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Relationship between semen quality and meat quality traits in Belgian Piétrain boars

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ABSTRACT

The main objective of this study was to assess the semen quality of Piétrain boars originating from Belgian AI centers and to correlate these results with their meat quality traits. Freshly diluted semen doses from 140 boars originating from 10 artificial insemination (AI) centers were used and stored for five days at 17 °C. Motility was assessed daily using a computer assisted semen analyzer (Hamilton-Thorne), while morphology and concentration were assessed on the day of semen collection (Day 0) by eosin-nigrosin staining and the Bürker counting chamber, respectively. These data were correlated with the lean meat percentage, loin eye depth and backfat thickness using linear mixed models taking into account the clustering of boars within each AI center and the daily measurements for each semen dose. The mean values (± SD) on Day 0 were: motility 79.7 ± 8.2%, live sperm 91.5 ± 4.3%, live normal sperm 83.6 ± 7.4%, and concentration 29.0 ± 10.6 ($\times 10^6$ sperm/mL). The average five-day motility across all AI centers was 77.7 ± 8.9%. None of the assessed semen quality traits were associated with lean meat percentage. Motility and progressive motility on Day 0 were positively associated with backfat thickness ($P < 0.05$), while no overall negative associations were elucidated between the latter semen quality traits and loin eye depth. The percentages of live and normal live sperm were not correlated with backfat thickness nor loin eye depth. To conclude, selection of terminal Belgian Piétrain boars for reduced backfat thickness might negatively influence semen motility, whereas selection for increased lean meat percentage and loin eye depth would not necessarily compromise semen quality traits.

1. Introduction

In pigs, the vast majority of the registered artificial inseminations (AI) worldwide are conducted using semen preserved in liquid form, also referred to as 'fresh semen'. The production efficiency of liquid stored doses and their efficient use for AI is important (Wagner and Thibier, 2000; Knox, 2014; Riesenbeck et al., 2015).

Traditionally, selection of boars has focused on economically important traits that need to be transmitted to the progeny, such as lean meat yield, feed efficiency and growth rates. Until now, little or no attention has been given to semen quality traits. Several publications have highlighted the importance of including semen quality traits into the genetic evaluation of boars (Robinson and Buhr, 2005; Oh et al., 2006; Flowers, 2008; Safranski, 2008; Pinart and Puigmulé, 2013). The rationale is that decreasing the amount of total sperm/dose, will result in a 'win-win' situation for both the AI centers and the commercial breeding herds. Profitability of AI centers will increase by utilizing fewer boars and the commercial breeding herds will be able to utilize semen doses from boars of higher genetic merit as the genetic lag affecting the chosen sire lines will be decreased (D' Allaire and Leman, 1990; Feitsma, 2009; Roca et al., 2011). Under field conditions, one of the basic requirements for the use of semen doses of lower sperm concentration is the availability of boars producing high quality ejaculates. One approach to achieve this is by selecting boar lines not only for meat and quality traits, but also for semen quality. This approach requires knowledge of the genetic and phenotypic correlations between

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meat quality and semen quality traits (Robinson and Buhr, 2005; Safranski, 2008; Pinart and Puigmulé, 2013).

Oh et al. (2006) reported that selection of boars for increased muscle depth and reduced backfat may result in reduced ejaculate volume and fewer sperm per ejaculate. Nevertheless, genetic correlation of traits is independent of their heritability (Gillespie and Flanders, 2010; Dube et al., 2012). This implies that estimations of genetic correlations between semen and meat quality traits could only be indicative and may not always predict phenotypic performance.

In the Belgian pig industry, Piétrain boars are indispensable as terminal sire and constitute the largest group of boars kept by AI centers. Belgian Piétrain boars and semen have been exported worldwide and are commonly used through various levels of the swine breeding pyramid, to produce purebred or crossbred grandparent to parent terminal sire lines (McGlone and Pond, 2003; Kyriazakis and Whittemore, 2006; Gillespie and Flanders, 2010; Luc et al., 2013). Selection of these boars is mainly based on performance and meat quality traits of the fattening pigs, with no emphasis on semen quality of the boars (Burlot and Naveau, 2003; Dufranse et al., 2011; Dufranse, 2014). The design of an efficient selection program that incorporates semen quality traits requires elucidation of whether the current narrowly focused selection for meat quality traits has had a negative effect on the semen quality of Belgian Piétrain boars. The main objective of this study was to assess the semen quality of Piétrain boars originating from Belgian AI centers and to correlate the results with the meat quality traits of these boars. Additionally, the storability of the semen doses during five consecutive days was investigated.

2. Materials and methods

2.1. Study design and semen sample collection

Freshly diluted semen doses from 147 randomly selected Piétrain boars, originating from 10 commercial AI centers (AI 1–10), were obtained on the day (Day 0) of semen collection (one dose per boar). Nine of the AI centers contributed semen doses from 15 boars each and one AI center (AI 10) contributed semen doses from 12 boars. Semen doses with a percentage of motile sperm (MOTILE%) below 60% on Day 0 ($n = 7$; one from AI 1, three from AI 2, two from AI 5 and one from AI 9) were not considered for further analysis, since a MOTILE% higher than 60% is considered the minimum requirement for a fertile dose (Martin-Rillo et al., 1996; Britt et al., 1999; Johnson et al., 2000; Vyt et al., 2004).

All semen samples were collected *via* the gloved-hand technique, within the regular collection interval of each AI center (ranging on average between 5.1 and 9.8 days; Table 1). Five of the AI centers used the short-term extender Beltsville thawing solution (BTS; Minitüb, Tiefenbach, Germany or Cobiporc, Saint-Gilles, France), while the other five AI centers used long-term extenders (Gedil®, Genes Diffusion-IMV Technologies, Douai, France or Vitasem®, Magapor, Zaragoza, Spain) (Table 1). All boars were fit for semen collection, with no fertility issues after multiple use. The trial lasted for 10 weeks, from 12th December 2014 until 20th February 2015. Each week, semen doses from one AI center were collected on Monday. The doses were transported in a climate box at 17 °C (Schippers UK Ltd., UK) to the semen laboratory of the Department of Reproduction, Obstetrics and Herd Health at the Faculty of Veterinary Medicine, Ghent University, Belgium and stored for five consecutive days (Day 0–Day 4) in a MS Climate Box at 17 °C (Table 2).

2.2. Semen sample analysis

Motility of the diluted semen doses was assessed daily, from Day 0 to Day 4, using a computer assisted semen analysis system (CASA, HTR Ceros 12.3 semen analyzer, Hamilton-Thorne research, Beverly, USA). Prior to the CASA analysis, the diluted semen samples were pre-warmed at 37 °C for 20 min in an incubator (IN, Memmert GmbH + Co.KG, Germany) and then, each sample was evaluated five times by the same procedure, using counting chambers and software settings as described by López Rodríguez et al. (2010). The motility parameters determined by the CASA system were MOTILE% and PROGR% (percentage of progressively moving sperm, having average path velocity > 45 µm/s and straightness > 45%). Morphology and concentration (CONC) were assessed only on Day 0, using eosin-nigrosin staining (Shipley, 1999) and the Bürker counting chamber, respectively. Specifically, morphology for each semen dose was assessed by counting 300 cells and then calculating the percentage of normal (NLS%) and dead sperm, and sperm with abnormal heads, proximal and distal droplets and abnormal tails (as a mean of three counts of 100 cells). The NLS% was then added to the percentage of sperm with abnormal heads, proximal and distal droplets, and abnormal tails, to calculate the percentage of live sperm (LS%).

2.3. Meat quality records

From each AI center, meat quality records were obtained for the lean meat percentage (LMP, %), backfat thickness (BFT, mm) and loin eye depth (LED, *M. longissimus dorsi* thickness in mm) of every boar involved in this study. In one AI center (AI 6) no records were available for the LMP and LED of the boars (Table 3). In Belgium, the documentation of the LMP, BFT and LED of every pedigree boar kept by the AI centers is mandatory. According to the law, BFT and LED should be measured at 230 days of age, using the PIGLOG 105 (SKF-Technology, Soborg, Denmark) ultrasound apparatus (Vettenburg, 2004). For that reason, for each boar involved in the study, BFT was measured at two points and LED at one point (Piglog 105 User''s Guide, 1991): 1)

Table 1
General description of the 10 artificial insemination (AI) centers (AI 1–10) that participated in the study.

	AI 1	AI 2	AI 3	AI 4	AI 5	AI 6	AI 7	AI 8	AI 9	AI 10
Number of serving boars	150	65	122	104	66	200	175	88	34	31
Total semen doses produced/year	200,000	50,000	150,000	105,000	65,000	260,000	185,000	112,000	55,000	30,000
Age of boars[a]	28.9 ± 10.3	28.5 ± 21.2	34.0 ± 11.0	29.6 ± 17.3	35.9 ± 24.1	43.6 ± 19.0	25.8 ± 12.3	34.9 ± 25.7	32.5 ± 18.6	31.0 ± 16.0
Days to previous collection[b]	9.8 ± 3.2	8.9 ± 6.1	6.9 ± 1.0	7.6 ± 1.2	5.8 ± 0.8	6.9 ± 1.1	5.1 ± 0.5	7.0 ± 1.1	7.6 ± 1.1	6.9 ± 3.6
Semen extender	BTS	Gedil®	Vitasem®	BTS	Vitasem®	Vitasem®	BTS	Vitasem®	BTS	BTS
Sperm concentration ($\times 10^9$/dose)[c]	2.0–2.5	2.5–3.0	2.0–2.5	2.0	> 3.0	2.0–2.5	2.5–3.0	> 3.0	2.5–3.0	2.0–2.5
Sperm quality analysis by AI center										
Concentration[d]	CASA	Photom.	CASA	CASA	Photom.	CASA	Photom.	Photom.	Photom.	Colorim.
Motility & morphology	CASA	Visual	CASA	CASA	Visual	CASA	Visual	Visual	Visual	Visual

[a] Average (± SD) age in months calculated only for the boars participating in the study ($n = 140$).
[b] Days to previous collection calculated only for the boars participating in the study ($n = 140$).
[c] Referring to the dilution target set by each AI center.
[d] Photom. and Colorim. constitute abbreviations for the terms photometer and colorimeter, respectively.

Table 2
Average (± SD) five-day motility and progressive motility of the semen doses (n = 140) of each artificial insemination (AI) center together with their average (± SD) values for the morphological parameters and the concentration assessed on Day 0.

	AI 1	AI 2	AI 3	AI 4	AI 5	AI 6	AI 7	AI 8	AI 9	AI 10	(AI 1–10)[a]
Five-day MOTILE%[b]	74.5 ± 10.4	67.4 ± 8.7	73.4 ± 8.3	82.4 ± 5.9	80.1 ± 5.3	77.0 ± 4.0	77.9 ± 7.8	80.6 ± 4.0	78.4 ± 13.3	85.4 ± 5.8	77.7 ± 8.9
Five-day PROGR%[c]	26.9 ± 10.0	18.5 ± 7.2	33.1 ± 11.0	25.5 ± 7.3	35.6 ± 7.4	39.2 ± 7.0	36.8 ± 9.8	41.1 ± 7.3	39.0 ± 13.5	41.0 ± 11.4	33.8 ± 11.5
LS%[d]	94.4 ± 3.0	92.5 ± 4.1	91.3 ± 2.2	92.2 ± 2.9	89.9 ± 4.7	90.0 ± 2.7	92.3 ± 3.2	91.2 ± 3.9	93.7 ± 6.4	86.9 ± 5.0	91.5 ± 4.3
NLS%[e]	90.9 ± 6.2	81.2 ± 8.5	82.9 ± 5.5	84.4 ± 6.9	79.3 ± 9.3	82.5 ± 5.3	84.9 ± 3.6	83.7 ± 4.7	86.3 ± 10.8	79.2 ± 5.3	83.6 ± 7.4
Abnormal morphological parameters[f]											
Dead (%)	5.6 ± 3.0	7.5 ± 4.1	8.7 ± 2.2	7.8 ± 2.9	10.1 ± 4.7	10.0 ± 2.7	7.7 ± 2.9	8.8 ± 3.6	6.3 ± 6.4	13.1 ± 5.0	8.5 ± 4.3
Abnormal heads (%)	–[g]	5.2 ± 4.1	5.7 ± 4.7	1.2 ± 2.0	0.1 ± 0.4	0.5 ± 0.7	0.6 ± 1.1	1.4 ± 1.4	0.4 ± 0.8	0.2 ± 0.5	1.5 ± 2.9
Proximal droplets (%)	1.5 ± 1.7	2.2 ± 1.6	0.8 ± 1.5	1.3 ± 1.6	3.9 ± 4.3	3.1 ± 2.9	2.1 ± 1.2	0.9 ± 1.0	2.8 ± 4.4	2.2 ± 2.8	2.1 ± 2.6
Distal droplets (%)	2.0 ± 2.6	1.4 ± 1.1	0.6 ± 0.7	2.9 ± 4.2	2.4 ± 2.4	2.0 ± 1.4	1.9 ± 1.8	1.9 ± 1.4	1.0 ± 1.0	2.6 ± 2.0	1.9 ± 2.1
Abnormal tails (%)	–[g]	2.5 ± 5.2	1.3 ± 1.6	2.4 ± 2.9	4.2 ± 6.2	1.9 ± 1.8	2.8 ± 2.3	3.3 ± 3.8	3.2 ± 5.8	2.7 ± 2.2	2.4 ± 4.0
CONC[h]	48.0 ± 13.3	20.3 ± 4.8	32.4 ± 7.8	32.7 ± 5.8	30.3 ± 5.3	28.5 ± 6.2	30.8 ± 6.8	25.4 ± 5.2	19.3 ± 2.8	19.3 ± 8.0	29.0 ± 10.6

[a] (AI 1–10): Average (± SD) across all AI centers participating in the study.
[b] MOTILE%: percentage of motile sperm.
[c] PROGR%: percentage of progressively moving sperm, having average path velocity > 45 μm/s and straightness > 45%.
[d] LS%: percentage of live sperm.
[e] NLS%: percentage of normal live sperm.
[f] Dead (%): percentage of dead sperm; Abnormal heads (%): percentage of sperm with abnormal heads; Proximal droplets (%): percentage of sperm with proximal droplets; Distal droplets (%): percentage of sperm with distal droplets; Abnormal tails (%): percentage of sperm with abnormal tails.
[g] No sperm with such morphological abnormality was found.
[h] CONC: concentration expressed as number of sperm × 10^6 / mL of semen dose.

between the 3rd and the 4th last lumbar vertebrae and 7 cm from the back medial line (x_1 for BFT in mm), 2) 10 cm from the last rib towards the cranial part and 7 cm from the back medial line (x_3 for BFT in mm). The LED was also measured at this second point (x_2 for LED in mm). The LMP (Y) was calculated by the PIGLOG 105 apparatus *via* the following in-coded formula that takes into account the BFT and LED measurements:

$$Y = 64.39 – 0.28x_1 + 0.14x_2 – 0.55x_3$$

2.4. Statistical analysis

Basic descriptive statistics were used to explore the outcome variables (MOTILE%, PROGR%, LS%, and NLS%) and predictor variables (meat quality traits LMP, BFT and LED, age of the boars and the type of semen extender). Descriptive results are presented as averages and standard deviations (average ± SD).

To model possible associations between the outcome and predictor variables linear mixed models were fitted. A random AI center effect was included to account for clustering of boars within each AI center. For MOTILE% and PROGR%, the daily measurements for each semen dose were taken into account by assuming an unstructured covariance matrix for the residual variance. Null models (no predictor variables except the effect of time) were fitted to estimate the average MOTILE% and PROGR% from Day 0 to Day 4. Next, univariable associations between the outcome and predictor variables were examined to determine the order in which predictor variables were entered during the multivariable model building process. Pearson correlation coefficients were estimated to avoid multicollinearity in the next steps. If the absolute value of the correlation coefficient between two selected independent variables was higher than |0.60|, the one with the highest statistical significance was withheld for further analysis. Then, the retained independent variables (including two-way interactions) were used to build a multivariable linear mixed model by a manual stepwise forward model building procedure. Nested models were compared using a likelihood ratio test at a 5% significance level. Normal probability plots of residuals and plots of residuals *versus* predicted values were generated to check whether the assumptions of normality and homogeneity of variance had been fulfilled. Statistical analyses were performed using SAS 9.4 (SAS Inst. Inc., Cary, NC, USA).

3. Results

3.1. Descriptive results

Table 1 provides descriptive data of each AI center that participated in the study. The average age of the boars was 32.6 ± 18.2 months (range: 6.5–99.0). The average five-day MOTILE% and PROGR% of the semen of the selected boars from each AI center are provided/summarized in Table 2. Figs. 1 and 2 present the average MOTILE% and PROGR% of the semen doses of each AI for each day of assessment, respectively. The null models showed a significant decrease over time in the average daily MOTILE% and PROGR% across the semen doses of all AI centers ($P < 0.001$) (Table 4). The random effect for AI center was significant, denoting presence of significant variations between AI centers in the MOTILE% and the PROGR%.

The average LS% and NLS% across the semen doses of all AI centers on Day 0 were 91.5 ± 4.3 and 83.6 ± 7.4, respectively. Table 2 provides the average LS% and NLS% as well as the percentage of abnormal sperm in the semen doses of each AI center (*i.e.* dead sperm and sperm with abnormal heads, proximal and distal droplets, and abnormal tails). Additionally, Table 2 provides the average CONC values of the semen doses of all AI centers.

Table 3
Average (± SD) lean meat percentage, backfat thickness and loin eye depth of the selected boars in each artificial insemination (AI) center.

	AI 1	AI 2	AI 3	AI 4	AI 5	AI 6	AI 7	AI 8	AI 9	AI 10	(AI 1–10)[a]
Lean meat percentage (LMP, %)	69.6 ± 0.8	70.1 ± 0.7	69.4 ± 0.8	69.7 ± 0.5	69.7 ± 0.8	n/a[b]	69.2 ± 0.7	69.6 ± 1.0	69.8 ± 0.7	69.4 ± 0.9	69.6 ± 0.8
Backfat thickness (BFT, mm)	6.3 ± 2.1	5.0 ± 0.6	5.2 ± 0.7	4.7 ± 0.6	4.9 ± 0.5	5.3 ± 0.6	5.3 ± 0.4	5.0 ± 0.7	4.6 ± 0.6	5.2 ± 0.9	5.2 ± 1.0
Loin eye depth (LED, mm)	85.3 ± 4.6	85.4 ± 3.9	82.0 ± 3.5	81.1 ± 3.3	82.8 ± 3.2	n/a[b]	81.5 ± 4.4	83.2 ± 3.0	81.6 ± 3.1	82.0 ± 4.9	82.8 ± 4.0

[a] (AI 1–10): Average (± SD) across all AI centers participating in the study.
[b] No data available.

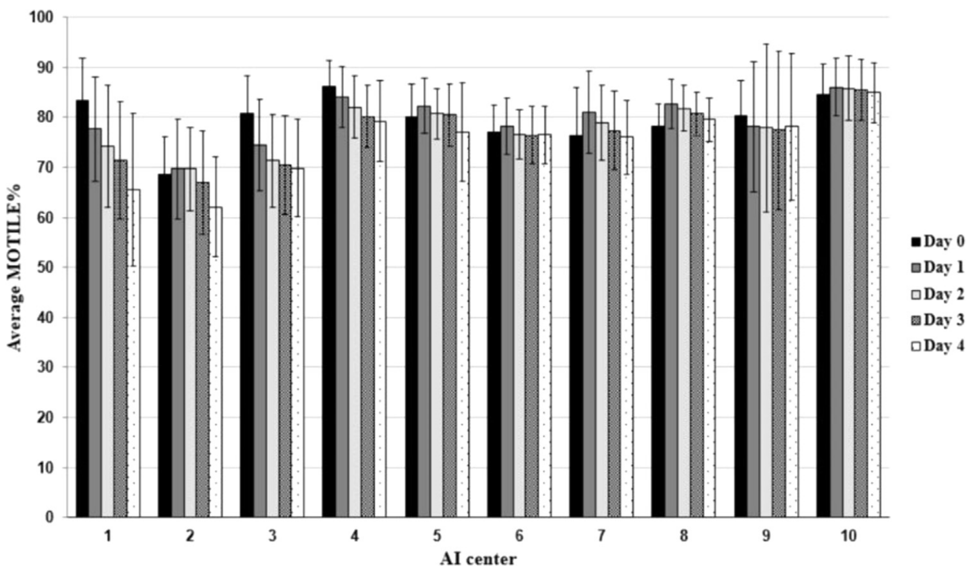

Fig. 1. Sperm motility. Average percentage of motile sperm (MOTILE%) in the semen doses of each AI center for every single day of assessment (from Day 0 to Day 4). Lines on top of each bar indicate standard deviation (± SD).

3.2. Relationship between the MOTILE% and semen age, boar age and meat quality traits

The final multivariable model included the meat quality traits BFT and LED together with their two-way interaction (Table 5). As there were no available data for the LED of 16 boars, the model was based on 124 boars. The predictor variables boar age and type of semen extender were not significantly associated with the MOTILE% (P = 0.11 and P = 0.20, respectively). Since LMP was highly correlated with BFT (r = − 0.74) and not significantly associated with the MOTILE%, after univariable analysis this variable was excluded from the multivariable model (P = 0.22).

Compared to Day 0 a significantly lower MOTILE% was noticed on Days 2, 3 and 4. To ease the interpretation of the two-way interaction, the variables BFT and LED were centered by subtracting the average BFT and LED, respectively. As such, when LED was kept centered at the average value (with the interaction cancelled out), a positive association between BFT and the MOTILE% was evident on Day 0, where for every 1 mm of extra BFT the MOTILE% increased on average by 2.08% (95% CI [0.37; 3.80]; P = 0.02). Compared to Day 0, no significant differences were noticed during the following days of assessment ($P > 0.05$). For boars with an average BFT, a significant increase of the association between LED and the MOTILE% was observed on Day 2 (0.40% higher MOTILE% for every 1 mm of extra LED; 95% CI [0.04; 0.77], P = 0.03) and Day 3 (0.51% higher MOTILE% for every 1 mm of extra LED; 95% CI [0.14; 0.89]; P = 0.01) when compared to Day 0. The two-way interaction indicated that the effect of BFT and LED on the MOTILE% depended on each other. This effect became more prominent over time, but was only significant on Day 4 ($P < 0.001$).

Table 4
Overview of the average percentage of motile sperm (MOTILE%) and percentage of progressively moving sperm (PROGR%) on each day of the five-day storage period, across the semen doses (n = 140) from the 10 artificial insemination centers. The estimates are based on a null-model including only the effect of time and are reported with their 95% confidence interval.

	Day 0	Day 1	Day 2	Day 3	Day 4
MOTILE%	79.58 [76.65; 82.52]	79.41 [76.36; 82.46]	77.84 [74.73; 80.95]	76.64 [73.51; 79.78]	74.91 [71.68; 78.13]
PROGR%	36.28 [31.49; 41.07]	35.75 [30.92; 40.58]	32.91 [28.17; 37.65]	32.14 [27.36; 36.92]	31.31 [26.54; 36.09]

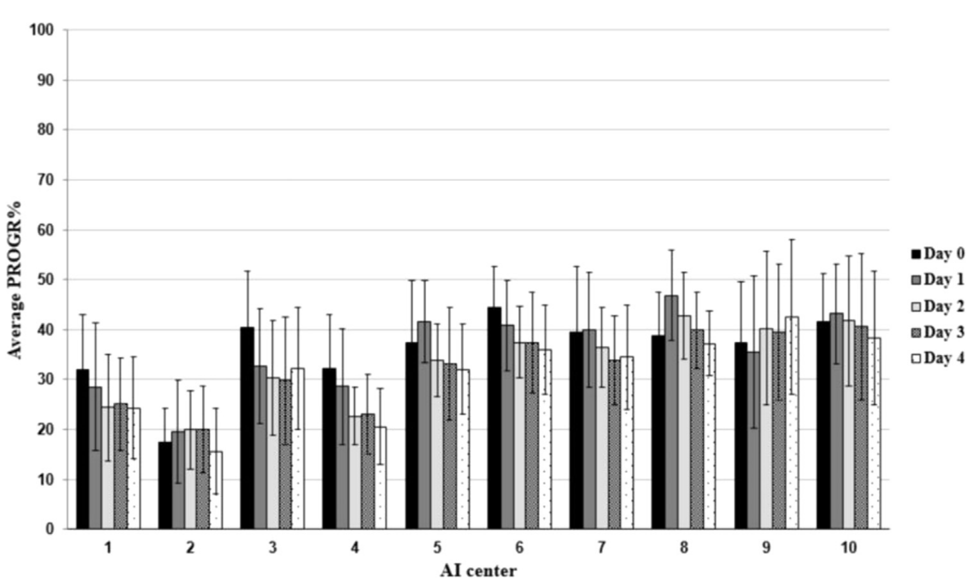

Fig. 2. Sperm progressive motility. Average percentage of progressively moving sperm (PROGR%) in the semen doses of each AI center for every single day of assessment (from Day 0 to Day 4). Lines on top of each bar indicate standard deviation (± SD).

Table 5
Parameter estimates of the multivariable linear mixed model with the percentage of motile sperm (MOTILE%) as the outcome variable. The estimates are reported with their 95% confidence interval (only significant associations between the MOTILE% and the predictors are described).

	Day 0	Day 1	Day 2	Day 3	Day 4
Time		− 0.09 [− 1.26; 1.07]	− 1.58 [− 2.99; − 0.18]	− 2.81 [− 4.25; − 1.37]	− 4.22 [− 5.75; − 2.69]
	Ref.[d]	$P = 0.88$	$P = 0.03$	$P < 0.001$	$P < 0.001$
Backfat thickness (BFT)[a]	2.08 [0.37; 3.80]	− 0.53 [− 2.00; 0.94]	− 0.55 [− 2.33; 1.22]	− 1.39 [− 3.21; 0.42]	− 1.47 [− 3.40; 0.46]
	Ref.[d]	$P = 0.48$	$P = 0.54$	$P = 0.13$	$P = 0.14$
Loin eye depth (LED)[b]	− 0.19 [− 0.55; 0.16]	0.29 [− 0.02; 0.59]	0.40 [0.04; 0.77]	0.51 [0.14; 0.89]	0.25 [− 0.15; 0.65]
	Ref.[d]	$P = 0.06$	$P = 0.03$	$P = 0.01$	$P = 0.22$
BFT*LED[c]	− 0.08 [− 0.34; 0.18] $P = 0.55$	− 0.24 [− 0.56; 0.09] $P = 0.16$	− 0.28 [− 0.64; 0.08] $P = 0.13$	− 0.34 [− 0.71; − 0.02] $P = 0.06$	− 0.70 [− 1.08; − 0.32] $P < 0.001$

[a] BFT: one unit of BFT is one mm.
[b] LED: one unit of LED is one mm.
[c] BFT*LED: variable describing the interaction between BFT and LED, denoting their total effect on a specific day. The variables BFT and LED were centered to enhance the interpretation of the interaction term.
[d] The reported estimates and P-values compare the effect of a predictor on a given day with the effect of that predictor at Day 0, which is the reference (Ref.).

3.3. Relationship between the PROGR% and semen age, boar age and meat quality traits

The final multivariable model included the meat quality traits BFT and LED (Table 6). As there were no available data for the LED of 16 boars, this model was based on 124 boars. The predictor variables boar age and type of semen extender were not significantly associated with the PROGR% ($P = 0.23$ and $P = 0.57$, respectively). Since LMP was highly correlated with BFT ($r = -0.74$) and not significantly associated with the PROGR%, after univariable analysis this variable was excluded from the multivariable model ($P = 0.14$).

Compared to Day 0 the model showed a significant decrease in the PROGR% on Days 2, 3 and 4. A positive association between BFT and the PROGR% was observed on Day 0 (2.89% higher PROGR% for every 1 mm of extra BFT; 95% CI [0.69; 5.09]; $P = 0.01$). This association between BFT and the PROGR% attenuated significantly over time (overall $P = 0.05$). A significant positive difference compared to Day 0 was noticed for the association between LED and the PROGR% during the remaining days of semen assessment (overall $P = 0.03$).

3.4. Relationship of the LS% and NLS% with semen age, boar age and meat quality traits

None of the univariable linear mixed models showed presence of any significant associations between the LS% or the NLS% and each of the independent variables, ($P > 0.20$ across all models).

4. Discussion

This study investigated the presence of phenotypic associations between semen and meat quality traits in Belgian Piétrain boars originating from 10 commercial AI centers. The fact that BFT was positively associated with both the MOTILE% and the PROGR% suggests that selecting Belgian Piétrain lines for reduced BFT might have an adverse effect on semen motility. As there was no overall negative association between LMP or LED and both the MOTILE% and the PROGR%, selection for increased LMP or LED would not necessarily compromise semen motility. None of the morphological parameters assessed were found to be associated with LMP, BFT or LED. Based on this information, it can be proposed that BFT should continue being an assessment criterion for meat quality in newly introduced Belgian Piétrain boars, not only for its impact on the economic performance of the offspring produced, but also for its effect on semen quality. Thus, continuous monitoring of BFT data from the meat quality records of those boars is necessary.

The presence of significant positive differences in the association between LED and the MOTILE%, and also in the association between LED and the PROGR%, when comparing Day 0 to the remaining days of semen assessment, suggests that the higher the LED the better semen motility is preserved during long-term semen storage periods. The significant negative interaction between BFT and LED on their effect on the MOTILE% on Day 4 means that when comparing two groups of boars with a different BFT, the positive effect of LED on the MOTILE% is more pronounced in the group of boars with the lower BFT. Consequently, one of the ways to achieve better motility preservation in

Table 6
Parameter estimates of the multivariable linear mixed model with the percentage of progressively moving sperm (PROGR%) as the outcome variable. The estimates are reported with their 95% confidence interval (only significant associations between the PROGR% and the predictors are described).

	Day 0	Day 1	Day 2	Day 3	Day 4
Time		− 0.15 [− 1.71; 1.42]	− 2.97 [− 4.63; − 1.31]	− 3.85 [− 5.48; − 2.22]	− 4.61 [− 6.33; − 2.90]
	Ref.[c]	$P = 0.85$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Backfat thickness (BFT)[a]	2.89 [0.69; 5.09]	− 1.57 [− 3.25; 0.10]	− 2.55 [− 4.31; − 0.78]	− 2.59 [− 4.33; − 0.86]	− 2.33 [− 4.16; − 0.50]
	Ref.[c]	$P = 0.07$	$P = 0.004$	$P = 0.004$	$P = 0.01$
Loin eye depth (LED)[b]	− 0.53 [− 1.08; 0.02]	0.60 [0.17; 1.03]	0.67 [0.21; 1.12]	0.71 [0.27; 1.16]	0.66 [0.19; 1.13]
	Ref.[c]	$P = 0.006$	$P = 0.004$	$P = 0.002$	$P = 0.006$

[a] BFT: one unit of BFT is one mm.
[b] LED: one unit of LED is one mm.
[c] The reported estimates and P-values compare the effect of a predictor on a given day with the effect of that predictor at Day 0, which is the reference (Ref.).

Belgian Piétrain lines with low BFT might be the selection for higher LED. This is of practical significance as under field conditions part of the already diluted doses can be stored over the weekend if semen collection has occurred on Friday (Khalifa et al., 2014).

The present study is the first to report on phenotypic associations between semen and meat quality traits for Belgian Piétrain terminal boars. Previous studies utilizing semen doses from different Piétrain hybrid boars have reported different results concerning the association between LMP and semen quality traits. Milewska (2007) reported a negative association between the LS% and LMP as well as ejaculate volume and LMP, while Szostak et al. (2016) reported a positive association between LMP and the latter semen quality traits. The results presented by Milewska (2007) and Szostak et al. (2016) indicate that there is breed-specific variability in the phenotypic associations between LMP and semen quality traits.

The above mentioned breed-specific variability could be attributed to different genes affecting common pathways involved in the production of boar taint compounds and essential sex hormones (Grindflek at al, 2011; Van den Broeke et al., 2015). Those genes also seem to be associated with muscling (Kim et al., 2000). Given the high heritability values for androstenone in plasma and fat 0.39–0.73; Varona et al., 2005; Grindflek et al., 2011), the long-term selection of Belgian Piétrain terminal sires for increased LMP, could have possibly resulted in the indirect selection for increased boar taint. This in turn may have resulted in increased levels of production of sex steroid hormones in some of those terminal sires, thus resulting in better semen quality. At this point, it should be noted that Xue et al. (1996) reported differences in tissue levels of boar taint compounds and in taint assessed by a sensory panel across four different boar breeds. Those differences might also explain the breed-specific variability in the phenotypic associations between LMP and semen quality traits.

Another interplaying parameter that can influence the phenotypic associations between semen and meat quality traits are the different management practices applied by the AI centers. In the present study, significant variations between AI centers in the MOTILE% and the PROGR% were detected, but not for the morphological parameters. Nevertheless, the presence of such significant variations did not seem to have a profound effect on the statistical power of the study as BFT was positively associated with both the MOTILE% and the PROGR%, and on the same time none of the morphological parameters assessed were found to be associated with LMP, BFT or LED. Although stronger associations could be obtained if a combination of less AI centers contributed the same amount of participating boars (*i.e.* semen doses), a negative implication of such study design would be that the conclusions drawn would reflect a much narrower proportion of the different Belgian Piétrain lines kept by the Belgian AI centers. According to the report of De Praeter et al. (2015), the current study utilized semen doses from eight of the largest AI centers in Belgium (8/15 largest in total; in terms of boars kept).

Smital (2009) proposed a composite semen quality trait that includes ejaculate volume, the MOTILE%, the percentage of morphologically deviant sperm and total concentration. This trait could be assessed throughout the entire breeding scale producing terminal sire lines. The results of the current study indicate that the trait proposed by Smital (2009) could constitute a selection criterion for Belgian Piétrain boars as there was no overall negative association between LMP or LED and any of the semen quality traits assessed, and BFT was only correlated with the MOTILE% and the PROGR%.

Although the relationship between semen quality traits and field fertility results can be tenuous (Broekhuisje et al., 2015), this might be associated with numerous interplaying factors, such as the storage of the semen doses and their sperm concentration, nutritional and seasonal effects and also the different AI techniques applied (Pinart and Puigmulé, 2013). Semen quality traits such as the MOTILE%, the PROGR%, the LS% and the NLS% remain the main criteria for the assessment of the fertility potential of the semen doses distributed by the commercial AI centers, since in many cases doses with poor motility and morphology constitute the principal etiological diagnosis of infertility and subfertility (Briz and Fábrega, 2013). Potentially, more substantial conclusions on the relationship between semen quality traits and field fertility results could be drawn if fertility records could be obtained from the herds utilizing semen doses from the boars that participated in this study or if *in vitro* fertilization results existed. At this point though, one should consider that the latter two investigations have significant limitations. Firstly, in many commercial breeding herds inseminations have been heterospermic (Morrow et al., 1992) and secondly, *in vitro* fertilization has not been shown to correlate well with field fertility results (Rodríguez-Martínez, 2007). Additionally, this study aimed to provide information that can assist the selection of Belgian Piétrain boar lines with favorable positive associations between semen and meat quality traits, and not to investigate associations with field fertility rates. The reason is that quite often AI centers cull boars that have excellent body conformation and meat quality traits, but poor semen quality (Robinson and Buhr, 2005; Pinart and Puigmulé, 2013). Profit losses are also magnified by the fact that the semen quality of the boars can only be assessed when they reach puberty (Broekhuisje et al., 2015).

In this study, a significant reduction in the MOTILE% and the PROGR% was noticed over time, more specifically towards the last three days of storage. The results suggest that a more efficient use of semen doses can be achieved within the first two to three days of storage. Furthermore, the type of semen extender used (short term or long term) did not significantly affect the MOTILE%, the PROGR% and the morphological parameters assessed. These results are similar to those of Kadirvel et al. (2005) and Estienne et al. (2007). However, other studies have reported that for similar storage period long term extenders offer significantly better preservation than the short term ones (Dubé et al., 2004; Karageorgiou et al., 2016). Possible explanations for this variability may be differences in the buffering capacity (ability to prevent changes in pH) among extenders and the presence of interactions between the particular boar breed used and the response of its semen quality traits to the extender (Vyt et al., 2004; Estienne et al., 2007).

The overall average five-day MOTILE% across the semen doses of all AI centers was 77.7 ± 8.9%. Together with the fact that semen doses with a MOTILE% below 60% at Day 0 were not considered for further analysis, this finding proves that the semen doses utilized in this study were suitable for commercial use. Regarding the average CONC of the semen doses used in this study, it became obvious that there were several deviations from the dilution targets set by some AI centers. More specifically, 3/10 AI centers diluted far less than the targets set by them, 4/10 diluted according to their target and 3/10 diluted far more than originally planned. Possible reasons might be inadequate personnel training, use of inappropriate glass chambers or settings during CASA analysis or as proposed by Vyt et al. (2007) due to the fact that when photometers are used there might be accidental confusion of particles of a size similar to that of sperm with actual sperm.

To conclude, the results of the present study suggest that continuing selection of terminal Belgian Piétrain boars for increased LMP and LED would not affect the MOTILE%, the PROGR%, the LS% and the NLS%. On the other hand, selection for reduced BFT might have an adverse effect on semen motility. The interaction between LED and BFT implies that in Belgian Piétrain lines having low BFT, selection for increased LED may improve preservation of semen motility. More studies are needed in order to further identify the semen quality traits that could be included into the genetic evaluation of Belgian Piétrain terminal boars and also influence selection decisions applied across the whole breeding pyramid. Proper personnel training and a systematic monitoring of the CONC of the diluted semen doses using a counting chamber could be beneficial in AI centers where large deviations from their initial semen dilution target have been observed.

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