# Unraveling the actual background of second litter syndrome in pigs: based on Large White data

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## ARTICLE INFO

## ABSTRACT

Second litter syndrome (**SLS**) in sows is when fertility performance is lower in the second parity than in the first parity. The causes of SLS have been associated with lactation weight loss, premature first insemination, short lactation length, short weaning to insemination interval, season, and farm of farrowing. There is little known about the genetic background of SLS or if it is a real biological problem or just a statistical issue. Thus, we aimed to evaluate risk factors, investigate genetic background of SLS, and estimate the probability of SLS existing due to the statistical properties of the trait. The records of 246 799 litters (total number born, **TNB**) from 46 218 Large White sows were used. A total of 15 398 sows had SLS. Two traits were defined: first a binominal trait if a sow had SLS or not (biSLS) and second a continuous trait (Range) created by subtracting the total number of piglets born in the first parity (TNB1) from the piglets born in the second parity (TNB2). Lactation length, farm, and season of the farrowing had significant effects on SLS traits when tested as fixed effects in the genetic model. These effects are farm management-related factors. The age at first insemination and weaning to insemination interval were significant only for other reproduction traits (e.g., TNB1, TNB2, litter weight in parity 1 and 2). The heritability of biSLS was 0.05 (on observed scale), whereas heritability of Range was 0.03. To verify the existence of SLS data with records of 50 000 sows and 9 parities was simulated. The simulations showed that the average expected frequency of SLS across all the parities was 0.49 ($\pm$0.05) while the observed frequency in the actual data was 0.46 ($\pm$0.04). We compared this to SLS frequencies in 67 farms and only 2 farms had more piglets born in the first parity compared to the second. Therefore, on the individual sow level SLS is likely due to statistical properties of the trait, whereas on the farm level SLS is likely due to farm management. Thus, SLS should not be considered an abnormality nor a syndrome if on average the herd litter size in parity 2 is larger than in parity 1.

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## Implications

We show that second litter syndrome, that is, lower reproductive performance in the second parity compared to the first, should not be considered a syndrome or abnormality as long average litter size in parity 2 is larger than in parity 1. Incidences of second litter syndrome on the sow level are likely a statistical matter, whereas on the herd level a high frequency of second litter syndrome suggests a farm management issue. Comparing a farm's observed second litter syndrome frequency to its expected level based on normal distribution, mean, and standard deviation of all farms together can be used as a tool to identify second litter syndrome.

## Introduction

Between 20 and 60% of sows have lower reproductive performance (litter size and/or farrowing rate) in the second parity compared to the first parity (e.g., Penny et al., 1971; Morrow et al., 1989; Hoving et al., 2011b; Segura Correa et al., 2013). This is called second litter syndrome (**SLS**; Hoving et al., 2010) and many of its aspects are already quite well known and described in literature. The reduced litter size in the second parity is attributed to weight loss during the first lactation (Morrow et al., 1989; Schenkel et al., 2005; Thaker and Bilkei, 2005; Hoving et al., 2010), premature first insemination (Clowes et al., 2003a, Clowes et al., 2003b), short first lactation length (Morrow et al., 1992; Segura Correa et al., 2013), short weaning to insemination interval (Le Cozier et al., 1997; Boulot et al., 2013; Segura Correa et al., 2013), or seasonal effects on the second farrowing (Boulot et al., 2013; Segura Correa et al., 2013). Optimal management of sows can eliminate or reduce many of these effects. For example, adjusting the diet of highly productive sows can stop weight loss. Other causes such

as the age at first insemination have consequences that remain for the sow's lifetime reproductive performance.

Moreover, there is little known about the potential genetic background of SLS. Several studies reported the genetic correlation between the first and second parity (e.g., 0.62 in Large White and Landrace, Hermesch et al., 2000; 0.83 in Landrace, Hanenberg et al., 2001), but did not investigate SLS directly. Recently, a study applied random regression to analyze the genetic variation between parities of a sow (Sell-Kubiak et al., 2019). A non-unity genetic correlation between parity 1 and 2 may indicate genetic variation in SLS. In case the existence of SLS is heritable, then it would be possible to address it with selective breeding.

Second litter syndrome may, however, be only a statistical artifact caused by partitioning the sows into SLS and non-SLS groups. It is, therefore, important to investigate to what extent the observed frequency of SLS can be explained by the statistical properties of litter size, that is, the statistical properties of the trait distribution.

Thus, the objectives of this study are 1) to evaluate the potential risk factors associated with SLS, 2) to investigate the potential genetic background of SLS in Large White pigs, and 3) to determine the probability of SLS due to the statistical properties of the trait.

## Material and methods

### Data

Data for the genetic analysis were previously used in Sell-Kubiak et al. (2015, 2019). Litter size observations were collected between February 1998 and July 2014 on multiplication farms of Topigs Norsvin (Vught, the Netherlands). Overall 263 088 total number born (**TNB**) records were available from 69 238 Large White sows. The sows produced purebred and crossbred litters. The average TNB for all sows was 13.6 (±3.4). An average TNB in each parity is in Supplementary Table S1. For statistical analysis, only the sows with TNB recorded in both parity 1 (TNB1) and parity 2 (TNB2) were kept. This gave 92 436 litters from 46 218 sows with 15 398 SLS and 30 820 non-SLS sows. Also, the number born alive (**NBA**) and litter weight (LW) in parity 1 (NBA1, LW1) and parity 2 (NBA2, LW2) were available. The average for NBA traits was 11.67 (±2.98) and 12.63 (±3.23), whereas for LW traits 15.17 kg (±4.3) and 17.40 kg (±4.7), respectively, in parity 1 and 2. Based on all available TNB records, the average lifetime TNB (mTNB) per sow was estimated.

To estimate the heritability of SLS, we defined two traits. First, the binomial trait (biSLS) indicating whether the sow had the SLS (biSLS = 1) or not (biSLS = 0). The biSLS was 1 when the litter size in parity 2 was lower than in parity 1. The second trait (Range) was linear and described the difference between litter size in parity 1 and parity 2, and was normally distributed with a range from −18 to 18 (Fig. 1).

In the genetic analyses, we used five generations of pedigree if available. The total pedigree included 83 571 animals.

### Culling reasons

Out of 15,398 sows that had SLS, 12,826 were kept in herds for at least one more parity, whereas the remaining 2339 were culled after the second parity. Culling reason is difficult to record, because usually several factors affect the decision. In this population of Large White pigs, 75 sows were removed from the herds because litter size was too small and 661 due to various reproduction problems (e.g., poor lactation, problems with insemination, high piglet mortality) or maternal behavior problems (e.g., biting or crushing piglets). Other main reasons for culling were leg and mobility issues (N = 271) or various health problems, for example, heart or kidney failure, sudden death (N = 823). SLS was never listed as one of the culling reasons. The remaining 509 sows had no reason of culling recorded.

### Evaluation of risk factors

The available data were also evaluated based on the possible risk factors for SLS presented in the literature. Factors included farm-year-season of the second farrowing, the age at the first insemination (Age_ins), age at first successful fertilization (Age_fer), first lactation length (Lactation), and weaning to insemination interval (Interval) between parity 1 and 2. Interval was the actual number of days (Supplementary Table S2) or nine classes created by grouping certain number of days following the definition of the Topigs Norsvin breeding program: 0–2, 3–5, 6–7, 8–9, 10–11, 12–17, 18–23, 24–28, and 28+ days (Supplementary Table S3). For 665 sows, Age_ins and Age_fer were not available, whereas for 3881 sows Age_ins and Age_fer differed. Lactation was not available for 337 sows. If the sows did not have an observation for one of the risk factors, the record was included in the analysis but marked as missing. Descriptive statistics of the risk factors are presented in Table 1.
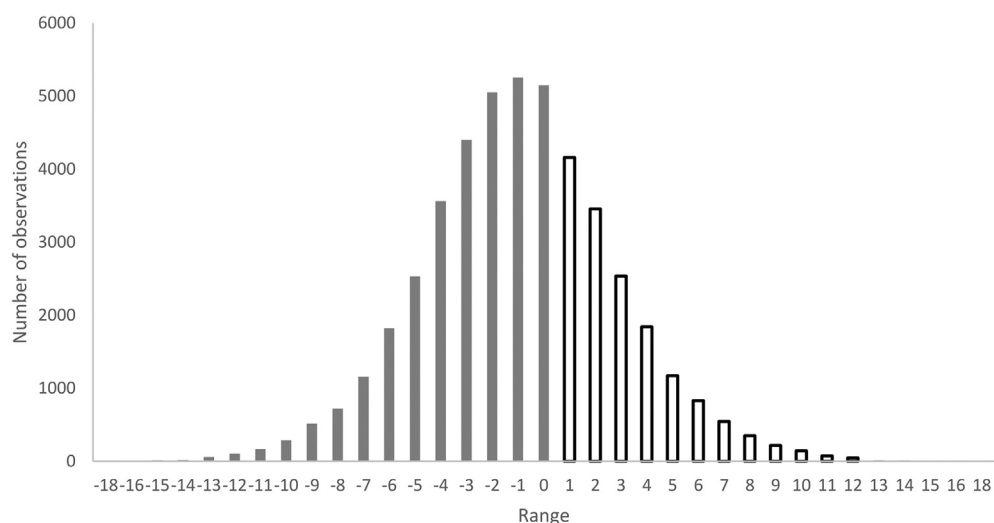


Fig. 1. Distribution of Range, the difference between litter size in parity 1 and litter size in parity 2 in Large White sows population. The grey columns indicate the sows without second litter syndrome and white columns indicate the sows with second litter syndrome.

**Table 1**

Descriptive statistics of risk factors: average age at first insemination, age at first successful fertilization, the first lactation length, and weaning to insemination interval for sows with or without second litter syndrome (SLS).

| Risk factor | | Non-SLS sows | SLS sows |
|---|---|---|---|
| Age at first insemination | N | 30 391 | 15 162 |
| | % | 67 | 33 |
| | Mean | 246.43 | 245.51 |
| | SD | 28.27 | 27.72 |
| | Min | 123 | 155 |
| | Max | 364 | 364 |
| Age at first fertilization | N | 30 391 | 15 162 |
| | % | 67 | 33 |
| | Mean | 249.97 | 249.08 |
| | SD | 31.99 | 31.56 |
| | Min | 123 | 157 |
| | Max | 688 | 521 |
| Lactation length | N | 25 379 | 12 185 |
| | % | 68 | 32 |
| | Mean | 25.73 | 25.31 |
| | SD | 4.47 | 4.03 |
| | Min | 21 | 21 |
| | Max | 64 | 65 |
| Weaning to insemination interval (actual values) | N | 28 544 | 14 554 |
| | % | 66 | 34 |
| | Mean | 5.73 | 5.48 |
| | SD | 4.04 | 3.55 |
| | Min | 0 | 0 |
| | Max | 25 | 25 |

*Heritability of second litter syndrome*

In the genetic analyses, the variance components for biSLS and Range were estimated with ASReml 4.1 (Gilmour et al., 2015). The available risk factors were fitted as fixed effects in the genetic model for biSLS and Range to test if they had significant effects on the trait. Each risk factor was tested as linear, linear and quadratic, and as a class variable (with the same number of levels as the available values per factor). Farm-year-season of the second farrowing and Lactation were added to the models for biSLS and Range (Table 2).

For the binomial trait biSLS, the logistic link was used in the generalized linear mixed model:

$$biSLS = \mathbf{X}_{biSLS}\mathbf{b}_{biSLS} + \mathbf{Z}_{biSLS}\mathbf{a}_{biSLS} + \mathbf{e}_{biSLS}$$

where biSLS is a vector of observations on SLS; $\mathbf{X}$ and $\mathbf{Z}$ are incidence matrices relating observations to effects; $\mathbf{b}_{biSLS}$ is a vector of fixed effects of farm-year-season of the second farrowing and Lactation on biSLS;

$\mathbf{a}_{biSLS}$ is a vector of random additive genetic effects, with $\mathbf{a}_{biSLS} \sim N(\mathbf{0}, \mathbf{A}\sigma^2_{abiSLS})$, where $\sigma^2_{abiSLS}$ is the additive genetic variance of biSLS, and $\mathbf{e}$ is a vector of residual, with $\mathbf{e}_{biSLS} \sim N(\mathbf{0}, \mathbf{I}_{ebiSLS}\sigma^2_{ebiSLS})$, where $\sigma^2_{ebiSLS}$ is the variance of residuals of biSLS. $\mathbf{I}_{ebiSLS}$ is the identity matrix of the appropriate dimensions and $\mathbf{A}$ is the numerator relationship matrix. In the analysis of binomial traits, the residual variance is by default fixed to 1.0 to avoid over/underestimation of variance components.

The trait Range was analyzed with the following model:

$$Range = \mathbf{X}_{Range}\mathbf{b}_{Range} + \mathbf{Z}_{Range}\mathbf{a}_{Range} + \mathbf{e}_{Range}$$

where Range is a vector of observations of a difference between TNB1 and TNB2; $\mathbf{b}$ is a vector of fixed effects of farm-year-season of the second farrowing and Lactation on Range; $\mathbf{a}$ is a vector of random additive genetic effects for Range, with $\mathbf{a}_{Range} \sim N(\mathbf{0}, \mathbf{A}\sigma^2_{aRange})$, where $\sigma^2_{aRange}$ is the additive genetic variance of Range; and $\mathbf{e}$ is a vector of residuals, with $\mathbf{e}_{Range} \sim N(\mathbf{0}, \mathbf{I}_{eRange}\sigma^2_{eRange})$, where $\sigma^2_{eRange}$ is the variance of residuals of Range. $\mathbf{I}_{eRange}$ is the identity matrix of the appropriate dimensions.

*Analysis of other traits*

Traditional reproduction traits and risk factors were analyzed with ASReml 4.1. These were TNB1, TNB2, NBA1, NBA2, LW1 and LW2, Age_ins, Lactation, and Interval. The analysis was performed similarly to the one performed for Range, but used farm-years-season of the first farrowing instead of the second farrowing for traits related to parity 1 (TNB1, NBA1, LW1). Traits from parity 1 did not include Interval and Lactation as a fixed effect because it was recorded in parity 2. In the model for Age_ins, only one fixed effect of sow's birth farm-year-season was used, since Lactation and Interval could not affect it.

*Genetic correlations*

The genetic correlations between SLS and traditional reproduction traits or risk factors were estimated using bivariate models in ASReml 4.1. The fixed effects for each trait are presented in Table 2 except Age_ins, which only had birth farm-year-season of a sow as a fixed effect. All bivariate models used the model below, which includes biSLS and TNB1 as examples:

$$\begin{bmatrix} biSLS \\ TNB1 \end{bmatrix} = \begin{bmatrix} \mathbf{X}_{biSLS} & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_{TNB1} \end{bmatrix} \begin{bmatrix} \mathbf{b}_{biSLS} \\ \mathbf{b}_{TNB1} \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_{biSLS} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_{TNB1} \end{bmatrix} \begin{bmatrix} \mathbf{a}_{biSLS} \\ \mathbf{a}_{TNB1} \end{bmatrix} + \begin{bmatrix} \mathbf{e}_{biSLS} \\ \mathbf{e}_{TNB1} \end{bmatrix}$$

where biSLS and TNB1 are vectors of observations as described above; $\mathbf{b}_{biSLS}$ and $\mathbf{b}_{TNB1}$ are vectors of fixed effects as described above; $\mathbf{a}_{biSLS}$

**Table 2**

The significance level of the fixed effect age at first insemination, age at first successful fertilization, weaning to insemination interval, and lactation length for traits: total number born in parity 1 (TNB1), total number born in parity 2 (TNB2), number born alive in parity 1 (NBA1), number born alive in parity 2 (NBA2), second litter syndrome (SLS), range between TNB1 and TNB2 (Range), mean litter size per sow (mTNB), litter weight in parity 1 (LW1), litter weight in parity 2 (LW2), lactation length as a trait (Lactation), and weaning to insemination interval as a trait (Interval).

| Trait | Age at first insemination | | | Age at first fertilization | | | Lactation length | | | Weaning to insemination interval (class) | Weaning to insemination interval (days) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Linear | Quadratic | Class | Linear | Quadratic | Class | Linear | Quadratic | Class | Class | Linear | Class |
| TNB1 | <0.001 | 0.009 | <0.001 | <0.001 | <0.001 | <0.001 | – | – | – | – | – | – |
| TNB2 | <0.001 | 0.051 | 0.007 | <0.001 | 0.002 | n.s.[1] | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| NBA1 | <0.001 | 0.001 | <0.001 | <0.001 | <0.001 | <0.001 | – | – | – | – | – | – |
| NBA2 | <0.001 | 0.003 | 0.003 | <0.001 | 0.004 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| mTNB | 0.002 | n.s. | 0.033 | 0.017 | n.s. | n.s. | <0.001 | <0.001 | <0.001 | n.s. | <0.001 | n.s. |
| SLS | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | <0.001 | n.s. | <0.001 | n.s. | n.s. | n.s. |
| Range | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | <0.001 | <0.001 | <0.001 | n.s. | n.s. | n.s. |
| LW1 | <0.001 | n.s. | <0.001 | <0.001 | 0.012 | <0.001 | – | – | – | – | – | – |
| LW2 | <0.001 | 0.001 | 0.010 | <0.001 | <0.001 | 0.034 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Interval | <0.001 | 0.004 | 0.002 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | – | – | – |
| Lactation | <0.001 | <0.001 | <0.001 | <0.001 | 0.001 | <0.001 | – | – | – | – | – | – |

[1] Not significant.

and $\mathbf{a}_{TNB1}$ are vectors of random additive genetic effects with $\begin{bmatrix} \mathbf{a}_{biSLS} \\ \mathbf{a}_{TNB1} \end{bmatrix} \sim$

$N\left(\mathbf{0} \begin{bmatrix} \sigma^2_{abiSLS} & \sigma_{abiSLS,aTNB1} \\ \sigma_{abiSLS,aTNB1} & \sigma^2_{aTNB1} \end{bmatrix} \otimes \mathbf{A}\right)$ where $\sigma^2_{aTNB1}$ is the additive ge-

netic variance of TNB1 and $\sigma_{abiSLS, aTNB1}$ is the covariance between addi-

tive genetic effects of biSLS and TNB1; and $\mathbf{e}_{biSLS}$ and $\mathbf{e}_{TNB1}$ are vectors of

residuals with $\begin{bmatrix} \mathbf{e}_{biSLS} \\ \mathbf{e}_{TNB1} \end{bmatrix} \sim N\left(\mathbf{0} \begin{bmatrix} \sigma^2_{ebiSLS} & \sigma_{ebiSLS,eTNB1} \\ \sigma_{ebiSLS,eTNB1} & \sigma^2_{eTNB1} \end{bmatrix} \otimes \mathbf{I}\right)$ where

$\sigma^2_{eTNB1}$ is the residual variance of TNB1 and $\sigma_{ebiSLS, eTNB1}$ is the covariance

between residuals of biSLS and TNB1.

### Verification of second litter syndrome

To investigate whether SLS is a real biological problem or just a statistical issue, two analyses were performed. First, we performed a simulation study that assumed the absence of any physiological factors that could induce SLS (i.e., there was no apparent reason for SLS to be present in the data). Second, we compared the frequency of SLS of each farm in the real data with the expectations based on the assumption of the normal distribution of the analyzed trait. In these analyses, we used TNB.

### Simulation

To verify the existence of SLS or any next parity syndrome (NLS), that is, a drop in TNB in the next parity in comparison to the previous one, we performed a simplistic stochastic simulation. Total number born for 50 000 unrelated sows was simulated over 10 parities (for simulation scheme, see Fig. 2). The number of piglets (TNB) in each parity was drawn from a multivariate normal distribution using mvtnorm package (Genz and Bretz, 2009) available in R software (R Core Team, 2019). The means and SD for each parity as well as phenotypic correlations between the parities were from the dataset used in this study (see Supplementary Tables S1 and S4). The simulation was replicated 100 times and the results averaged over all the replications.

The next litter syndrome is reported when the number of piglets in the next litter is lower than in the previous one: $TNB_i > TNB_{i+1}$. We split the simulated data set into two groups: 1) $TNB_i > TNB_{i+1}$ and 2) $TNB_i \le TNB_{i+1}$, where the first group had NLS. This division was done separately six times for the six parity pairs 1&2, 2&3, 3&4, 4&6, 6&7, 7&8.

### Expected frequency

The expected frequency of NLS or $TNB_i > TNB_{i+1}$ was assessed using properties of the normal distribution. A farm's observed frequency of SLS can be compared to its expected frequency using the normal distribution, mean, and standard deviation of all farms together. Therefore,
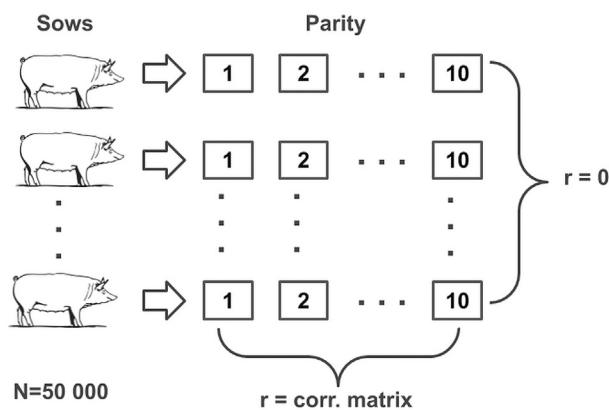
using function pnorm available in R software (R Core Team, 2019), we calculated the cumulative distribution function to predict the probability that TNB1 will be lower or equal to TNB2 as:

$$F_x(X) = P(X \le x)$$

where $x$ is average TNB1 for a particular farm and $X = N(\mu_{TNB2}, \sigma_{TNB2})$, $\mu_{TNB2}, \sigma_{TNB2}$ were based on the all farms in the dataset.

### Comparison of expected and observed second litter syndrome frequency

The data from individual herds were used to indicate the existence of SLS by comparing the observed frequency of SLS with the deterministically predicted frequency of TNB (Supplementary Table S5). In total, 67 herds with 21 to 3774 sows were analyzed. The observed frequency SLS was on average 0.33 ($\pm$0.07) and ranged from 0.16 to 0.60.

## Results

### Second litter syndrome in Large White population

The sows used for the genetic analysis had the smallest litters in early and late parities and the highest in parities 3 to 6. Evaluating only the average litter size will overlook the incidences of SLS in the population since it is only observed in sows that deviate from the average parity curve.

It was observed that 13 301 sows had SLS for both TNB and NBA, from which 8228 sows had TNB1 = NBA1. There were 2097 unique SLS sows for TNB caused by many stillborn piglets in TNB1, which decreased NBA1 enough to be lower than NBA2, and 2109 unique SLS sows for NBA caused by many stillborn piglets in TNB2, which decreased NBA2 enough to be lower than NBA1. Based on NBA, 15 410 sows were assigned as SLS sows, which is a very similar number to SLS based on TNB.

Average litter size and number of records for SLS sows and non-SLS sows are presented in Fig. 3. Second litter syndrome sows had more piglets ($P < 0.001$) than non-SLS sows with 14.03 ($\pm$2.58) piglets in the first litter and 10.86 ($\pm$2.96) piglets in the second compared to non-SLS sows that had 11.62 ($\pm$2.92) in the first parity and 14.61 ($\pm$2.83) piglets in the second parity (Fig. 3). In the remaining parities, SLS sows had on average 0.11 to 0.47 piglets less than non-SLS sows; these differences were not statistically significant. This indicates that later performance is unaffected by SLS.

The 12 826 sows with SLS were kept for later parities so if SLS has genetic component this syndrome could remain in the population.

### Risk factors

The Age_ins, the Age_fer, and Interval were not significant for biSLS and Range but were significant for other reproduction traits (Table 2). Only Lactation affected biSLS and Range ($P < 0.001$), with longer lactation reducing chances of SLS.

Farm-year-season effect of the farrowing was significant for biSLS and Range and was included in the models for those traits. There were no seasons or farms with SLS significantly different to the average of 33% of SLS cases, even in harsh seasons.

### Heritability of second litter syndrome

The additive genetic variance was 0.10 ($\pm$0.02) for biSLS and 0.48 ($\pm$0.07) for Range. The residual variance was 1.0 for biSLS (the residual variance in binomial analysis in ASReml 4.1 is 1 by default) and 13.57 ($\pm$0.09) for Range. Heritability for biSLS was 0.09 ($\pm$0.02) on the underlying scale and 0.05 on the observed scale (calculated based on Gilmour et al., 1985), whereas the heritability of Range was 0.03 ($\pm$0.005).



**Fig. 2.** Simulation scheme for litter size in pigs with correlations (r) between litter size in different parities.
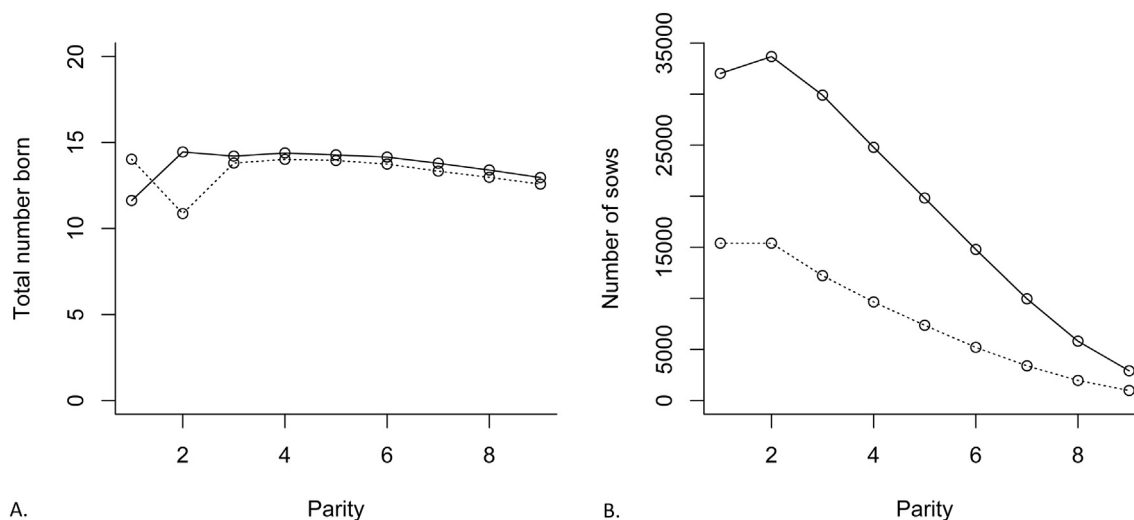
**Fig. 3.** A. Average litter size (TNB) across parities of Large White sows with second litter syndrome (dashed line) and sows without second litter syndrome (solid line). B. Number of observations across parities of Large White sows with second litter syndrome (dashed line) and sows without second litter syndrome (solid line).

*Genetic and phenotypic relationship between traits and risk factors*

The phenotypic and genetic correlations are presented in Table 3. The correlations were significant between SLS traits and traditional reproduction traits (TNB, NBA, LW in parity 1 or 2, and mTNB). The biSLS and Range are derived from TNB1 and TNB2, which are correlated with NBA1 and NBA2 as well as LW1 and LW2. Thus, existence of those correlations should be expected by default. The correlations between risk factors and SLS traits are close to "0" or have a very high SE suggesting insignificance of relationship between those traits (Table 3).

*Simulation results*

Dividing the dataset to simulate the next litter syndrome into NLS and non-NLS sows always resulted in a split between the average TNB observed in the two adjacent parities. The average TNB in the remaining parities was always equal in both groups of sows (see Fig. 4). For example, in case when the two first parities were analyzed (Fig. 4a), NSL sows in parity 1 had lower average TNB2 than non-SLS sows. This gives an impression of the existence of SLS and suggests a problem with highly performing sows in parity 1.

The average expected frequency across multiple parities ($0.49 \pm 0.05$) from the simulation was slightly higher than observed frequencies averaged for all the replicates ($0.46 \pm 0.02$; Table 4). This comparison

established that the null hypothesis of no presence of NSL in the data was accepted.

Data from individual herds were used to investigate if some farms have higher frequency of SLS beyond the null hypothesis of no NSL (Supplementary Table S5). The expected frequency of SLS was calculated using $\mu_{TNB_1}$ from each herd, and $\mu_{TNB_2}$ and $\sigma_{TNB_2}$ were based on the all herds. The expected frequency of SLS was on average 0.39 ($\pm 0.14$) and ranged from 0.08 to 0.65. The correlation between the expected and observed frequencies was weak ($-0.03$) and not significantly different from 0. The average difference between the observed and expected frequencies was $-0.06$ ($\pm 016$.). Out of 67 farms, 27 had lower expected SLS frequency than observed. This discrepancy was because of properties of the normal distribution and farms' average TNB1 and TNB2 and their SD rather than a SLS. Among the 27 farms with lower expected SLS frequency, 4 had lower average TNB2 than TNB1 (farm numbers 62, 63, 66, and 67; Supplementary Table S5). Two of those farms (63 and 67) had low number of records available which might have affected the frequencies. Thus, only farms 62 and 63 present a real issue with lower average TNB2 than TNB1. We checked all the records for these two farms, but found no clear indications of biological or management issues. Nonetheless, those farms should be observed more closely as they do not perform as expected. The comparison of the observed and expected frequencies of SLS used in this study can identify the presence of NLS in a herd.

**Table 3**
Estimates of phenotypic and genetic (additive genetic effect and residuals) correlations between second litter syndrome as binomial trait (biSLS) or difference between litter size in the first and second parity (Range) with: total number born in parity 1 (TNB1), total number born in parity 2 (TNB2), number born alive in parity 1 (NBA1), number born alive in parity 2 (NBA2), mean litter size per sow (mTNB), litter weight in parity 1 (LW1), litter weight in parity 2 (LW2), age at first insemination (Age_ins), weaning to insemination interval (Interval), and lactation length (Lactation).

| Correlation | | TNB1 | TNB2 | NBA1 | NBA2 | mTNB | LW1 | LW2 | Age_ins | Interval | Lactation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| biSLS | Phenotypic | 0.37 | −0.52 | 0.35 | −0.50 | 0.25 | −0.35 | −0.11 | −0.02 | −0.03 | −0.06 |
| | Additive genetic effects | 0.64 (0.06) | −0.61 (0.05) | 0.48 (0.08) | −0.57 (0.06) | −0.07 (0.02) | 0.29 (0.04) | −0.23 (0.02) | 0.12 (0.08) | −0.14 (0.15) | −0.21 (0.13) |
| | Residuals | 0.41 (0.01) | −0.56 (0.01) | 0.38 (0.003) | −0.51 (0.002) | −0.37 (0.02) | 0.53 (0.03) | −0.42 (0.04) | −0.001 (0.004) | −0.03 (0.003) | −0.03 (0.004) |
| Range | Phenotypic | 0.53 | −0.65 | 0.50 | −0.62 | 0.36 | −0.43 | −0.13 | −0.02 | −0.04 | −0.08 |
| | Additive genetic effects | 0.27 (0.03) | −0.46 (0.04) | 0.28 (0.07) | −0.39 (0.07) | −0.08 (0.03) | 0.29 (0.04) | −0.17 (0.02) | −0.002 (0.08) | −0.09 (0.14) | −0.11 (0.11) |
| | Residuals | 0.63 (0.06) | −0.70 (0.07) | 0.57 (0.004) | −0.65 (0.004) | −0.32 (0.04) | 0.47 (0.03) | −0.52 (0.06) | 0.007 (0.006) | −0.04 (0.006) | −0.03 (0.006) |
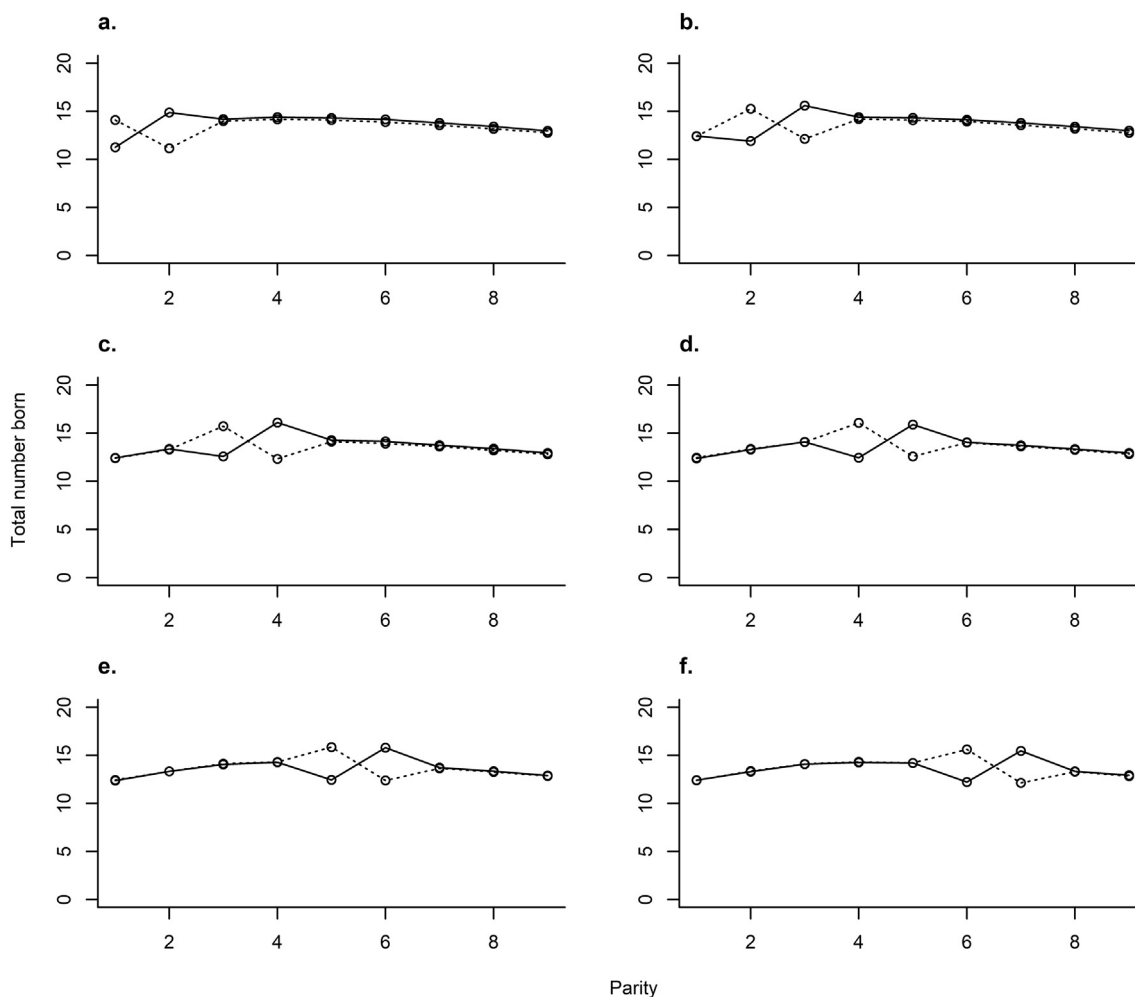
**Fig. 4.** Average number of piglets born in the consecutive simulated parities. Dashed line represents averages for sows that had less piglets in the parity n + 1 than in parity n; solid line is for the remaining sows. Letters a. to f. denote parity numbers in which the condition was checked – parity 2–7, respectively.

**Table 4**
Frequency of expected and observed sows with larger litter in the preceding parity than in the next one, analyzed for parities 1–6 and averaged over all simulated repetitions.

| Frequency | Parity | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 |
| Expected | 0.45 | 0.42 | 0.48 | 0.51 | 0.52 | 0.54 |
| Observed | 0.43 | 0.44 | 0.44 | 0.47 | 0.47 | 0.49 |

## Discussion

The objectives of this study were to evaluate the potential risk factors associated with SLS, to investigate the genetic background of SLS in Large White pigs, and to determine the probability of SLS in the population existing due to the statistical properties of the trait.

### Second litter syndrome's risk factors

First, we investigated the risk factors that could potentially cause SLS. Lactation length, however, was the only significant risk factor for SLS traits when used as fixed effect in the genetic model. This risk factor was not confirmed by the phenotypic and genetic correlations between Lactation and SLS traits. The quadratic relationship between Lactation and Range was significant which could explain why no correlation existed. The quadratic relationship between Lactation and biSLS, however, was not significant. Morrow et al. (1992) indicated that longer lactation leads to larger second litter because young sows had more time to recover after first gestation. However, not all researchers confirmed the Lactation association with SLS (e.g., Segura Correa et al., 2013). Nonetheless, this effect was highly significant for all reproduction traits analyzed in our study. Lactation length is highly management related because the breeder decides when to wean the piglets. Therefore, longer lactations would be recommended to help with overall reproductive performance in the herd.

Age at first insemination, age at first fertilization, and weaning to insemination interval did not contribute to SLS traits, but did to traditional reproduction traits. This confirms what is already known from literature. Age_ins and Age_fer affect overall reproductive performance of the sow (e.g., Schukken et al., 1994; Sterning et al., 1998; Tummaruk et al., 2001), whereas an inadequate Interval affects second parity traits (i.e., TNB2, NBA2, LW; Segura Correa et al., 2013).

Another biological risk factor present in the literature is large lactation weight loss after the first farrowing of the sow (Morrow et al., 1989; Morrow et al., 1992; Whittemore, 1996; Kemp and Soede, 2004; Schenkel et al., 2005; Thaker and Bilkei, 2005; Hoving et al., 2010). In general, the conclusion is that primiparous sows are more susceptible to the lactation weight loss causing incomplete recovery at weaning and low ovulation rate or increased embryonic mortality. Those findings were followed up by the experiments showing how SLS could be

avoided with an adjusted diet (Whittemore, 1996; Hoving et al., 2011a, 2011b; Soede et al., 2013) or hormonal treatment (Vargas et al., 2006; Everaert et al., 2007). We did not have the information about lactation weight loss because it is not routinely collected on Topigs Norsvin farms. However, based on a study done by Bergsma (2011) on data collected between October 2001 and December 2005 for 363 commercial crossbred sows from Institute for Pigs Genetics (currently Topigs Norsvin Research Center, Beuningen, the Netherlands) there was no effect of lactation weight loss on subsequent reproductive performance. Thus, reduced reproduction on the individual sow level in highly performing, primiparous sows can be prevented by adjusting the diet of those sows to help them recover before their second parity. However, on a herd level the lactation weight loss is not associated with lower reproductive performance.

The last risk factor indicated in the literature for the SLS is the season and farm of the farrowing (Boulot et al., 2013; Segura Correa et al., 2013). The effect of farm-year-season of the farrowing was significant for all traits including biSLS and Range. Boulot et al. (2013) suggested that SLS can happen more often on bigger farms than on smaller farms because there is not enough time spent per animal to adjust diets. We did not see such relationship and the farm effect accounted for more aspects than just farm size, such as feed fluctuations or management in general. The significance of the effect of year and season was not surprising since insemination of pigs is all year long with some farrowing in more difficult conditions than others such as winter and summer, or drought years. Some breeding programs consider the harsh environmental conditions (Herrero-Medrano et al., 2015) or even disease occurrence (Rashidi et al., 2014) to select for robust animals performing well under changing conditions. We did not observe any differences between seasons in the incidence of SLS.

In summary, farm management can control significant SLS risk factors Lactation and farm-year-season, whereas adjusting the diet of primiparous, highly performing sows controls the most common risk factor of lactation weight loss. Therefore, the farm management can avoid reduced performance in second parity, and as long the average TNB2 is higher than TNB1 the breeder should not be alarmed.

*Heritability of second litter syndrome and genetic correlations*

The heritability of biSLS (on observed scale) and Range is lower than other reproductive traits. There is genetic variation within the parity curve for litter size in pigs (Sell-Kubiak et al., 2019), indicated by non-unity correlations between parities. Thus, phenotypic and genetic fluctuations between the parities of sows are expected. The way SLS was defined in our study as a binomial trait (biSLS) or a difference between TNB1 and TNB2 (Range) is another approach to study variation between parities. However, to our knowledge there is no other possibility to define SLS for statistical analysis.

Although our study presents existence of genetic relationships between traditional reproductive traits and SLS traits, those correlations are expected. The biSLS and Range are derived from TNB1 and TNB2, which are highly correlated with NBA1 and NBA2 and litter weight in parities 1 and 2. The remaining correlations between mTNB and risk factors are close to "0" or have very high SE indicating not significant results.

*Probability of second litter syndrome occurrence*

Dividing the dataset to simulate the next litter syndrome into NLS and non-NLS sows always resulted in a split between the average TNB observed in the two adjacent parities. This gives an impression of the existence of SLS and suggests a problem with highly performing sows in parity 1. Furthermore, the pattern observed for second parity syndrome was very similar across the remaining parities for the next parity syndrome. Thus, it could be expected that the observed pattern of decrease in performance in the next parity is visible due to the statistical

properties of the trait's (i.e., litter size) distribution rather than a biological phenomenon. Assuming that SLS and NLS are just statistical properties of the traits, the observed number of NLS sows will be determined by the means and standard deviations of parity n and parity n + 1. For instance, as the difference in means increases the frequency of NLS decreases. Similarly, a lower standard deviation in parity n + 1 decreases the expected frequency of NLS. A higher difference in means and a lower standard deviation will reduce the potential overlap between distributions of the trait between consecutive parities. For example, if $\mu_{TNB_2} = 10$ and $\sigma_{TNB_2} = 5$, while $\mu_{TNB_1} = 9$ then in parity 2 we will find 42% sows that have a lower TNB in parity 2 than in parity 1, that is, SLS. As the condition for NLS is $TNB_n > TNB_{n+1}$, by definition $\mu_{TNB_{n+1}}$ has to be higher than $\mu_{TNB_n}$. If, $\mu_{TNB_2} = 12$ only 27% sows have a lower TNB in parity 2 than in parity 1. The simulation confirmed also that NLS instances are related to the means and variance of the litter size and not necessarily to any physiological or management issues. Furthermore, these characteristics could be changed through breeding.

*Is second litter syndrome an actual problem in pig breeding?*

The SLS is present in the literature already for quite some time (Penny et al., 1971; Morrow et al., 1989) and is mentioned as one of the reasons for removing sows from herds (Hoving et al., 2011a). Surprisingly, SLS is not a common reason to cull sows after the first parity, as the literature suggests. At least not on commercial farms of Topigs Norsvin, where nearly 13 000 sows with SLS were kept for further breeding purposes. Moreover, on average the performance of the sows in our study is as expected physiologically: the sows had the smallest litters in early and late parities and the highest in parities 3 to 6 (Sell-Kubiak et al., 2019). This was also observed in Japanese herds (Saito et al., 2010). Only after dividing the sows into two groups, the SLS exists (Saito et al., 2010 and this study).

## Conclusion

The observed difference between TNB1 and TNB2 and heritability of biSLS and Range suggest that SLS is a real biological phenomenon. However, the rest of our results presented no relationship between potential risk factors for SLS that have different source than management. Furthermore, the simulations showed that any next litter syndrome is as probable as SLS due to the properties of the TNB distribution. Therefore, SLS on an individual sow level is not a physiological issue, but an event that can be expected since litter size is a trait following a multivariate normal distribution. Only abnormalities on a herd level, that is, a drop in the average litter size for parity 2 compared to parity 1 in a given time or a batch, should be used by a farmer to adjust their management. In any other case, a drop in TNB2 in comparison to TNB1 on an individual sow level can be ignored. The frequency of SLS within the population can also be lowered by breeding. Avoiding selection pressure on litter size in parity 1 (Sell-Kubiak et al., 2019) will help to slow down the increase of litter size in the first parity, which negatively affects reproductive performance of young sows. Alternatively, breeders can put more selection pressure on increasing litter size in parity 2. Both can be simultaneously addressed by selective breeding.

## Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2020.100033

## Ethics approval

Not applicable.

## ORCHID

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## References

Bergsma, R., 2011. Genetic aspects of feed intake in lactating sows. PhD Thesis. Wageningen University, Wageningen, the Netherlands.
Boulot, S., Despres, Y., Badouard, B., Salle, E., 2013. Le "syndrome de 2eme portee" dans le'elevages francais: prevalence de differents profils et facteurs de risqué. Journal of Recherche Porcine 45, 79–80.
Clowes, E.J., Aherne, F.X., Foxcroft, G.R., Baracos, V.E., 2003a. Selective protein loss in lactating sows is associated with reduced litter growth and ovarian function. Journal of Animal Science 81, 753–764.
Clowes, E.J., Aherne, F.X., Schaefer, A.L., Foxcroft, G.R., Baracos, V.E., 2003b. Parturition body size and body protein loss during lactation influence performance during lactation and ovarian function at weaning in first-parity sows. Journal of Animal Science 81, 1517–1528.
Everaert, N., Vanderhaeghe, C., Mateusen, B., Dewulf, J., Van Soom, A., de Kruif, A., Maes, D., 2007. Effects of post-weaning altrenogest treatment in primiparous sows. Vlaams Diergeneeskundig Tijdschrift 76, 293–299.
Genz, A., Bretz, F., 2009. Computation of multivariate normal and t probabilities. volume 195 of Lecture Note Series. Springer, Heidelberg, Germany.
Gilmour, A.R., Anderson, R.D., Rae, A.L., 1985. The analysis of binomial data by a generalised linear mixed model. Biometrika 72, 593–599.
Gilmour, A.R., Gogel, B.J., Cullis, B.R., Welham, S., Thompson, R., 2015. ASReml user guide release 4.1 structural specification. VSN Int. Ltd., Hemel Hempstead, UK.

Hanenberg, E., Knol, E.F., Merks, J.W.M., 2001. Estimates of genetic parameters for reproduction traits at different parities in Dutch landrace pigs. Livestock Production Science 69, 179–186.
Hermesch, S., Luxford, B.G., Graser, H.-U., 2000. Genetic parameters for lean meat yield, meat quality, reproduction and feed efficiency traits for Australian pigs: 3. Genetic parameters for reproduction traits and genetic correlations with production, carcase and meat quality traits. Livestock Production Science 65, 261–270.
Herrero-Medrano, J.M., Mathur, P.K., ten Napel, J., Rashidi, H., Alexandri, P., Knol, E.F., 2015. Estimation of genetic parameters and breeding values across challenged environments to select for robust pigs. Journal of Animal Science 93, 1494–1502.
Hoving, L.L, Soede, N.M., Graat, E.A.M., Feitsma, H., Kemp, B., 2010. Effect of live weight development and reproduction in first parity on reproductive performance of second parity sows. Animal Reproduction Science 122, 82–89.
Hoving, L.L, Soede, N.M., Graat, E.A.M., Feitsma, H., Kemp, B., 2011a. Reproductive performance of second parity sows: relations with subsequent reproduction. Livestock Science 140, 124–130.
Hoving, L.L, Soede, N.M., Van Der Peet-Schwering, C.M.C., Graat, E.A.M., Feitsma, H., Kemp, B., 2011b. An increased feed intake during early pregnancy improves sow body weight recovery and increases litter size in young sows. Journal of Animal Science 89, 3542–3550.
Kemp, B., Soede, N.M., 2004. Reproductive problems in primiparous sows. Proceedings of the 18th Congress of International Pig Veterinary Society, 27 June – 1 July 2004, Hamburg, Germany, pp. 843–848.
Le Cozier, Y., Dagorn, J., Dourmad, J.Y., Johansen, S., 1997. Effect of weaning-to-conception interval and lactation length on subsequent litter size in sows. Livestock Production Science 51, 1–11.
Morrow, W.E.M., Leman, A.D., Williamson, N.B., Moser, R., Pijoan, C., 1989. Improving parity-two litter size in swine. Journal of Animal Science 67, 1707–1713.
Morrow, W.E.M., Leman, A.D., Williamson, N.B., Morrison, R.B., Robinson, R.A., 1992. An epidemiological investigation of reduced second-litter size in swine. Preventive Veterinary Medicine 12, 15–26.
Penny, R.H.C., Edwards, M.J., Mulley, R., 1971. The reproductive efficiency of pigs in Australia with particular reference to litter size. Australian Veterinary Journal 47, 194–202.
R Core Team, 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria https://www.R-project.org/.
Rashidi, H., Mulder, H.A., Mathur, P., van Arendonk, J.A.M., Knol, E.F., 2014. Variation among sows in response to porcine reproductive and respiratory syndrome. Journal of Animal Science 92, 95–105.
Saito, H., Sasaki, Y., Hoshino, Y., Koketsu, Y., 2010. The occurrence of decreased numbers of pigs born alive in parity 2 sows does not negatively affect herd productivity in Japan. Livestock Science 128, 189–192.
Schenkel, A.C., Kummer, R., Schmidt, A.C.T., Fries, H.C.C., Bernardi, M.L., Bortolozzo, F.P., 2005. Caracterização da síndrome do segundo parto em suínos. Proceedings of XII Congresso Brasileiro de Veterinários Especialistas em Suínos, 4–7 October 2005, Fortaleza, Brazil, pp. 252–253.
Schukken, Y.H., Buurman, J., Huirne, R.B., Willemse, A.H., CVernooy, J., Van den Broek, J., Verheijden, J.H., 1994. Evaluation of optimal age at first conception in gilts from data collected in commercial swine herds. Journal of Animal Science 72, 1387–1392.
Segura Correa, J.C., Alzina-López, A., Santos-Ricalde, R.H., 2013. Risk factors associated with the occurrence of the second-litter syndrome in sows in southeastern Mexico. Scientific World Journal 2013, 969402.
Sell-Kubiak, E., Duijvesteijn, N., Lopes, M.S., Janss, L.L.G., Knol, E.F., Bijma, P., Mulder, H.A., 2015. Genome-wide association study reveals novel loci for litter size and its variability in a Large White pig population. BMC Genomics 16, 1049.
Sell-Kubiak, E., Knol, E.F., Mulder, H.A., 2019. Selecting for changes in average "parity curve" pattern of litter size in Large White pigs. Journal of Animal Breeding and Genetics 136, 134–148.
Soede, N.M., Hoving, L.L., van Leeuwen, J.J., Kem, B., 2013. The second litter syndrome in sows; causes, consequences and possibilities of prevention. Proceedings of the 9th international conference on pig reproduction, 9–12 June 2013, Olsztyn, Poland, pp. 28–34.
Sterning, M., Rydhmer, L., Eliasson-Selling, L., 1998. Relationships between age at puberty and interval from weaning to estrus and between estrus signs at puberty and after the first weaning in pigs. Journal of Animal Science 76, 353–359.
Thaker, M.Y.C., Bilkei, G., 2005. Lactation weight loss influences subsequent reproductive performance of sows. Animal Reproduction Science 88, 309–318.
Tummaruk, P., Lundeheim, N., Einarsson, S., Dalin, A., 2001. Effect of birth litter size, birth parity number, growth rate, backfat thickness and age at first mating of gilts on their reproductive performance as sows. Animal Reproduction Science 66, 225–237.
Vargas, A.J., Bernard, M.L., Wentz, I., Neto, G.B., Bortolozzo, F.P., 2006. Time of ovulation and reproductive performance over three parities after treatment of primiparous sows with PG600. Theriogenology 66, 2017–2023.
Whittemore, C.T., 1996. Review: nutrition reproduction interactions in primiparous sows. Livestock Production Science 46, 65–83.