

# Linear growth model analysis of factors affecting boar semen characteristics in Southern China<sup>1</sup>

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**ABSTRACT:** This study investigated the factors affecting the semen traits of boars in Southern China. A total of 172,408 ejaculates of boars from 9 AI centers were collected from January 2013 to May 2016. A linear growth model was used to analyze the effects of level 1 (boar breed, age, season, and boar age at herd entry) and level 2 (housing type) factors on semen quality. The intraclass correlation coefficients of semen volume, total sperm number, functional sperm number, sperm concentration, motility, and abnormal sperm were 0.62, 0.62, 0.61, 0.60, 0.54, and 0.70, respectively. Boars reared in ordinary houses had lower total and functional sperm numbers than those reared in air filtration houses ( $P < 0.05$ ). The functional sperm number of Duroc boars was

lower than that of Landrace and Yorkshire boars ( $P < 0.05$ ). The total and functional sperm numbers were lowest from May to September and peaked at the age of 34.1 and 37.7 mo, respectively. Furthermore, boars aged 8 and 9 mo at herd entry had greater functional sperm numbers than those aged 5, 6, 7, and 12 mo at herd entry ( $P < 0.05$ ), whereas no significant difference was observed between boars aged 8 mo and boars aged 9 mo at herd entry ( $P > 0.05$ ). In conclusion, the linear growth model is suitable for longitudinal data analysis. To improve boar breeding, sunstroke prevention in the early spring should be given greater attention. Importantly, 8 mo appears to be the most suitable age for boar introduction, especially for Duroc boars.

**Key words:** boar, influencing factor, linear growth model, semen characteristics

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## INTRODUCTION

Semen volume and sperm concentration, motility, and morphology affect sow litter size and the economic profitability of boar herds (Flowers, 2002; Robinson and Buhr, 2005). However, poor semen

quality, which is the main reason for boar culling, decreases the longevity of boars by 12.3 mo (Knox et al., 2008; Wang et al., 2017). Therefore, for boar production, it is vital to ascertain the factors influencing boar semen quality.

Regarding boar semen quality, there have been numerous studies of the effects of heritability (Smital et al., 2005; Wolf, 2009; Wolf and Smital, 2009), breed and season (Ciereszko et al., 2000; Savić et al., 2013; Knecht et al., 2014), age (Huang et al., 2010; Knecht et al., 2017b), testis size (Clark et al., 2003), nutrition (Wilson et al., 2004), and collection interval (Smital, 2009; Knecht et al., 2017a). However, few single studies have considered multiple influencing factors, and it may be difficult to simultaneously satisfy numerous conditions in actual production situations. On the other hand, although linear and animal models have been used to estimate some factors affecting semen traits (Smital, 2009; Wolf and Smital, 2009), these

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methods ignored the longitudinal quality of the semen production data and did not consider time dependency (Oh et al., 2006). Furthermore, there may be some other factors influencing semen traits, such as housing condition and age at herd entry, besides the factors mentioned above (Kunavongkrit et al., 2005; Savić et al., 2014).

Based on the above considerations, we hypothesized that the linear growth model, which can reduce the SE compared with the traditional estimation methods, could be used to analyze the hierarchical and longitudinal production data of boar semen (Goldstein et al., 2002). Therefore, this study used the linear growth model to investigate the effects of boar breed, age at collection, semen collection season, age at herd entry, and housing type on semen quality.

## MATERIALS AND METHODS

Animal Care and Use Committee approval was not obtained for this study because the data were obtained from an existing database of a single large integrated pork production company.

### *Herds and Animals*

This study was conducted in 9 AI centers owned by a single large integrated pork production company located in Southern China. The inventory for these AI centers comprised 50 to 1,000 boars, with purebred Duroc, Landrace, and Yorkshire boars the only 3 breeds raised in these AI centers. The nutritional level of the boar feed examined in the integrated pork production company was as follows: 3.25 Mcal/kg DE, 16% CP, 0.75% Lys, 0.55% Met + cystine, 0.80% total Ca, and 0.70% total phosphate. The 2 housing types in the 9 AI centers comprised either an individual pen without an air filtration pattern (ordinary house, 7 of the 9 AI centers, with dimensions from 2.0 by 2.5 m to 2.8 by 3.2 m) or an individual stall with an air filtration pattern (house with air filtration, 2 of the 9 AI centers, with dimensions from 0.7 by 2.0 m to 0.8 by 2.4 m). Furthermore, an Automated Production System (Automated Production Systems, Assumption, IL), which included a positive pressure ventilation system, automatic feeding system, and heating system, was used in an individual stall with an air filtration pattern to control the indoor environment. To present the climate characteristics, we chose Nanning as a representative city in Southern China. Daily temperature records were collected from January 2013 to May 2016. The Kingdee software (Kingdee International Software Group Co., Ltd., Shenzhen, P. R. China) and data recording system was used to record and store data in all AI centers.

### *Data Collection and Selection Criteria*

The producers in these AI centers were requested to submit their initial data on boar semen quality (from January 2013 to May 2016) to the College of Animal Science and Technology, Huazhong Agricultural University (Wuhan, P. R. China). For boar semen collection, sperm-rich ejaculate fractions were collected by the gloved-hand technique during the ejaculation of boars, and the gelatinous fraction was strained from the ejaculate through 4 layers of cotton-mesh gauze. Four basic semen traits were examined: semen volume, spermatozoa concentration, sperm motility, and abnormal spermatozoa. Because the 9 AI centers were owned by a single large integrated pork production company, the equipment for assessing semen quality was the same. The methods for evaluating boar semen quality were described in our previous study (Wang et al., 2016). Briefly, the semen volume was measured by weighing each ejaculate and considering 1 g of semen to be equal to 1 mL. A sperm density meter was used to estimate the sperm concentration of fresh semen (Fujihira Industry Co., Ltd., Tokyo, Japan). Computer-aided semen analysis with a phase-contrast microscope (ML-210JZ; Nanning SongJinTianLun Biological Technology Co., Ltd., Nanning, P. R. China) was used to examine sperm motility and morphology. In addition, the total sperm number and functional sperm number were calculated according to the formulae described by Smital et al. (2004) and Wolf and Smital (2009).

To obtain the representative data, we established some selection criteria for boar age at collection, semen volume, sperm concentration, sperm motility, percentage of abnormal sperm, and boar age at herd entry. First, ejaculates from boars younger than 8 mo of age or older than 50 mo were excluded from the data set. Then, we selected data on semen volume (from 50 to 600 mL), sperm concentration (from 0 to  $1 \times 10^9$ /mL), sperm motility, and percentage of abnormal sperm (from 0 to 99%). Additionally, boars with an age at herd entry from 5 to 12 mo and with complete information were also included in the final data set for analysis. Therefore, 172,408 ejaculates from 2,688 boars of the Duroc, Landrace, and Yorkshire breeds were included in the data analysis.

### *Statistical Analysis*

Based on the boar semen records, 7 factors were analyzed, including farm (divided into 2 housing types), boar breed, boar age at collection, semen collection date (ejaculate season), and boar age at herd entry. Housing type was considered a fixed high-level effect (level 2). The low level (level 1) factors included boar breed, boar age at collection, ejaculate season, and boar age at herd

**Table 1.** Intraclass correlation coefficients (ICC) of 6 boar semen traits

Item <sup>1</sup>	Variance sources			ICC
	Within-group variance	Between-group variance	Total variance	
VO	2,220.00	1,356.02	3,576.02	0.62
NO <sub>T</sub>	261.44	159.68	421.12	0.62
NO <sub>C</sub>	182.92	119.21	302.13	0.61
CO	4,168.21	2,721.74	6,889.95	0.60
MO	64.96	55.13	120.09	0.54
AB	44.24	19.13	63.37	0.70

<sup>1</sup>VO = semen volume; NO<sub>T</sub> = total sperm number; NO<sub>C</sub> = functional sperm number; CO = sperm concentration; MO = sperm motility; AB = abnormal sperm.

entry. Therefore, a 2-level linear growth model was constructed. The procedure can be described briefly as follows. First, the intraclass correlation coefficient (ICC) was calculated in the null model. Any significant within-group correlations in the null model indicated the presence of significant between-group variability. Therefore, a random intercept growth model was constructed. The next step incorporated the contextual variable (housing type). If the *t*-test was significant ( $P < 0.05$ ), the factors included in level 1 were incorporated one by one. To distinguish the difference at the 2 levels, we controlled for individual background covariates in the growth model. Considering the time effect, we also added time-varying covariates into the model. All analyses were completed in SAS (version 9.4; SAS Inst. Inc., Cary, NC). The parameters were estimated by using the REML method, and the residual variance/covariance was set as unstructured. Three information criteria—Akaike's information criterion, the finite-sample corrected version of Akaike's information criterion, and the Bayesian information criterion—were used for model testing. Therefore, the final model was as follows:

$$y_{ij} = \beta_{00} + \beta_{01}\text{var1} + \beta_{02}\text{var2} + \beta_{03}\text{var3} + \beta_{04}\text{var4} + \beta_{05}\text{var4} \times \text{var4} + \beta_{06}\text{var5} + (u_{0j} + e_{ij}),$$

in which  $y_{ij}$  is the value of the given *j*th housing type with the *i*th boar;  $\beta_{00}$  and  $\beta_{01}$  are the outcome measure of var1 under control at level 2; the coefficients of  $\beta_{02}$ ,  $\beta_{03}$ ,  $\beta_{04}$ ,  $\beta_{05}$ , and  $\beta_{06}$  represent the slopes of var2 to var5;  $\beta_{01}\text{var1} + \beta_{02}\text{var2} + \beta_{03}\text{var3} + \beta_{04}\text{var4} + \beta_{05}\text{var4} \times \text{var4} + \beta_{06}\text{var5}$  represent the fixed effects in the model; and  $u_{0j} + e_{ij}$  represent the residual effects.

**Table 2.** Type III tests of fixed effects

Item	Semen traits <sup>1</sup> ( <i>P</i> -value)					
	VO	NO <sub>T</sub>	NO <sub>C</sub>	CO	MO	AB
Level 2						
Housing type	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Level 1						
Breed	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Age	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Season	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Age at herd entry	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

<sup>1</sup>VO = semen volume; NO<sub>T</sub> = total sperm number; NO<sub>C</sub> = functional sperm number; CO = sperm concentration; MO = sperm motility; AB = abnormal sperm.

## RESULTS

### Model Testing

As presented in Table 1, the ICC of semen volume, total sperm number, functional sperm number, sperm concentration, sperm motility, and abnormal sperm in the linear growth model were 0.62, 0.62, 0.61, 0.60, 0.54, and 0.70, respectively. The results of the Type III Tests of Fixed Effects (SAS) are summarized in Table 2. The effects of housing type, boar breed, age at collection, semen collection season, and boar age at herd entry on semen traits were all significant in the linear growth model ( $P < 0.05$ ).

### Effects of Housing Type (Level 2) on Semen Traits

As indicated in Table 3, housing type had a significant impact on semen traits ( $P < 0.05$ ). Boars reared in an ordinary house had lower semen volume, total sperm number, and functional sperm number than those reared in a house with air filtration ( $P < 0.05$ ). Additionally, boars reared in an ordinary house also had more abnormal sperm than those reared in a house with air filtration ( $P < 0.05$ ).

### Effects of Boar Breed (Level 1) on Semen Traits

The results of breed effect on semen traits are presented in Table 4. The semen volume of Yorkshire boars was the highest followed by that of Landrace and Duroc boars, whereas the order of the sperm concentration in the 3 breeds was reversed ( $P < 0.001$ ). In addition, Duroc boars also had lower total and functional sperm numbers than Landrace and Yorkshire boars ( $P < 0.001$ ).

**Table 3.** Effect of housing type on boar semen traits

Item	Housing type		SEM <sup>1</sup>	P-value
	Ordinary house	House with air filtration		
No. of ejaculates	104,824	67,584		
Semen volume, mL	173.86	177.04	2.00	0.04
Sperm motility, %	83.6	83.8	0.39	0.4685
Abnormal sperm, %	12.5	12.0	0.24	0.04
concentration, 10 <sup>6</sup> /mL	273.6	279.3	2.82	0.02
Total sperm, 10 <sup>9</sup> /eja. <sup>2</sup>	47.2	48.5	0.69	0.03
Functional sperm, 10 <sup>9</sup> /eja.	35.4	36.4	0.59	0.04

<sup>1</sup>SEM = pooled SEM.<sup>2</sup>eja. = ejaculation.**Effect of Boar Age (Level 1) on Semen Traits**

As indicated in Fig. 1, the linear coefficients and quadratic coefficients of the 6 semen traits were all significant ( $P < 0.001$ ). The maximum values of semen volume, total sperm number, and functional sperm number were reached at the age of 30.2, 37.7, and 34.1 mo, respectively. Sperm motility of boars peaked at 18 mo. In addition, abnormal sperm levels increased after the age of 47 mo.

**Effect of Semen Collection Season (Level 1) on Semen Traits**

The results of seasonal effects on functional sperm number are shown in Fig. 2. The lowest functional sperm numbers were recorded in May, June, July, August, and September.

**Effect of Boar Age at Herd Entry (Level 1) on Semen Traits**

As presented in Table 5, the functional sperm number ranged from 31.8 to 39.1  $\times 10^9$ /ejaculation.

Boars aged 8 and 9 mo at herd entry had a greater functional sperm number than those aged 5, 6, 7, and 12 mo at herd entry ( $P < 0.05$ ), whereas no significant difference was observed between boars aged 8 mo and boars aged 9 mo at herd entry ( $P > 0.05$ ).

**DISCUSSION****General Discussion of the Linear Growth Model**

Because the semen data collected from different herds are hierarchical and longitudinal, the SE evaluated by the ordinary method (ordinary least square) was overestimated (Goldstein et al., 2002). The multilevel model, which can distinguish the layered structure between independent and dependent variables, improves the efficiency and scientific validity of the parameter estimation method. The basic principle for creating a linear growth model involves evaluation of the ICC of the data (Stawski, 2013). The ICC estimated in the present study were 0.62, 0.62, 0.61, 0.60, 0.54, and 0.70 for semen volume, total sperm number, functional sperm number, sperm concentration, motility, and abnormal sperm, respectively. This indicated that the housing type (level 2) accounts for approximately 62, 62, 61, 60, 54, and 70% of the total variance of the 6 semen traits. Therefore, the linear growth model was suitable for analyzing the hierarchical and longitudinal semen data.

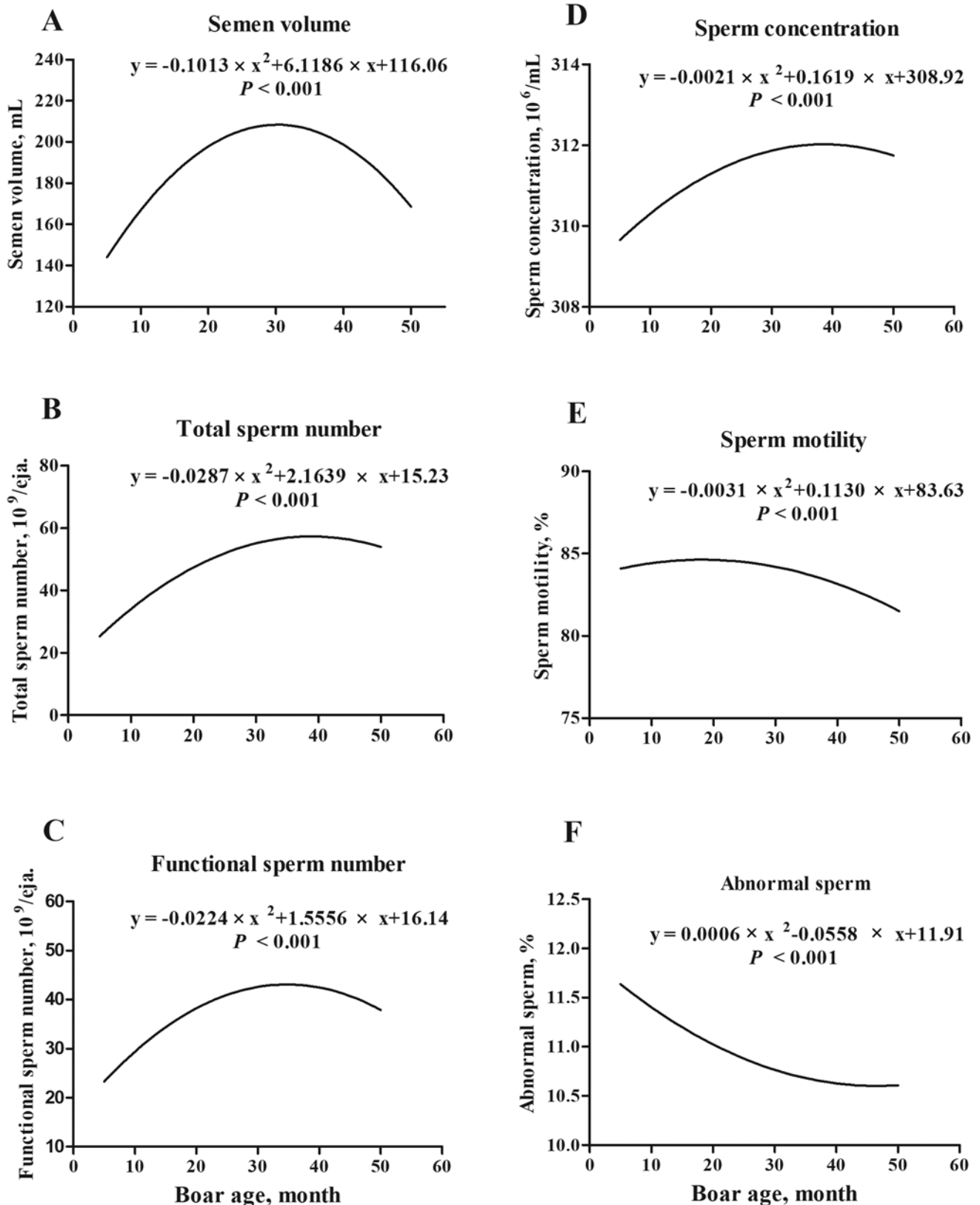
**Housing Condition Effects**

An ordinary house with a water curtain and a thermostatically controlled house with air filtration are the 2 main housing patterns in Southern China. In our current study, we found that boars reared in an ordinary house had a lower semen volume, total sperm number, and functional sperm number and greater abnormal sperm

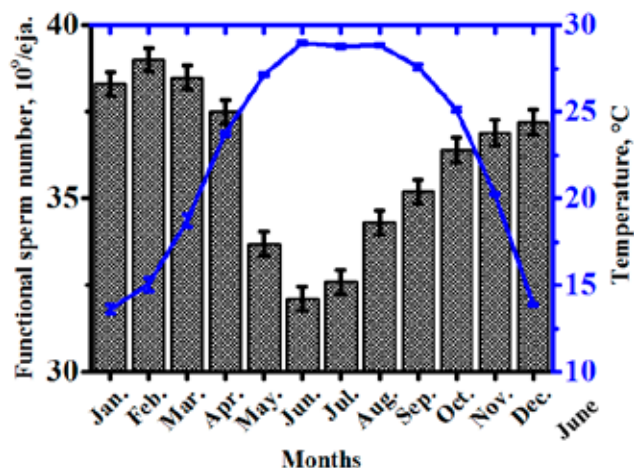
**Table 4.** Effect of boar breed on boar semen traits

Item	Breed			SEM <sup>1</sup>	P-value		
	Duroc	Landrace	Yorkshire		Duroc vs. Landrace	Duroc vs. Yorkshire	Landrace vs. Yorkshire
No. of boars	1,020	1,178	490				
Ejaculates	56,670	82,360	33,378				
Average ejaculate	56	70	68				
Semen quality							
Semen volume, mL	153.6	183.8	189.4	2.55	†	†	†
Sperm motility, %	83.7	83.8	83.7	0.49	NS <sup>2</sup>	NS	NS
Abnormal sperm, %	12.9	12.0	11.8	0.30	†	†	NS
Concentration, 10 <sup>6</sup> /mL	291.7	279.7	258.2	3.49	†	†	†
Total sperm, 10 <sup>9</sup> /eja. <sup>3</sup>	44.4	50.7	48.5	0.86	†	†	†
Functional sperm, 10 <sup>9</sup> /eja.	33.1	38.3	36.6	0.73	†	†	†

<sup>1</sup>SEM = pooled SEM.<sup>2</sup>NS = not significant.<sup>3</sup>eja. = ejaculation.† $P < 0.001$ .



**Figure 1.** Effects of boar age on semen volume (A), total sperm number (B), functional sperm number (C), sperm concentration (D), sperm motility (E), and abnormal sperm (F). The examined boars ranged from 8 to 50 mo of age. Results of the 6 semen traits are presented as least squares means. eja. = ejaculation.



**Figure 2.** Effect of semen collection season on boar functional sperm number ( $10^9$ /ejaculation [eja.]). The bar chart represents the results of the functional sperm number, corresponding to the left y-axis, and the curve chart shows temperature variation results, corresponding to the right y-axis. All values are presented as least squares means  $\pm$  SE.

levels than those reared in a house with air filtration. Furthermore, the functional sperm number from boars reared in a thermostatically controlled house showed smaller fluctuations than those reared in an ordinary house throughout the year (data not shown). This could be attributable to a less bacterial environment and more constant temperature provided by a thermostatically controlled house with air filtration, which are good for the health of the boar reproductive system (Quiñonero et al., 2009; Schulze et al., 2015). The bacterial contamination rates of AI boar studs were as high as 66.7% in Germany and Austria (Schulze et al., 2015). Sources can be classified as either animal or environmental (Althouse, 2008). Among several microorganisms detected as contaminants of boar semen, *Escherichia coli* and *Staphylococcus aureus* were those more commonly identified (Althouse and Lu, 2005). The presence of *S. aureus* led to a decrease in total sperm number and sperm motility and changes in sperm morphology (Emokpae et al., 2009). Additionally, an appropriate testicular and scrotal temperature is paramount for spermatogenesis. Increased scrotal temperatures cause sperm output and motility decreases in mice and in men working in high-temperature environments (Pérez-Crespo et al., 2008; Ahmad et al., 2012). The reproductive system anatomy of boars is similar to that of mice and adult males. When boars are exposed to an elevated ambient temperature, the germ cells in the seminiferous epithelium, which are sensitive to heat, might be damaged, reducing semen quality (Li et al., 2015). The results of our study suggest that a steady temperature (around 18.8 to 26.9°C during the investigation period) and less bacterial environment offered

**Table 5.** Effect of boar age at herd entry on functional sperm number

Item	No.	LSM <sup>1</sup>	SE	Significance of age at herd entry							
				5	6	7	8	9	10	11	12
5	28,638	34.9	0.50		*	†	**	†	NS <sup>2</sup>	NS	*
6	53,468	36.4	0.32			NS	*	†	NS	NS	†
7	43,518	37.0	0.35				*	†	NS	0.09	†
8	24,361	38.7	0.43					NS	NS	NS	†
9	12,006	39.1	0.71						**	†	†
10	5,473	36.5	1.06							NS	**
11	2,635	34.4	1.52								**
12	2,309	31.8	1.34								

<sup>1</sup>LSM = least squares mean.

<sup>2</sup>NS = not significant.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; † $P < 0.001$ .

by a thermostatically controlled house with air filtration have a positive impact on boar semen traits.

### Boar Breed Effects

In general, crossbred boars show better semen parameters than purebred boars (Smital et al., 2005; Wolf and Smital, 2009). However, because only 3 purebred breeds—Duroc, Landrace, and Yorkshire boars—were housed in Southern China, we have discussed the differences in semen traits among these 3 breeds. According to most authors dealing with boar semen, no breed excels in all basic semen characteristics (Smital, 2009). In a Canadian study comparing the semen characteristics of 5 breeds, Hampshire boars showed the largest semen volume, Duroc boars showed the highest sperm concentration, and Yorkshire boars showed the highest motility score (Kennedy and Wilkins, 1984). In our present study, Landrace boars by far exceeded all other breeds in all investigated semen characteristics. The very poor sperm output of the Duroc boars found in our current analysis is consistent with the results of previous studies (Buchanan, 1987; Smital, 2009; Wolf and Smital, 2009). Although the reason for our present findings cannot yet be explained, the valuable information we obtained suggests that producers should pay greater attention to Duroc boar production.

### Effect of Boar Age at Collection

The fertility of boars is time-dependent and changes considerably during their development (Smital, 2009). In our present investigation, we found that sperm output rose first and then fell during boar lifespan and that the optimum sperm production in terms of total sperm and functional sperm number was reached at ages between 30.2 and 38.5 mo. In agreement with our current results, a previous study reported a dramatic increase in aver-

age total sperm numbers from 8 to 10 mo up to 14 mo of age with little change thereafter (Clark et al., 2003). Another study also observed a rapid increase in sperm output with boar age but in that study, maximum output was achieved at a later time (3.5 yr of age; Smital, 2009). These findings indicate that boar fertility is significantly affected by the age at collection, and this may be because the increase in sperm output with age is probably mainly caused by testis growth and development.

### Seasonal Effects

Seasonal factors, which are mainly defined in temperate climates by the photoperiod and temperature, affect the process of spermatogenesis in boars (Claus and Weiler, 1985; Rivera et al., 2005; Zasiadczyk et al., 2015). Sperm production of boars may fluctuate as much as 25 to 30% throughout the year (Colenbrander and Kemp, 1990). Studies of the effects of seasonal factors on boar semen quality are mainly classified into 2 categories: by season (Knecht et al., 2014; Zasiadczyk et al., 2015; Fraser et al., 2016) and by month (Ciereszko et al., 2000; Smital, 2009; Wolf and Smital, 2009). The results of the former concluded that sperm output was reduced in spring or summer, whereas the latter showed reduced sperm output from April to September. Similarly, we found that sperm output significantly decreased from May to September. This may be related to the significantly elevated temperatures, which were above 27°C from May to September during the 3.5 yr of the survey in the present study (Fig. 2). In North America, evaporative and mechanical cooling systems are the predominant methods of thermal control (Knox et al., 2008). Compared with the temperature of a conventional system house, that of the evaporative cooling system house dropped between 4.4 and 6.2°C, significantly increasing the sperm output from boars maintained in such houses (Suriyasomboon et al., 2004; Kunavongkrit et al., 2005). Therefore, in contrast to other parts of China, use of a cooling system to prevent sunstroke from May onward is necessary for boar production in Southern China.

### Effect of Boar Age at Herd Entry

An early onset of reproductive activity in young boars will result in reduced fertility not only during the entire sexual life but also at the end of reproductive activity (Smital, 2009). This may be attributable to the negative effect of the premature introduction of boars to reproduction on libido and subsequent sexual activities (Savić et al., 2014). D'Allaire and Leman (1990) and D'Allaire et al. (1992) also reported that premature ejaculation in young boars may decrease boar lon-

gevity due to increased reproductive problems. In our present study, we found that boars aged 8 and 9 mo at herd entry had a greater functional sperm number than those aged 5, 6, 7, 10, 11, or 12 mo at herd entry. This indicates that 8 mo is a more suitable age for boar introduction, which can increase sperm output in the full production life. Premature introduction of boars to reproduction has a negative impact on boar sperm output in subsequent life.

### Conclusions

In conclusion, the multilevel method should be used when there is an ICC between hierarchical data. However, some other factors, such as environment, feeding, and management, were not measured in the present study. Nevertheless, our overall findings still provide valuable information for producers. In particular, sunstroke prevention should be taken into account from May onward for boar studs in Southern China. Additionally, 8 mo of age at herd entry is the most suitable age for boar introduction.

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