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Mixing aggression intensity is associated with age at first service and floor type during gestation, with implications for sow reproductive performance

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ABSTRACT

Aggression resulting from mixing to establish a dominance hierarchy is a major welfare concern for group-housed sows. The associated stress can negatively impact aspects of reproductive performance. Objectives of this study were to investigate associations between 1) age at first service (**AFS**) and mixing aggression intensity in first parity sows, 2) mixing aggression intensity and reproductive performance within and between parity one (P1) and parity two (P2), and 3) mixing aggression intensity, floor type during gestation and reproductive performance. Gilts ($n = 160$, hereafter referred to as sows) were mixed into stable groups of eight unfamiliar individuals approximately 4 days after artificial insemination, housed on fully slatted concrete (**CON**; $n = 80$) floor uncovered or covered with rubber slat mats (**RUB**; $n = 80$), and followed through two parities. Skin lesions (**SLMIX**; a proxy for the intensity of mixing aggression), were scored post mixing in each parity according to severity (0 = no lesions to 5 = severe lesions) on five body regions (ear, neck, hindquarter, rump, and belly) on the left and right sides, and at the tail/anogenital region. Total SLMIX score was calculated for each sow. Data on reproductive performance traits were acquired retrospectively from farm records for both parities. Two analyses were performed: 1) data from each parity were analysed separately and 2) SLMIX score in P1 was used to predict reproductive performance in P2. Lower AFS was associated with a lower SLMIX score in P1 ($P = 0.031$). There was no association between SLMIX score and reproductive performance in P1, while sows with higher SLMIX score in P2 had a higher proportion of piglets dead during lactation ($P = 0.027$) and a longer cycle length ($P = 0.003$) in P2. Sows with higher SLMIX scores in P1 had more non-productive days ($P < 0.001$) in P2. Concrete sows had a higher SLMIX score than RUB sows in P1 ($P = 0.015$), but not in P2. In addition, CON sows had a higher proportion of piglets born dead ($P = 0.013$) compared with RUB sows in P2. Mixing aggression has a negative influence on reproductive performance within parities, and it may also have a long-term negative carry-over effect on reproductive performance in subsequent parities. Serving gilts at younger ages could help to minimize the intensity of aggression at mixing, while housing on rubber flooring has beneficial implications for their reproductive performance.

Implications

Aggression resulting from the mixing of unfamiliar sows compromises welfare and could have detrimental effects on reproductive performance. Results of this study suggest that serving gilts at a younger age could potentially improve lifetime reproductive performance and animal welfare, as a consequence of the reduced intensity of mixing aggression. In addition, our results suggest that housing sows on rubber flooring could reduce the intensity of mixing aggression, and positively affect reproductive performance. Thus, serving gilts at younger ages and providing rubber floors can improve sow performance and welfare.

Introduction

Mixing aggression resulting from fighting to establish a dominance hierarchy has a negative impact on sow welfare and reproductive performance (Munsterhjelm et al., 2008). Direct effects on sow welfare include skin lesions [which can be used as a proxy for aggression (de Koning, 1984, Turner et al., 2006)], and other injuries, lameness and fear (Maes et al., 2016; Martinez-Miro et al., 2016). Mixing aggression is also associated with stress reflected in increased cortisol levels (Arey and Edwards, 1998). This in turn mediates the negative effects

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on reproductive performance (Einarsson et al., 2008) such as impaired pre-ovulatory oestrogen surges (Turner et al., 2002). Ultimately, this is associated with increased embryonic losses (Kranendonk et al., 2006) and lower litter performance (Tonepohl et al., 2013). In addition, the associated prenatal stress is linked to lower the overall number of piglets born alive per litter, lower litter size (Einarsson et al., 2008; Greenwood et al., 2019), increases in mummified foetuses and piglets born dead (Turner et al., 2005), and an overall decrease in reproductive success at farrowing (Salak-Johnson, 2017). Moreover, performance in parity one predicts sow performance in subsequent parities (Gruhot et al., 2017). This could be partly mediated by a potential detrimental, long-term effect of aggression experienced in parity one on performance in subsequent parities (Turner et al., 2005). Mixing aggression could also contribute to chronic stress, as animals that receive and/or inflict high levels of aggression at mixing, will continue to receive and/or inflict high levels of aggression later in life (Turner et al., 2009).

Age at first service (**AFS**) affects reproductive performance, with gilts served at a younger age staying in the herd longer, having more piglets born alive per litter and producing more pigs over a lifetime (Cottney et al., 2012; Koketsu et al., 2020). While this is mainly a result of extended reproductive life (Cottney et al., 2012; See and Knauer, 2019), it can also be due to improved conception and farrowing rates (Koketsu et al., 1999), as well as reduced wean-to-first-service intervals of younger gilts (Holm et al., 2005). Given the implications which stress has for reproductive performance in sows, this could also be mediated by an effect of AFS on the intensity of aggression at mixing. Younger gilts tend to be smaller in size, which reduces their ability to inflict damage on others (Clark and D'Eath, 2013), and they also tend to break away from fights sooner (Pitts et al., 2000).

Floor-type could affect mixing aggression and reproductive performance. Certain floor types such as rubber flooring are more slippery than concrete (**CON**), which may discourage sows from engaging in prolonged fights at mixing due to poor foothold (Boyle and Llamas Moya, 2003; Palmer et al., 2010). While this could conceivably delay fights at mixing, thus delaying the establishment of the dominance hierarchy (Barnett et al., 1993), there could be positive implications for sow reproductive performance (Einarsson et al., 2008). Poor foothold and associated fear of slipping are arguably negative for sow welfare; however, there are studies showing improvements to sow welfare associated with rubber flooring, possibly mediated by improved comfort (Elmore et al., 2010; Calderón Díaz et al., 2013). Specifically, rubber flooring has protective effects on claw and limb health, and on lameness incidence (Calderón Díaz et al., 2013). This could help to reduce the risk of piglets being crushed by sows in farrowing crates (Pfeiffer et al., 2019). It may also mean that sows experience less pain and distress (Heinonen et al., 2013), and thereby show improved reproductive performance. We hypothesized that 1) gilts served at a younger age are exposed to less aggression at mixing, and that this results in improved reproductive performance and welfare, 2) that reduced mixing aggression can have a positive effect on reproductive performance, and 3) that rubber flooring is associated with less aggression at mixing. Hence, the objectives of this study were to investigate possible associations between 1) AFS and mixing aggression intensity, 2) mixing aggression intensity and reproductive performance within and between parity one and two, and 3) mixing aggression intensity, floor type (CON vs rubber), and reproductive performance.

Material and methods

Care and use of animals

Data used for this study were originally collected for a project investigating the use of rubber flooring on sow welfare with a special focus on limb and claw health. Data were collected from October 2010 to February 2012 on a 1 000 sow farrow-to-finish commercial Irish pig farm with weekly farrowing batches. Details regarding animal husbandry practices and results for the associations between floor type, locomotory ability, claw, limb, and skin lesions were previously described in Calderón Díaz et al. (2013). In brief, the study followed 160 (119 Large White × Landrace, and 41 Landrace) replacement gilts during two consecutive parities. None of the authors had input into animal management decisions, and thus, farm staff were in charge of performing overall checks as per routine practice. This included oestrus detection, pregnancy determination, and overall health status checks.

Assigning animals to trial and management during the first parity

Gilts were home reared, produced from the nucleus of purebred Landrace sows present on the farm. They were identified by an ear notch at birth, and at approximately 24 weeks of age were transferred to gilt rearing accommodation. Gilts were housed in groups of 10 to 12 animals in fully slatted pens, and were dry fed with *ad libitum* access to wheat-barley-soy-bean-meal-based gilt diet until they were approximately 150 kg. Gilts were then moved to the service house and kept in groups of eight in fully slatted pens, and were exposed daily to a rotation of two mature vasectomised boars using direct single boar contact, and were also observed for signs of standing oestrus. On average, gilts were first served at 244.4 ± 23.68 days of age indicating that they were not artificially inseminated at their pubertal oestrus, and were likely served on their second oestrus as per farm practice. However, it was not possible to verify if indeed they were served on their second oestrus. Gilts were artificially inseminated, immediately after confirming oestrus by applying the back-pressure test, and also 24 h after the first service. Oestrus synchronization was not practiced on the farm. Gilts remained in the same pen in the service house, and once eight gilts with similar body condition score (**BCS**) were served, they were moved to the experimental pens in the gestation house within 1 week after service, where they were kept in stable groups of eight until 1 week before their expected farrowing date. Gilts returning to oestrus were inseminated in the gestation pen and remained in the same groups.

The farm followed a rotational arrangement to allocate animals to different pens in the gestation house. During gestation, gilts (hereafter referred to as sows) were housed in pens with free access feeding stalls (1.51 m length × 0.75 m width × 1.23 m height) and an unobstructed area behind (2.40 m length × 2.94 m width) for exercise and dunging. Pens had fully slatted CON floors which were either uncovered ($n =$ 80 sows), or covered with 10-mm thick rubber slat mats (**RUB**; $n =$ 80 sows; EasyFix Rubber Products, Ballinasloe, County Galway, Ireland). The RUB consisted of a two-strip system with circular-shaped patterns on the surface and wedges underneath for fixation to the CON slats [for more details see Calderón Díaz et al., 2013]. In total, RUB were installed in 16 pens randomly distributed throughout the gestation house. Sows were kept in stable groups of eight where they were free to move about the pen at all times. Due to the low number of rubber pens available compared with the number of CON pens, and to avoid interfering with farm management practices, CON gilts went on trial between October 2010 to March 2011, and RUB gilts went on trial between October 2010 and May 2011. In total, 59 gilts were inseminated in autumn, 61 gilts were inseminated in winter, and 40 gilts were inseminated in spring.

On day 110 of gestation, sows were moved to the farrowing accommodation, where they were kept in conventional individual farrowing crates with plastic-coated woven wire floors. Sows were weaned approximately 28 days *post partum*. Twenty-three sows were culled/died during parity one (12 CON and 11 RUB). Sows were culled due to leg problems (10 CON sows and one RUB sow), six sows were culled due to reproductive failure (one CON sow and five RUB sows) and six sows were culled or died due to other reasons (one CON sow and five RUB sows).

Management during the second parity

At weaning, sows were moved to the service house where they were kept in gestation stalls (2.10 m length × 0.55 m width × 1.06 m height) with fully slatted CON floors. They were inseminated after confirming standing oestrus by applying the back-pressure test, and also 24 h after the first service. In total, 80 sows were inseminated in spring, 50 sows were inseminated in summer, and 7 sows were inseminated in autumn. Sows were transferred into the same gestation accommodation within 1 week of service where they remained until 1 week before farrowing, after which they were transferred to the farrowing accommodation. Sows returning to oestrus were inseminated in the gestation pen and remained in the same groups. It is important to note that although sows were housed on the same floor type in both parities, group composition changed within flooring type between parity one and parity two due to service returns. Therefore in the second parity, sows were mixed with unfamiliar experimental sows as well as with non-experimental sows. The non-experimental sows were generally, but not necessarily, second parity animals; however, they were likely similar in terms of BCS, as older sows that were particularly thin or compromised in some other way, were sometimes mixed with the younger sows. However, as the identification of the non-experimental animals in the pens was not recorded, we cannot be 100% certain that all non-experimental animals were second parity sows. Nonetheless, the overall effect of re-mixing was likely similar between floor treatments, as the ratio between experimental to non-experimental sows (1:1.4 on CON and 1:1.2 on RUB) and an average number of first parity groups from which second parity groups originated (2.4 for CON and 2.6 for RUB) was similar between floor types. During the second parity, one RUB sow was removed (i.e. was culled or died) due to unknown reasons.

Measurements

All the measurements were taken by one trained observer to avoid inter-observer variation. The observer was trained to use the scoring systems by an experienced researcher over a period of approximately 4 weeks. Training involved repeated measurements of 20 sows by both Laura Ann Boyle and Julia Adriana Calderón Díaz, and continued until at least 90% intra- and inter-observer scores for repeatability were achieved.

Body condition score

Body condition was scored at service in both parities using a five-point scale where 1 = emaciated: hip and backbone visible, bone structure apparent; 2 = thin hips, backbone noticeable and easily felt, and ribs and spine can be felt; 3 = normal: hips and backbone only felt with firm palm pressure, body tube-shaped; 4 = fat: hips and backbone cannot be felt, body tending to bulge; and 5 = overly fat: hips and backbone covered, body shape bulbous.

Skin lesion scores

Skin lesion scores were recorded for two consecutive parities. Sows were individually inspected for skin lesions at service, post mixing (1.6 ± 0.96 days post mixing in parity one and 1.4 ± 0.86 days post mixing in parity two), mid-pregnancy (58.1 ± 4.72 days of gestation in parity one and 54.3 ± 10.19 days of gestation in parity two) and before farrowing (101.9 ± 5.71 days of gestation in parity one and 103.7 ± 7.69 days of gestation in parity two). Skin lesions were examined on five body regions (ear, neck, hindquarter, rump, and belly) on the left and right sides, along with the examination of the tail/anogenital region. Skin lesions were scored as follows: 0 = no lesions; 1 = one small (approximately 2 cm), superficial lesion; 2 = more than one small or just one red (deeper than score 1) but still superficial lesion; 3 = one or several big (2 to 5 cm) and deep lesions; 4 = one very big (> 5 cm), deep, red lesion or many big, deep, red lesions; and 5 = many very big, deep, red lesions. The summation of scores across all examination sites yielded a total skin lesion score for each sow per inspection. The maximum total skin lesion score per inspection was 55. Mean ± SD for the total skin lesion score per inspection for each parity are presented in Fig. 1.

Reproductive performance traits

Data on reproductive performance were retrospectively acquired from farm records. For each sow, traits including AFS (days), cycle length (i.e. days from artificial insemination to weaning in parity one, and days from weaning-to-weaning in parity two), wean-to-first-service interval (days), non-productive days (i.e. days where a sow was neither pregnant nor nursing, measured as days from weaning to successful mating), litter size (i.e. the sum of piglets born alive, born dead, and mummified), number of piglets born alive, born dead, and piglet mortality during lactation (total number of piglets dead), and the reasons for death (i.e. number of piglets crushed) were collected.

Statistical analysis

To account for the change in the composition of the groups in the second parity, data from the first and second parity were analysed separately. All statistical analyses were performed in SAS v9.4 (SAS Inst. Inc., Cary, NC, USA) with pen as the experimental unit and sow as the observational unit. Residuals were tested for normality using the Shapiro test and by examining the quantile–quantile plot. Residuals were non-normally distributed, except for residuals of AFS. For all analyses, statistical differences were reported when $P < 0.05$, while statistical trends were reported when $P > 0.05$ and $P < 0.10$.

Associations between predictor variables

First, Spearman's rank correlation test was used to check for correlations between skin lesion scores on the different inspection days within each parity. Correlations were detected (Table 1), and therefore only skin lesion scores post mixing (**SLMIX**) were used in the analysis. Then, univariable generalized linear mixed models in PROC GLIMMIX were used to investigate the relationship between predictor variables to check for collinearity. Associations between 1) SLMIX score and floor type and 2) SLMIX score and BCS within each parity were investigated using the following model:

$$Y \tilde{} Gamma(\mu, v)$$

$$\log(\mu) = \beta_0 + \beta X + Z\gamma + \varepsilon$$

where $\log(\mu)$ = SLMIX for each sow; β_0 = constant; βX = floor type or BCS (as categorical fixed effects); $Z\gamma$ = pen random effect; and ε = error term.

The association between AFS and floor type, and AFS and BCS were investigated using the following model:

$$Y = \beta X + Z\gamma + \varepsilon$$

where Y = AFS; βX = floor type or BCS (as categorical fixed effects); $Z\gamma$ = pen random effect; and ε = error term. Results for categorical fixed effects are reported as means ± SEM.

Finally, due to a low number of sows with BCS≥3, sows with BCS = 3 were grouped with sows of BCS = 2 into a single group (i.e. BCS≥2) in parity two. The association between BCS and floor was investigated as follows:

$$Y \tilde{} Binomial(\beta_0, \rho)$$

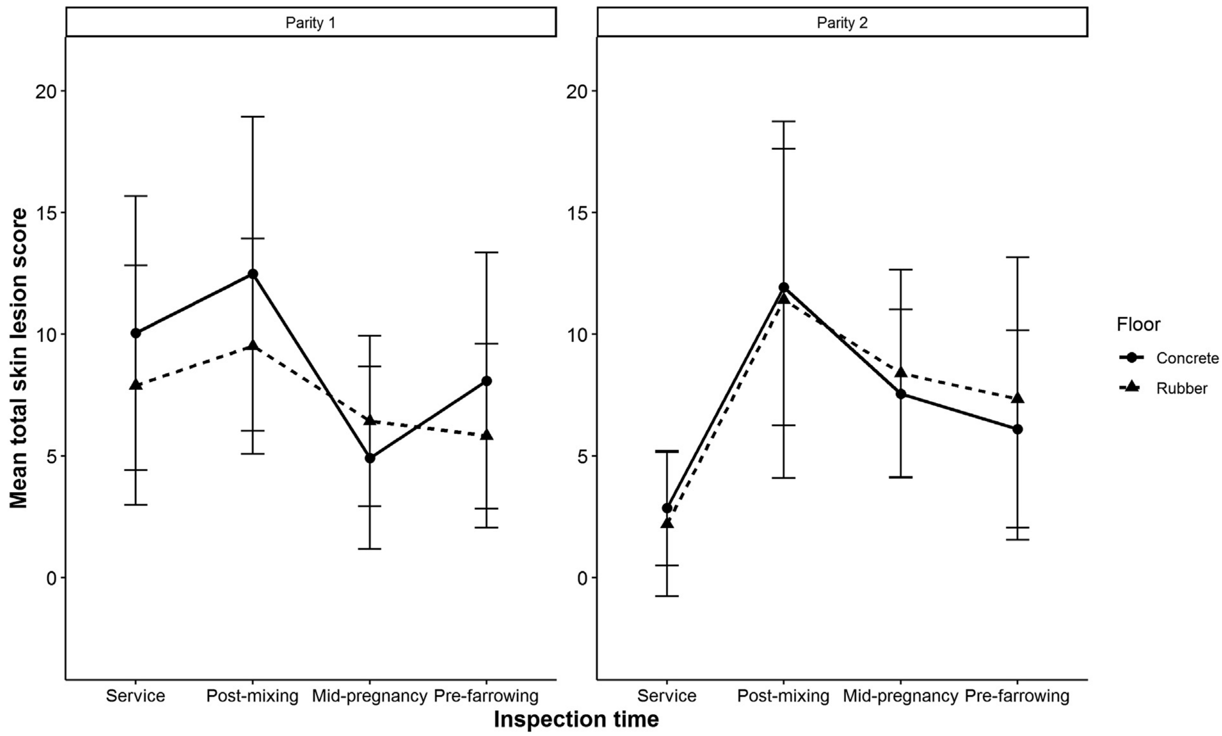

Fig. 1. Mean ± SD for the total skin lesion score per inspection period in parity one and parity two of 160 group-housed sows on concrete ($n = 80$) or rubber ($n = 80$) floor, where skin lesion scores were recorded based on the severity from 0 = no lesions to 5 = severe lesions, on five body regions (ear, neck, hindquarter, rump, and belly), on the left and right side of the body, including the examination of the tail/anogenital region. The summation of skin lesion scores across all examination sites yielded a total score for each sow, with 55 as the maximum possible score per inspection. Sows were individually inspected for skin lesions at service, post mixing (1.6 ± 0.96 days post mixing in parity one and 1.4 ± 0.86 days post mixing in parity two), mid-pregnancy (58.1 ± 4.72 days of gestation in parity one and 54.3 ± 10.19 days of gestation in parity two) and before farrowing (101.9 ± 5.71 days of gestation in parity one and 103.7 ± 7.69 days of gestation in parity two).

$$\text{logit}(\rho) = \beta_0 + \beta X + Z\gamma + \varepsilon$$

where logit(ρ) = BCS for each sow; β_0 = constant; βX = floor type (as a categorical fixed effect); $Z\gamma$ = pen random effect; and ε = error term. Results are reported as odds ratios (**OR**) with the associated 95% confidence interval (**CI**).

Only SLMIX in parity one was associated with floor type, and thus the variance inflation factor for a model with SLMIX, floor type, and BCS score was calculated in PROC REG. Variance inflation factor was approximately 1 for all predictors (i.e. one time larger than it would be if predictors were not associated), indicating that variance inflation would not be a problem when including all predictors in a single model.

Table 1
Spearman's rank correlations between skin lesion scores[1] at four different time points during the reproductive cycle of 160 sows in parity one and parity two.

	Service	Post mixing[2]	Mid-pregnancy[3]	Pre-farrowing[4]
Parity one				
Service	1.0			
Post mixing	0.34***	1.0		
Mid-pregnancy	0.05	0.17***	1.0	
Pre-farrowing	0.12	0.32***	0.28***	1.0
Parity two				
Service	1.0			
Post mixing	0.12	1.0		
Mid-pregnancy	−0.01	0.24**	1.0	
Pre-farrowing	−0.11	0.09	0.11	1.0

Probability levels are indicated by ** and *** for $P < 0.01$ and $P < 0.001$, respectively.
[1] Skin lesion scores recorded based on the severity from 0 = no lesions to 5 = severe lesions, on five body regions (ear, neck, hindquarter, rump, and belly), on the left and right side of the body, including the examination of the tail/anogenital region. The summation of scores across all examination sites yielded a total skin lesion score for each sow per inspection.
[2] 1.6 ± 0.96 days post mixing in parity one and 1.4 ± 0.86 days post mixing in parity two.
[3] 58.1 ± 4.72 days of gestation in parity one and 54.3 ± 10.19 days of gestation in parity two.
[4] 101.9 ± 5.71 days of gestation in parity one and 103.7 ± 7.69 days of gestation in parity two.

Factors associated with skin lesion score at mixing

The following model was used to investigate the associations between SLMIX score in parity one and two and AFS:

$$Y \tilde{Gamma}(\mu, v)$$

$$\log(\mu) = \beta_0 + \sum \beta X + Z\gamma + \varepsilon$$

where log(μ) = SLMIX for each sow within parity; β_0 = constant; βX = floor type, BCS (as categorical fixed effects) within parity and AFS (as a continuous predictor); $Z\gamma$ = pen random effect; and ε = error term.

Associations between reproductive performance traits and skin lesion scores post mixing within each parity

Data from each parity were analysed separately to investigate the effect of within parity SLMIX score on reproductive performance traits. Generalized linear mixed models were used in PROC GLIMMIX as follows:

$$Y \tilde{Poisson}(\beta_0 \times \rho)$$

$$\log(\mu) = \beta_0 + \sum \beta X + Z\gamma + \varepsilon$$

where log(μ) = count of reproductive performance traits within each parity (i.e. number of piglets born alive, litter size); β_0 = constant; βX = fixed effects within parity [i.e. floor type, BCS (as categorical fixed effects) and SLIMX (continuous predictor)]; $Z\gamma$ = pen random effect; and ε = error term,

$$Y \tilde{} Gamma(\mu, v)$$

$$\log(\mu) = \beta_0 + \sum \beta X + Z\gamma + \varepsilon$$

where log(μ) = cycle length (days); β_0 = constant; βX = fixed effects within parity [i.e.floor type, BCS (as categorical fixed effects) and SLIMX (continuous predictor)]; Zγ = pen random effect; and ε = error term.

$$Y \tilde{} Binomial(\beta_0, \rho)$$

$$\text{logit}(\rho) = \beta_0 + \sum \beta X + Z\gamma + \varepsilon$$

where logit(ρ) = proportion of piglets born dead, proportion of piglets dead during lactation, and proportion of piglets crushed during lactation per litter; β_0 = constant; βX = fixed effects within parity [i.e. floor type, BCS (as categorical fixed effects) and SLIMX (continuous predictor)]; Zγ = pen random effect; and ε = error term.

Associations between reproductive performance traits in parity two and skin lesion scores post mixing in parity one

SLMIX score in parity one was used to investigate the effect of aggression intensity received as a first parity sow on reproductive performance later in life using generalized linear mixed models in PROC GLIMMIX as follows:

$$Y \tilde{} Poisson(\beta_0 \times \rho)$$

$$\log(\mu) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + Z\gamma + \varepsilon$$

where log(μ) = count of reproductive performance traits in parity two [i.e. number of piglets born alive, litter size, non-productive days, and wean-to-first-service interval (days)]; β_0 = constant; $\beta_1 X_1$ and $\beta_2 X_2$ = floor type and BCS (as categorical fixed effects) in parity one, and $\beta_3 X_3$ = SLIMX in parity one (as a continuous predictor); Zγ = pen random effect; and ε = error term,

$$Y \tilde{} Gamma(\mu, v)$$

$$\log(\mu) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + Z\gamma + \varepsilon$$

where log(μ) = cycle length (days); β_0 = constant; $\beta_1 X_1$ and $\beta_2 X_2$ = floor type and BCS (as categorical fixed effects) in parity one, $\beta_3 X_3$ = SLIMX in parity one (as a continuous predictor); Zγ = pen random effect; and ε = error term.

$$Y \tilde{} Binomial(\beta_0, \rho)$$

$$\text{logit}(\rho) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + Z\gamma + \varepsilon$$

where logit(ρ) = proportion of piglets born dead, proportion of piglets dead during lactation, and proportion of piglets crushed during lactation per litter; β_0 = constant; $\beta_1 X_1$ and $\beta_2 X_2$ = floor type and BCS (as categorical fixed effects) in parity one, and $\beta_3 X_3$ = SLIMX in parity one (as a continuous predictor); Zγ = pen random effect; and ε = error term.

For reproductive performance traits, results for categorical fixed effects are reported as the back-transformed means ± SEM with their associated 95% CI. Means and 95% CI were back-transformed to the original data scale using the *ilink* (i.e. inverse link transformation) function of PROC GLIMMIX. Results for continuous predictor variables are reported as their regression coefficient (**REG**) ± SE, which is given on the log scale.

Results

Associations between predictor variables

Age at first service did not differ between floor types (242 ± 5.3 days on CON and 245 ± 5.3 days on RUB floor; $F_{1,140}$ = 0.17; *P* = 0.679). Similarly, AFS was not different between BCS classifications ($F_{1,131}$ = 0.0; *P* = 0.978). Body condition score did not differ between floors in parity one (OR = 2.38; 95% CI = 0.59 to 9.53; $F_{1,132}$ = 1.55; *P* = 0.216) or in parity two (OR = 1.10; 95% CI = 0.55 to 2.23; $F_{1,126}$ = 0.08; *P* = 0.781).

Factors associated with skin lesion score at mixing

At mixing during the first parity, there was an increase in SLMIX score with every 1 day increase in AFS (REG = 0.004 ± 0.0020; $F_{1,147}$ = 4.77; *P* = 0.031), but not in parity two ($F_{1,120}$ = 0.03; *P* = 0.853). Concrete sows had higher SLMIX score (12.0 ± 0.95; 95% CI = 10.2 to 14.0) than RUB sows (9.4 ± 0.69; 95% CI = 8.1 to 10.9) in parity one ($F_{1,147}$ = 6.05; *P* = 0.015). However, there were no differences in SLMIX scores between floors in parity two (11.5 ± 1.14; 95% CI = 9.5 to 14.0 on CON vs 10.5 ± 1.18; 95% CI = 8.4 to 13.1 on RUB; $F_{1,95}$ = 0.41; *P* = 0.525). Similarly, SLMIX score was not associated with BCS in parity one ($F_{1,146}$ = 0.01; *P* = 0.907) or parity two ($F_{1,95}$ = 0.69; *P* = 0.409).

Reproductive performance traits

Model 1: There were no observed associations between SLMIX score and reproductive performance traits in parity one or in parity two, except for a tendency for a higher proportion of piglets dead during lactation associated with higher SLMIX score in parity one ($F_{1,125}$ = 2.79; *P* = 0.097), and an increase in the proportion of piglets dead during lactation ($F_{1,91}$ = 5.08; *P* = 0.027) and cycle length ($F_{1,90}$ = 9.42; *P* = 0.003) in parity two with increasing SLMIX score in the same parity (Table 2). Floor-type had no effect on reproductive performance traits in parity one. In parity two, CON sows had a higher proportion of piglets born dead ($F_{1,91}$ = 6.47; *P* = 0.013) compared with RUB sows (Table 3). There were no observed associations between BCS and reproductive performance traits in parity one or in parity two. Model 2: Non-productive days in parity two increased with increasing SLMIX scores in parity one ($F_{1,117}$ = 126.66; *P* < 0.001; Table 2). Lower BCS in parity one was associated with shorter wean-to-first-service interval (7.1 ± 0.59 BCS of 2 vs 9.5 ± 0.99 BCS of 3; $F_{1,117}$ = 11.46; *P* = 0.001).

Discussion

Mixing aggression, reproductive performance and welfare are interlinked (Arey and Edwards, 1998). Aggression is a major source of stress for sows, with hormonally mediated knock-on effects on both reproductive performance and welfare (Einarsson et al., 2008), which could become chronic in nature (Turner et al., 2005). In this study, skin lesion score was used as a proxy for the intensity of mixing aggression (de Koning, 1984; Turner et al., 2006) during the gestation period. Skin lesion score at mixing was selected because this was the time point where higher lesion scores were observed, and also because moderate correlations were observed between skin lesion score at mixing and subsequent inspections at mid-pregnancy and before farrowing. Additionally, skin lesion score at mixing likely reflects aggressive encounters associated with the mixing of unfamiliar animals that fight to establish a dominance hierarchy within the pen (Turner et al., 2006). As study sows were housed in static groups, aggressive interactions after mixing were likely related to competition for resources such as feed or space.

In this study, we found an association between levels of mixing aggression and sow performance within and between parities. Specifically, piglet mortality during lactation and cycle length in parity two increased with increasing SLMIX score in parity two. These results are

Table 2
Associations (regression coefficient ± SE[1]) between skin lesion scores post mixing[2] (SLMIX) and reproductive performance traits within and between parities one and two, in 160 sows group-housed on concrete slats either uncovered or covered by rubber slat mats, as a proxy for the acute and chronic effects of mixing aggression on reproductive performance.

Reproductive performance traits	Skin lesion score post mixing			
	Regression coefficient	SE	F-statistic	*P*-value
Within parity[3]				
Parity one				
Born alive (n)	0.001	0.0046	$F_{1,126} = 0.06$	0.805
Born dead (proportion)	−0.03	0.023	$F_{1,126} = 2.06$	0.153
Litter size (n)	−0.001	0.0045	$F_{1,126} = 0.05$	0.828
Cycle length (days)	−0.0002	0.00048	$F_{1,127} = 0.11$	0.742
Piglets dead (proportion)	0.03	0.019	$F_{1,125} = 2.79$	0.097
Crushed (proportion)	0.02	0.028	$F_{1,125} = 0.73$	0.393
Parity two				
Born alive (n)	0.003	0.0041	$F_{1,91} = 0.48$	0.490
Born dead (proportion)	0.02	0.021	$F_{1,91} = 1.35$	0.248
Litter size (n)	0.004	0.0040	$F_{1,91} = 1.04$	0.310
Cycle length (days)	0.005	0.0015	$F_{1,90} = 9.42$	0.003
Piglets dead (proportion)	0.04	0.019	$F_{1,91} = 5.08$	0.027
Crushed (proportion)	−0.004	0.0293	$F_{1,91} = 0.02$	0.889
Between parity analysis[4]				
Born alive (n)	−0.003	0.0048	$F_{1,112} = 0.41$	0.525
Born dead (proportion)	0.007	0.0229	$F_{1,112} = 0.09$	0.768
Litter size (n)	−0.002	0.0049	$F_{1,112} = 0.16$	0.689
Cycle length (days)	0.002	0.0020	$F_{1,111} = 1.52$	0.221
Piglets dead (proportion)	−0.02	0.024	$F_{1,112} = 0.71$	0.402
Crushed (proportion)	−0.03	0.030	$F_{1,112} = 0.70$	0.404
Non-productive days	0.07	0.006	$F_{1,117} = 126.66$	<0.001
Wean-to-first-service interval (days)	−0.004	0.0076	$F_{1,117} = 0.27$	0.606

[1] Regression coefficient ± SE is given on the log scale.
[2] Skin lesion scores recorded based on the severity from 0 = no lesions to 5 = severe lesions, on five body regions (ear, neck, hindquarter, rump, and belly), on the left and right side of the body, including the examination of the tail/anogenital region. The summation of skin lesion scores across all examination sites yielded a total score for each sow. Lesions were scored 1.6 ± 0.96 days post mixing in parity one and 1.4 ± 0.86 days post mixing in parity two.
[3] Within parity analysis, where each parity was analysed separately to investigate the effect of within parity SLMIX score on reproductive performance traits, with reproductive performance traits included in the model as predicted variables, SLMIX score as a continuous predictor variable, and body condition score and floor as categorical fixed effects.
[4] Between parity analysis, where SLMIX score in parity one was used to investigate the effect of mixing aggression intensity received as a gilt on reproductive performance traits in parity two, with reproductive performance traits included in the model as predicted variables, SLMIX score as a continuous predictor variable, and body condition score and floor as categorical fixed effects.

in contrast to the findings of Verdon et al. (2016), but these authors used a ranking system (i.e. dominant vs submissive) to quantify aggression, while we used skin lesion scores. In addition, Verdon et al. (2016) used a different range of reproductive performance measures to the ones employed in our study.

Our results also support the possibility that mixing aggression causes chronic stress, with long-lasting, detrimental consequences for reproductive performance in subsequent parities. For example, we found that the number of non-productive days in parity two was associated with SLMIX score in parity one only. This finding is in agreement with the results of other studies showing that chronic stress in sows is associated with negative effects on reproductive performance, including lower total piglets born per sow (Einarsson et al., 2008). This is thought to be mediated by the negative effects of prenatal stress on embryo survival (Kranendonk et al., 2006) and offspring viability (Tuchscherer et al., 2002), which is manifested in future parities. In our study, more

Table 3
Differences (means[1] ± SEM) and their associated 95% confidence interval (CI) in reproductive performance traits of 160 sows group-housed on concrete slats either uncovered ($n = 80$) or covered by rubber slat mats ($n = 80$) during their first two parities.

Reproductive performance traits	Concrete	95% CI	Rubber	95% CI	SEM	F-statistic	*P*-value
Parity one[2]							
Born alive (n)	11.8	10.9 to 12.8	11.3	10.4 to 12.2	0.45	$F_{1,126} = 0.94$	0.333
Born dead (proportion)	5.7	3.7 to 8.7	4.8	3.1 to 7.4	1.14	$F_{1,126} = 0.32$	0.571
Litter size (n)	12.8	11.8 to 13.8	12.0	11.1 to 12.9	0.47	$F_{1,126} = 1.60$	0.209
Cycle length (days)	141.3	139.8 to 142.8	141.1	139.7 to 142.6	0.73	$F_{1,127} = 0.03$	0.862
Piglets dead (proportion)	7.5	4.6 to 12.1	6.6	4.1 to 10.5	1.72	$F_{1,125} = 0.18$	0.675
Crushed (proportion)	2.7	1.6 to 4.6	2.2	1.3 to 3.8	0.67	$F_{1,125} = 0.35$	0.554
Parity two[2]							
Born alive (n)	12.3	11.4 to 13.2	11.5	10.6 to 12.4	0.45	$F_{1,91} = 1.47$	0.228
Born dead (proportion)	5.2	3.6 to 7.3	2.3	1.3 to 3.9	0.77	$F_{1,91} = 6.47$	0.013
Litter size (n)	13.0	12.2 to 14.0	11.9	11.0 to 12.9	0.46	$F_{1,91} = 3.04$	0.085
Cycle length (days)	153.6	147.5 to 159.9	155.6	148.4 to 163.1	3.42	$F_{1,90} = 0.17$	0.682
Piglets dead (proportion)	5.3	3.1 to 8.7	5.3	2.8 to 9.6	1.50	$F_{1,91} = 0.004$	0.999
Crushed (proportion)	2.7	1.7 to 4.5	1.4	0.7 to 2.9	0.60	$F_{1,91} = 2.93$	0.131

[1] Means were back-transformed to the original data scale using the *ilink* function in PROC GLIMMIX of SAS v9.4.
[2] Each parity was analysed separately to investigate the effect of within parity skin lesion post mixing (SLMIX) score on reproductive performance traits. Reproductive performance traits were included in the model as predicted variables, body condition score and flooring type as categorical fixed effects, and SLMIX as a continuous predictor variable. Lesions were scored 1.6 ± 0.96 days post mixing in parity one and 1.4 ± 0.86 days post mixing in parity two.

non-productive days could be related to impaired pre-ovulatory oestrogen surges caused by chronic stress (Turner et al., 2002), and a subsequent failure to conceive.

Correlations between skin lesion scores at different inspections in this study suggest a mechanism through which mixing aggression in early life could contribute to chronic stress. It seems that, in line with Turner et al. (2009), animals that experienced intense aggression at first mixing are more likely to continue to receive more intense aggression or to be more aggressive throughout pregnancy. Such animals thus suffer chronically increased levels of stress resulting from their continuous involvement in aggressive behaviour. Another explanatory mechanism for the chronic stress effects resulting from mixing aggression relates to skin lesions resulting from aggression. Skin lesions are painful and it is possible that the pain they generate may negatively influence reproductive performance in subsequent parities (Martinez-Miro et al., 2016). It is important to note that although our results suggest a chronic stress effect on reproductive performance, physiological measures of stress such as cortisol concentrations were not recorded. Therefore, results must be treated with caution, given the possibility of other factors, including animal genetics (Koketsu et al., 2017), affecting reproductive performance. Future studies investigating the relationship between chronic stress and reproductive performance should include measures of chronic stress (e.g. hair cortisol concentrations, ACTH challenge) which would provide support for the effects of stress on reproductive performance. Moreover, it is possible that the current study did not have sufficient statistical power to detect other meaningful differences in reproductive performance based on skin lesion score, as the original calculations were performed to determine the power needed to investigate the use of rubber flooring to improve sow leg health. We therefore acknowledge this as a limitation to our study.

We found the optimal BCS of 3 in parity one to be associated with a longer wean-to-first-service interval in parity two. This is in contrast to the general consensus, whereby this score is linked to shorter wean-to-first-service intervals (Koketsu et al., 2017). We are unable to explain this contradictory result. Moreover, future studies should use more objective measures for body condition and/or composition of gilts at first mating such as BW, back fat content, and muscle depth.

This study showed that AFS was associated with the intensity of mixing aggression, with gilts served at the youngest ages of the cohort showing lower SLMIX scores resulting from fights to establish a dominance hierarchy. Although Pitts et al. (2000) demonstrated that mixing piglets at younger ages resulted in fights of shorter duration and fewer injuries, to the best of our knowledge, no previous study reported such an effect for sows. At the individual level, it is possible that younger/smaller gilts are more timid and less inclined to challenge larger individuals, therefore both incurring and inflicting less physical damage (Clark and D'Eath, 2013). Although BCS was not associated with SLMIX score, it is possible that information on BW and/or body composition traits of gilts would have provided additional insight into the relationship between AFS and aggression intensity of gilts. Nonetheless, the association between younger age at first service and reduced intensity of mixing aggression observed in this study, coupled with findings of other studies showing physiological reproductive performance benefits of serving gilts at a younger age (Koketsu et al., 1999; Cottney et al., 2012; See and Knauer, 2019) provides further evidence for the benefits of serving gilts at younger ages. However, it is still important to adhere to guidelines for optimal gilt body condition and weight when serving gilts young (Kummer et al., 2006). Earlier AFS (e.g. <190 days) is not recommended (Koketsu et al., 2020). This is because at such early ages gilts may not yet have an adequate body composition, or have not yet reached sexual maturity (Malanda et al., 2019). This in turn could have adverse effects on reproductive performance, such as reduced farrowing performance and consequently increased risk of culling (Kummer et al., 2006; Malanda et al., 2019).

Our findings provide further evidence to support the improvement of sow welfare through the use of bedding or rubber mats (Calderón Díaz et al., 2013). We did not observe sow behaviour at mixing, so the true frequency and duration of the aggressive interactions are not known. However, lower SLMIX scores of sows on rubber flooring suggest that they experienced less intense aggression at mixing, which is a positive outcome for sow welfare (Munsterhjelm et al., 2008). The possibility that the intensity of mixing aggression was reduced because of the animals' reluctance to prolong fights on slippery rubber flooring (Boyle and Llamas Moya, 2003; Palmer et al., 2010) cannot be discounted, and this has negative connotations for animal welfare. Nevertheless, SLMIX scores in this study were not very high, and the difference in SLMIX scores between floors while significant, was small, and perhaps not biologically relevant. In spite of this, the possibility that more intense mixing aggression on CON floors contributed to higher levels of chronic stress during gestation cannot be ruled out. This in turn could help to explain the higher proportion of piglets born dead from sows on CON, possibly due to a prolonged farrowing process, which could be a consequence of chronic stress during gestation (Lawrence et al., 1992).

In conclusion, mixing aggression experienced by replacement gilts soon after service negatively influenced reproductive performance parameters not only within, but also between parities. This emphasizes the potential for long-term carry-over effects of a severe acute stressor experienced at this time (Turner et al., 2005; Einarsson et al., 2008). The findings of the current study also show how AFS and flooring type can influence mixing aggression intensity, with associated effects on reproductive performance. Moreover, based on the results of this study, there is evidence for a reduction in mixing aggression intensity in parity one with a lower AFS. Coupled with the results of previous studies showing the positive effects of serving gilts at younger ages on reproductive performance, the findings of the present study are important. This is because the implementation of service at a younger age in practice would result both in improved welfare and lifetime productivity as a consequence of lower levels of aggression at mixing. Nonetheless, this recommendation must be implemented with caution, with optimal gilt body condition and BW being the primary deciding factors for serving gilts at a younger age (Kummer et al., 2006). Results of this study also provide further validation for the use of rubber floors in sow gestation housing, with the positive influence of this flooring material on both aggression levels at mixing and on aspects of reproductive performance. The study did not measure physiological stress indicators such as cortisol concentrations, and did not include measurements of gilt BW, both of which could have been useful in the interpretation of the relationships reported in this study. Future research would benefit from the inclusion of such measures to clarify the relationship between gilt AFS, skin lesion scores, and reproductive performance.

Ethics approval

The farm on which this experiment was conducted was in compliance with Statutory Instrument number 311 of 2010 European Communities (Welfare of Farmed Animals) Regulations 2000. The experiment did not require licencing under the European Communities (Amendment of Cruelty to Animals Act, 1876) Regulations (2002), as no invasive measures were used.

Data and model availability statement

None of the data were deposited in an official repository. Datasets used for the results presented in this study are available from the corresponding author upon reasonable request.

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Declaration of interest

None.

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