}
November	3.1 ± 0.83^{ab}	2.9 ± 0.59	3.8 ± 1.25
December	2.8 ± 0.85^{ab}	1.8 ± 0.60	3.1 ± 1.01

^{a,b}Statistically significant difference between the months (values in the columns) for the specific breed with $P < 0.05$; ^{x,y}Statistically significant difference between the breeds (values in the rows) for the specific month with $P < 0.05$

Table 6. The effect of the month and breed on the percentage of spermatozoa with tail defects

Factor	Tail defects		
Month	Duroc	Landrace	Large White
January	2.0 ± 0.34	3.5 ± 0.77^{ab}	2.8 ± 0.90^{ab}
February	2.5 ± 0.67	3.6 ± 0.98^{ab}	2.6 ± 0.80^{a}
March	2.0 ± 0.69	3.6 ± 0.95^{ab}	3.4 ± 0.95^{ab}
April	2.3 ± 0.62	4.0 ± 0.50^{ab}	3.3 ± 0.81^{ab}
May	2.1 ± 0.66	3.8 ± 1.28^{ab}	3.4 ± 0.97^{ab}
June	3.5 ± 0.82	3.6 ± 0.85^{ab}	2.8 ± 0.91^{ab}
July	2.8 ± 0.76	6.7 ± 1.90^{b,y}	3.1 ± 1.02^{x}
August	3.4 ± 0.96	4.6 ± 0.88^{ab}	3.8 ± 1.13^{ab}
September	3.3 ± 1.84^{x}	4.0 ± 1.73^{ab,xy}	6.5 ± 1.46^{b,y}
October	4.1 ± 0.90	3.7 ± 1.05^{ab}	5.4 ± 1.45^{ab}
November	3.6 ± 1.06	3.5 ± 0.76^{ab}	3.9 ± 0.93^{ab}
December	3.1 ± 1.38	2.3 ± 0.53^{a}	3.4 ± 0.74^{ab}

^{a,b}Statistically significant difference between the months (values in the columns) for the specific breed with $P < 0.05$; ^{x,y}Statistically significant difference between the breeds (values in the rows) for the specific month with $P < 0.05$

LW boars contained a significantly lower percent concentration of PCD than the semen of the D boars ($P < 0.05$).

Table 6 presents the effect of the breed and month on the occurrence of tail defects. It is noticeable that the differences in the percentage concentration of this defect were found only in the L and LW breeds, while there were no significant differences for the D breed. The highest concentration of tail defects was observed in July for the L boars and in September for the LW boars ($P < 0.05$).

DISCUSSION

The study evaluated the relationship between the occurrence of abnormal sperm cells in boars, their breed and the temperature in the stable. The results reveal that the concentration of the morphological defects depend on the breed and ambient temperature during the year. In the L boars, the MAS values started to increase in July and this growth was mainly caused by an increased concentration of spermatozoa with tail defects. On the contrary, in the LW and D boars, increased MAS values did not appear until September. The D boars showed a higher percent concentration of spermatozoa with PCD during the autumn months, while the semen of the LW boars contained more sperm cells with tail abnormalities. The concentration of acrosome abnormalities increased in August in the LW boars and in October in the L boars.

The seasonal occurrence of MAS can be commented on with lower concentrations in the first half of the year (January–June) and higher concentrations in the second half of the year (July–December). Similar tendencies were described by Lipensky et al. (2010), whose study was also performed on boars kept in the Czech Republic. Our study reported that the semen of the L boars contained more MAS in the summer than the semen of the D boars. On the contrary, studies of Okere et al. (2005) and Huang et al. (2000) present that the L boars have lower MAS concentrations in the summer than the D and the LW boars. Data on the range of the thermal comfort zone for boars differ depending on the climatic zone. According to Horky et al. (2015) and Hansen (2009), the upper limit of the thermal optimum for boars kept in the conditions of Central Europe is 25 °C. The results of our study show that the L boars are the most sensitive to this temperature, with the onset of an increased occurrence of abnormalities in July, i.e., one month after the temperature in the stable reached 25 °C. The results indicate that the LW and the D boars are more resistant to high tempera-

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tures, since they showed increased MAS concentrations later on, one month after the temperatures rose to 28–30 °C. This assumption can be related to the conclusions of Stone (1982), who state that the critical temperature for the LW boars is 29 °C, above this temperature, the boars are no longer able to produce normal spermatozoa. As regards to the period with the lowest MAS concentrations, for the L and the LW boars, it was winter, which corresponds to the findings of Petrocelli et al. (2015).

In the present study, higher concentrations of the acrosome abnormalities were observed in August for the LW boars and in October for the L boars. According to Oyeyemi and Ubiogoro (2005), the abnormalities of acrosome are caused by a defect in the spermatogenesis, which suggests that this defect will occur in the ejaculate with a time delay from the onset of high temperatures. The percentage of spermatozoa with acrosome defects increased in the LW boars after two months from the onset of temperatures around 25 °C, in the L boars, this did not happen until two months from the onset of temperatures between 28 °C to 30 °C. This finding confirms that limits of the thermal optimum and sensitivity to high temperatures differ in different pig breeds. Cassuto et al. (2012) state that during spermatogenesis, adverse factors including a high ambient temperature can influence the chromatin condensation and DNA integrity, which results in abnormal head shapes. Therefore, increased concentrations of head defects caused by a high ambient temperature should be expected. In the present study, this hypothesis was confirmed only for the D boars with an increased concentration of head defects observed in July. The cytoplasmic droplet is an organelle formed during the last (maturation) phase of spermatogenesis (Aitken 2004). Its normal morphology and localisation correlates to the motility potential during maturation in the epididymis, thus, to the period of passage of the spermatozoa from the caput to the corpus epididymis (Cooper and Yeung 2003) and the increased occurrence of PCD is due to a failure of the normal maturation in the epididymis (Cooper 2005). According to Lovercamp et al. (2007), this defect may originate in abnormal spermatogenesis during the months with high temperatures. The heat stress during spermatogenesis can affect the germinal cells in the testicles, which could explain the increased concentration of PCD in the D boars after two months from the onset of temperatures above 28 °C. Sancho et al. (2004) also stated an increased occurrence of PCD in the autumn months. On the contrary, the study of Andersson et al. (1998) showed an increased concentration of proximal droplets in the spring and summer. According to Briz et al. (1996), tail abnormalities are formed during the passage of the sperm through the epididymis, due to a disrupted construction of tail fibrils (Chemes et al. 1998) and the effect of adverse factors can be expected after the onset of high temperatures. In this study, such an assumption was confirmed in the L boars, for which the percentage of the spermatozoa with tail defects increased immediately in July, when temperatures exceeded 25 °C.

The above-mentioned results of our study and the studies of other authors indicate that the ambient temperature affects the spermatogenesis and sperm maturation in the epididymides in some boar breeds, which may be negatively reflected in the sperm morphology. According to Setchell (2006), the processes of spermatogenesis and the subsequent sperm maturation are very sensitive to heat. As stated by Momen et al. (2010), an impaired spermatogenesis may be associated with the increased temperature of the testes and epididymides. Setchell (2006) distinguishes between the direct effect of heat on the testicles (on specific cell types in the testicles) and the indirect effect of heat on the testicles (a result of cellular changes in the testicles caused by heating).

The data presented in this study show that the sperm morphology differs depending on the breed and ambient temperature, which may indicate a different sensitivity of the boars of different breeds to high temperatures. In the Landrace boars, the concentration of morphologically abnormal spermatozoa and tail defects increased in July, one month after the ambient temperatures increased above 25 °C. In the Large White and Duroc boars, the concentration of morphologically abnormal spermatozoa increased in August and September, which is one month after onset of the temperatures in the range of 28–30 °C, although the boars were not exposed to these temperatures very long. In the Large White boars, August and September were characterised by increased tail defects, in the Duroc boars, the concentration of the spermatozoa with proximal cytoplasmic droplets increased. Taking these seasonal and interbreed variations into account may help to ensure the stable and continuous production of quality insemination doses throughout the year.

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Conflict of interest

The authors declare no conflict of interest.

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