The implications of chronic stress during gestation for sow reproductive performance and welfare

Konsekwencje przewlekłego stresu podczas ciąży dla wydajności reprodukcyjnej i dobrostanu loch

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Robert Frost

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Summary

Group-housed pregnant sows continuously face numerous challenges associated with the physical and social aspects of their environments, many of which induce chronic stress. While the detrimental effects of chronic stress experienced by sows during pregnancy on their welfare and reproductive performance are now well-established, the specific risk factors for it remain understudied. Similarly, the effects of sow gestational chronic stress mediated prenatally on the welfare and resilience of future offspring require further elucidation. Meaningful improvements in sow welfare will rely on the identification of chronic stress risk factors and effective methods of their mitigation or removal from sow environments. Hence, research conducted as part of this thesis reviewed the potential of various factors to cause chronic stress throughout gestation, investigated the implications of it for sow welfare and reproductive performance, and attempted to identify potential methods of identifying and mitigating chronic stress in pregnant sows.

The literature was reviewed to assess the potential of a range of factors to contribute to chronic stress in pregnant sows. As chronic stress is likely to be of detriment to the developing offspring, the mechanisms of action of both chronic and prenatal stress were also discussed. A number of factors associated with the physical and social (space allowance, group size and type, feeding level and system, lameness, pen design, enrichment and rooting material, floor type, the quality of stockmanship, environmental conditions) aspects of sow gestational environments, as well as with individual sow factors (body weight variation, parity, coping style) were identified as potential chronic stress risk factors. However, several of the identified factors require further investigation to determine the extent of their contribution to sow chronic stress, and also to prenatal stress.

Building on the well-known acute stress effects of mixing aggression on sow welfare and reproductive performance, the second study investigated the potential of mixing aggression to induce chronic stress. In addition, the effect of age at first service and housing on rubber floors during gestation were investigated as potential methods to reduce mixing aggression intensity. The effect of aggression intensity (skin lesion scores) at first mixing on reproductive performance over two parities was tested to determine the potential of this stressor to have a long-term, carry-over effect. Indeed, sows with higher skin lesion scores at first mixing had more non-productive days in parity 2. The results also indicated that service at a younger age is likely to reduce mixing aggression intensity, with gilts served younger having lower skin lesion scores at mixing. Furthermore, sows housed on rubber floors not only had lower skin lesion scores at mixing, but also had fewer piglets that were born dead. Our results suggest that serving gilts at younger ages could be a potential method of reducing mixing aggression intensity. Our results also provide further evidence for the improvement of sow welfare and reproductive performance on rubber floors.

In the third study, the potential of sustained aggression to induce chronic stress and to impair sow reproductive performance was investigated. Skin lesion counts recorded 3 weeks post-mixing (proxy for the level of sustained aggression) in combination with sow hair cortisol

concentrations were tested for their potential to indicate chronic stress. Higher number of mummified piglets and higher IUGR scores in piglets born to sows with higher 3 week skin lesion counts support the detrimental effect of sustained aggression on sow reproductive performance. This was likely mediated by chronic stress. As mummification and IUGR are also piglet-based measures, the negative effect of sustained aggression on these measures also implies an associated prenatal stress effect. While skin lesion counts showed potential as a chronic stress indicator, this was not the case for hair cortisol concentrations. We found no associations between hair cortisol concentrations and reproductive performance measures. Further research into the validity of the combination of hair cortisol concentrations and skin lesion counts as a useful indicator of chronic stress should consider the wide range of factors which can influence hair cortisol concentrations.

The literature review conducted as part of this thesis identified lameness as one of the risk factors for chronic stress. Furthermore, previous work in our department highlighted the ineffectiveness of existing locomotion scoring systems, and identified the need for a more detailed and a sensitive system. Consequently, a novel locomotion scoring system in the form of a visual analogue scale (VAS) was developed in the fourth study, to enable early detection of locomotion disorders. The novel VAS consisted of a number of locomotion components, and was also used to identify a single component with potential to act as a proxy for overall locomotory ability, and a possible candidate for simplified on-farm use. The novel VAS proved more effective at early detection of developing lameness disorders than existing locomotion scoring systems. Combined with the identification of several locomotion components with potential to act as proxies for overall locomotory ability this could contribute to better on-farm locomotion assessments and earlier prevention of lameness. Such findings are important, as results of the fourth study also showed that even slight locomotion disorders are likely to mediate chronic stress, with associated negative effects on reproductive performance. This highlights the need to treat and prevent lameness early.

Streszczenie

Prośne lochy utrzymywane w systemie grupowym w trakcie całego cyklu produkcyjnego mierzą się z licznymi wyzwaniami związanymi z fizycznymi i społecznymi aspektami ich środowiska, z których wiele powoduje przewlekły stres. Chociaż szkodliwy wpływ przewlekłego stresu doświadczanego przez lochy w czasie okresu prośności na ich dobrostan i wydajność reprodukcyjną jest obecnie dobrze znany, poszczególne czynniki mające udział w tym mechanizmie pozostają niedostatecznie zbadane. Podobnie, wpływ przewlekłego stresu u lochy prośnej na dobrostan i żywotność jej potomstwa nie jest wystarczająco zbadany. Możliwość poprawy dobrostanu loch zależy od identyfikacji czynników przyczyniających się do ich przewlekłego stresu, skutecznych metod ich kontroli lub usunięcia ze środowiska produkcyjnego, co stanowiło cel badawczy tej pracy doktorskiej. Ponadto, dokonano przeglądu piśmiennictwa opisującego przyczyny przewlekłego stresu w okresie prośności lochy, zbadano konsekwencje przewlekłego stresu dla ich dobrostanu i wydajności reprodukcyjnej, oraz podjęto próbę zidentyfikowania potencjalnych metod wykrywania i łagodzenia przewlekłego stresu u loch prośnych.

Przegląd piśmiennictwa został przeprowadzony w celu oceny potencjalnego wpływu szeregu czynników na przewlekły stres u loch prośnych. Opisano również mechanizmy działania zarówno przewlekłego, jak i prenatalnego stresu, ponieważ przewlekły stres może być szkodliwy dla rozwijającego się potomstwa lochy poddanego jego działaniu. Szereg czynników związanych z fizycznymi i społecznymi (przydział przestrzeni, wielkość i typ grupy, system i poziom karmienia, kulawizna, konstrukcja kojca, obecność materiałów wzbogacających środowisko loch, rodzaj podłoża, jakość hodowli, warunki środowiskowe) aspektami środowiska w którym utrzymywane są lochy prośne, a także z indywidualnymi cechami (różnorodność masy ciała, liczba wyproszeń, styl radzenia sobie ze stresem/temperament) tych loch zidentyfikowano jako potencjalne czynniki przyczyniające się do ich przewlekłego stresu. Część zidentyfikowanych czynników wymaga dalszych badań, aby określić wielkość ich wpływu na przewlekły stres u loch prośnych, a także na stres prenatalny u rozwijającego się potomstwa.

W drugiej publikacji niniejszej pracy doktorskiej opisano wyniki badań nad przyczynami występowania stresu przewlekłego u loch prośnych, głównie agresji pomiędzy osobnikami spowodowanej łączeniem nieznajomych loch w grupy, wpływających na ich dobrostan i wydajność reprodukcyjną. Ponadto zbadano wpływ wieku loch w momencie pierwszej inseminacji oraz utrzymywania ich na gumowanym podłożu w okresie prośności, jako potencjalnych metody pozwalających kontrolować intensywność agresji podczas tworzenia grup spośród nieznajomych loch. Zbadano też zależność pomiędzy intensywnością agresji (mierzonej na podstawie oceny obrażeń skóry) przy pierwszym mieszaniu osobników w grupach na ich wydajność reprodukcyjną z dwóch wyproszeń, jako efekt długoterminowy. U loch z poważniejszymi obrażeniami skóry przy pierwszym mieszaniu zaobserwowano więcej dni nieproduktywnych podczas drugiego wyproszenia. Wyniki wskazują również, że pierwsza inseminacja w młodszym wieku prawdopodobnie zmniejsza intensywność agresji związanej z

mieszaniem osobników w grupach, ponieważ loszki inseminowane w młodszym wieku wykazały mniejsze obrażenia skóry podczas mieszania. Co więcej, lochy utrzymywane na gumowanym podłożu miały nie tylko mniejsze obrażenia skóry podczas mieszania, ale także charakteryzował je niższy wskaźnik martwych prosiąt. Uzyskane wyniki sugerują, że inseminowanie loszek w młodszym wieku może być potencjalną metodą zmniejszania intensywności agresji związanej z mieszaniem. Wyniki te dostarczają również dalszych dowodów na to że utrzymywanie loch na gumowanym podłożu przyczynia się do poprawy dobrostanu i wydajności reprodukcyjnej loch.

W trzeciej publikacji niniejszej rozprawy doktorskiej zbadano zależność pomiędzy długotrwałą, nieprzerwaną agresją a przewlekłym stresem i osłabieniem potencjału reprodukcyjnego loch. Zbadano potencjał wskaźników: liczby obrażeń skóry 3 tygodnie po mieszaniu loch oraz poziomu stężenia kortyzolu w ich szczecinie do pomiaru przewlekłego stresu. Zaoobserwowana większa liczba zmumifikowanych prosiąt i wyższe wyniki hipotrofii wewnątrzmacicznej (IUGR) prosiąt urodzonych przez maciory z wyższą liczbą obrażeń skóry odnotowanych 3 tygodnie po mieszaniu wskazują na szkodliwy wpływ długotrwałej agresji pomiędzy lochami prośnymi na ich wydajność reprodukcyjną. Prawdopodobnie występowanie przewlekłego stresu u loch było ważnym elementem powyższego mechanizmu. Ponieważ mumifikacja i poziom hipotrofii wewnątrzmacicznej (IUGR) są również wskaźnikami żywotności prosiąt, negatywny wpływ długotrwałej agresji pomiędzy lochami na te wskaźniki implikuje również możliwość działania stresu prenatalnego spowodowanego długotrwałą agresją. Chociaż liczba obrażeń skóry loch okazała się efektywnym wskaźnikiem przewlekłego stresu, nie dotyczyło to stężenia kortyzolu w szczecinie zwierząt. Nie znaleziono zależności między stężeniem kortyzolu w szczecinie, a pomiarami wydajności reprodukcyjnej. Dalsze badania zależności poziomu stężenia kortyzolu w szczecinie loch i liczby obrażeń ich skóry z poziomem przewlekłego stresu powinny obejmować też analizę czynników, które mogą wpływać na stężenie kortyzolu w szczecinie.

Przegląd piśmiennictwa przeprowadzony w ramach niniejszej pracy doktorskiej zidentyfikował kulawiznę jako jeden z ważnych czynników powodujących przewlekły stres u loch prośnych. Ponadto, wcześniejsze badania uwydatniły nieskuteczność istniejących systemów oceny lokomocji i wskazały potrzebę stworzenia bardziej szczegółowej metody takiej oceny. W związku z tym, w czwartej publikacji opracowano taki system oceny lokomocji loch oparty na wizualnej skali analogowej (VAS), umożliwiający wczesne wykrywanie zaburzeń lokomocji u zwierząt. Zaproponowany system VAS ocenia poszczególne elementy opisujące poziom poprawności lokomocji zwierząt, które mogą również zastąpić ogólną ocenę jakości lokomocyjnej dotychczas używanej na farmach. Nowy system VAS okazał się bardziej skuteczny do wczesnego wykrycia rozwijających się zaburzeń lokomocji w porównaniu do istniejących systemów oceny lokomocji. System ten może poprawić jakość oceny lokomocji na farmach i wcześniejszego zapobiegania kulawiznom u trzody chlewnej. Wyniki uzyskane w czwartej publikacji wykazały również, że nawet niewielkie zaburzenia lokomocji mogą powodować przewlekły stres, co wiąże się z negatywnym wpływem na zdolności reprodukcyjne. Wskazuje to na potrzebę wczesnego leczenia i zapobiegania kulawiźnie zwłaszcza u loch.

1. Introduction

The debate on what constitutes appropriate treatment of, and the level of moral consideration that humans should afford animals stretches over hundreds of years, and is fundamental to the developments in the area of animal welfare (Alonso et al., 2020). While we now live in a world where many non-human animal species are accepted as beings worthy of moral consideration, this acceptance does not always translate in practice. This is reflected in the poor welfare standards still experienced by many species used to human ends. Modern moral philosophers such as Lori Gruen put down the mismatch between our moral beliefs and the reality of how we treat animals to the fact that in general, society is disconnected from the natural world, including animals (Singer, 2013). For instance, most people will never see the stages their food goes through before it is ready for sale at the supermarket. Thus, Gruen surmises that "most people are shielded from the consequences of their actions", which can hamper improvements in the area of animal welfare (Singer, 2013).

Nevertheless, societal attitudes are now changing across a range of areas, bringing a greater focus onto the importance of good animal welfare. People are becoming more environmentally and health-conscious, wanting more transparency on the food they consume, as well as on the effects its production has on our planet. More people want to ensure that the food they consume is safe and ethically produced (Alonso et al., 2020). This attitude was intensified by the emergence of the Covid-19 pandemic, highlighting the need for increased food security, which goes hand in hand with high standards of animal welfare (Alonso et al., 2020, Buller et al., 2018). The idea that healthy, psychologically-sound animals raised to high welfare standards produce higher quality meat products is not only a human perception, but a fact supported by scientific research (Smulders et al., 2006, Warriss, 1998, Boyle and O'Driscoll, 2011, Dawkins, 2019). Similarly, good animal welfare is now more readily recognised as a crucial contributor to sustainable governance (Sebo et al., 2022). This is reflected in the recent calls to include animal welfare as a target in the 2030 Agenda for Sustainable Development (Sebo et al., 2022). The Covid-19 pandemic also highlighted the vulnerability of highly intensive livestock production systems to any kind of disruption to the production chain (Marchant-Forde and Boyle, 2020). The pig industry was a prime example of this, with Covid-19 outbreaks among processing plant staff resulting in 45% decrease in processing capacity in the United States (Marchant-Forde and Boyle, 2020). This undoubtedly compromised pig welfare due to overcrowding and the need to cull animals on farms in ways that likely caused suffering (Marchant-Forde and Boyle, 2020, Hashem et al., 2020). The need to reinforce highly intensive livestock production systems to ensure their resilience and flexibility in preparation for potential future challenges became clear (Marchant-Forde and Boyle, 2020). The improvement of animal welfare was highlighted as a crucial part of this endeavour (Marchant-Forde and Boyle, 2020).

Moreover, global concern for animal welfare continues to grow (Galli et al., 2021). There are now increased consumer demands for improved pig welfare which show that this species is no exception to the trend (End the Cage Age, 2018). Such demands are timely and long-awaited by pig welfare experts, many of whom would describe pigs as “the losers in the animal welfare debate” (Mance, 2022). The conditions in which pigs are kept commercially are a testament to their welfare still being largely overlooked. There is no doubt that the changing attitudes towards pigs are a result of continuously emerging evidence of pig intelligence, sentience, capacity for emotions and so much more (Mance, 2022). Furthermore, the changing pattern of emerging research in the area of pig welfare reflects this, with an overall increase in scientific studies investigating gestating sow welfare, employing a broader range of welfare measures (Galli et al., 2021). The sow, being the breeding animal, and thus the driver of the pig industry, is at the centre of said consumer demands. It features prominently in “End the Cage Age”, a European Citizens’ initiative, in its North American equivalent, “Proposition 12” (Proposition 12, 2022), and also in the “Farm to Fork Strategy”, all of which will enforce additional pig welfare legislation in the coming decade (Boyle et al., 2022). For example, this will encompass a ban on the use of stalls and farrowing crates for sows (End the Cage Age, 2018), to further increase their freedom of movement during gestation and lactation. However, such improvements are only the start, as sows face many other challenges associated with aspects of their physical (group type and size, flooring, feeding systems) and social (stocking density, mixing strategy, individual sow factors) environments, which must also be addressed to achieve higher welfare standards (**Publication 1**; Lagoda et al., 2022). For instance, sows on commercial farms experience overcrowding, aggression (both at mixing and post-hierarchy establishment), hot temperatures, feed restriction, inability to forage and express natural behaviours, uncomfortable floors, lameness, and poor handling (Martinez-Miro et al., 2016, Salak-Johnson, 2017, Spoolder et al., 2009).

Many such challenges are likely to induce chronic stress in sows (**Publication 1**; Lagoda et al., 2022) which is highly detrimental to their welfare and productivity (Martinez-Miro et al., 2016, Olsson et al., 1999, Salak-Johnson and McGlone, 2007). Sow chronic stress is also detrimental to the welfare and resilience of their piglets, mediated prenatally (Braastad, 1998). Lower litter size, irregular rebreeding and longer weaning-to-oestrous interval are among some of the known consequences of chronic stress on sow production parameters (Einarsson et al., 2008). Chronic stress can also suppress immune function (Morrow-Tesch et al., 1994, Tuchscherer et al., 2009, Wrona et al., 2001), which consequently results in production diseases (Proudfoot and Habing, 2015). This increases the need for treatment with antibiotics, and in turn exacerbates the risk of antimicrobial resistance development (Xu et al., 2018). Overall, the negative effects of chronic stress in sows threaten the sustainability of the pig industry and decrease societal acceptability of pig production (**Publication 1**; Lagoda et al., 2022).

Addressing the above challenges to achieve a reduction in chronic stress levels experienced by gestating sows is therefore needed to improve sow welfare (Spoolder et al., 2009). Furthermore, such reductions would also be invaluable to the pig industry, given the difficult situation it currently finds itself in. Poor longevity and high sow replacement rates (National Pig Herd Performance Report; Teagasc, 2021), as well as the rising threat of antimicrobial resistance (Rodrigues da Costa and Diana, 2022) are among some of the issues the pig industry is faced with. Such issues further exacerbate the current pig industry crisis related to low pig prices and high production costs (especially driven by rising feed costs). Moreover, improvements in sow welfare can lead to improvements in sow reproductive performance, health (Dawkins, 2019, Einarsson et al., 2008, Spoolder et al., 2009, Martinez-Miro et al., 2016), and also in the resilience and productivity of their offspring (Merlot et al., 2013, Quesnel et al., 2019, Rault et al., 2013). Consequently, improved sow welfare has the potential to safeguard the sustainability and profitability of the pig industry.

Despite its postulated negative implications for sow reproductive performance and welfare, the specific risk factors for chronic stress remain understudied (**Publication 1**; Lagoda et al., 2022). In addition, few studies actively investigated the removal of specific chronic stress sources from the gestational environments (Bernardino et al., 2016, Merlot et al., 2019, Parada Sarmiento et al., 2021, Quesnel et al., 2019, Tatemoto et al., 2019). Yet meaningful improvements in sow welfare will depend on the identification of specific chronic stress risk factors and methods of their elimination from sow environments. Hence, it is important to determine the potential risk factors for chronic stress associated with the physical and social aspects of sow environments, and with individual sow factors. Further elucidation of the effects of chronic stress on sow reproductive performance and welfare, and on the welfare and resilience of sow offspring is also needed. Finally, potential methods of identifying and measuring sow chronic stress, as well as methods of its reduction should also be given additional attention.

2. Hypotheses

Publication 2

- Gilts served at a younger age are exposed to less aggression at mixing, and this results in improved reproductive performance and welfare
- Reduced mixing aggression can have a positive effect on reproductive performance
- Mixing aggression has the potential to cause chronic stress
- Rubber flooring is associated with less aggression at mixing

Publication 3

- Higher total skin lesion counts 24hr and 3 weeks post-mixing (sustained aggression) are associated with increased hair cortisol concentrations and impaired sow reproductive performance
- Sustained aggression has the potential to cause chronic stress and impair reproductive performance
- Skin lesions recorded 3 weeks post-mixing and hair cortisol can act as potential biomarkers of chronic stress in gestating sows

Publication 4

- A novel visual analogue scale (VAS) allows to detect slight deviations from optimal gilt locomotion over time more effectively than an existing categorical scoring system

- A single component of locomotion which can provide a quick insight into the gilt's overall locomotory ability exists
- Impaired locomotion is associated with chronic stress and reduced reproductive performance of sows

3. Objectives

Publication 1

- Discuss potential risk factors for chronic stress in pregnant sows, the mechanisms of action of both chronic and prenatal stress, as well as the effects of the latter on offspring
- Outline gaps in existing research and provide recommendations for future work

Publication 2

- Investigate possible associations between 1) age at first service (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 (concrete vs. rubber), and reproductive performance

Publication 3

- Investigate associations between total skin lesion counts 24hr and 3 weeks post-mixing, hair cortisol concentrations and sow reproductive performance
- Elucidate the effects of chronic stress on reproductive performance
- Determine the congruence between skin lesion counts 3 weeks post-mixing and hair cortisol as potential biomarkers of chronic stress in gestating sows

Publication 4

- Develop a novel VAS to assess both overall locomotory ability and individual aspects of gilt locomotion.
- Compare effectiveness of a novel VAS at detecting slight deviations from optimal gilt locomotion over time to that of an existing categorical scoring system
- Identify a single component of locomotion which can provide a quick insight into the gilt's overall locomotory ability
- Detect chronic stress levels associated with impaired locomotion and predict reproductive performance of sows

4. Materials and Methods

4.1. Ethical approval

Commercial farms on which experiments described in **publication 2** and **publication 3** were conducted were in compliance with Statutory Instrument number 311 of 2010 European Communities (Welfare of Farmed Animals) Regulations 2000. These experiments did not require licencing under the European Communities (Amendment of Cruelty to Animals Act, 1876) Regulations (2002), as no invasive measures were used. The research farm on which the experiment described in **publication 4** was conducted also complied with Statutory Instrument number 311 of 2010 European Communities (Welfare of Farmed Animals) Regulations 2000. Experimental work described in **publication 4** was authorized by the Teagasc Animal Ethics Committee (Approval No: TAEC219-2019).

4.2. Animals and housing

The experiment described in **publication 2** was conducted on a 1000 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 Calderon Diaz 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. Gilts used in this experiment 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 vasectomized 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 hours after the first service. Oestrus synchronisation 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 one week after service, where they were kept in stable groups of eight until one 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 concrete floors which were either uncovered (CON; n = 80 sows), or covered with 10-mm thick rubber slat mats (RUB; n = 80 sows; EasyFix Rubber Products, Ballinasloe, County Galway, Ireland). The rubber slat mats consisted of a two-strip system with circular shaped patterns on the surface and wedges underneath for fixation to the concrete slats [for more details see Calderon Diaz et al. (2013)]. In total, rubber slat mats 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 concrete 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). 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 hours 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 one week of service where they remained until one 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 returns to service. 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, it is likely that the overall effect of re-mixing was 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 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.

The experiment described in **publication 3** was conducted on a commercial 2000-sow farrow-to-finish farm in Co. Cork, Ireland between March and July 2018 (see Table 1, **publication 3** for experimental schedule dates). The study used 264 sows (parity 1-5). Oestrous synchronisation was not practiced on the farm. Sows were artificially inseminated and immediately thereafter locked into individual full-length feeding stalls (2.3 m length × 0.65 m width) within 11 fully slatted gestation pens (7.8 m length × 7 m width; roaming area behind feeding stalls 7.8 m length × 2.4 m width) each with two rows of 12 stalls. A vasectomised boar was walked behind the sows while still restrained in the feeding stalls three weeks post-service, to check for returns to oestrous. Sows were released from the stalls in groups of 24 per pen and allowed to mix, once they were approximately 25 days post-service (24.8 ± 3.14 days post-service). This occurred over a four week period in March (week 1: 72 sows, 3 pens; week 2: 96 sows, 4 pens; week 3: 48 sows, 2 pens; week 4: 48 sows, 2 pens). Sows were fed a liquid diet twice per day and had *ad libitum* access to water via two nipple drinkers at one end of the pen. Sows were transferred into conventional farrowing crates with fully slatted floors one week before farrowing, and were weaned at approximately 28 days post-farrowing.

The experiment described in **publication 4** took place on a 200-sow research unit at the Teagasc Pig Development Department in Moorepark, Fermoy, Co. Cork, Ireland, between May 2019 and March 2020. In total, 51 gilts in eight replicate groups were used. Gilts were purchased from a breeder and thus had to undergo a six-week quarantine before entering the research unit at approximately 210 days of age. Upon completion of the quarantine period, gilts entered the main pig unit and were housed in fully slatted pens (3.2 m × 2.6 m) in groups of four, fed from a long-trough, and were treated with Altresyn for oestrus synchronisation. Gilts were served twice in service stalls by artificial insemination, first at the onset of standing oestrus, and then within 24hrs. Each replicate was served between three to nine weeks apart, depending on the availability of new gilts entering the breeding pool as replacements (see Table 1, **publication 4** for experimental schedule). Approximately five days after service gilts were moved back into their home pens in the same groups as before service, where they stayed until day 30 of pregnancy. They were then mixed into a larger dynamic group with other pregnant gilts (see Table 1, **publication 4** for number of gilts present at the time of mixing) where they were fed by an electronic sow feeder (ESF; Schauer Feeding System; Prambachkirchen, Austria) set to a 23 h cycle, starting at 17:00 daily. The ESF recognised each gilt by a transponder tag programmed to her individual daily allowance of a standard gilt diet. Water was available *ad libitum* from a single-bite drinker inside the ESF, and from a drinker bowl in the pen. The group pen (68.11 m2) comprised of fully slatted concrete floors in the group area, with four insulated solid concrete bays for lying. Gilts had a wooden block suspended from a chain as enrichment. Approximately one week prior to farrowing (day 108), they were moved to the farrowing accommodation and housed in standard individual farrowing crates (pen dimensions: 2.5m × 1.8m), with cast-iron fully slatted floors within the farrowing crate, plastic fully slatted floors around the crate, and a solid plastic heated mat for piglets. Weaning took place approximately 28 days post-partum.

4.3. Measurements

4.3.1. Body condition score

In **publication 2**, 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; 5 = overly fat: hips and backbone covered, body shape bulbous.

4.3.2. Skin lesions

In **publication 2**, 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 2) and before farrowing (101.9 ± 5.71 days of gestation in parity one and 103.7 ± 7.69 days of gestation in parity 2). 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/ano-genital 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 ± standard deviation (SD) for the total skin lesion score per inspection for each parity are presented in Figure 1 (**Publication 2**).

In **publication 3**, skin lesion counts were used. Sows were inspected for skin lesions 24hr post-mixing (i.e. one day after release from the stalls), and three weeks post-mixing, using a method validated by Turner et al. (2006). In brief, skin lesions were counted on the anterior (head, neck, shoulders and front legs), middle (flanks and back), and posterior (rump, hind legs and tail). Counts included fresh skin lesions only, identified by colour and the estimated age of scabbing. No weighting was given to account for the length or diameter of skin lesions. The summation of counts across all examination sites yielded a total skin lesion count for each sow per inspection.

4.3.3. Back fat depth

One week prior to farrowing, sows used in the **publication 3** experiment were locked into the feeding stalls to enable back fat measurement. The back fat measurement site (dorso-lumbar region) was identified (by measuring 6.5 cm left and right from the mid-point at the spine marked by the position of the last rib), and shaved to facilitate measurements. Back fat depth (mm) was measured at the two identified sites using a Renco LEAN-MEATER® device, and an average back fat depth figure was then calculated.

4.3.4. Hair collection and subsequent hair cortisol concentration analysis

In **publication 3**, hair collection for cortisol determination was performed while sows were locked into the feeding stalls, one week prior to farrowing, while in **publication 4**, this was performed while gilts were inside the weighing scales immediately prior to mixing into the dynamic group (day 30 of pregnancy), and on the day of entry to the farrowing crates (day 108; late pregnancy) during their first pregnancy. Hair is hypothesised to be a suitable medium for quantifying chronic stress levels, due to the long-term accumulation of cortisol within the shaft (Davenport et al., 2006, Heimburge et al., 2019, Meyer and Novak, 2012). Therefore, in **publication 4**, the shave/re-shave method (first shave on day 30, then re-shave performed in late pregnancy) allowed determination of the concentration of cortisol which accumulated during the period between hair shavings. Thus, hair cortisol concentration measured in late pregnancy was used in the analysis as an indicator of chronic stress corresponding to approximately the last two-thirds of the pregnancy.

The site of hair collection is important and can have a bearing on the resulting cortisol concentrations (Heimburge et al., 2019). The back fat measurement site (dorso-lumbar region) was selected as the most appropriate region for hair collection (both in **publication 3** and **publication 4**) to ensure adequate measurement of cortisol levels. The dorso-lumbar region is outside of reach of the tail, and thus at a lower risk of chewing by other pigs. It is also away from the neck which is most at risk of aggressive attacks involving bites. This means that the hair of the dorso-lumbar region is at lower risk of contamination by saliva and blood, and is therefore at lower risk of contamination with exogenous cortisol which can diffuse into the hair shaft from both fluids (Otten et al., 2020). Moreover, hair in this region is also at a lower risk of exogenous cortisol contamination coming from urine and faeces, as it does not usually come into contact with the floor surface when the animal is lying down (Otten et al., 2020). The dorso-lumbar region was also chosen for convenience, as hair had to be shaved to allow for the back fat measurement, as well as due to the abundance of hair in this region (Casal et al., 2017). Hair was thus shaved from the back fat measurement site, and placed into plastic zip-lock bags and frozen at -20ºC until hair cortisol analysis.

Hair sample preparation and cortisol extraction were based on the procedure described by Davenport et al. (2006), with certain modifications. In brief, hair samples were defrosted for one hour prior to preparation procedures, then washed by placing 300 mg of hair into a 10 ml polypropylene tube along with 5 ml of isopropanol, and mixing gently on a shaker for 3 min. This was repeated using fresh isopropanol for the second wash. Washed hair samples were left inside the wash tubes and placed inside a protected fume hood to dry overnight. Samples prepared in this way were then individually ground into a fine powder using a Retsch mixing mill (MM200; 10 ml stainless steel grinding jars, single 12 mm stainless steel grinding ball) for 4 min at 25 Hz. Approximately 50 mg of ground hair sample was weighed out and placed in a 2 ml tube along with 1 ml of methanol, which was followed by incubation of the sample for 24hr at room temperature with constant gentle agitation (shaker setting 3; approximately 95 rpm) for cortisol extraction. Following the 24hr incubation period, 0.6 ml of the cortisol extract in methanol was removed (taking care not to disturb the settled hair powder at the bottom of the tube) using an Eppendorf pipette and transferred to a clean 1.5 ml tube for methanol evaporation, which was performed using a stream of nitrogen gas at 38ºC. Cortisol extract samples were frozen at –20ºC pending EIA analysis. Extracted cortisol samples were analysed using Salimetrics® Expanded Range, High Sensitivity Salivary Cortisol EIA kit, which was validated for the analysis of hair cortisol concentrations (Casal et al., 2017, Davenport et al., 2006, Moya et al., 2013), and is valid for use in a range of species, including swine (Davenport et al., 2006, Fürtbauer et al., 2019, Otten et al., 2020). Frozen cortisol extract samples along with the EIA kit were brought to room temperature 1.5 hr prior to being reconstituted with 0.4 ml of phosphate buffer (assay diluent) provided with the EIA kit. Reconstituted extracts (n = 125, **publication 3;** and n = 102, **publication 4**) were analysed for cortisol concentration levels in duplicate using four assays, following the protocol provided with the EIA kit. Inter- and intra-assay CV in were 8.8 and 7.8% in **publication 3**, and 24.1 and 8.7% in **publication 4**, respectively.

4.3.5. Locomotion scoring

In **publication 4**, locomotion was scored visually while gilts walked on solid concrete along the corridor outside of the home pen, taking at least six strides (distance of approximately 30 m). Locomotion was scored on three occasions during the first pregnancy: three days before service (service), in mid-pregnancy (approximately day 57), and on the day of entry to the farrowing crates (day 108; late pregnancy). Sows were also scored at weaning of their first litter. Scoring was performed by a single trained observer who practiced until at least 90% intra-observer scores for repeatability were achieved.

Categorical locomotion scoring (CAT)

Each gilt was assigned a locomotion score (0 to 5) using the gait component of the categorical locomotion scoring system developed by Main et al. (2000).

Visual analogue scales

- Overall locomotion scoring

Overall locomotory ability (OVERALL) was assessed using a VAS consisting of a 150 mm horizontal line, with the left end (0 mm) representing perfect locomotion, and the very right end (150 mm) representing severely impaired locomotion. Locomotory ability was scored by marking a point along the scale, with increasing impairment represented by a mark further to the right of the line. The distance from the left-hand end of the scale was measured and the value for each recorded in millimetres. Thus, the greater the number, the more impaired the locomotory ability. As a guide, the VAS was also divided into descriptive sublevels, to aid with consistency of locomotion scoring [(Averbuch and Katzper, 2004, Lansing et al., 2003, Nalon et al., 2014); e.g., Figure 1; **publication 4**)]. The sublevels were selected based on previous literature on pig and dairy cow locomotion scoring (Bos et al., 2016, Flower and Weary, 2006, Main et al., 2000, Nalon et al., 2014).

- Component locomotion scoring

As well as the overall locomotory ability, several components of locomotion (Table 2; **publication 4**) were assessed using an individual VAS for each component. These components were selected based on previous literature on pig and dairy cow locomotion scoring (Bos et al., 2016, Flower and Weary, 2006, Main et al., 2000, Nalon et al., 2014) and upon feedback gathered during a pilot trial whereby two authors (L.A.B and K.O) assessed locomotion in a number of sows. As in the case of OVERALL, the VAS for each of the individual locomotion components was also divided into descriptive sublevels to aid with consistency of scoring (Bos et al., 2016, Flower and Weary, 2006, Main et al., 2000, Nalon et al., 2014). Different numbers of sublevels were applied to each locomotion component, based on severity levels reported on in the pig locomotion assessment literature [(Bos et al., 2016, Nalon et al., 2014); see **publication 4**, Appendix A, Figure A1)].

4.3.6. Reproductive performance

For **publication 2**, 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. 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.

For **publication 3**, sow reproductive performance was recorded by the farm staff and included the following measures: number of piglets born alive, born dead, mummified, and total born. Piglets from 75 sows were available for more detailed measures, namely, to study the relationship between skin lesions, hair cortisol and measures of piglet development. The sows were selected on the basis of being recently farrowed, or in the process of farrowing, and for which farm sow cards were not yet updated with performance details when the research team arrived on the farm each day. The coefficient of variation (CV) for the representation of the 11 pens by the 75 sows was 31.3%. Piglets were tagged, weighed and scored for vitality and intra-uterine growth retardation (IUGR). Vitality was scored according to criteria shown in Table 2 (**publication 3**), modified from Schmitt et al. (2019) and Rooney et al. (2020). The summation of scores for each criterion yielded a total vitality score, with the maximum (best) possible score of 4 per piglet. The level of IUGR was estimated by scoring the presence/absence of nose wrinkles, cone-shaped head, and bulging eyes, based on a method of Hales et al. (2013). For all three measures a piglet scored 0 if the trait was absent, and 1 if it was present; therefore the maximum total IUGR score a piglet could receive was 3.

For **publication 4**, reproductive performance records were acquired from the sow management system (PigChamp) used on the farm, to ascertain the number of piglets born alive, born dead, mummified, and total born over four parities (parity 1 to 4).

4.4. Statistical analysis

All statistical analyses described in this thesis were performed in SAS v9.4 (SAS Inst. Inc., Cary, NC).

4.4.1. Publication 2

To account for the change in the composition of the groups in the second parity as described in **publication 2**, data from the first and second parity were analysed separately. Pen was used 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; **publication 2**), and therefore only skin lesion scores post-mixing (SLMIX) were used in the analysis. Then, univariable generalised 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, with pen as a random effect. The associations between AFS and floor type, and AFS and BCS were also investigated with pen as the random effect. Results for categorical fixed effects are reported as means ± standard error of the mean (SEM). 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. Finally, the association between BCS and floor was also investigated with pen as the random effect, and 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.

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:

$$Y \sim Gamma(\mu, v)$$
$$log(\mu) = \beta_0 + \sum \beta X + Z\gamma + \varepsilon$$

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); Zγ = 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. Generalised linear mixed models were used in PROC GLIMMIX as follows:

$$Y \sim Poisson(\beta_0 \times \rho)$$

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

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)]; Zγ = pen random effect; and ε = error term,

$$Y \sim Gamma(\mu, v)$$

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

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.

$$Y \sim Binomial\ (\beta_0, \rho)$$

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

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 generalised linear mixed models in PROC GLIMMIX as follows:

$$Y \sim Poisson(\beta_0 \times \rho)$$

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

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; β_1X_1 and β_2X_2 = floor type and BCS (as categorical fixed effects) in parity one, and β_3X_3 = SLIMX in parity one (as a continuous predictor); Zγ = pen random effect; and ε = error term,

$$Y \sim Gamma(\mu, v)$$

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

where log(μ) = cycle length (days); β_0 = constant; β_1X_1 and β_2X_2 = floor type and BCS (as categorical fixed effects) in parity one, and β_3X_3 = SLIMX in parity one (as a continuous predictor); Zγ = pen random effect; and ε = error term.

$$Y \sim Binomial\ (\beta_0, \rho)$$

$$logit(\rho) = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + Z\gamma + \varepsilon$$

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; β_1X_1 and β_2X_2 = floor type and BCS (as categorical fixed effects) in parity one, and β_3X_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) ± standard error (SE), which is given on the log scale.

4.4.2. Publication 3

In **publication 3**, sow was used as the experimental unit. For all analyses statistical differences were reported when $P \leq 0.05$, while statistical trends were reported when $P > 0.05$ and $P \leq 0.10$. Pearson's correlation test was initially used to check for correlations between total skin lesion counts at both 24hr and at 3 weeks post-mixing, and hair cortisol concentrations. Correlations were not detected ($P > 0.05$), and therefore total skin lesion counts recorded at both 24hr and 3 weeks post-mixing, as well as hair cortisol concentration were used as predictor variables in a single model for all analyses. Due to a low number of sows in parity 5, sows in parity 5 were grouped into a single group with sows in parity 4 (i.e. parity ≥ 4). Results for independent continuous variables are reported as their regression coefficient (REG) ± standard error (SE). Results for categorical fixed effects are reported as least square means ± SE with their associated 95% Confidence Intervals (CI).

Sow reproductive performance and back fat depth

In the analysis of sow reproductive performance, independent variables included total skin lesion counts 24hr and 3 weeks post-mixing, and hair cortisol concentration, while parity was included in all models as a fixed effect. Parity was included as a fixed effect, as groups of sows were formed as per routine farm practice, and were therefore not homogeneous in terms of parity. We accounted for clustering of sows within a group by using pen as the random effect. In the case of birth weight, piglet was nested within sow using the repeated statement of PROC MIXED to account for repeated piglet measurements for individual sows, and the total number of piglets born was included as a continuous covariate in this model. In the case of vitality and IUGR, sow was used as an additional random effect to account for repeated piglet measurements for individual sows in PROC GLIMMIX. Residuals were checked for normality using the Shapiro test, and by examining the quantile-quantile plot. For normally distributed residuals (born alive, total born, back fat, birth weight) linear mixed model equations were built in PROC MIXED, while non-normally distributed residuals (the number of piglets born dead, mummified, vitality, and IUGR) were analysed using generalised linear mixed model equations built in PROC

GLIMMIX, and fitted with the Poisson distribution in the case of piglets born dead and mummified, and the multinomial distribution in the case of vitality and IUGR. All models were tested with and without an interaction between 24hr and 3 week total skin lesion counts, with the interaction being removed upon a lack of a significant result in the case of all dependent variables, except for back fat.

4.4.3. Publication 4

In **publication 4**, sow was also used as the experimental unit. Differences were reported when $P \leq 0.05$, while statistical trends were reported when $P > 0.05$ and $P \leq 0.10$. Results for independent continuous variables are reported as their regression coefficient (REG) ± standard error (SE).

Comparison of scoring methods over time
A repeated measures analysis was carried out to investigate the effect of time of locomotion scoring (n = 4) on locomotion scores recorded using OVERALL, locomotion components, and CAT. Residuals were checked for normality using the Shapiro test, and by examining the quantile-quantile plot. For variables with normally distributed residuals (OVERALL, and the components: caudal sway, stride length, fluidity of movement, and reluctance to bear weight while walking), linear mixed model equations were built in PROC MIXED. For variables with non-normally distributed residuals (CAT, and the components: abduction and adduction) generalised linear mixed model equations were built in PROC GLIMMIX and fitted with either the Poisson (abduction and adduction score) or the multinomial distribution (CAT). For model equations built in PROC MIXED, time was included as a repeated measure, with sow ID as subject, while for model equations built in PROC GLIMMIX, time was included as an additional random effect to account for repeated sow ID measures. Replicate was included as a random effect in all models.

Associations between OVERALL and locomotion components
A repeated measures regression analysis was performed to investigate the association between OVERALL (dependent variable) and the individual components of locomotion (included as continuous independent variables; PROC MIXED) across all scoring days together, and also on each scoring day separately. The latter was completed as it is important to consider the relationship on the different days, since the changing shape and weight of the gilt with progressing pregnancy could potentially impact the way she walks. Residuals were checked as described previously to confirm the suitability of the models. Time was included as a repeated measure, and replicate was included as a random effect.

Associations between OVERALL, hair cortisol concentration, and reproductive performance
Separate regression analyses were performed to investigate the association between OVERALL at each of the three recording occasions during pregnancy, and hair cortisol concentration in late pregnancy. A separate regression analysis was also carried out to investigate the association between OVERALL at each of the three recording occasions

and the following measures: the number of piglets born alive, born dead, mummified, and the total number born over four parities. Residuals were checked as before. Hair cortisol concentration, number of piglets born, and piglets born alive were analysed using linear mixed models (PROC MIXED), and the number of piglets mummified or born dead were analysed using generalised linear mixed models (PROC GLIMMIX) and fitted with the Poisson distribution. For the analysis of hair cortisol concentration, an EIA assay plate was included as an additional random effect. For the measures of reproductive performance which had model equations built in PROC MIXED, parity was included as a repeated measure, with sow ID as subject, while for model equations built in PROC GLIMMIX, parity was included as an additional random effect to account for repeated sow ID measures. Replicate was included as a random effect in all models.

5. Results

5.1. Publication 2

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 one day increase in AFS (REG = 0.004 ± 0.0020; $F_{1,147} = 4.77$; $P = 0.031$), but not in parity two ($F_{1,120} = 0.03$; $P = 0.853$). CON 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, **publication 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, **publication 2**). 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, **publication 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; $F_{1,117} = 11.46$; $P = 0.001$).

5.2. Publication 3

The final number of sows included in the study was 251. Table 3 (**publication 3**) shows numbers of sows available for each of the reproductive performance measures. The mean ± standard deviation (SD) for total skin lesion count 24hr post-mixing was 31.0 ± 26.77 ($n = 250$; range 0 to 157), and 19.2 ± 16.62 ($n = 248$; range 0 to 105) for the total skin lesion count 3 weeks post-mixing. The mean ± SD for cortisol was 0.200 ± 0.0835 µg/dL [microgram/decilitre, ($n = 125$; range 0.058 to 0.452 µg/dL)], and 15.2 ± 3.08 mm ($n = 218$; range 6 to 25 mm) for back fat depth.

There were no associations ($P > 0.05$) between total skin lesion counts 24hr and 3 weeks post-mixing or hair cortisol concentrations with most reproductive performance traits (Table 3, **publication 3**). There was a positive association between the total skin lesion counts 3 weeks post-mixing and the number of piglets born mummified ($P = 0.045$; Table 3, **publication 3**), and a positive association between the total skin lesion counts 3 weeks post-mixing and IUGR scores ($P = 0.018$; Table 3, **publication 3**). Thus, sows with higher skin lesion counts 3 weeks post mixing had a higher likelihood of having mummified piglets, or piglets born with a higher IUGR score. The total number of piglets born was negatively associated with birth weight (REG = -0.04 ± 0.008; $F_{1,536} = 25.54$; $P < 0.001$); parity (included as a fixed effect) also had an effect on birth weight (Parity 1: 1.4 ± 0.06, 95% CI = 1.25 to 1.47; Parity 2: 1.5 ± 0.06, 95% CI = 1.39 to 1.64; Parity 3: 1.6 ± 0.07, 95% CI = 1.44 to 1.71; Parity 4: 1.2 ± 0.08, 95% CI = 1.10 to 1.40; $F_{1,536} = 3.83$; $P = 0.010$). The interaction between total skin lesion count 24hr post-mixing and total skin lesion count 3 weeks post-mixing was negatively associated with back fat depth (REG = -0.002 ± 0.0010; $F_{1,117} = 4.06$; $P = 0.046$). There was no correlation between total skin lesion count either 24hr (Rho = 0.10; $P > 0.05$) or 3 weeks post-mixing (Rho = -0.02; $P > 0.05$) and hair cortisol concentrations.

5.3. Publication 4

Gilts were considered lame if they received a score of 2 or higher (≥ 2) on the CAT scale (n = 5 gilts throughout entire study), and if they scored 60 mm or higher (≥ 60) on the VAS for OVERALL (based on the descriptive sublevel overlying the VAS, whereby visible signs of obvious lameness such as limping and shortened stride are described for the first time; n = 6 gilts throughout entire study). The mean ± standard deviation (SD) for the CAT locomotion score throughout the entire study was 0.2 ± 0.50 (median = 0; range 0 to 3). The mean ± SD for the OVERALL locomotion score throughout the entire study was 17.1 ± 14.47 mm (median = 15; range 1 to 72 mm).

Comparison of scoring methods over time

There was an effect of time of scoring on OVERALL ($F_{3,145} = 2.70$; $P \leq 0.05$), and on some of the components of locomotion, namely, caudal sway ($F_{3,144} = 2.92$; $P \leq 0.05$), stride length ($F_{3,145} = 3.04$; $P \leq 0.05$), and fluidity of movement ($F_{3,145} = 3.82$; $P \leq 0.05$; Figure 2, **publication 4**). No effect of time of scoring on reluctance to bear weight while walking ($P > 0.05$) was found, while abduction ($F_{3,194} = 2.47$; $P = 0.063$) and adduction ($F_{3,194} = 2.24$; $P = 0.086$; Figure 2, **publication 4**) tended to change over time. As shown in Figure 2 (**publication 4**), the pattern of locomotory ability over time which was most similar to OVERALL was that of stride length, with the least similar being caudal sway and abduction. Locomotion scores estimated using CAT tended to change over time (mean (median); at service = 0.18 (0); mid-pregnancy = 0.12 (0); late pregnancy = 0.37 (0); weaning = 0.20 (0); $F_{3,195} = 2.45$; $P = 0.065$; Figure 3, **publication 4**).

Associations between OVERALL and locomotion components

There were positive associations between the OVERALL VAS score and the scores for caudal sway, stride length, fluidity of movement, and reluctance to bear weight while walking across all scoring days together (Table 3, **publication 4**), with the highest regression coefficients for the latter three measures. Indeed, although the association between caudal sway and OVERALL locomotion score across all scoring days together was positive, when considered on each scoring day separately, this association did not always hold true (e.g., service: $P < 0.001$; mid-pregnancy: $P = 0.056$; late pregnancy: $P = 0.006$; weaning: $P = 0.103$; see Appendix A, Figure A2, **publication 4**, for graphs representing the relationship between OVERALL and locomotion components on each scoring day). This suggests that gilts with a higher OVERALL locomotion score also had higher caudal sway scores across all scoring days together, despite the pattern of increase/decrease being different for OVERALL and caudal sway locomotion scores on any given day. On the other hand, the associations between OVERALL locomotion score and stride length, fluidity of movement, and reluctance to bear weight while walking across all scoring days together were reflected by the associations found when each scoring day was considered separately ($P < 0.001$ for stride length, fluidity of movement, and reluctance to bear weight while walking on each scoring day; Appendix A, Figure A2, **publication 4**).

Associations between OVERALL, hair cortisol concentration, and reproductive performance

The OVERALL locomotion score both at service (REG = 0.003 ± 0.0012; $F_{1,48} = 4.25$; $P \leq 0.05$) and at mid-pregnancy (REG = 0.003 ± 0.0013; $F_{1,48} = 6.95$; $P \leq 0.05$) was positively associated with hair cortisol concentration in late pregnancy (i.e. the more impaired locomotory ability was during early to mid-pregnancy, the greater the accumulation of cortisol in the hair shaft by end of the pregnancy). No association between OVERALL locomotion score in late pregnancy and hair cortisol concentration in late pregnancy was found ($P > 0.05$).

The OVERALL locomotion score at service was positively associated with the number of piglets born dead (REG = 0.01 ± 0.006; $F_{1,36} = 4.24$; $P \leq 0.05$), and the total born (REG =

0.1 ± 0.03; $F_{1,120} = 4.88$; $P \leq 0.05$), and tended to be positively associated with the number of piglets born alive (REG = 0.1 ± 0.03; $F_{1,120} = 3.17$; $P = 0.078$) and piglets mummified (REG = 0.01 ± 0.008; $F_{1,24} = 2.97$; $P = 0.098$).

The OVERALL locomotion score in late pregnancy tended to be positively associated with the number of piglets born alive (REG = 0.04 ± 0.024; $F_{1,119} = 3.06$; $P = 0.083$) and total born (REG = 0.1 ± 0.03; $F_{1,119} = 3.84$; $P = 0.053$). There were no associations between OVERALL locomotion score at mid-pregnancy and any aspect of reproductive performance ($P > 0.05$).

6. General Discussion

This discussion summarises some of the main findings and their implications as outlined in full in the publications comprising this thesis. Furthermore, data collected, but not included in this thesis (**publications in preparation**) will be given some attention, and suggestions for future research are provided.

Tackling gestational chronic stress in sows by addressing its sources within their housing systems is key to improving sow welfare. This in turn can help safeguard the sustainability and profitability of the pig industry. To achieve this, we must identify risk factors for chronic stress. The literature review (**Publication 1**) conducted as part of this thesis confirmed that there are several risk factors with potential to induce chronic stress in sow gestational environments, namely space allowance, group size and type (stable/dynamic), feeding level, lameness, pen design, feed system, enrichment and rooting material, floor type, the quality of stockmanship, environmental conditions, and individual sow factors. The literature review also confirmed the detrimental effects of chronic stress on sow welfare and reproductive performance, but also on her offspring, mediated by prenatal stress. Finally, the review highlighted the need to develop methods of effective chronic stress reduction.

Given the need to determine specific risk factors for chronic stress acting on gestating sows, **publication 2** investigated the potential of mixing aggression to act as a chronic stressor. Mixing aggression is a well-known acute stressor for sows (Arey and Edwards, 1998), mediating negative effects on reproductive performance (Einarsson et al., 2008, Kranendonk et al., 2006, Turner et al., 2002). Results of our study (**publication 2**) further confirmed this. We found an association between levels of mixing aggression [with skin lesion score used as a proxy for the intensity of mixing aggression (De Koning, 1984, Turner et al., 2006)] and sow performance within parity, representing the acute stress effect of mixing aggression. Specifically, piglet mortality during lactation and cycle length in parity two increased with increasing SLMIX score in parity two. Our results are in contrast to 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, and also used different reproductive performance measures.

In addition to inducing acute stress, Turner et al. (2009) and Salak-Johnson (2017) suggested that mixing aggression in early life could also contribute to chronic stress, as animals that receive and/or inflict high levels of aggression at mixing, will often continue to receive and/or inflict high levels of aggression later in life. Indeed, our results in **publication 2** support this possibility. Correlations between skin lesion scores at different inspection times found in this study are in line with the mechanism proposed by Turner et al. (2009). Namely, it seems that gilts which experienced intense aggression at first mixing were more likely to continue to receive more intense aggression, or to be more aggressive throughout pregnancy. Such sows are then likely to 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 accumulated during aggressive encounters. 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). Moreover, our results confirmed the detrimental effect of chronic stress associated with mixing aggression on sow reproductive performance. We found that the number of non-productive days in parity two was positively associated with SLMIX score in parity one only. This result is in agreement with other studies which demonstrated similar findings, including lower total piglets born per sow (Einarsson et al., 2008). In our study, more 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.

As highlighted in the literature review (**publication 1**), there is a need to develop methods of reducing chronic stress, by eliminating chronic stress risk factors from sow environments. Our results in **publication 2** provide evidence for two methods with potential to reduce mixing aggression intensity, and hence the associated chronic stress. Firstly, we showed that age at first service was associated with the intensity of mixing aggression, whereby gilts served at the youngest ages of the cohort had lower SLMIX score resulting from fights to establish a dominance hierarchy. While mixing piglets at younger ages resulted in shorter fight duration and fewer injuries (Pitts et al., 2000), to our knowledge, no previous study reported such an effect for sows. At 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). Moreover, this finding further adds to the known benefits of serving gilts at younger ages, namely, improved physiological reproductive performance and longevity (Cottney et al., 2012, Koketsu et al., 1999, See and Knauer, 2019). Nonetheless, guidelines for optimal gilt body condition and weight should still be observed when serving gilts young (Kummer et al., 2006). For instance, serving at less than 190 days of age is not recommended (Koketsu et al., 2020), as gilts may not yet have an adequate body composition, or have not yet reached sexual maturity (Malanda et al., 2019). This in turn could impair reproductive performance, leading to reduced farrowing performance and an increased risk of culling (Kummer et al., 2006, Malanda et al., 2019).

Secondly, we found lower SLMIX scores in sows on rubber flooring, suggesting those animals experienced less intense aggression at mixing, which is a positive outcome for sow welfare (Munsterhjelm et al., 2008, Calderon Diaz et al., 2013). In this case, the possibility that intensity of mixing aggression was reduced because of the animals' reluctance to prolong fights on slippery rubber flooring (Boyle and Llamas Moya, 2003) cannot be discounted. The driving factors behind this finding require further investigation to fully understand its implications for sow welfare. While this could have negative implications for animal welfare, it did not translate into impaired sow reproductive performance. In contrast, the possibility that more intense mixing aggression on concrete floors contributed to higher levels of chronic stress during gestation was evident in this study. Namely, there was a higher proportion of piglets born dead from sows on concrete (**publication 2**), likely due to a prolonged farrowing process, which could be a consequence of chronic stress experienced during gestation (Lawrence et al., 1992). Moreover, with clear benefits to reproductive performance and welfare of sows housed on rubber floors, our findings should encourage a more widespread use of this flooring type in sow housing.

Aggression among sows does not cease after the establishment of the dominance hierarchy at mixing. Indeed, it continues at lower levels throughout gestation due to bullying and competition for limited resources (Hemsworth et al., 2013, Spoolder et al., 2009). Consequently, sustained aggression is another potential risk factor for chronic stress (Salak-Johnson, 2017). Results in **publication 3** provide some evidence that sustained aggression has a negative effect on sow reproductive performance, likely mediated by chronic stress. Hence, these results indicate that skin lesion counts recorded post-hierarchy establishment have the potential to act as an indicator of chronic stress. Given the ease and speed of skin lesion recording, this is a valuable finding which could aid fast identification of animals at risk of chronic stress. Specifically, we found an increase in the number of piglets born mummified with higher skin lesion counts 3 weeks post-mixing (whereby skin lesion counts 3 weeks post-mixing acted as a proxy for sustained aggression). This is a novel finding.

A possible mechanism behind this result could be related to calcification and skeleton development which in the pig foetus does not begin until day 38 to 45 of gestation (Flower and Weary, 2006, Flowers, 2019). Furthermore, the sow reabsorbs any foetus that dies in utero prior to day 38 (Flower and Weary, 2006, Flowers, 2019). Sows in our study (**publication 3**) were mixed approximately 25 days post-service, so the number of skin lesions 3 weeks post-mixing was counted at approximately 49 days of pregnancy. Thus, the presence of skin lesions at this time indicates that sows were experiencing aggression, and therefore stress, at a time when foetuses that die are not re-absorbed, but persist as 'mummies' (Flower and Weary, 2006, Flowers, 2019). A similar negative effect of late-gestation stress on foetal losses was recorded in dairy cows (Santolaria et al., 2010).

The increase in IUGR scores with higher skin lesion counts 3 weeks post-mixing is another novel finding (**publication 3**), which also implies a negative effect of sustained aggression experienced by sows during gestation on reproductive performance. In this case, energy metabolism and nutrient allocation to developing foetuses by sows could be a likely explanation. Rooney et al. (2020) found higher IUGR scores in piglets born to sows fed low energy diets in late gestation, and suggested that maternal protein or energy intake deficits are associated with reduced allocation of nutrients to foetal development. This could in turn exacerbate the incidence of IUGR in offspring. Indeed, chronic stress associated with rough handling and heat stress can increase plasma protein and glucose levels, and also alter sow energy metabolism and nutrient digestion (Barnett et al., 1983, He et al., 2019). This could consequently lead to lower amounts of nutrients to be available for allocation to foetal development (Barnett et al., 1983, He et al., 2019). It is therefore possible that chronic stress associated with sustained aggression could have a similar effect on sow energy metabolism, with potentially negative consequences for offspring development and incidence of IUGR.

While the number of mummified piglets and IUGR scores are more commonly treated as sow reproductive performance indicators, they are also piglet-based measures. The fact that both were associated with sustained aggression, and potentially, the consequent chronic stress in sows (**publication 3**) suggests a prenatal stress effect was also at play. Further support for the detrimental effects of impaired sow welfare and consequent gestational chronic stress acting prenatally on offspring comes from our unpublished work (**article in preparation**). We found associations between gilt welfare status and offspring behaviour later in life. Namely, gilts which avoided agonistic encounters (as indicated by lower skin lesion counts) and performed higher levels of stereotypic behaviour (a likely indicator of chronic stress) throughout gestation had offspring which vocalised more during the back test. Similarly, gilts of seemingly subordinate status (as indicated by delayed entry to, and longer durations spent inside the ESF), and therefore those experiencing increased levels of social stress, produced offspring with a proactive-like personality profile (**article in preparation**). Furthermore, results of our most recent work more specifically show the detrimental effects of sow chronic stress acting prenatally to impair the health and resilience of future offspring. For instance, we found a lower incidence of scour in suckling piglets born to sows which experienced lower gestational chronic stress as a result of being housed in a physically more comfortable (rubber floors within free-access feeding stalls) and enriched (straw provided in racks, and natural rope suspended in stalls) system. Lower levels of sow chronic stress were reflected in lower frequencies of sow oral stereotypic behaviours and lower eye tear stain scores. This in turn translated into a lower risk of prenatal stress and improved offspring health and resilience (**article in preparation**). Such results highlight the significance of prenatal stress and the consequent need for further research into strategies for chronic stress mitigation in gestating sows.

We found no association between hair cortisol concentrations and skin lesion counts, nor between hair cortisol concentrations and indicators of sow reproductive performance in **publication 3**. Moreover, hair cortisol concentrations did not prove a useful indicator of chronic stress in **publication 3**. As outlined in **publication 1**, hair cortisol concentrations are now more commonly used as an indicator of chronic stress in several species (Burnard et al., 2016, Heimbürge et al., 2020), however, much is still unclear regarding the incorporation of cortisol into the hair shaft (Casal et al., 2017, Heimburge et al., 2019, Otovic and Hutchinson, 2015). For example, Heimbürge et al. (2020) compared hair cortisol concentrations following a period of ACTH injections in cattle and pigs, and found differences between treatments for cattle, but not for pigs. Similarly, an increase in wool cortisol following a stress-inducing treatment (extensive brushing and dexamethasone injection) was found in sheep (Salaberger et al., 2016). Given the clear effects of stress on hair cortisol concentrations in cattle and sheep, it is possible that hair cortisol has a reduced reliability as a chronic stress indicator for pigs (Heimbürge et al., 2020). Heimbürge et al. (2020) suggest that this could be due to lower systemic cortisol response following ACTH administration in this species. However, it could also be possible that ACTH is metabolised faster in pigs, and that the ACTH injection and cortisol detection protocols used by those authors did not span an appropriate period to capture a difference in hair cortisol concentration.

It is also possible that the lack of association between hair cortisol and skin lesions in **publication 3** is due to the phenomenon of habituation to chronic stress (Grissom and Bhatnagar, 2009). This occurs through continuous exposure to a particular stressor, leading to a blunted HPA axis response (Grissom and Bhatnagar, 2009, Meyer and Novak, 2012). Skin lesion counts recorded 3 weeks post-mixing in **publication 3** were a proxy for sustained aggression, and it is likely that study sows habituated to this stressor over the course of gestation. The effects of various confounding factors which could affect concentrations of cortisol entering the hair shaft should also be considered when validating the use of hair cortisol as a chronic stress indicator (Otten et al., 2020, Salaberger et al., 2016). For instance, Otten et al. (2020) found that endogenous hair cortisol concentrations may be substantially altered by exogenous cortisol entering the hair shaft by diffusion from media such as urine, faeces and saliva, which commonly contaminate the outside of sow hair in on-farm settings. The body region from which hair is collected is therefore important (Heimburge et al., 2019). The selection of the dorso-lumbar region as the site of hair collection in **publication 3** was based on its lower chance of contamination with urine, faeces, and saliva (Casal et al., 2017, Otten et al., 2020). Despite this, the possibility of some of those contaminants still being present at various points throughout gestation cannot be ruled out. Thus, it is clear that the usefulness of hair cortisol as an indicator of chronic stress is dependent upon various factors which must be controlled in order to ensure accuracy of results. Consequently, the lack of associations between hair cortisol concentration and other parameters investigated in **publication 3** must be treated with caution.

The literature review conducted in **publication 1** emphasized the potential of lameness to act as a chronic stress risk factor, with detrimental effects on sow reproductive performance and welfare (Briene et al., 2021, Lagoda et al., 2022). To safeguard both, lameness must be treated early (for greater treatment effectiveness), or prevented from developing altogether. This in turn relies on its early detection as signified by slight deviations from optimal locomotion (Conte et al., 2015, Heinonen et al., 2013).

Previous work involving locomotion scoring of sows within our research department highlighted the inefficiency of existing locomotion scoring systems, and identified the need for a novel, improved system. Based on our own observations (**publication 4**) and on previous research (d'Eath, 2012, Nalon et al., 2014, Tuyttens et al., 2009), a scoring system in the form of a visual analogue scale emerged as a likely candidate for a more sensitive and a detailed locomotion scoring system. Indeed, the VAS developed for the purpose of **publication 4** enabled early detection of slight deviations from optimal locomotion over time, and as hypothesised, it was more effective at achieving this than the categorical system developed by Main et al. (2000).

As expected, gilt locomotion scores increased as pregnancy progressed (**publication 4**). This is because as pregnancy advances, gilts gain weight, which in turn puts more pressure on their limbs and could result in a deterioration in leg health and therefore higher locomotion scores (Pluym et al., 2011). Furthermore, the longer sows spend in a group, the greater the likelihood of fights and consequent injuries to the limbs (Anil et al., 2009, Calderon Diaz et al., 2013). In addition, sows are most commonly housed on fully slatted concrete floors (as was the case in the study described in **publication 4**), which are rough and uncomfortable, and a risk factor for lameness (Calderon Diaz et al., 2013, Spoolder et al., 2009). The longer sows spend on this type of floor, the greater the likelihood of increased locomotion scores as a result of leg discomfort experienced by the animals.

Publication 4 is the first study that we are aware of which investigated variation in individual components of gilt stride. Looking at specific components of locomotion can provide a more detailed picture of locomotory ability throughout pregnancy, and give insights into the underlying causes of lameness. Hence, this can help ameliorate lameness risk factors, and could in turn be important when deciding on the best form of treatment. Understanding such variation can also aid in the identification of a single locomotion component which could act as a reliable measure of the animal's overall locomotory ability (Hoffman et al., 2014). It could speed up and potentially make on-farm locomotion assessment more accurate by simplifying the methodology for the farmer (Hoffman et al., 2014). As an example from the dairy cow literature, it is commonly accepted that the degree of back arch displayed by a cow provides insight into overall locomotory ability/lameness status, as the two are positively associated (Hoffman et al., 2014, Poursaberi et al., 2010).

Caudal sway, stride length, fluidity of movement, and reluctance to bear weight while walking were positively associated with the overall locomotion score assessed using a VAS, demonstrating potential to simplify sow locomotion assessment on-farm (**publication 4**). A good proxy for OVERALL locomotion should have a consistent relationship with it across all stages of pregnancy and management. Stride length, fluidity of movement, and reluctance to bear weight while walking all had a consistent relationship with OVERALL across and on each scoring day separately, and thus have potential to be used as proxies for OVERALL locomotory ability. In contrast, this was not the case for caudal sway, which made it unsuitable to act as a proxy for OVERALL (**publication 4**). The lack of a positive association on each recording day could be a consequence of the changing weight and shape of the gilt as pregnancy progresses, which in turn could alter the degree of her caudal sway. A similar phenomenon was noted in dairy cows by O'Driscoll et al. (2010), whereby the degree of abduction and adduction recorded in the animals differed depending on the fullness of their udders.

We recommend fluidity of movement and reluctance to bear weight while walking as the most appropriate proxy options for farmers. Fluidity of movement is a measure of the overall smoothness/ease of an animal’s walking ability, where any deviations away from the norm are easy to observe. Reluctance to bear weight while walking requires the observer to identify whether the animal is reluctant to place any of its limbs on the floor, and the degree to which this occurs, to determine the severity of the phenomenon. Abnormal weight bearing is easily spotted, thus, similar to fluidity of movement, any deviations away from the norm are easy to identify.

Results presented in **Publication 4** confirm the existence of a relationship between impaired locomotion and reproductive performance of sows. Namely, we found positive associations between the VAS locomotion score of gilts around their first service and the total number of piglets born, as well as a trend for a positive association with piglets born alive, over the first four parities. There was also a positive association between the VAS locomotion score at this time and the number of piglets born dead, and a trend for a positive association with mummified piglets. Locomotory ability later on in pregnancy was related to long-term reproductive performance to a much lesser extent, with just a trend for a positive association between the VAS locomotion score and the total born and born alive piglet numbers. Thus, it appears that assessing locomotory ability around the time of first service is likely the optimal time to estimate how it could affect lifetime performance.

As higher scores indicate worsening locomotory ability, this implies that gilts that deviated more from the ‘ideal’ stride around the time of first service were more productive across their first four parities. These findings conflict with the existing literature. In the studies of Anil et al. (2009) and Iida et al. (2020), lame sows had lower numbers of piglets born alive, thus demonstrating a detrimental effect of lameness on reproductive performance. However, these studies utilised locomotion scores recorded at different stages of pregnancy to those used in the current study (e.g. only on the way to the farrowing rooms). Moreover, it is possible that this difference to our findings relates to the fact that the above studies considered effects of clinical lameness, rather than a slight impairment in locomotion, as was the case in **publication 4**. It is possible that as clinical lameness is a more severe condition, this led to much higher chronic/acute stress levels, and consequently had a more marked effect on reproductive performance parameters (Anil et al., 2009, Iida et al., 2020). In turn, a possible explanation for our findings regarding associations between locomotory ability and reproductive performance could relate to energy resource distribution in sows. Redirection of energy resources away from non-crucial physiological processes towards reproduction is a known phenomenon in mammals, as this strategy maximizes reproductive performance (Speakman, 2008). It is possible that our study sows redirected their energy resources towards reproductive functions in a likewise manner, with positive impacts on the number of total born and born alive piglets. In consequence, this could have left fewer energy resources available for the maintenance of leg health, resulting in slight deviations from optimal locomotion.

Moreover, we speculate that the slightly compromised leg health (as marked by slight deviations from optimal locomotion) experienced even at this early stage in the reproductive cycle generated elevated stress levels, which persisted chronically. This is supported by our finding of higher hair cortisol concentrations in late pregnancy (reflecting chronic stress levels experienced by gilts throughout pregnancy) with higher overall locomotion scores both at service and in mid-pregnancy (**publication 4**). The elevated stress levels could in turn have detrimental knock-on effects on prenatal mortality. Hence this could explain the positive association between locomotion scores at service and the numbers of piglets born dead, and the trend for a higher number of piglets mummified with increasing overall locomotion score. This finding is in line with Hartnett et al. (2020); in that study, replacement gilts reared with entire males had impaired leg health in terms of higher hoof lesion scores. These gilts went on to have higher numbers of piglets born dead over their first five parities, which the authors hypothesised was due to the elevated stress levels associated with impaired leg health (Hartnett et al., 2020). Thus, it is possible that even slightly impaired leg health/locomotion could generate sufficient chronic stress levels to impair certain aspects of reproductive performance. Future research is necessary to further elucidate the mechanisms involved in the impairment of reproductive performance by slightly impaired locomotory ability, with a focus on the extent to which chronic stress associated with slightly impaired locomotion is involved in this process.

7. Conclusions

- There are a number of factors with potential to contribute to chronic stress in gestating sows. Development of chronic stress mitigation strategies can lead to positive effects on sow welfare and reproductive performance, and on the health and resilience of their offspring. Several of the identified chronic stress risk factors require further investigation to determine the extent of their contribution to sow chronic stress, and prenatal stress in their offspring.
- Mixing aggression experienced by replacement gilts has the potential for carry-over, chronic stress effects, resulting in impaired reproductive performance. Service at a younger age resulted in a reduction of mixing aggression intensity, with associated positive effects on reproductive performance. Similarly, the use of rubber floors in gestation housing had a positive influence on mixing aggression intensity and reproductive performance.
- Higher levels of sustained aggression experienced by sows were associated with impaired reproductive performance, with more mummified piglets and higher IUGR scores. This implies a sow chronic stress effect, with a knock-on prenatal stress effect

on offspring. While hair cortisol concentrations did not prove a useful indicator of chronic stress, skin lesion counts recorded post-hierarchy establishment showed such potential.

- Early detection and prevention of developing lameness disorders is possible by implementing a novel visual analogue scale in sow locomotion scoring. This can in turn reduce the risk of chronic stress induced by lameness in sows, and safeguard welfare and reproductive performance. Identification of several components of locomotion with potential to act as proxies for overall locomotion could simplify on-farm locomotion assessment.

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9. Appendices

A. Percentage contribution of authors to each publication

I hereby give the percentage of each author’s contribution to the publication entitled:

Lagoda, M.E., Marchewka, J., O'Driscoll, K. and Boyle, L.A., 2022. Risk Factors for Chronic Stress in Sows Housed in Groups, and Associated Risks of Prenatal Stress in Their Offspring. *Frontiers in Veterinary Science*, *9*, pp.883154-883154.

Co-author name	**Percentage of author’s contribution**	**Contribution to the publication**
Martyna Lagoda	60%	Conceptualization, Validation, Writing – original draft preparation, Writing – review and editing
Joanna Marchewka	10%	Validation, Writing – review and editing
Keelin O’Driscoll	15%	Conceptualization, Validation, Writing – review and editing
Laura Boyle	15%	Conceptualization, Validation, Writing – review and editing

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I hereby give the percentage of each author’s contribution to the publication entitled:

Lagoda, M.E., Boyle, L.A., Marchewka, J. and Calderón Díaz, J.A., 2021. Mixing aggression intensity is associated with age at first service and floor type during gestation, with implications for sow reproductive performance. *Animal*, *15*(3), p.100158.

Co-author name	**Percentage of author’s contribution**	**Contribution to the publication**
Martyna Lagoda	50%	Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Visualization.
Laura Boyle	15%	Conceptualization, Methodology, Investigation, Writing – review& editing, Supervision, Project administration, Funding acquisition
Joanna Marchewka	5%	Writing – review & editing, Supervision
Julia Calderon Diaz	30%	Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization, Supervision, Project administration.

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I hereby give the percentage of each author’s contribution to the publication entitled:

Lagoda, M.E., O'Driscoll, K., Marchewka, J., Foister, S., Turner, S.P. and Boyle, L.A., 2021. Associations between skin lesion counts, hair cortisol concentrations and reproductive performance in group housed sows. *Livestock Science*, *246*, p.104463.

Co-author name	**Percentage of author’s contribution**	**Contribution to the publication**
Martyna Lagoda	50%	Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Visualization.
Keelin O’Driscoll	15%	Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Supervision, Project administration.
Joanna Marchewka	10%	Writing – review & editing.
Simone Foister	5%	Data curation, Methodology, Investigation.
Simon Turner	5%	Conceptualization, Methodology, Writing – review & editing
Laura Boyle	15%	Conceptualization, Methodology, Investigation, Writing –review & editing, Supervision, Project administration, Funding acquisition.

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I hereby give the percentage of each author's contribution to the publication entitled:

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Co-author name	**Percentage of author's contribution**	**Contribution to the publication**
Martyna Lagoda	50%	Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration
Laura Boyle	15%	Conceptualization, Methodology, Validation, Resources, Writing – review and editing, Supervision, Project administration, Funding acquisition
Joanna Marchewka	10%	Validation, Writing – review and editing, Supervision
Keelin O'Driscoll	25%	Conceptualization, Methodology, Software, Validation, Formal analysis, Resources, Writing – review and editing, Supervision, Project administration

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B. Publications

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REVIEW
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Risk Factors for Chronic Stress in Sows Housed in Groups, and Associated Risks of Prenatal Stress in Their Offspring

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Chronic stress has a detrimental effect on sow welfare and productivity, as well as on the welfare and resilience of their piglets, mediated prenatally. Despite this, the specific risk factors for chronic stress in pregnant sows are understudied. Group-housed pregnant sows continuously face numerous challenges associated with aspects of the physical (group type and size, flooring, feeding system) and social (stocking density, mixing strategy) environment. There are many well-known potent stressors for pigs that likely contribute to chronic, physiological stress, including overcrowding, hot temperatures, feed restriction, inability to forage, uncomfortable floors, and poor handling. Some of these stressors also contribute to the development of production diseases such as lameness, which in turn are also likely causes of chronic stress because of the associated pain and difficulty accessing resources. The aim of this review is to discuss potential risk factors for chronic stress in pregnant sows such as space allowance, group size and type (stable/dynamic), feeding level, lameness, pen design, feed system, enrichment and rooting material, floor type, the quality of stockmanship, environmental conditions, and individual sow factors. The mechanisms of action of both chronic and prenatal stress, as well as the effects of the latter on offspring are also discussed. Gaps in existing research and recommendations for future work are outlined.

Keywords: swine, piglet, gestation, prenatal, chronic, stress, welfare, risk

INTRODUCTION

Implementation of Couuncil Directive 2001/88/EC, 2001 saw the transition from confinement of sows in individual stalls during gestation to group housing in the European Union. This is a trend mirrored in pig producing countries worldwide [e.g., Proposition 12 in the United States (1, 2)]. Group housing systems are considered more welfare-friendly as they allow sows a greater degree of freedom of movement and an opportunity for social interactions (3) compared to confinement in stalls. However, group housing comes at a price of other challenges, including sustained aggression among sows due to the competition for limited resources, social conflicts caused by continuous re-mixing, subordination/isolation of individuals, as well as suboptimal physical environments, all of which could lead to long-term (chronic) stress (4–6).

There are numerous studies investigating the types and physiological consequences of acute stressors that sows experience (7–9). One of the most commonly studied acute stressors is mixing of unfamiliar individuals, which results in fighting to establish a dominance hierarchy. This is associated with high levels of stress, manifested as elevated heart rate, plasma catecholamines (10), and cortisol levels. In fact, mixing is also a major acute stressor for weaner, grower, and finisher pigs (11, 12), with studies showing evidence of profound physiological and behavioural changes following mixing (11, 13). Overall, this confirms that mixing is a highly stressful event (11). Other examples of acute stressors include transport, social isolation, and physical restraint (7–9). For instance, Bradshaw et al. (7) and Soler et al. (9) found higher salivary cortisol levels in pigs shortly after they experienced rough transport conditions. Higher serum amyloid A and cortisol concentrations were shown in pigs isolated for short periods of time (9). In addition, increased cortisol and serum amyloid A concentrations were also recorded in pigs subjected to physical restraint (8).

While most work on stress in pigs focuses on weaner and finisher pigs, generally, there is limited knowledge on stress in pregnant sows (other than while in the farrowing crate), with even less information on chronic stress (14–17). This is despite the fact that some acute stressors could contribute to chronic stress if experienced repeatedly, such as repeated remixing of unfamiliar individuals (18), or competition for limited resources and the associated aggression (4). Therefore, it is evident that chronic stress could be experienced by sows to a greater extent, as the additive negative effects of repeated acute stressors were not previously considered as contributing factors (19–21). This is a major cause for concern as stress experienced by the mother throughout gestation has negative effects not only for the sow herself, but also on foetal development, with the potential to persist into the offspring's adulthood (22). This is known as prenatal stress, and could have negative implications for offspring resilience to disease, welfare challenges, productivity and performance.

It is possible to make inferences about levels of chronic stress experienced by animals based on performance, behavioural, and physiological parameters (23). For instance, impaired reproductive performance can be a symptom of chronic stress, as energy resources are redirected away from maintenance and developmental processes, including pregnancy (24, 25), and diverted towards processes aimed at ensuring survival (26). Likewise, stereotypic behaviours can become established in situations where animals are chronically stressed (6). Other behaviours indicative of chronic stress in pigs include abnormal levels of vocalisations, urination/defecation, and inactivity (6, 27). Chronic stress also leads to immunosuppression, which in turn results in higher disease incidence (28, 29). Other physiological indicators of chronic stress include increased levels of cortisol [e.g., in hair (30)], and altered patterns of cortisol concentrations in faeces, blood plasma, and saliva (31).

The medium in which cortisol is measured can have an effect on the resulting concentration. Hence, the choice of medium must be considered carefully, to ensure it is appropriate for the specific type of stress under investigation, i.e., acute or chronic. For instance, there is increasing focus on measurement of cortisol in hair as an indicator of chronic stress due to the long-term accumulation of cortisol within the growing hair shaft (32–34). While hair collection is a non-invasive procedure with potential to give insight into stress levels over weeks or months (30, 32), there are also many confounding factors (collection site, hair colour, age, sex, stage of gestation, cleanliness) that affect cortisol levels in hair, and which must therefore be controlled for when using this method to determine chronic stress levels (30, 32, 33). On the other hand, cortisol levels in saliva, blood plasma, urine or faeces are "point samples" strongly influenced by time of day (circadian rhythm pattern), food intake and environmental disturbances [including stress associated with the blood sampling procedure in particular; (6, 32)]. As a result, such mediums are mainly used to quantify short-term stress levels (6, 32). Saliva and blood plasma capture stress levels experienced over minutes, while urine and faeces capture slightly longer periods which might span days (6, 32, 35). However, it is still possible to use such mediums to quantify chronic stress levels, provided they are measured consistently over time [Davenport et al. (32)]. Doing so allows patterns to be identified, which in turn can reveal deviations from the norm, indicative of chronic stress (31, 32, 34).

Although there are numerous indicators that allow researchers to make inferences about levels of chronic stress, there are no confirmed risk factors within the sow's environment. Given the postulated negative effect of chronic stress on both the sow and her offspring (21, 22), there is an urgent need for additional research to identify the potential risk factors for chronic stress experienced by pregnant sows. It is also necessary to ascertain the potential for such factors to contribute to prenatal stress and associated reduced resilience in the offspring of chronically stressed sows. There are numerous aspects of the physical and social environment to which sows are continuously subjected throughout gestation, which could act as potential risk factors for chronic stress. These include space allowance (36, 37), group size and type [stable/dynamic; (38)], feeding level (39, 40), lameness (30, 41, 42), pen design (4, 43), feed system (23, 44), enrichment and rooting material (1, 45), floor type (46–48), quality of stockmanship (49, 50), environmental conditions (51), and certain individual sow factors (52, 53). The aim of this review is to discuss such factors in terms of their ability to induce chronic stress in sows. The mechanisms of action of both chronic and prenatal stress, as well as the effects of the latter on offspring are also discussed. Gaps in existing research and recommendations for future work are outlined.

CHRONIC AND PRENATAL STRESS–MECHANISMS OF ACTION

Mechanisms Underlying Chronic Stress

Stress is a phenomenon defined as a "non-specific response of the body to any demand" (54, 55). Stressors which drive this response are of variable nature, and can be both physical and psychological (54, 56). While stressors act on many different regions of the nervous system to induce appropriate responses (54), the most prominent features of the stress response involve the activation of the autonomic nervous system and the hypothalamo-pituitary-adrenocortical (HPA) axis (54, 57, 58). At its most basic, this involves the synthesis of cortisol (glucocorticoid stress hormone) by the adrenal cortex in response to adrenocorticotropic hormone (ACTH) (57). This in turn has numerous knock on effects on a range of internal processes (57, 58). Moreover, these effects differ depending on whether the stressor is acute (lasting minutes or hours), persists chronically (for days, weeks, or even months), or whether the organism is repeatedly exposed to acute stressors [chronic intermittent stress; (18)], as well as depending on the severity of the stressor (57, 58). In fact, the results from existing research on HPA axis activity are conflicting (59). Some authors argue that HPA axis activation does not always reflect stressful conditions, as it is known that it can be either upregulated or downregulated in response to chronic stress, depending on the situation and the individual involved (34, 59, 60). Others suggest that a generalised endocrine profile of a chronically stressed animal does not exist, as there is so much variation in the stress response of individual animals (61). Furthermore, while accounting for individual differences in animal biology was overlooked due to the historical focus on group as the experimental unit, many still highlight the importance of considering individual animals in the design of experiments (62, 63). Not all animals in the group respond in the same way to stressors, or indeed to their overall environment (63). This is highlighted by research investigating animal personalities and coping styles, defined as "alternative response patterns in reaction to a stressor" (63). For instance, animals that respond to a stressor with high levels of offensive, aggressive behaviour are said to adopt a proactive (active) coping style, while animals responding with low levels of offensive aggressive behaviour are said to adopt a reactive (passive) coping style (63, 64). Moreover, the resulting variation in responses could differentially impact offspring. Following on from this, Herman et al. (31) state that chronic stress-induced, protracted activation of the HPA axis takes many forms, including prolonged basal hypersecretion of glucocorticoids, sensitised stress responses, and even adrenal exhaustion, and that this can depend on the duration of the stressor, as well as its intensity, frequency and modality (31). Thus, caution must be exercised when interpreting chronic stress levels based on HPA axis activity patterns alone (59, 60).

Effects of Chronic Stress

Nonetheless, evidence exists of the negative effects of prolonged activation of the HPA axis during the experience of chronic stress. This includes immunosuppression by cortisol (predominant mediator of stress in situations whereby stressful stimuli are prolonged), resulting in increased susceptibility to disease, due to decreased numbers of lymphocytes, cytokines and immunoglobulins in the blood of chronically stressed animals (6). This can in turn mean that energy resources in stressed animals are redirected away from maintenance and developmental processes, including pregnancy (24, 25), impairing reproductive performance (28, 29). Chronic stress can also impair reproductive performance by inhibiting the release of both luteinizing hormone and progesterone (6).

Chronically stressed animals can also have an enhanced or a diminished response to acute stressors (65–69). In terms of effects on behaviour, frustration associated with an animal's inability to cope with a challenge, or having no control over its immediate environment/social situations can lead to chronic stress, which in turn can stimulate the development of stereotypic behaviours (6). Research into the functional significance of stereotypic behaviours suggests that their performance acts to reduce the stress associated with the situation which initially caused it [i.e., acting as a stress coping mechanism; (70)]. This is supported by evidence from studies demonstrating reduced heart rates in stereotyping equines (71, 72), increased plasma cortisol concentration in horses prevented from stereotyping (72, 73), and a decrease in faecal corticoids in stereotyping macaques (74). Consequently, while stereotypic behaviour is indicative of suboptimal environments and the chronic stress associated with them [either past or present; (75)], it may not be an accurate indicator of current physiological stress as measured by heart rate or glucocorticoid levels (72). On the other hand, neurotransmitters such as serotonin are implicated in the pathology underlying stereotypic behaviour, with lower basal levels found in stereotyping animals (72). Therefore, measuring serotonin levels could be a better method of assessing the pathological nature of stereotypic behaviours (72).

Prenatal Stress–Mechanism of Transfer to the Offspring

Based on research into the effects of acute prenatal stress events on offspring, it is now known that prenatal stress is mostly hormonally mediated in many mammalian species, including guinea pigs, mice, rats, and swine (14, 76). Moreover, chronic maternal stress experienced during gestation can also cause chemical changes in the mother's body, which in turn can lead to increases in cortisol levels, and associated negative consequences for the developing offspring [guinea pig (14); mouse, rat, swine (70)]. Depending on the species, there are different types of placenta, with structural differences (77). Ultimately, the placenta acts as an interface between the mother and foetus (78). While the placenta forms a barrier to many chemicals, some, including glucocorticoids, will still pass through and have an effect on the developing foetus (15). For instance, Welberg et al. (69) demonstrated that acute stress can upregulate the chemical activity of placental 11β-hydroxysteroid dehydrogenase type 2 (component of the foetal-placental barrier to maternal corticosteroids) in rats, thus protecting the foetus against elevated maternal cortisol levels. However, under chronic stress conditions, the capacity to upregulate placental 11β-hydroxysteroid dehydrogenase type 2 activity in the face of an acute stressor is reduced by 90%. Thus, maternal exposure to chronic stress diminishes the placental capacity to protect the foetus from elevated maternal cortisol levels, with negative effects on the developing offspring (69). Maternal glucocorticoids can activate the foetal HPA axis and alter its development, with consequences for offspring stress coping mechanisms later in life [demonstrated in primates, guinea pigs, sheep, cattle, goats, pigs, rats and mice (15)]. For instance, prenatal stress dysregulates functionality of the HPA axis in species of monkeys and rodents in a way that leads to decreased feedback inhibition of corticotropin releasing hormone, causing prolonged elevation of circulating glucocorticoids in response to stress in later life (79).

Besides glucocorticoids, other maternal circulating hormones and chemicals such as catecholamines, also mediate prenatal stress (80). For instance, Kapoor et al. (15) found that increased maternal catecholamine concentrations in rats resulted in constriction of placental blood vessels, causing foetal hypoxia. This in turn caused the activation and reprogramming of the foetal sympathetic system, again resulting in altered offspring physiological responses to stress later in life (15).

More recently, the maternal vaginal microbiome was proposed as another potential mediator of prenatal stress (81). Vaginal microbiota harvested and transplanted from chronically stressed mouse dams into their naïve offspring delivered by caesarean section had effects which resembled those seen in naturally prenatally stressed offspring. These effects included changes in the foetal intestinal transcriptome and in hypothalamic gene expression (81).

Prenatal Stress in Swine Offspring

The stage of gestation during which a stressor occurs is also an important factor to consider (82–86), because various systems of the developing embryo/foetus are vulnerable to stress at different times throughout prenatal development (83). For example, early gestation (day 10 to day 17) is a critical period for pig embryo establishment and development. Couret et al. (85) showed that early gestational stress in the form of a social stressor led to an increased adrenal weight, while late gestational stress resulted in an increased proliferation index of blood cells in sow offspring. Omtvedt et al. (87) demonstrated differential effects of heat stress experienced by pregnant sows in early, mid and late gestation on the prenatal development of offspring. For example, heat stress experienced in early gestation interfered with embryo development and implantation (87). Likewise, Lucy et al. (88) showed that heat stress increased embryo mortality during early gestation, but led to a higher number of stillborn piglets if experienced later in gestation. Mixing is also a major stressor for sows, and also an example of a stressor with different effects on prenatal development depending on the stage of gestation during which it occurs (89). Mixing in early gestation generates sufficient prenatal stress to increase embryonic mortality and decrease the future litter size, in contrast to mixing during the fourth week of gestation (90). Lagoda et al. (91) demonstrated that mixing in early gestation can generate stress which persists chronically, with detrimental effects on reproductive performance in subsequent parities. It is thus possible that stress associated with early mixing, acting prenatally, could have long-term, carry-over effects on the affected offspring that survive to birth. Overall, it is clear that irrespective of the type of stressor which causes the maternal stress response, experience of prenatal stress in early gestation is especially detrimental to the developing offspring.

RISK FACTORS FOR CHRONIC STRESS IN PREGNANT SOWS

Space Allowance

Space allowance encompasses the physical space which the animal occupies and needs to change posture, stand up or lie down, as well as the additional space it needs to exercise and maintain muscle tone (92). When investigating the effects of space allowance on sow stress levels, certain confounding factors must be considered. For instance, both quality and quantity of the available space are important factors that can influence the stress levels. Often, factors such as the amount of "free" shared space available to group-housed sows, or whether extra space is required by larger sows, are not considered (93). Moreover, some authors advise caution when interpreting the effects of different space allowances, as group size can act as a confounder (93), while others show few or no interactions between group size and space allowance (94).

The feeding system in use can also impact the space available to the animals (1). Individual feeding stalls take up more space than a single electronic sow feeder (ESF), and thus the stocking density of the sows must be considered in relation to the actual space allowance available to each sow (1).

Animals also require adequate space for social interactions, such as establishment of a dominance hierarchy, avoidance of aggression, and performance of natural behaviours for which they are highly motivated (92). As such, restriction of space is associated with chronic stress in all species [i.e., fish: Sundh et al. (95); birds: Selvam et al. (96); cattle: Schubach et al. (97)]. Indeed, the behavioural diversity of sows housed at lower space allowances can be curtailed (98), and inability to perform a full behavioural repertoire is a source of frustration and stress for animals (6). Following on from this, inadequate space allowance can lead to overcrowded conditions, exacerbating agonistic interactions between pen mates (99, 100), which leads to elevated cortisol levels, indicative of stress (38).

It is also possible that adequate space allowance is crucial to the animal's ability to maintain personal space. For instance, Greenwood et al. (101) demonstrated benefits of increased space allowance at mixing, especially in the case of low ranking sows. In that study, sows in the highest space allowance treatment also had the highest cortisol concentrations. The authors explain this to be a consequence of increased levels of activity within this treatment, rather than a consequence of increased aggression or stress (101). On the other hand, Hemsworth et al. (37) and Barnett et al. (36) confirmed negative effects of reduced space allowance as indicated by chronically elevated cortisol levels in sows housed at a low space allowance. Lower space allowance was also associated with a lower percentage of gilts in oestrus, suggesting an impairment of sexual behaviour and reproductive performance at lower space allowances (37). Not meeting space allowance requirements therefore exerts stress on the animals, which can potentially act as a risk factor for chronic stress.

Group Size and Type (Stable/Dynamic)

Elucidating the effects of group size on sow stress levels is difficult because of confounding factors influencing levels of aggression in a group. These include the effects of the group type (dynamic vs. static) or space allowance, given that the optimal space allowance for sows at times of high aggression is unknown (94). The effect that group type has on aggression could mask the effect of group size on stress levels in sows. For example, in dynamic groups, the addition of new individuals continuously disrupts the dominance hierarchy, resulting in an increased intensity of fighting to establish the rank order (102, 103). This is in contrast to static groups, where the dominance hierarchy is established once, after which the intensity of fighting diminishes. Therefore, dynamic groups themselves could act as a potential risk factor for chronic stress. Moreover, following on from the constant addition of new individuals into dynamic groups, the size of dynamic groups is often larger than that of static groups, resulting in more hierarchy conflicts to resolve, and leading to higher levels of aggression (104). Consequently, sows housed in dynamic groups have higher cortisol levels compared to sows in stable groups (105).

However, such conclusions warrant a degree of caution, as it is not clear whether increased aggression levels in dynamic groups result from larger group size, or from the constant disruption of the dominance hierarchy due to the addition of new individuals (104). Most likely, it is a combination of both. However, Misra et al. (106) showed lower levels of aggression in large stable groups compared to small stable groups of finisher pigs. Therefore, it is possible that levels of aggression are also lower in large compared to small groups of pregnant sows provided the groups are stable. Unfortunately, investigations of effects of group size on aggression often test the same group sizes at different space allowances. Hence, while Hemsworth et al. (94) showed that in static groups, smaller group sizes ($n = 10$ sows) were associated with fewer injuries than in large groups ($n = 30$ or $n = 80$ sows), Taylor et al. (107) showed no effect of group size on skin injuries. Due to differences in space allowance, such results cannot be compared, and thus the effect of group size is unclear. Nonetheless, with more hierarchy conflicts to resolve in large groups, and therefore increased aggression levels, large group size is a plausible candidate risk factor for the development of chronic stress in sows.

Feeding Level

Sows in commercial systems are feed-restricted during pregnancy to ensure optimal body condition when it comes to production of viable piglets (108, 109). The aim is to optimise reproductive performance and ensure correct timing of return to estrus after weaning (40, 110, 111). While the restricted feed ration is sufficient to meet general maintenance requirements, ensure good health and performance, and adequate maternal and embryonic tissue growth, it does not ensure satiety (112). Providing feed restricted sows with high fibre diets allows to minimise the negative effects of restrictive feeding (40, 109, 113, 114). High fibre diets including roughage materials such as straw and grass silage, or bulky materials such as beet pulp promote a feeling of satiety, and thus reduce the motivation to continue feeding, and ameliorate associated frustration and hunger (40, 112, 114).

Moreover, in the absence of roughage, feed restricted sows remain highly motivated to eat. For instance, when tested in an operant task, feed restricted pigs were highly motivated to continue feeding by accessing extra feed (112, 115). This research revealed that the restricted ration typically allocated to pigs accounted for only 60–70% of the quantity of food they were capable of eating *ad libitum* (112, 115). Thus, the motivation to feed persists, resulting in chronic hunger, frustration, and increased stress levels as indicated by elevated cortisol concentrations (39, 40), as well as increased stereotypic behaviour performance (109, 116). As a consequence, there is competition for feed resources among feed restricted sows, leading to high levels of and more intense aggression (40, 117). Several studies found associations between restricted feeding and stereotypic behaviours (109, 116), which signal increased stress levels of feed restricted sows (118). This, combined with the high motivation to continue feeding, makes for compelling evidence that feed restriction is a risk factor for chronic stress for pregnant sows.

Nevertheless, it is difficult to quantify the levels of chronic stress associated with restricted feeding regimes. In contrast to Amdi et al. (39) who found elevated cortisol levels in feed-restricted sows, certain studies showed no changes in cortisol concentrations, and thus in stress levels of feed restricted animals (109, 119, 120). Although measuring cortisol levels is the standard when it comes to quantifying stress in animals, it is also possible that cortisol may not be a suitable physiological indicator of stress associated with hunger (121). This is because corticosteroids are affected by metabolic rates, which in turn relate to the state of hunger, potentially acting as a confounder (121).

Lameness

Lameness in sows is a common cause of reduced welfare and economic losses to pig producers (122–124). As a consequence, lame sows are often culled prematurely, reducing their longevity (122), and increasing the need to purchase replacement gilts (122).

The presence of both injuries and claw/hoof lesions, and unhygienic environments can exacerbate the development of lameness (47, 123, 125). Lameness occurs when an animal adjusts its posture or gait to minimise the experience of pain. Indeed studies investigating pain thresholds and the use of analgesics confirm that lameness is associated with pain (47, 122, 125–127). Lameness persists chronically as it often goes unnoticed due to the difficulties associated with identification of its early stages (122). Any associated long-term pain could contribute to stress both physiologically and psychologically (30, 41, 122). Physiological stress resulting from lameness is evident in studies which measured cortisol (salivary, hair), acute phase protein levels, and various salivary stress biomarker proteins (salivary α-amylase, salivary lactate dehydrogenase), with significantly higher levels of such stress related indicators in lame than non-lame animals (30, 41, 42). In addition, lameness also reduces reproductive performance (122); lame sows displayed delays in post-weaning oestrous, and had smaller litter sizes compared to non-lame sows (128).

Lameness may also contribute to psychological stress in sows, in a similar way to that reported for human patients suffering from chronic rheumatoid arthritis (129). For example, the pain and discomfort associated with lameness could render sows less successful during aggressive encounters with unfamiliar individuals when establishing a dominance hierarchy (52). This is an important component of the social behaviour of this species (52), and not being able to defend oneself from aggressors could lead to stress (93). Thus, lameness is a good candidate for a potential chronic stressor.

Pen Design

Under commercial conditions where space is limited, pigs likely benefit from places to hide and to avoid or escape from an aggressive interaction (4, 130–132). Indeed a lack of barriers within a pen is associated with higher levels of aggression (4, 132). Barriers reduce visual contact between the aggressor and the victim (23, 133–135), reducing fear/anxiety levels in sows, as indicated by a reduction in cortisol levels (43). This suggests that the lack of barriers within a pen, particularly in the case of dynamic groups whereby dominance hierarchy is continuously disrupted, can be a risk factor for chronic stress.

Feed System

Pigs prefer to synchronise their feeding behaviour (136), and feed restricted sows are highly motivated to access feed (39, 40). Hence competition for access to feed can cause severe aggression at feeding time (40, 136). Moreover, as feeding systems differ in the level of protection they provide to the feeding animal (23), this can affect the level of aggression that sows experience at feeding, and any associated stress (23). Protection while feeding can reduce aggression and the associated injury, stress, and disruptions to feed intake (4, 132). Feeding systems with such potential include protected ESF systems and individual full length feeding stalls, followed by troughs with barriers to separate the feeding animals. However, in the case of the latter, the level of protection depends on the length of the barriers (23). Feeding systems such as troughs without barriers, as well as floor feeding, do not provide protection during feeding time, and can thus exacerbate and prolong aggression within a group (4).

Despite providing protection at feeding and the added benefit of allowing a tailored feed allowance and diet for each individual (23), there are also certain negative aspects to protected feeding systems such as that offered by ESF systems. For example, any potential break down in the ESF system can result in sows not being fed. While the technology associated with ESF systems improved over the last decade, breakdowns are still possible and could majorly disturb the group dynamics. Another risk is that of aggression occurring as sows queue up to enter the ESF (109, 117, 137). However, this can be minimised by strategically placing the ESF away from busy pen areas and resources of interest (137, 138). It can also be ameliorated to an extent as sows establish a feeding order, with dominant sows feeding first, followed by subordinate sows (23, 139). However, due to feed restriction and the resulting chronic hunger, dominant sows continue to return to the feeder despite having eaten their daily ration (117). This results in frustration which can be expressed as vulva biting by sows waiting in the queue (109), and it also disrupts the feeding order, leading to aggression being directed towards subordinate sows still in the queue (23).

In order to avoid aggression associated with queuing to gain entry to the ESF, protected individual, free-access feeding stalls could be a useful alternative, provided that all or nearly all sows have access to a feeding stall. Indeed, Bahnsen et al. (44) showed that sows housed with protected feeding stalls had lower salivary cortisol levels compared to sows housed with an ESF system. This confirms the benefit of protected feeding stalls on sow stress levels.

Enrichment and Rooting Material

Domestic pigs are a highly intelligent species, requiring an appropriate level of cognitive stimulation in order to maintain mental and physical wellbeing (140). In addition, domestic pigs retain a high motivation to perform exploratory behaviour, including rooting behaviour which evolved in their wild counterparts (141). The inability to perform this natural behaviour within a commercial setting due to the lack of suitable materials at which it could be directed results in frustration, and is linked to the development of damaging behaviours (142). Providing pigs with appropriate enrichment allows for cognitive stimulation, and depending on the type of substrate used, it allows the animals to fulfil their behavioural needs, including the performance of rooting behaviour (141).

The provision of enrichment also has the potential to reduce sustained levels of aggression by keeping sows occupied and less likely to get involved in aggressive behaviour [demonstrated for spent mushroom compost (143); peat (144)]. Indeed, it must be noted that this depends on the enrichment type provided. For instance, Horback et al. (145) demonstrated that while enrichment items such as ropes and wooden blocks could satisfy behavioural needs of individual sows, they could not reduce the overall levels of aggression in the pen. While its provision is not panacea when it comes to eliminating stress, there are nonetheless multiple positive effects of enrichment on sow behaviour which can reduce their stress levels (1). This explains the association between the provision of enrichment and a higher frequency of behaviours indicative of good welfare, i.e., sleeping (144). Moreover, sows housed with deep straw bedding had lower cortisol concentrations and reduced immune stimulation (lower total white blood cells) compared to sows housed without straw bedding (45). These studies support that lack of appropriate enrichment is a risk factor for the development of chronic stress in pregnant sows.

Floor Type

Floor type can act as a risk factor for chronic stress both directly and indirectly. Its direct effect could be associated with discomfort due to lying on concrete slatted floors without bedding (23, 123). It could also directly contribute to the fear of falling and injury in instances where smooth concrete floors are slippery because of urine and faeces (46, 48, 93, 123). Additionally, indirect effects are associated with the injuries and lameness arising from certain floor types [e.g., fully slatted concrete floors (122, 123); see Lameness].

In contrast, rubber flooring can reduce the risk of claw lesion and lameness incidence, as well as improve comfort during resting and ease of changing posture (123, 146–149). In line with this, sows with access to stalls with rubber mats had lower cortisol concentrations on day 28 of gestation than sows in standard pens with concrete-floored stalls (150). In addition, sows housed on rubber floors also had improved reproductive performance (91). This finding confirms the potential of rubber flooring to reduce stress in pregnant sows (150).

On the other hand, rubber floors can become slippery, thus providing poor foothold, which may discourage sows from engaging in aggression (46, 48). In support of this, Lagoda et al. (91) showed that sows housed on rubber floors had lower skin lesion scores following mixing compared to sows on concrete slatted floors, suggesting reduced intensity of mixing aggression. Persistent slipperiness of rubber floors could also reduce aggression in the long term, as a result of fear of slipping and the consequent reluctance to engage in fights. Clearly, while slippery rubber floors may reduce the intensity of aggression, they should not be used intentionally for that purpose. Using the fear of slipping to prevent sows from fighting has its own negative connotations for welfare. Not being able to fight in order to settle dominance conflicts, as well as the constant fear of slipping would undoubtedly contribute to chronic stress (46, 151).

Quality of Stockmanship

The quality of stockmanship is determined by the stockperson's personality, attitude, and behaviour (50, 152), and has a substantial effect on stress levels in farm animals (49, 50). Hayes et al. (153) showed the potential for positive handling to reduce fear of humans in sows, while Dokmanovic et al. (154) showed numerically lower cortisol concentrations in gently handled pigs, compared to those which were handled roughly. In contrast, Manteca and Jones (50), Hemsworth and Boivin (155) showed compromised reproductive performance resulting from rough handling and fear of humans in sows.

Despite the process of domestication and living in close proximity to humans, the initial response of farm animals to humans is still that of fear (156). This is worsened when animals are exposed to rough handling and poor quality stockmanship, and when no effort is made towards the establishment of a neutral or a positive connexion with the animals (50). This effect is exacerbated by the increasing automation of the animal production sector, which gives stock people fewer opportunities to interact with the animals in their care (50). This means that it is more difficult for animals to habituate to the presence of humans (50).

Moreover, rough handling of sows can result in a lasting aversion towards certain or all humans through classical conditioning (157). Sows handled aversively by a single stock person can learn to associate such handling with all people, thus developing a learned fear of people in general (157). Therefore, the fear of humans is not only an acute stressor which occurs at the time of handling by an abusive person. In fact, it is a lasting issue and a potential chronic stressor. This effect can be exacerbated as human handling is still inevitable at various stages of a production animal's life (50). For example, in the case of group-housed sows, handling by stock people is necessary at vaccination or when moving sows from one location to another during different stages of gestation (158). Although such handling instances are interspersed throughout gestation, for the sows with a lasting aversion and fear of humans, even intermittent handling can be extremely difficult, as well as dangerous for the stock people involved (50, 155). Fearful sows are therefore at a continuous risk of being handled adversely due to their responses to humans (158). This in turn can be associated with intense acute stress (158) occurring intermittently and contributing to chronic stress (18). Poor stockmanship is therefore a potential risk factor for chronic stress, with known detrimental consequences for sows (153).

Environmental Conditions

Dust, gases such as ammonia, and inappropriate ambient temperature levels are just some of the environmental challenges in pig farm environments (159). Although electronic management of the farm environment strives to maintain constant conditions, fluctuations in the levels of the above listed environmental variables are still inevitable at various times throughout animals' lives (160). This is particularly evident in the case of environmental temperatures. Pigs are especially sensitive to heat stress, as they lack functional sweat glands and have a thick layer of adipose tissue which acts as insulation (51). Combining this vulnerability with the prolonged periods of increased environmental temperatures which sows experience in many pork producing regions (161), heat stress has the potential to act as a true chronic stress risk factor (51). In addition, with global warming on the rise, heat stress may become a problem of an increasingly chronic nature in places where until now it acted as an intermittent stressor. Moreover, heat stress in pregnant sows is linked with markedly reduced productivity and impaired reproductive performance [irregular expression of oestrous, reduced farrowing rates, increased abortion rates, and reduced litter size (88, 162)], greater inflammatory response at farrowing, and insulin resistance during lactation, all of which are indicative of a heightened chronic stress response (88).

Individual Sow Factors

As outlined in Mechanisms underlying chronic stress, individual sows differ in personalities and coping styles and in how they adapt in response to stressors (63, 64). As different coping styles are associated with differential physiological responses to stress [e.g., higher expression of glucocorticoid receptors in proactive pigs, vs. higher oxytocin receptor expression in reactive pigs (163)], it is possible that each personality or coping style may act as a risk factor for the experience of chronic stress to a different extent (163). Indeed, personalities/coping styles exhibit temporal stability (164), and therefore those styles which are associated with a heightened stress response are an especially likely candidate to act as a chronic stress risk factor.

Little variation in body weight between sows within a group is another proposed risk factor for chronic stress, mediated by the potential to sustain high levels of aggression (52). Although the extent to which this is the case depends on the degree of body weight variation between sows, as well as other factors such as group size. Housing sows of unequal body weights together leads to reduced aggression levels at mixing (53), whereas sows of similar body weights could take longer to settle dominance conflicts, due to their evenly matched strength and fighting ability (165). Animals are able to assess the fighting ability (resource holding potential) of conspecifics, and based on the information gathered, decide whether to attack or withdraw (166). In general, smaller animals tend to avoid conflicts with larger individuals (52). It is therefore possible that a strategy of mixing sows of a range of sizes could also reduce long-term, sustained aggression levels. However, there is no research investigating the implications of sows housed with pen mates of equal or unequal body weights for cortisol concentrations.

The variation in parity of sows within a group must also be considered. Specifically, housing younger sows (parity 1 and 2) with older, multiparous sows (parity > 2) exacerbates aggression experienced by the former, generally subordinate and more vulnerable animals (167). Indeed, first parity sows housed with multiparous sows had lower farrowing rates compared to gilts housed with first parity sows only (167). This reflects the detrimental effect of housing sows of different parities on reproductive performance, mediated by the resulting increased aggression levels, and associated chronic stress.

Finally, the lack of familiarity between sows at mixing into groups may increase levels of aggression and therefore chronic stress (168). Previous studies demonstrate that improving familiarity among sows *via* pre-mixing (and thus sub-group establishment) prior to mixing reduces levels of aggression at mixing (168–170). For instance, no major disruption to social organisation and lower levels of aggression were observed when familiar sows were mixed together (168). This in turn could reduce levels of chronic stress (168).

PRENATAL EFFECTS OF CHRONIC STRESS IN SOWS ON THEIR OFFSPRING

Various studies show the potential effects of prenatal stress on swine offspring, whether physiological or psychological (behaviour/personality). Specific physiological effects reported to date include altered development of the HPA axis (76), and associated decreased (86), or increased levels of basal circulating cortisol (171). Increased offspring hippocampal glucocorticoid receptors, decreased serum immunoglobulin G concentrations, and decreased lymphocyte proliferation (86) are also reported. Such effects can result in reduced immunity, higher susceptibility to disease, and greater mortality among prenatally stressed piglets (17). A potential effect of prenatal stress on offspring resilience was reported as a result of repeated nose sling restraint applied to sows during gestation, which more than doubled the mortality of neonatal piglets during the sucking period (17, 172). Similarly, Kanitz et al. (86) showed a higher frequency of disease and higher mortality during lactation in prenatally stressed piglets (born to sows restrained daily for 5 min, for a period of 5 weeks in late gestation), than in non-stressed piglets.

Prenatal stress can also have profound psychological effects on offspring, manifested as altered behaviour immediately after birth and throughout adult life (173), sometimes with sex-specific differences (174). Jarvis et al. (174) showed an effect of prenatal stress (a consequence of mixing stress imposed on pregnant sows) on female offspring, which displayed abnormal maternal behaviour later in life. This included restlessness and more responsiveness towards piglets that approached the sow's head, as well as a tendency to bite more at the piglets (174). Others showed that mothers that experienced pre-natal stress *in utero* spent more time lying ventrally following the birth of their first piglet, more time standing, and made more postural changes (175). These mothers also spent longer visually attending to their piglets compared to non-stressed mothers (175). This is suggestive of a pro-anxiety phenotype resulting from altered brain development during foetal life, as a consequence of mixing stress being imposed on the pregnant sow (175).

Other studies found effects of prenatal stress on behaviour regardless of sex. This included a heightened behavioural response to acute pain and injury such as tail docking (176), or decreased exploratory behaviour in a novel environment shown by prenatally stressed piglets born to sows repeatedly mixed throughout gestation to impose stress (177). Brajon et al. (177) also found decreased locomotion play and fighting play in prenatally stressed piglets, indicative of compromised welfare (178). In addition, the coping behaviour of prenatally stressed offspring is similar to the coping behaviour of humans with depression, suggesting that prenatally stressed offspring may also be at risk of developing depression-like symptoms (79).

Some studies investigating the effects of prenatal stress used models that artificially induce a stress response in sows, for example, through adrenocorticotrophic injections or cortisol administration (179, 180). In addition, many studies investigate only individual acute stressors (86, 175, 180), with a lack of focus on chronic stressors. While useful in providing knowledge on prenatal stress mechanisms, such studies lack on-farm applicability, and therefore their results cannot fully represent real-life scenarios.

Investigating the potential for chronic stress to result in prenatal stress is more applicable to real life situations that sows might experience. The risk factors for chronic stress discussed above are commonly found on-farm, often in combination, and are thus likely to be a realistic risk for prenatal stress in offspring. For instance, piglets of sows housed in barren environments had higher pre-weaning mortality (45, 181), and reduced neonatal survival (182), compared to piglets of sows housed with enrichment (deep straw bedding; manipulable wood materials and straw pellets). In the study of Quesnel et al. (183), piglets born to sows housed in non-enriched environments showed reduced maturity in terms of various physiological indicators at birth, compared to piglets born to sows from enriched housing, which is likely less stressful for the mother. Likewise, Tatemoto et al. (184) showed a beneficial effect of providing enrichment to sows during gestation on offspring behaviour outcomes. In that study, offspring born to sows from enriched environments showed less aggression and less nosing behaviour (184). In addition, female offspring specifically showed more exploratory behaviour and less fear during a novel object test (184).

As discussed above, lameness in sows is another potential chronic maternal stressor. Offspring born to lame sows have altered weight gain, aggressiveness, and also vocalisation levels during open field and novel object tests compared to piglets from non-lame sows (185, 186). Likewise, the offspring of restrictively fed sows on a low fibre diet showed more aggressive behaviour prior to weaning compared to the offspring of sows fed a high fibre diet (187). Heat stress also generates sufficient chronic stress in pregnant sows to lead to developmental damage to their offspring *in utero* (88); this includes altered offspring thermoregulatory ability (188), carcass composition (189), as well as sex-specific effects, such as reduced numbers of functional ovarian oocytes in female offspring (189), and reduced sperm number and quality in male offspring (190). Such findings confirm that exposing sows to a range of potential chronic stressors experienced during gestation does indeed cause a level of prenatal stress that has long-term, negative effects on offspring (183).

FUTURE RESEARCH

The aim of this review was to discuss the potential for several aspects of the sow physical and social environment to act as risk factors for chronic stress. Moreover, the review considered these risk factors in terms of their potential to cause prenatal stress in offspring. With increasing focus on improving animal welfare in recent years, the study of chronic stress and its consequences for the sow and her offspring is an area warranting urgent investigation. Chronic stress in gestation not only has immediate negative consequences for the sow [e.g., immunosuppression and associated morbidity (28, 29); and reduced reproductive performance (23, 24)], but also has long-term consequences for their offspring in terms of susceptibility to disease (191). Such negative effects not only reduce sow and piglet welfare (23, 191), but also threaten the sustainability of the pig industry. This is the case due to diminished sow reproductive performance (23), reduced piglet growth efficiency, and an increased need for the use of antimicrobials to treat disease in piglets, which has consequences for antibiotic resistance (192). Nevertheless, the extent to which a number of the factors discussed in the current review contribute to chronic stress in sows is still poorly understood, with even less research into their potential to contribute to prenatal stress and their postulated consequences for the offspring.

Due to numerous confounders (i.e., group size, quality and quantity of available space, or whether extra space is required by larger sows) which must be considered when investigating the effects of different space allowances on stress levels (93), an optimal space allowance, as well as the contribution of inadequate space allowances to chronic stress are not yet established for sows (94). Future study designs should take such confounders into consideration to ensure that results are meaningful in a broad context.

The absence of high fibre diet provision can exacerbate the chronic hunger effect and stress associated with restrictive feeding (40, 193, 194). Moreover, effective “off the floor” methods of roughage material delivery should be investigated. However, caution must be exercised when adopting “off the floor” methods such as straw racks, as such structures increased aggression associated with competition for access to the racks (195).

Enrichment materials are of interest and value to sows, and if not enough of them are provided, or they are difficult to access, they are usually monopolised by dominant animals. Further research should identify a method of enrichment delivery that ensures all animals have access, and which does not induce competition and stimulate aggression (145, 195). This will help to ensure that the positive effects of enrichment provision on sow stress levels are not counteracted by the negative effects of the aggression associated with competition for it.

There is potential in housing sows of unequal body weights together to reduce sustained aggression levels and the associated consequences related to chronic stress. Specifically, physiological measures such as cortisol concentration and immune status of individuals housed in groups with varying degrees of body weight variation should be measured to ascertain this possibility.

Given the benefits of rubber flooring to sow welfare overall (123, 146–149), indications of a reluctance to interact on such flooring, possibly attributable to slipperiness and fear of slipping/falling should be elucidated.

Studies investigating the link between stress resulting from the sow’s fear of humans and the prenatal stress risks for her offspring are limited (64). Research in this area is needed to further highlight the importance of good quality stockmanship, and more effort must be committed to training of stock people to ensure their knowledge of this area (50).

CONCLUSION

Chronic stress during gestation is not only detrimental to sow welfare and productivity, but also to their offspring, mediated by prenatal stress. The current review flagged a number of factors with potential to contribute to chronic stress in sows. There is an existing body of knowledge on methods to improve sow welfare during gestation, which could be used as a starting point to encourage pig industry stakeholders to adopt strategies to minimise levels of chronic stress experienced by sows. This could lead not only to positive associated effects for sow welfare and productivity, but also for the resilience and health of the offspring, and to increased societal acceptability of pig production. Nevertheless, several of the potential risk factors which can contribute to chronic stress still require additional research to determine the extent of their contribution, and also their potential to induce prenatal stress in offspring. Further investigation into these factors would also help to decide which sources of stress should be prioritised. Likewise, the impact of multiple concurrent chronic stressors also requires further investigation, as it is unlikely that any of the above reviewed chronic stress risk factors exist in isolation, with sows potentially experiencing multiple stressors at once. Although challenging, system based studies could be a potentially useful way of addressing this gap. Furthermore, such knowledge would help to determine whether to target sources of stress individually or in combination for an improved effect. Overall, novel research in the areas outlined in this review will be beneficial to sow and piglet welfare and productivity, with economically positive consequences for the pig industry.

AUTHOR CONTRIBUTIONS

ML: planned and organised the article, as well as drafted the manuscript. LB, JM, and KO'D: revised the manuscript. All authors contributed to the article and approved the submitted version.

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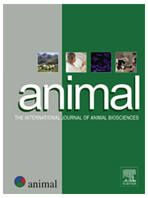

Mixing aggression intensity is associated with age at first service and floor type during gestation, with implications for sow reproductive performance

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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 and parity two, 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 parity one was used to predict reproductive performance in parity two. Lower AFS was associated with a lower SLMIX score in parity one ($P = 0.031$). There was no association between SLMIX score and reproductive performance in parity one, while sows with higher SLMIX score in parity two had a higher proportion of piglets dead during lactation ($P = 0.027$) and a longer cycle length ($P = 0.003$) in parity two. Sows with higher SLMIX scores in parity one had more non-productive days ($P < 0.001$) in parity two. Concrete sows had a higher SLMIX score than RUB sows in parity one ($P = 0.015$), but not in parity two. In addition, CON sows had a higher proportion of piglets born dead ($P = 0.013$) compared with RUB sows in parity two. 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 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 (**CON**; $n = 80$ 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 RUB 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 seven 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:

$$\mathrm{YGa\tilde{m}ma}(\mu, v)$$

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

where log(μ) = SLMIX for each sow; β_0 = constant; βX = floor type or BCS (as categorical fixed effects); Zγ = 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); Zγ = 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:

$$\mathrm{YBino\tilde{m}ial}\ (\beta_0, \rho)$$

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

where logit(ρ) = BCS for each sow; β_0 = constant; βX = floor type (as a categorical fixed effect); Zγ = pen random effect; and ε = error term.

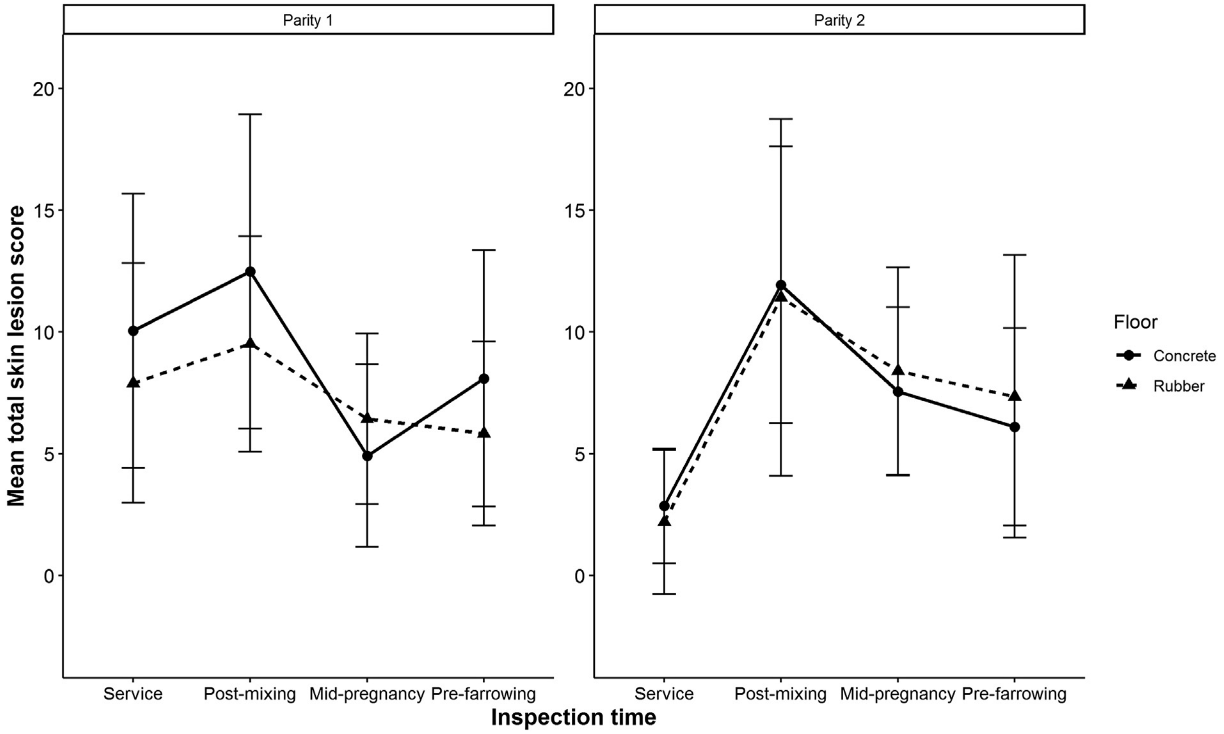

Fig. 1. Mean ± SD for the total skin lesion score per inspection period in parity one and parity two of 160 group-housed sows on concrete ($n = 80$) or rubber ($n = 80$) floor, where skin lesion scores were recorded based on the severity from 0 = no lesions to 5 = severe lesions, on five body regions (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).

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.

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.

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:

$$\mathrm{YGa\tilde{m}ma}(\mu, v)$$

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

where $\log(\mu) =$ SLMIX for each sow within parity; $\beta_0 =$ constant; $\beta X =$ floor type, BCS (as categorical fixed effects) within parity and AFS (as a continuous predictor); $Z\gamma =$ pen random effect; and $\varepsilon =$ 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:

$$\mathrm{YPoi\tilde{s}son}(\beta_0 \times \rho)$$

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

where $\log(\mu) =$ count of reproductive performance traits within each parity (i.e. number of piglets born alive, litter size); $\beta_0 =$ constant; $\beta X =$

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,

$$\text{YGa}\tilde{\text{m}}\text{ma}(\mu, v)$$

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

where $\log(\mu)$ = cycle length (days); 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.

$$\text{YBin}\tilde{\text{o}}\text{mial}\ (\beta_0, \rho)$$

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

where $\text{logit}(\rho)$ = 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)]; $Z\gamma$ = 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:

$$\text{YPois}\tilde{\text{s}}\text{on}(\beta_0 \times \rho)$$

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

where $\log(\mu)$ = 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); $Z\gamma$ = pen random effect; and ε = error term,

$$\text{YGa}\tilde{\text{m}}\text{ma}(\mu, v)$$

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

where $\log(\mu)$ = 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); $Z\gamma$ = pen random effect; and ε = error term.

$$\text{YBin}\tilde{\text{o}}\text{mial}\ (\beta_0, \rho)$$

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

where $\text{logit}(\rho)$ = 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); $Z\gamma$ = 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 in contrast to the findings of Verdon et al. (2016), but these authors

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.

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 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.

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.

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 body weight, 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 body weight 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 body weight 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 body weight, 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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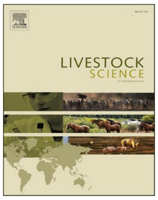

Associations between skin lesion counts, hair cortisol concentrations and reproductive performance in group housed sows

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HIGHLIGHTS

- Skin lesions 3 weeks post-mixing were associated with sow reproductive performance.
- Skin lesions 3 weeks post-mixing could be associated with chronic stress.
- Hair cortisol was not associated with skin lesions or sow reproductive performance.

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ABSTRACT

The effects of acute stress on sow reproductive performance are well established, but we know less about the implications of chronic stress for sow performance. This study investigated associations between total skin lesion counts 24hr and 3 weeks post-mixing, hair cortisol concentrations at the end of pregnancy, and reproductive performance of sows. Sows ($n = 264$; parity 1-5) were artificially inseminated and locked into individual feeding stalls within 11 fully slatted gestation pens, immediately after service. Sows were released from the stalls at approximately 25 days post-service, allowed to mix, and thereafter had free access to the stalls. Skin lesions were counted 24hr post-mixing (i.e. one day after release from the stalls), and 3 weeks post-mixing on the anterior (head, neck, shoulders and front legs), middle (flanks and back), and posterior (rump, hind legs and tail). The sum of counts across all sites yielded a total skin lesion count for each sow. Back fat depth measurement and hair collection were carried out one week prior to farrowing. Sow reproductive performance measures included the number of piglets born alive, born dead, mummified, and total born. Piglets from 75 sows were tagged, weighed and scored for vitality (0 = least vital, to 4 = perfect) and intra-uterine growth retardation (IUGR; 0 = none, to 3 = severe). There was a positive association between total skin lesion counts 3 weeks post-mixing and both the number of mummified piglets ($P = 0.045$), and IUGR scores ($P = 0.018$). There was no correlation between total skin lesion count either 24hr (Rho = 0.10; $P > 0.05$) or 3 weeks post-mixing (Rho = -0.02; $P > 0.05$) and hair cortisol concentrations. There was also no association between hair cortisol concentrations and measures of sow reproductive performance ($P > 0.05$). Higher skin lesion counts 3 weeks post-mixing were associated with aspects of sow reproductive performance. This suggests that chronic stress caused by sustained aggression had a negative impact on the reproductive system. Nevertheless, given the lack of significant associations between hair cortisol, skin lesion counts, and measures of reproductive performance, this was not supported by the findings for hair cortisol.

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1. Introduction

Aggression and the associated fear and injury are among the main sources of stress, and a major welfare concern for group housed sows (Munsterhjelm et al., 2008; Maes et al., 2016; Martinez-Miro et al., 2016). Aggression impacts negatively on reproductive performance (Turner et al., 2005; Einarsson et al., 2008; Munsterhjelm et al., 2008), causing impaired pre-ovulatory oestrogen surges (Turner et al., 2002) and lower litter performance (Tonepohl et al., 2013). Mediated prenatally, stress associated with sow aggression is also detrimental to the offspring (Kranendonk et al., 2008); prenatal stress can reduce the number of piglets born alive per litter and litter size (Einarsson et al., 2008; Greenwood et al., 2019). It also has negative effects on offspring behaviour, stress-coping abilities and immune function, with effects persisting throughout adult life (Jarvis et al., 2006; Rutherford et al., 2009; Brajon et al., 2017).

Group housed sows can experience high levels of aggression over prolonged periods (Hemsworth et al., 2013). Not only does aggression occur at mixing when sows meet unfamiliar individuals and fighting ensues to establish a dominance hierarchy, it may continue once the hierarchy is established. This can be due to bullying and competition for resources such as feed and space (Spoolder et al., 2009), although this type of aggression is not as intense as when animals meet for the first time. Nevertheless, if it persists throughout gestation, it can contribute to chronic stress, with potentially negative effects on sow and offspring performance and welfare (Spoolder et al., 2009).

Since the move to group housing in the EU, sow related research focused on risk factors for (Stevens et al., 2015; Verdon and Rault, 2018) and methods of mitigating/minimising aggression (Peden et al., 2018). Specifically, the focus is on acute stress resulting from mixing aggression and its consequences for sow welfare and performance. However, research investigating the consequences of chronic stress resulting from sustained aggression for the sow and her offspring is scarce (Emack et al., 2008; Kranendonk et al., 2008; Salak-Johnson, 2017). This is perhaps due to the difficulties associated with quantifying chronic stress levels using conventional physiological measures (Spoolder et al., 2009). The research surrounding hypothalamo-pituitary-adrenocortical (HPA) axis activity in response to stress is inconclusive (Otovic and Hutchinson, 2015). The way in which the HPA axis responds to stress depends on the type of stress exposure, severity of stressor, and its duration (Grissom and Bhatnagar, 2009). Some authors even argue that HPA axis activation does not always reflect stressful conditions (Otovic and Hutchinson, 2015). This is based on the finding that HPA axis activity can be either upregulated or downregulated under chronic stress conditions (Miller et al., 2007). For example, Mayer and Novak (2012) showed blunted HPA axis activity (hypoactivity) in chronically stressed animals. Thus, in this case, baseline concentrations of stress hormones such as cortisol may be lower than in acutely stressed, or non-stressed animals (Mayer and Novak, 2012). Consequently, the interpretation of stress levels based on HPA axis activity patterns can be misleading (Miller et al., 2007; Otovic and Hutchinson, 2015). In addition, capturing long-term patterns of cortisol synthesis is challenging (Davenport et al., 2006).

In recent years, hair cortisol concentrations were used as an indicator of chronic stress. Cortisol accumulates in the hair during growth, and as such could theoretically provide insight into stress hormone levels over weeks or months (Davenport et al., 2006; Carroll et al., 2018). Nonetheless, the process of cortisol accumulation within the hair shaft is not completely understood (Meyer and Novak, 2012), and the best method of hair collection (plucking versus shaving to avoid hair follicle inclusion) is still debated (Meyer and Novak, 2012; Burnard et al., 2017). Therefore, inferring chronic stress from measures of hair cortisol concentration is accompanied with caveats, and ideally should be supported by other measures. Skin lesions are a proxy for the amount of aggression a sow experiences, and thus potentially for the associated stress (de Koning, 1984; Turner et al., 2006a).

It must be noted that the aggressive strategy adopted by sows at mixing does not always predict the strategy a sow will adopt later on (i.e. once dominance relationships are established; Turner et al., 2017). Thus, skin lesions resulting from mixing aggression may not always predict the degree to which sows (in static groups) will be involved in aggression later on. In contrast, skin lesions recorded after the dominance hierarchy is established could be used as a reference for sustained aggression (Turner et al., 2017). This in turn suggests that they could be associated with levels of chronic stress. This study employed skin lesions recorded 24hr and 3 weeks post-mixing as two separate points of reference for the aggression experienced by gestating sows.

Given the postulated negative effects of chronic stress on both sow and offspring welfare and performance (Salak-Johnson, 2017), research into methods of identifying and measuring chronic stress effectively is warranted. The objective of the present study was to investigate associations between total skin lesion counts 24hr and 3 weeks post-mixing, hair cortisol concentrations and sow reproductive performance. In doing so, we hoped to elucidate the effects of chronic stress on reproductive performance, and to determine the congruence between skin lesion counts 3 weeks post-mixing and hair cortisol as potential biomarkers of chronic stress in gestating sows.

2. Materials and methods

2.1. Ethical approval

The farm on which this experiment was conducted complied with Statutory Instrument number 311 of 2010 European Communities (Welfare of Farmed Animals) Regulations 2000. The Teagasc Animal Ethics Committee approved the experiment (TAEC218-2019), however, it did not require licensing under the European Communities (Amendment of Cruelty to Animals Act, 1876) Regulations (2002), as no invasive measures were used.

2.2. Animals and housing

The study was conducted on a commercial 2000-sow farrow-to-finish farm in Co. Cork, Ireland between March and July 2018 (see Table 1 for experimental schedule dates). The study used 264 sows (parity 1-5). Oestrous synchronisation was not practiced on the farm. Sows were artificially inseminated and immediately thereafter locked into individual full-length feeding stalls (2.3 m length × 0.65 m width) within 11 fully slatted gestation pens (7.8 m length × 7 m width; roaming area behind feeding stalls 7.8 m length × 2.4 m width) each with two rows of 12 stalls. A vasectomised boar was walked behind the sows while still restrained in the feeding stalls three weeks post-service, to check for returns to oestrous. Sows were released from the stalls in groups of 24 per pen and allowed to mix, once they were approximately 25 days post-service (24.8 ± 3.14 days post-service). This occurred over a four week period in March (week 1: 72 sows, 3 pens; week 2: 96 sows, 4 pens; week

Table 1
Experimental schedule dates.

	Release from stalls (mixing)	24hr post-mixing skin lesion count	3 week post-mixing skin lesion count	Hair collection and back fat measurement	Due to farrow date (piglet data collection)
Week 1	06/03/2018	07/03/2018	28/03/2018	24/05/2018	01/06/2018
Week 2	13/03/2018	14/03/2018	04/04/2018	30/05/2018	08/06/2018
Week 3	20/03/2018	21/03/2018	11/04/2018	07/06/2018	16/06/2018
Week 4	27/03/2018	28/03/2018	18/04/2018	12/06/2018	24/06/2018

3: 48 sows, 2 pens; week 4: 48 sows, 2 pens). Sows were fed a liquid diet twice per day and had *ad libitum* access to water via two nipple drinkers at one end of the pen. Sows were transferred into conventional farrowing crates with fully slatted floors one week before farrowing, and were weaned at approximately 28 days post-farrowing.

2.3. Skin lesion counts

Sows were inspected for skin lesions 24hr post-mixing (i.e. one day after release from the stalls), and three weeks post-mixing, using a method validated by Turner et al. (2006a). In brief, skin lesions were counted on the anterior (head, neck, shoulders and front legs), middle (flanks and back), and posterior (rump, hind legs and tail). Counts included fresh skin lesions only, identified by colour and the estimated age of scabbing. No weighting was given to account for the length or diameter of skin lesions. The summation of counts across all examination sites yielded a total skin lesion count for each sow per inspection. Lesion counts were conducted by two trained observers who practiced until at least 90% intra- and inter-observer scores for repeatability were achieved.

2.4. Back fat depth and hair collection

One week prior to farrowing sows were locked into the feeding stalls to enable back fat measurement and hair collection from each individual. The site of hair collection is important and can have a bearing on the resulting cortisol concentrations (Heimbürge et al., 2019). The back fat measurement site (dorso-lumbar region) was selected as the most appropriate region for hair collection to ensure adequate measurement of cortisol levels. The dorso-lumbar region is outside of reach of the tail, and thus at a lower risk of chewing by other pigs. It is also away from the neck which is most at risk of aggressive attacks involving bites. This means that the hair of the dorso-lumbar region is at lower risk of contamination by saliva and blood, and is therefore at lower risk of contamination with exogenous cortisol which can diffuse into the hair shaft from both fluids (Otten et al., 2020). Moreover, the hair in this region is also at a lower risk of exogenous cortisol contamination coming from urine and faeces, as it does not usually come into contact with the floor surface when the animal is lying down (Otten et al., 2020). The dorso-lumbar region was also chosen for convenience, as hair had to be shaved to allow for the back fat measurement, as well as due to the abundance of hair in this region (Casal et al., 2017). Hair was therefore shaved from the back fat measurement site (dorso-lumbar region; identified by measuring 6.5 cm left and right from the mid-point at the spine marked by the position of the last rib), placed into plastic zip-lock bags and frozen at -20°C until hair cortisol analysis. Back fat depth (mm) measurements were taken at the two identified sites using a Renco LEAN-MEATER® device, and an average back fat depth figure was then calculated.

2.5. Sow reproductive performance traits

Sow reproductive performance was recorded by the farm staff and included the following measures: number of piglets born alive, born dead, mummified, and total born. Piglets from 75 sows were available for more detailed measures, namely, to study the relationship between skin lesions, hair cortisol and measures of piglet development. The sows were selected on the basis of being recently farrowed, or in the process of farrowing, and for which farm sow cards were not yet updated with performance details when the research team arrived on the farm each day. The coefficient of variation (CV) for the representation of the 11 pens by the 75 sows was 31.3%. Piglets were tagged, weighed and scored for vitality and intra-uterine growth retardation (IUGR). Vitality was scored according to criteria shown in Table 2, modified from Schmitt et al. (2019) and Rooney et al. (2020). The summation of scores for each criterion yielded a total vitality score, with the maximum (best) possible score of 4 per piglet. The level of IUGR was estimated by scoring the presence/absence of nose wrinkles, cone-shaped head, and bulging eyes, based on a method of Hales et al. (2013). For all three measures a piglet scored 0 if the trait was absent, and 1 if it was present; therefore the maximum total IUGR score a piglet could receive was 3.

Table 2
Vitality scoring system used for piglets at birth (Schmitt et al., 2019; Rooney et al., 2020).

Vitality indicators	Vitality score	
	0	1
Reaction to handling		
Vocalisation	Piglet does not scream during handling	Piglet screams
Escape	Piglet does not attempt to escape during handling	Piglet attempts to escape
Muscle tone	Leg muscles are soft when pressed against handler's palm	Leg muscles are firm and piglet pushes back against handler's palm
Initial position on return to the farrowing crate	Piglet is on its back or lies on its side without trying to right itself	Piglet is immediately up on its four legs and moves away

2.6. Hair cortisol extraction and analysis

Hair sample preparation and cortisol extraction were based on the procedure described by Davenport et al. (2006), with certain modifications. In brief, hair samples were defrosted for one hour prior to preparation procedures, then washed by placing 300 mg of hair into a 10 ml polypropylene tube along with 5 ml of isopropanol, and mixing gently on a shaker for 3 min. This was repeated twice, using fresh isopropanol for the second wash. Washed hair samples were left inside the wash tubes and placed inside a protected fume hood to dry overnight. Samples prepared in this way were then individually ground into a fine powder using a Retsch mixing mill (MM200; 10 ml stainless steel grinding jars, single 12 mm stainless steel grinding ball) for 4 min at 25 Hz. Approximately 50 mg of ground hair sample was weighed out and placed in a 2 ml tube along with 1 ml of methanol, which was followed by incubation of the sample for 24hr at room temperature with constant gentle agitation (shaker setting 3; approximately 95 rpm) for cortisol extraction. Following the 24hr incubation period, 0.6 ml of the cortisol extract in methanol was removed (taking care not to disturb the settled hair powder at the bottom of the tube) using an Eppendorf pipette and transferred to a clean 1.5 ml tube for methanol evaporation, which was performed using a stream of nitrogen gas at 38°C. Cortisol extract samples were frozen at –20°C pending EIA analysis. Extracted cortisol samples were analysed using Salimetrics® Expanded Range, High Sensitivity Salivary Cortisol EIA kit, which was validated for the analysis of hair cortisol concentrations (Davenport et al., 2006; Moya et al., 2013; Casal et al., 2017), and is valid for use in a range of species, including swine (Davenport et al., 2006; Fürtbauer et al., 2019; Otten et al., 2020). Frozen cortisol extract samples along with the EIA kit were brought to room temperature 1.5hr prior to being reconstituted with 0.4 ml of phosphate buffer (assay diluent) provided with the EIA kit. Reconstituted extracts ($n = 125$) were analysed for cortisol concentration levels in duplicate using four assays, following the protocol provided with the EIA kit. Inter- and intra-assay CV were 8.8 and 7.8%, respectively.

2.7. Statistical analysis

All statistical analyses were performed in SAS v9.4 (SAS Inst. Inc., Cary, NC) with sow as the experimental unit. For all analyses statistical differences were reported when $P \leq 0.05$, while statistical trends were reported when $P > 0.05$ and $P \leq 0.10$. Pearson's correlation test was initially used to check for correlations between total skin lesion counts at both 24hr and at 3 weeks post-mixing, and hair cortisol concentrations. Correlations were not detected ($P > 0.05$), and therefore total skin lesion counts recorded at both 24hr and 3 weeks post-mixing, as well as hair cortisol concentration were used as predictor variables in a single model for all analyses. Due to a low number of sows in parity 5, sows in parity 5 were grouped into a single group with sows in parity 4 (i.e. parity ≥ 4). Results for independent continuous variables are reported as their regression coefficient (REG) ± standard error (SE). Results for categorical fixed effects are reported as least square means ± SE with their associated 95% Confidence Intervals (CI).

Sow reproductive performance and back fat depth. In the analysis of sow reproductive performance, independent variables included total skin lesion counts 24hr and 3 weeks post-mixing, and hair cortisol concentration, while parity was included in all models as a fixed effect. Parity was included as a fixed effect, as groups of sows were formed as per routine farm practice, and were therefore not homogeneous in terms of parity. We accounted for clustering of sows within a group by using pen as the random effect. In the case of birth weight, piglet was nested within sow using the repeated statement of PROC MIXED to account for repeated piglet measurements for individual sows, and the total number of piglets born was included as a continuous covariate in this model. In the case of vitality and IUGR, sow was used as an additional random effect to account for repeated piglet measurements for individual sows in PROC GLIMMIX. Residuals were checked for normality using the Shapiro test, and by examining the quantile-quantile plot. For normally distributed residuals (born alive, total born, back fat, birth weight) linear mixed model equations were built in PROC MIXED, while non-normally distributed residuals (the number of piglets born dead, mummified, vitality, and IUGR) were analysed using generalised linear mixed model equations built in PROC GLIMMIX, and fitted with the Poisson distribution in the case of piglets born dead and mummified, and the multinomial distribution in the case of vitality and IUGR. All models were tested with and without an interaction between 24hr and 3 week total skin lesion counts, with the interaction being removed upon a lack of a significant result in the case of all dependent variables, except for back fat.

3. Results

The final number of sows included in the study was 251. Table 3 shows numbers of sows available for each of the reproductive performance measures. The mean ± standard deviation (SD) for total skin lesion count 24hr post-mixing was 31.0 ± 26.77 ($n = 250$; range 0 to 157), and 19.2 ± 16.62 ($n = 248$; range 0 to 105) for the total skin lesion count 3 weeks post-mixing. The mean ± SD for cortisol was 0.200 ± 0.0835 μg/dL [microgram/decilitre, ($n = 125$; range 0.058 to 0.452 μg/dL)], and 15.2 ± 3.08 mm ($n = 218$; range 6 to 25 mm) for back fat depth.

There were no associations ($P > 0.05$) between total skin lesion counts 24hr and 3 weeks post-mixing or hair cortisol concentrations with most reproductive performance traits (Table 3). There was a positive association between the total skin lesion counts 3 weeks post-mixing and the number of piglets born mummified ($P = 0.045$; Table 3), and a positive association between the total skin lesion counts 3 weeks post-mixing and IUGR scores ($P = 0.018$; Table 3). Thus, sows with higher skin lesion counts 3 weeks post mixing had a higher likelihood of having mummified piglets, or piglets born with a higher IUGR score. The total number of piglets born was negatively associated with birth weight (REG = -0.04 ± 0.008; $F_{1,536} = 25.54$; $P < 0.001$); parity (included as a fixed effect) also had an effect on birth weight (Parity 1: 1.4 ± 0.06, 95% CI = 1.25 to 1.47; Parity 2: 1.5 ± 0.06, 95% CI = 1.39 to 1.64; Parity 3: 1.6 ± 0.07, 95% CI = 1.44 to 1.71; Parity 4: 1.2 ± 0.08, 95% CI = 1.10 to 1.40; $F_{1,536} = 3.83$; $P = 0.010$). The interaction between total skin lesion count 24hr post-mixing and total skin lesion count 3 weeks post-mixing was negatively associated with back fat depth (REG = -0.002 ± 0.0010; $F_{1,117} = 4.06$; $P = 0.046$). There was no correlation between total skin lesion count either 24hr (Rho = 0.10; $P > 0.05$) or 3 weeks post-mixing (Rho = -0.02; $P > 0.05$) and hair cortisol concentrations.

4. Discussion

Aggression is one of the main welfare challenges for pregnant sows housed in groups even after the establishment of the dominance hierarchy (Hemsworth et al., 2013), and they are consequently at risk of chronic stress (Salak-Johnson, 2017). Results of the present study provide some evidence that chronic aggression has a negative effect on sow reproductive performance. Specifically, we found an increase in the number of piglets born mummified with higher skin lesion counts 3 weeks post-mixing. There are no previous reports of this in the literature.

Skin lesion counts recorded in the current study were substantially higher than injuries recorded by other authors in the context of sustained aggression (e.g. grower stage pigs, Turner et al., 2009; sows, Tonepohl et al., 2013). Hence, it is possible that our study sows

Table 3
Associations (regression coefficient ± standard error; SE) between total skin lesion count 24hr and 3 weeks post-mixing[1], hair cortisol concentrationand sow reproductive performance traits, in 251 sows group housed in 11 fully-slatted pens in stable groups of 24.

Reproductive performance trait (n)[2]	Total skin lesion count 24hr post-mixing[1]				Total skin lesion count 3 weeks post-mixing[1]				Hair cortisol			
	Regression coefficient	SE	*F*-statistic	*P*-value	Regression coefficient	SE	*F*-statistic	*P*-value	Regression coefficient	SE	*F*-statistic	*P*-value
Born alive (209)	0.01	0.018	$F_{1,115} =$ 0.23	0.631	-0.01	0.032	$F_{1,111} =$ 0.10	0.748	2.1	4.73	$F_{1,117} =$ 0.20	0.659
Born dead (209)	0.01	0.005	$F_{1,110} =$ 1.60	0.209	-0.01	0.009	$F_{1,110} =$ 2.42	0.122	-1.6	1.44	$F_{1,110} =$ 1.22	0.271
Mummified (209)	0.01	0.010	$F_{1,110} =$ 1.34	0.250	0.03	0.014	$F_{1,110} =$ 4.10	0.045	3.1	2.53	$F_{1,110} =$ 1.46	0.230
Total born (209)	0.02	0.020	$F_{1,116} =$ 0.65	0.421	-0.01	0.035	$F_{1,110} =$ 0.15	0.701	1.6	5.22	$F_{1,117} =$ 0.09	0.760
Birth weight (75)	0.0001	0.00170	$F_{1,536} =$ 0.00	0.950	0.003	0.0029	$F_{1,536} =$ 1.15	0.283	-0.3	0.37	$F_{1,536} =$ 0.68	0.410
Vitality (75)	-0.005	0.0087	$F_{1,534} =$ 0.33	0.565	-0.02	0.015	$F_{1,534} =$ 2.18	0.141	-0.7	1.84	$F_{1,534} =$ 0.16	0.686
IUGR (75)	-0.004	0.0117	$F_{1,535} =$ 0.12	0.728	0.05	0.023	$F_{1,535} =$ 5.62	0.018	-1.1	2.61	$F_{1,535} =$ 0.18	0.672

[1] Skin lesions counted on the anterior (head, neck, shoulders and front legs), middle (flanks and back), and posterior (rump, hind legs and tail). No weighting was given to account for the length or diameter of skin lesions. The summation of counts across all examination sites yielded a total skin lesion count for each sow per inspection.

[2] Number of sows for which reproductive performance data were available are shown in parentheses.

experienced higher levels of stress associated with sustained aggression than might be found on other farms, therefore resulting in a more marked negative effect on reproductive performance. However, it should be noted that lesion counting involves a certain degree of subjectivity dependent upon the lighting level on the farm, and the minimum size of lesion deemed worthy of recording. Hence, direct comparisons between studies should be made with caution.

Calcification and skeleton development does not begin in the pig foetus until day 38 to 45 of gestation, and thus the sow reabsorbs any foetus that dies in utero prior to day 38 (Flowers, 2019; Flowers, 2020). Sows in this study were mixed approximately 25 days post-service, so the number of skin lesions 3 weeks post-mixing was counted at approximately 49 days of pregnancy. Thus, the presence of skin lesions at this time indicates that the sows were experiencing aggression, and therefore stress, at a time when foetuses that die would not be re-absorbed, but would persist as 'mummies' (Flowers, 2019; Flowers, 2020). A similar negative effect of late-gestation stress on foetal losses was recorded in dairy cows (Santolaria et al., 2010).

The increase in IUGR scores with higher skin lesion counts 3 weeks post-mixing is a novel finding, which also suggests a negative effect of sustained aggression experienced by sows during gestation. Energy metabolism and nutrient allocation to developing foetuses by sows could explain this result. Rooney et al. (2020) found higher IUGR scores in piglets born to sows fed low energy diets in late gestation, and suggested that maternal protein or energy intake deficits are associated with reduced allocation of nutrients to foetal development. This could in turn exacerbate the incidence of IUGR in offspring. Indeed, chronic stress associated with rough handling and heat stress can increase plasma protein and glucose levels, and also alter sow energy metabolism and nutrient digestion (Barnett et al., 1983; He et al., 2019). This could consequently lead to lower amounts of nutrients to be available for allocation to foetal development (Barnett et al., 1983; He et al., 2019). It is therefore possible that chronic stress associated with sustained aggression could have a similar effect on sow energy metabolism, with potentially negative consequences for offspring development and incidence of IUGR. The association between skin lesion counts 3 weeks post-mixing and both the number of piglets mummified and IUGR scores, can be used as support for the potentially detrimental effects of chronic stress on sow reproductive performance. This adds to the validity of using skin lesion counts 3 weeks post-mixing as a potential indicator of chronic stress associated with sustained aggression. However, we acknowledge that this statement must still be regarded with a degree of caution, as mummification and IUGR scores were only two measures of reproductive performance out of many that were not associated with sustained aggression in this study.

The negative effect of the interaction between skin lesion counts 24hr and 3 weeks post-mixing on back fat depth is difficult to explain. It is also in contrast to the studies of Turner et al. (2006b), Desire et al. (2015), and Wurtz et al. (2017), who found no associations between skin lesions and back fat. Although not recorded in this study, sow weight and dominance status are associated with sow back fat depth and aggressive behaviour (Pacheco and Salak-Johnson, 2016). Such information could help to clarify this result, and should be included in future studies investigating the effects of sustained aggression on sow performance.

This study found no association between hair cortisol concentrations and skin lesion counts, nor between hair cortisol concentrations and indicators of sow reproductive performance. Hair cortisol concentrations, as recorded here, did not reflect the association between skin lesion counts 3 weeks post-mixing and the numbers of mummified piglets and IUGR scores. While measurements of hair cortisol concentrations are now more widely employed as an indicator of chronic stress in several species (Burnard et al., 2017; Heimbürge et al., 2020), much is still unclear regarding the incorporation of cortisol into the hair shaft (Otovic and Hutchinson, 2015; Casal et al., 2017; Heimbürge et al., 2019). Indeed Heimbürge et al. (2020) compared hair cortisol concentrations following a period of ACTH injections in cattle and pigs, and found differences between treatments for cattle, but not for pigs. Similarly, an increase in wool cortisol following a stress-inducing treatment (extensive brushing and dexamethasone injection) was found in sheep (Salaberger et al., 2016). Given the clear effects of stress on hair cortisol concentrations in cattle and sheep, it is possible that hair cortisol has a reduced reliability as a chronic stress indicator for pigs (Heimbürge et al., 2020). Heimbürge et al. (2020) suggest that this could be due to lower systemic cortisol response following ACTH administration in this species. However, it could also be possible that ACTH is metabolised faster in pigs, and that the ACTH injection and cortisol detection protocols used by those authors did not span an appropriate period to capture a difference in hair cortisol concentration. Besides the lower systemic cortisol response of the HPA axis noted in swine (Heimbürge et al., 2020), it is also possible that the lack of association between hair cortisol and skin lesions found in this study is due to a phenomenon of habituation previously noted in animals and humans (Grissom and Bhatnagar, 2009). In this case, habituation to stress occurs through continuous exposure to a particular stressor, leading to a blunted HPA axis response (Grissom and Bhatnagar, 2009; Mayer and Novak, 2012). Skin lesion counts recorded 3 weeks post-mixing in this study are a proxy for sustained aggression, and it is likely that study sows habituated to this stressor over the course of gestation. The effects of various confounding factors which could affect concentrations of cortisol entering the hair shaft should also be considered when validating the use of hair cortisol as a chronic stress indicator (Salaberger et al., 2016; Otten et al., 2020). For instance, Otten et al. (2020) found that endogenous hair cortisol concentrations may be substantially altered by exogenous cortisol entering the hair shaft by diffusion from media such as urine, faeces and saliva, which commonly contaminate the outside of sow hair in on-farm settings. The body region from which hair is collected is therefore important (Heimbürge et al., 2019). The selection of the dorso-lumbar region as the site of hair collection in this study was based on its lower chance of contamination with urine, faeces, and saliva (Casal et al., 2017; Otten et al., 2020). Despite this, the possibility of some of those contaminants still being present at various points throughout gestation cannot be ruled out. Thus the lack of associations between hair cortisol concentration and other parameters investigated in this study must be treated with further caution. In addition, age, pregnancy, hair colour, sex, and season affect hair cortisol concentrations (Heimbürge et al., 2019). Thus, it is clear that the usefulness of hair cortisol as an indicator of chronic stress is dependent upon various factors which must be controlled in order to ensure accuracy of results. Further research would be beneficial to improve hair collection and cortisol analysis protocols to minimise the effect of the various potential confounders.

5. Conclusion

Skin lesion counts 3 weeks post-mixing were associated with two aspects of sow reproductive performance; the incidence of mummified piglets and IUGR scores. This suggests that skin lesion counts recorded post-hierarchy establishment have the potential to act as an indicator of chronic stress. However, it must be highlighted that the higher incidence of mummified piglets and IUGR scores with increasing skin lesion counts 3 weeks post-mixing found in this study is associative rather than causal. Therefore, more detailed work quantifying the actual levels of aggression experienced by the animals (e.g. through behavioural observations) is needed to validate this result. Nevertheless, given the ease and speed of skin lesion scoring, it is an important finding which could aid fast identification of animals vulnerable to stress. In contrast, hair cortisol concentrations were not associated with any other measure, and thus did not prove a useful indicator of chronic stress in the current study. However, potential sources of bias were not controlled for in this study, and as such, our findings must be treated with caution. Further research into the validity of the combination of hair cortisol concentrations and skin lesion counts as a useful indicator of chronic stress should consider the wide range of factors which can influence hair cortisol concentrations.

Data statement

Data were not deposited in an official repository. Datasets used for the results presented in this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Martyna E. Lagoda: Conceptualization, Methodology, Data curation, Formal analysis, Writing - original draft, Visualization. **Keelin O'Driscoll:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - review & editing, Supervision, Project administration. **Joanna Marchewka:** Writing - review & editing. **Simone Foister:** Data curation, Methodology, Investigation. **Simon P. Turner:** Conceptualization, Methodology, Writing - review & editing. **Laura A. Boyle:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interests

The authors declare that there is no conflict of interest regarding the publication of this article.

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animals

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Article

Early Detection of Locomotion Disorders in Gilts Using a Novel Visual Analogue Scale; Associations with Chronic Stress and Reproduction

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Simple Summary: Lameness in sows causes pain and poor welfare. Early detection is crucial if treatment is likely to be effective. Locomotion scoring is the best way to achieve this, but existing scoring systems are not sensitive enough to detect subtle deviations from optimal locomotion. Our objective was to develop a new visual analogue scale (VAS) to measure the locomotory ability of sows over time. Effectiveness in detecting slight deviations was tested in young female pigs by comparing the scale to an existing categorical scoring system. The VAS detected slight deviations from optimal locomotion over time more effectively than the categorical locomotion scoring system. It was also positively associated with hair cortisol concentrations (chronic stress) and measures of reproductive performance. If used by farmers, the VAS could potentially help in lameness prevention and thereby improve sow welfare and performance.

Abstract: Locomotion scoring is crucial for the early detection of lameness, which reduces sow welfare and performance. Our objective was to test the effectiveness of a visual analogue scale (VAS) to measure locomotory ability (OVERALL) compared to a categorical scoring system (CAT) and to investigate associations with hair cortisol and reproductive performance. Locomotion was scored in gilts ($n = 51$) at service, on day 57 and day 108 of pregnancy, and at weaning, using a VAS (150mm line: 0 mm (perfect)–150 mm (severely lame)), and a CAT (1 (perfect)–5 (severely lame)). Hair cortisol concentration was measured on day 108 of pregnancy. Reproductive performance data (parity 1–4) were acquired from farm records. VAS detected deviations from optimal locomotion more effectively than the CAT ($F_{3,145} = 2.70$; $p \leq 0.05$ versus $F_{3,195} = 2.45$; $p = 0.065$). Higher OVERALL scores at service ($REG = 0.003 \pm 0.0012$; $F_{1,48} = 4.25$; $p \leq 0.05$) and on day 57 ($REG = 0.003 \pm 0.0013$; $F_{1,48} = 6.95$; $p \leq 0.05$) were associated with higher hair cortisol concentrations on day 108. Positive associations were detected between OVERALL at service and the number of piglets born dead ($REG = 0.01 \pm 0.006$; $F_{1,36} = 4.24$; $p \leq 0.05$), and total born ($REG = 0.1 \pm 0.03$; $F_{1,120} = 4.88$; $p \leq 0.05$). The VAS better facilitates early detection of lameness, which could help to prevent detrimental effects, possibly mediated by chronic stress, on reproductive performance.

Keywords: swine; mobility; lameness; welfare; productivity; cortisol

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1. Introduction

Lameness is a painful, multifactorial disorder, considered one of the main welfare issues for sows [1–3]. It also has economic implications for the farmer, as it remains one of the primary reasons for premature culling of sows [1–3]. Chronic lameness [1] contributes to elevated stress levels (swine [4]; dairy cows [5]), and consequently, impaired reproductive performance [6,7]. For instance, lame sows had lower numbers of piglets born alive in the study of Anil et al. [8]. In dairy cows, O'Connor et al. [9] showed that even slight deviations from optimal locomotion can have a negative impact on reproductive performance parameters, such as calving interval, as well as cow death on farms and the reasons for slaughter. Moreover, the early detection of slight deviations from optimal locomotion is important, potentially acting as an early warning sign of a developing lameness disorder [1,3]. Early detection would allow the application of preventative lameness treatment at a stage when it is likely to be more effective [2,10], consequently reducing the associated chronic stress and the risks to reproductive performance.

There are several published sow locomotion scoring systems [11], with that of Main et al. [12] being the most commonly used. However, most are not detailed/sensitive enough to detect slight deviations from optimal locomotion [1,3], as in general they consist of categories clustering several descriptors together [1,3]. In addition, scoring systems often measure locomotion on an ordinal scale, despite the fact that locomotion traits can change in a continuous manner [13–15]. This can also lead to missing important variation in locomotion [15]. Taken together, this results in a reduced level of detail that a system can retain [1,3], and as such, an animal with a slight deviation from optimal locomotion could be classified as sound, because not all descriptors within a category are met [1]. Indeed, the sensitivity of a scoring system and therefore its ability to detect slight deviations from optimal locomotion is affected by the number of categories it possesses [1,16], with fewer categories meaning less sensitivity.

Even though the rationale for developing scoring systems with fewer categories was to improve inter-observer reliability [1,3], there is evidence that the reliability of more detailed scoring systems may actually be superior [3]. Moreover, a scoring system which has a larger number of categories, or is continuous, could potentially capture a slight deviation from optimal locomotion, when a less detailed scoring system may not [3]. In addition to scoring overall locomotory ability in a detailed manner, a scoring system could also consider individual locomotion components separately [17,18]. Based on dairy cow literature, such an approach facilitates better insight into an animal's locomotory ability by revealing how different components may contribute to the overall locomotion score, and also aids in interpreting the causes of deviations from an 'ideal' stride [19–21].

Previous research suggests that visual analogue scales (VAS) could overcome these problems [1,3,15]. VASs assist human patients in rating their own pain experiences [22], and have been modified for use in animal locomotion assessment [13]. Many authors agree that VASs are more sensitive than categorical scoring systems, as they measure traits on a continuous scale, rather than restricting scores to discrete units [3,13,23]. Indeed, there is extensive use of VAS in the dairy cow literature [15,19,24,25]. There are advantages of measuring locomotion on a continuous scale when compared to categorical scoring systems [15,19]. However, to our knowledge, the use of a VAS in pigs is limited to two studies [3,26]. Thus, the aim of this study was to develop a novel VAS to assess both overall locomotory ability and individual aspects of gilt locomotion. We hypothesised that this VAS would allow us to (1) detect slight deviations from optimal gilt locomotion over time more effectively than a categorical scoring system; (2) identify a single component of locomotion which can provide a quick insight into the gilt's overall locomotory ability; (3) detect chronic stress levels associated with impaired locomotion and predict reproductive performance of sows.

2. Materials and Methods

2.1. Ethical Approval

The research farm on which this experiment was conducted complied with Statutory Instrument number 311 of 2010 European Communities (Welfare of Farmed Animals) Regulations 2000. Experimental work was authorized by the Teagasc Animal Ethics Committee (Approval No: TAEC219-2019).

2.2. Animals and Housing

This study took place on a 200-sow research unit at the Teagasc Pig Development Department in Moorepark, Fermoy, Co. Cork, Ireland, between May 2019 and March 2020. In total, 51 gilts in eight replicate groups were used. Gilts were purchased from a breeder and thus had to undergo a six-week quarantine before entering the research unit at approximately 210 days of age. Upon completion of the quarantine period, gilts entered the main pig unit and were housed in fully slatted pens (3.2 m × 2.6 m) in groups of four, fed from a long-trough, and were treated with Altresyn for oestrus synchronisation. Gilts were served twice in service stalls by artificial insemination, first at the onset of standing oestrus, and then within 24 h. Each replicate was served between three to nine weeks apart, depending on the availability of new gilts entering the breeding pool as replacements (see Table 1 for experimental schedule). Approximately five days after service gilts were moved back into their home pens in the same groups as before service, where they stayed until day 30 of pregnancy.

Table 1. Details of experimental schedule and design.

Replicate	Replicate Size	Date of Mixing	Group Size at Mixing	Interval between Mixing Events (Weeks)
1	12	07/05/2019	33	9
2	10	28/05/2019	32	3
3	8	30/07/2019	19	9
4	4	20/08/2019	15	3
5	8	10/09/2019	21	3
6	4	22/10/2019	15	6
7	8	12/11/2019	17	3
8	4	03/12/2019	16	3

They were then mixed into a larger dynamic group with other pregnant gilts (see Table 1 for number of gilts present at the time of mixing) where they were fed by an electronic sow feeder (ESF; Schauer Feeding System; Prambachkirchen, Austria) set to a 23 h cycle, starting at 17:00 daily. The ESF recognised each gilt by a transponder tag programmed to her individual daily allowance of a standard gilt diet. Water was available ad libitum from a single-bite drinker inside the ESF, and from a drinker bowl in the pen. The group pen (68.11 m^2) comprised of fully slatted concrete floors in the group area, with four insulated solid concrete bays for lying. Gilts had a wooden block suspended from a chain as enrichment. Approximately one week prior to farrowing (day 108), they were moved to the farrowing accommodation and housed in standard individual farrowing crates (pen dimensions: 2.5m × 1.8m), with cast-iron fully slatted floors within the farrowing crate, plastic fully slatted floors around the crate, and a solid plastic heated mat for piglets. Weaning took place approximately 28 days post-partum.

2.3. Locomotion Scoring

Locomotion was scored visually while gilts walked on solid concrete along the corridor outside of the home pen, taking at least six strides (distance of approximately 30 m). Locomotion was scored on three occasions during the first pregnancy: three days before service (service), in mid-pregnancy (approximately day 57), and on the day of entry to the farrowing crates (day 108; late pregnancy). Sows were also scored at weaning of their first litter. Scoring was performed by a single trained observer who practiced until at least 90% intra-observer scores for repeatability were achieved.

2.3.1. Categorical Locomotion Scoring (CAT)

Each gilt was assigned a locomotion score (0 to 5) using the gait component of the categorical locomotion scoring system developed by Main et al. [12].

2.3.2. Visual Analogue Scales

- Overall locomotion scoring

Overall locomotory ability (OVERALL) was assessed using a VAS consisting of a 150 mm horizontal line, with the left end (0 mm) representing perfect locomotion, and the very right end (150 mm) representing severely impaired locomotion. Locomotory ability was scored by marking a point along the scale, with increasing impairment represented by a mark further to the right of the line. The distance from the left-hand end of the scale was measured and the value for each recorded in millimetres. Thus, the greater the number, the more impaired the locomotory ability. As a guide, the VAS was also divided into descriptive sublevels, to aid with consistency of locomotion scoring ([3,27,28]; e.g., Figure 1). The sublevels were selected based on previous literature on pig and dairy cow locomotion scoring [3,12,19,26].

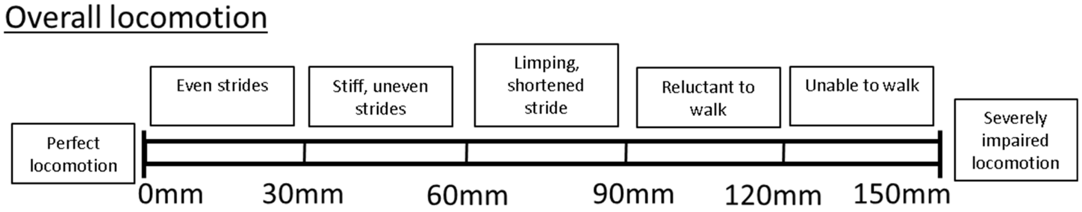

Figure 1. Example of a visual analogue scale for the scoring of overall locomotory ability developed for the purpose of this study.

- Component locomotion scoring

As well as the overall locomotory ability, several components of locomotion (Table 2) were assessed using an individual VAS for each component. These components were selected based on previous literature on pig and dairy cow locomotion scoring [3,12,19,26] and upon feedback gathered during a pilot trial whereby two authors (L.A.B. and K.O.) assessed locomotion in a number of sows. As in the case of OVERALL, the VAS for each of the individual locomotion components was also divided into descriptive sublevels to aid with consistency of scoring [3,12,19,26]. A different number of sublevels were applied to each locomotion component, based on severity levels reported on in the pig locomotion assessment literature ([3,26]; see Appendix A, Figure A1).

Table 2. Components of locomotion scored using a Visual Analogue Scale (VAS). The VAS ranged from 0 mm (perfect) to 150 mm (the most severe impairment possible).

Locomotion Component	Definition
Caudal sway	The side-to-side movement of the hindquarters
Stride length	The evenness of strides taken by the sow
Fluidity of movement	The overall ease with which the sow walks
Reluctance to bear weight while walking	Evidence of differences in weight bearing between the limbs, including shifting weight between hind/front legs, and intermittent placement of limbs on the floor
Abduction	Outward swinging of hind legs
Adduction	Inward swinging of hind legs

2.4. Hair Collection and Subsequent Hair Cortisol Concentration Analysis

Hair collection for cortisol determination was performed while gilts were inside the weighing scales immediately prior to mixing into the dynamic group (day 30 of pregnancy) and on the day of entry to the farrowing crates (day 108; late pregnancy) during their first pregnancy. Hair is hypothesised to be a suitable medium for quantifying chronic stress levels, due to the long-term accumulation of cortisol within the shaft [29–31]. Combined with this, the shave/re-shave method (first shave on day 30, then re-shave performed in late pregnancy) used in this study allowed determination of the concentration of cortisol which accumulated during the period between hair shavings. Thus, hair cortisol concentration measured in late pregnancy was used in the analysis as an indicator of chronic stress corresponding to approximately the last two-thirds of the pregnancy. The collection site can have an impact on cortisol concentrations found in hair [30,32], and thus, based on previous research, the dorso-lumbar region was selected as the most appropriate site for collection to best guarantee adequate measurement of cortisol concentration [32–34]. The dorso-lumbar site was identified by measuring 6.5 cm left and right from the mid-point at the spine marked by the position of the last rib; hair was shaved using an electric shaver, placed into plastic zip-lock bags, and frozen at −20 °C until hair cortisol analysis.

Hair sample preparation and cortisol extraction were based on the procedure described by Davenport et al. [29], with certain modifications described by Lagoda et al. [32]. In brief, hair samples were defrosted for one hour prior to preparation procedures, then washed by placing 300 mg of hair into a 10 mL polypropylene tube along with 5 mL of isopropanol, and mixing gently on a shaker for 3 min. This was repeated using fresh isopropanol for the second wash. Washed hair samples were left inside the wash tubes and placed inside a protected fume hood to dry overnight. Samples prepared in this way were then individually ground into a fine powder using a Retsch mixing mill (MM200; 10 mL stainless steel grinding jars, single 12 mm stainless steel grinding ball) for 4 min at 25 Hz. Approximately 50 mg of ground hair sample was weighed out and placed in a 2 mL tube along with 1 mL of methanol, which was followed by incubation of the sample for 24 h at room temperature with constant gentle agitation (approximately 95 rpm) for cortisol extraction. Following the 24 h incubation period, 0.6 mL of the cortisol extract in methanol was removed (taking care not to disturb the settled hair powder at the bottom of the tube) using an Eppendorf pipette and transferred to a clean 1.5 mL tube for methanol evaporation, which was performed using a stream of nitrogen gas at 38 °C. Cortisol extract samples were frozen at −20 °C pending EIA analysis. Extracted cortisol samples were analysed using Salimetrics® Expanded Range, High Sensitivity Salivary Cortisol EIA kit, which was validated for the analysis of hair cortisol concentrations [29,33,35], and is valid for use in a range of species, including swine [29,34,36]. Frozen cortisol extract samples along with the EIA kit were brought to room temperature 1.5 h prior to being reconstituted with 0.4 mL of phosphate buffer (assay diluent) provided with the EIA kit. Reconstituted extracts (n = 102) were analysed for cortisol concentration levels in duplicate using 4 assays, following the protocol provided with the EIA kit. Inter- and intra-assay CV were 24.1 and 8.7%, respectively.

2.5. Reproductive Performance

Reproductive performance records were acquired from the sow management system (PigChamp) used on the farm, to ascertain the number of piglets born alive, born dead, mummified, and total born over four parities (parity 1 to 4).

2.6. Statistical Analysis

SAS v9.4 was used for all statistical analyses (SAS Inst. Inc., Cary, NC, USA) with sows as the experimental unit. Differences were reported when $p \leq 0.05$, while statistical trends were reported when $p > 0.05$ and $p \leq 0.10$. Results for independent continuous variables are reported as their regression coefficient (REG) ± standard error (SE).

2.6.1. Comparison of Scoring Methods over Time

A repeated measures analysis was carried out to investigate the effect of time of locomotion scoring (n = 4) on locomotion scores recorded using OVERALL, locomotion components, and CAT. Residuals were checked for normality using the Shapiro test, and by examining the quantile-quantile plot. For variables with normally distributed residuals (OVERALL, and the components: caudal sway, stride length, fluidity of movement, and reluctance to bear weight while walking), linear mixed model equations were built in PROC MIXED. For variables with non-normally distributed residuals (CAT, and the components: abduction and adduction) generalised linear mixed model equations were built in PROC GLIMMIX and fitted with either the Poisson (abduction and adduction score) or the multinomial distribution (CAT). For model equations built in PROC MIXED, time was included as a repeated measure, with sow ID as subject, while for model equations built in PROC GLIMMIX, time was included as an additional random effect to account for repeated sow ID measures. Replicate was included as a random effect in all models.

2.6.2. Associations between OVERALL and Locomotion Components

A repeated measures regression analysis was performed to investigate the association between OVERALL (dependent variable) and the individual components of locomotion (included as continuous independent variables; PROC MIXED) across all scoring days together, and also on each scoring day separately. The latter was completed as it is important to consider the relationship on the different days, since the changing shape and weight of the gilt with progressing pregnancy could potentially impact the way she walks. Residuals were checked as described previously to confirm the suitability of the models. Time was included as a repeated measure, and replicate was included as a random effect.

2.6.3. Associations between OVERALL, Hair Cortisol Concentration, and Reproductive Performance

Separate regression analyses were performed to investigate the association between OVERALL at each of the three recording occasions during pregnancy, and hair cortisol concentration in late pregnancy. A separate regression analysis was also carried out to investigate the association between OVERALL at each of the three recording occasions and the following measures: the number of piglets born alive, born dead, mummified, and the total number born over four parities. Residuals were checked as before. Hair cortisol concentration, number of piglets born, and piglets born alive were analysed using linear mixed models (PROC MIXED), and the number of piglets mummified or born dead were analysed using generalised linear mixed models (PROC GLIMMIX) and fitted with the Poisson distribution. For the analysis of hair cortisol concentration, an EIA assay plate was included as an additional random effect. For the measures of reproductive performance which had model equations built in PROC MIXED, parity was included as a repeated measure, with sow ID as subject, while for model equations built in PROC GLIMMIX, parity was included as an additional random effect to account for repeated sow ID measures. Replicate was included as a random effect in all models.

3. Results

Gilts were considered lame if they received a score of 2 or higher (≥ 2) on the CAT scale (n = 5 gilts throughout entire study), and if they scored 60 mm or higher (≥ 60) on the VAS for OVERALL (based on the descriptive sublevel overlying the VAS, whereby visible signs of obvious lameness such as limping and shortened stride are described for the first time; n = 6 gilts throughout entire study). The mean ± standard deviation (SD) for the CAT locomotion score throughout the entire study was 0.2 ± 0.50 (median = 0; range 0 to 3). The mean ± SD for the OVERALL locomotion score throughout the entire study was 17.1 ± 14.47 mm (median = 15; range 1 to 72 mm).

3.1. Comparison of Scoring Methods over Time

There was an effect of time of scoring on OVERALL ($F_{3,145} = 2.70$; $p \leq 0.05$), and on some of the components of locomotion, namely, caudal sway ($F_{3,144} = 2.92$; $p \leq 0.05$), stride length ($F_{3,145} = 3.04$; $p \leq 0.05$), and fluidity of movement ($F_{3,145} = 3.82$; $p \leq 0.05$; Figure 2). No effect of time of scoring on reluctance to bear weight while walking ($p > 0.05$) was found, while abduction ($F_{3,194} = 2.47$; $p = 0.063$) and adduction ($F_{3,194} = 2.24$; $p = 0.086$; Figure 2) tended to change over time. As shown in Figure 2, the pattern of locomotory ability over time which was most similar to OVERALL was that of stride length, with the least similar being caudal sway and abduction. Locomotion scores estimated using CAT tended to change over time (mean (median); at service = 0.18 (0); mid-pregnancy = 0.12 (0); late pregnancy = 0.37 (0); weaning = 0.20 (0); $F_{3,195} = 2.45$; $p = 0.065$; Figure 3).

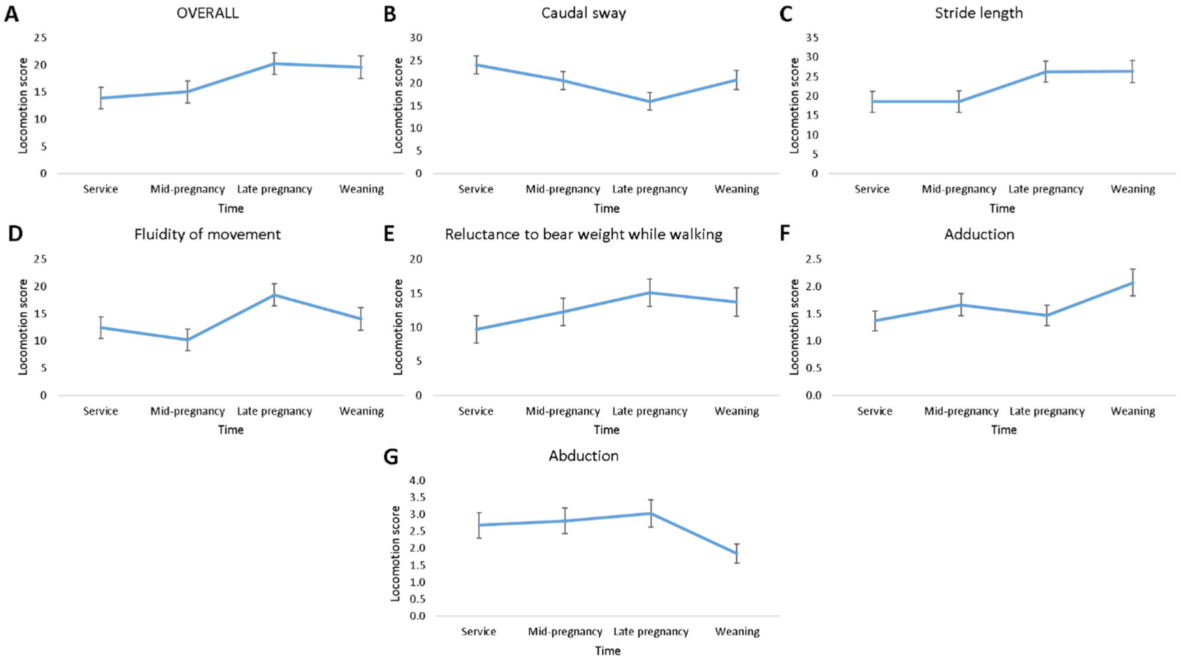

Figure 2. Plots of locomotion score least square mean ± standard error changes over time for OVERALL (**A**), caudal sway (**B**), stride length (**C**), fluidity of movement (**D**), reluctance to bear weight while walking (**E**), adduction (**F**), and abduction (**G**) in 51 gilts (n = 8 replicates).

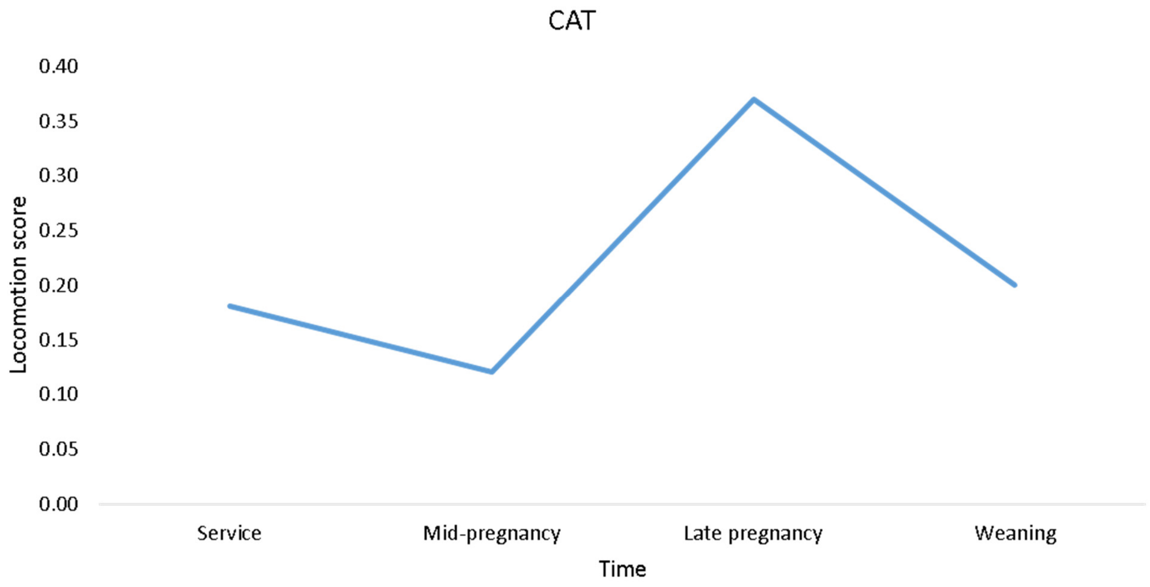

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Figure 3. Plot of CAT mean locomotion score changes over time in 51 gilts (n = 8 replicates).

3.2. Associations between OVERALL and Locomotion Components

There were positive associations between the OVERALL VAS score and the scores for caudal sway, stride length, fluidity of movement, and reluctance to bear weight while walking across all scoring days together (Table 3), with the highest regression coefficients for the latter three measures. Indeed, although the association between caudal sway and OVERALL locomotion score across all scoring days together was positive, when considered on each scoring day separately, this association did not always hold true (e.g., service: $p < 0.001$; mid-pregnancy: $p = 0.056$; late pregnancy: $p = 0.006$; weaning: $p = 0.103$; see Appendix A, Figure A2 for graphs representing the relationship between OVERALL and locomotion components on each scoring day). This suggests that gilts with a higher OVERALL locomotion score also had higher caudal sway scores across all scoring days together, despite the pattern of increase/decrease being different for OVERALL and caudal sway locomotion scores on any given day. On the other hand, the associations between OVERALL locomotion score and stride length, fluidity of movement, and reluctance to bear weight while walking across all scoring days together were reflected by the associations found when each scoring day was considered separately ($p < 0.001$ for stride length, fluidity of movement, and reluctance to bear weight while walking on each scoring day; Appendix A, Figure A2).

Table 3. Associations (regression coefficient and standard error; SE) between individual visual analogue scale (VAS) locomotion component scores and the VAS overall locomotion score in 51 gilts (n = 8 replicates), as a way of identifying a single locomotion component most associated with overall locomotory ability.

Individual Locomotion Component Score	Regression Coefficient	SE	F-Statistic	*p*-Value
Caudal sway	0.4	0.07	$F_{1,146} = 32.23$	<0.001
Stride length	0.7	0.02	$F_{1,147} = 1090.77$	<0.001
Fluidity of movement	0.8	0.04	$F_{1,147} = 328.75$	<0.001
Reluctance to bear weight while walking	0.8	0.04	$F_{1,147} = 390.90$	<0.001
Abduction	0.1	0.16	$F_{1,146} = 0.20$	0.652
Adduction	−0.02	0.649	$F_{1,146} = 0.00$	0.980

3.3. Associations between OVERALL, Hair Cortisol Concentration, and Reproductive Performance

The OVERALL locomotion score both at service (REG = 0.003 ± 0.0012; $F_{1,48} = 4.25$; $p \leq 0.05$) and at mid-pregnancy (REG = 0.003 ± 0.0013; $F_{1,48} = 6.95$; $p \leq 0.05$) was positively associated with hair cortisol concentration in late pregnancy (i.e., the more impaired locomotory ability was during early to mid-pregnancy, the greater the accumulation of cortisol in the hair shaft by end of the pregnancy). No association between OVERALL locomotion score in late pregnancy and hair cortisol concentration in late pregnancy was found ($p > 0.05$).

The OVERALL locomotion score at service was positively associated with the number of piglets born dead (REG = 0.01 ± 0.006; $F_{1,36} = 4.24$; $p \leq 0.05$), and the total born (REG = 0.1 ± 0.03; $F_{1,120} = 4.88$; $p \leq 0.05$), and tended to be positively associated with the number of piglets born alive (REG = 0.1 ± 0.03; $F_{1,120} = 3.17$; $p = 0.078$) and piglets mummified (REG = 0.01 ± 0.008; $F_{1,24} = 2.97$; $p = 0.098$).

The OVERALL locomotion score in late pregnancy tended to be positively associated with the number of piglets born alive (REG = 0.04 ± 0.024; $F_{1,119} = 3.06$; $p = 0.083$) and total born (REG = 0.1 ± 0.03; $F_{1,119} = 3.84$; $p = 0.053$). There were no associations between OVERALL locomotion score at mid-pregnancy and any aspect of reproductive performance ($p > 0.05$).

4. Discussion

The detrimental nature of lameness [37] warrants the need for its early detection [2,10]. The VAS developed for the purpose of this study enabled the detection of slight deviations from optimal locomotion and its individual components over time, and as hypothesised, it was more effective at this than the categorical system developed by Main et al. [12]. Thus, it holds promise to be a more effective research tool than the categorical scale.

As expected, gilt locomotion scores increased as pregnancy progressed. This is because as pregnancy advances, gilts gain weight, which in turn puts more pressure on their limbs and could result in a deterioration in leg health and therefore higher locomotion scores [38]. Furthermore, the longer sows spend in a group, the greater the likelihood of fights and consequent injuries to the limbs [8,39]. In addition, sows are most commonly housed on fully slatted concrete floors (as was the case in the current study), which are rough and uncomfortable, and a risk factor for lameness [39,40]. The longer sows spend on this type of floor, the greater the likelihood of increased locomotion scores as a result of leg discomfort experienced by the animals. Provision of more comfortable floor surfaces, such as rubber mats, could help to reduce lameness throughout pregnancy [41]. Rubber mats/floors are associated with greater ease of changing posture [42], fewer foot and claw injuries, and are more comfortable to rest on [39]. A reduction in lameness can also be achieved through the provision of bedding such as straw, as bedding can minimise the negative impact of rough concrete floors on sow feet and claws [43,44]. Additionally, early detection of locomotion issues is crucially important when attempting to reduce lameness, as treatment applied early can be more effective [2,10].

This is the first study that we are aware of which investigated variation in individual components of gilt stride. Information on specific components presents a more detailed picture of locomotory ability as pregnancy progresses, and mirrors similar work with dairy cows [20,21]. These authors were able to attribute higher overall locomotion scores in a proportion of dairy cows to higher scores for individual locomotion components such as “tracking up” [21], and abduction/adduction [20]. More importantly, they were able to relate these differences back to specific hypotheses developed in relation to the experimental treatments; for instance, O’Driscoll et al. [20] hypothesised that more ab/adduction in cows milked once daily compared to twice daily was due to the legs swinging out around an engorged udder. Looking at specific components of locomotion could therefore provide insights into the underlying causes of lameness, help to ameliorate its risk factors, and could in turn be important when deciding on the best form of treatment.

Farmers are not trained to assess locomotion [37]. Locomotion scoring is complex, as to do so reliably usually requires observing several aspects of locomotion simultaneously, which is challenging even for trained personnel [37,45]. Thus, identification of a single locomotion component which could act as a reliable measure of the animal's overall locomotory ability would therefore be extremely useful [45]. It could speed up and potentially make on-farm locomotion assessment more accurate by simplifying the methodology for the farmer [45]. As an example from the dairy cow literature, it is commonly accepted that the degree of back arch displayed by a cow provides insight into overall locomotory ability/lameness status, as the two are positively associated [45,46].

The current study identified caudal sway, stride length, fluidity of movement, and reluctance to bear weight while walking as being positively associated with the overall locomotion score assessed using a VAS, demonstrating potential to simplify sow locomotion assessment on-farm. However, when we compared patterns over time, and whether the relationship between OVERALL and each component on each day was similar, we found that caudal sway is likely not a suitable proxy for OVERALL locomotory ability. The lack of a positive association on each recording day could be a consequence of the changing weight and shape of the gilt as pregnancy progresses, which in turn could alter the degree of her caudal sway. A similar phenomenon was noted in dairy cows by O'Driscoll et al. [20], whereby the degree of abduction and adduction recorded in the animals differed depending on the fullness of their udders. A good proxy for OVERALL locomotion should have a consistent relationship with it across all stages of pregnancy and management. Stride length, fluidity of movement, and reluctance to bear weight while walking all had a consistent relationship with OVERALL across and on each scoring day separately, and thus have potential to be used as proxies for OVERALL locomotory ability.

While stride length requires a degree of familiarity and experience to be scored accurately [11,47], fluidity of movement and reluctance to bear weight while walking could be more appropriate options for farmers. Fluidity of movement is a measure of the overall smoothness/ease of an animal's walking ability, where any deviations away from the norm are easy to observe. Reluctance to bear weight while walking requires the observer to identify whether the animal is reluctant to place any of its limbs on the floor, and the degree to which this occurs, to determine the severity of the phenomenon. Abnormal weight bearing is easily spotted; thus, similar to fluidity of movement, any deviations away from the norm are easy to identify. Further work consisting of repeatability testing involving producers, advisors, and vets should examine both of these aspects in more detail to determine the ease with which they can be learned, and thus ascertain their suitability for on-farm use.

Lameness has detrimental effects on reproductive performance, potentially mediated by chronic stress [1,4,48,49]. The current study found positive associations between the VAS locomotion score of gilts around their first service and the total number of piglets born, as well as a trend for a positive association with piglets born alive, over the first four parities. There was also a positive association between the VAS locomotion score at this time and the number of piglets born dead, and a trend for a positive association with mummified piglets. Locomotory ability later on was related to long-term reproductive performance to a much lesser extent, with just a trend for a positive association between the VAS locomotion score and the total born and born alive piglet numbers. Thus, it appears that assessing locomotory ability around the time of first service is likely the optimal time to estimate how it could affect lifetime performance.

As higher scores indicate worsening locomotory ability, this implies that gilts that deviated more from the 'ideal' stride around the time of first service were more productive across their first four parities. These findings conflict with the existing literature. In the studies of Anil et al. [8] and Iida et al. [50], lame sows had lower numbers of piglets born alive, thus demonstrating a detrimental effect of lameness on reproductive performance.

However, these studies utilised locomotion scores recorded at different stages of pregnancy to those used in the current study (e.g., only on the way to the farrowing rooms). Moreover, it is possible that this difference to our findings relates to the fact that the above studies considered effects of clinical lameness, rather than a slight impairment in locomotion, as was the case in our study. It is possible that as clinical lameness is a more severe condition, this led to much higher chronic/acute stress levels, and consequently had a more marked effect on reproductive performance parameters [8,50].

A possible explanation for our findings regarding associations between locomotory ability and reproductive performance could relate to energy resource distribution in sows. Redirection of energy resources away from non-crucial physiological processes towards reproduction is a known phenomenon in mammals, as this strategy maximizes reproductive performance [51]. It is possible that our study sows redirected their energy resources towards reproductive functions in a likewise manner, with positive impacts on the number of total born and born alive piglets. In consequence, this could have left fewer energy resources available for the maintenance of leg health, resulting in slight deviations from optimal locomotion.

Following on from this, we speculate that the slightly compromised leg health (as marked by slight deviations from optimal locomotion) experienced even at this early stage in the reproductive cycle generated elevated stress levels, which persisted chronically. This is supported by our finding of higher hair cortisol concentrations in late pregnancy (reflecting chronic stress levels experienced by gilts throughout pregnancy) with higher overall locomotion scores both at service and in mid-pregnancy. The elevated stress levels could in turn have detrimental knock-on effects on prenatal mortality. Moreover, perhaps this could explain the positive association between locomotion scores at service and the numbers of piglets born dead, and the trend for a higher number of piglets mummified with increasing overall locomotion score. This finding is in line with Hartnett et al. [52]; in that study, replacement gilts reared alongside males had impaired leg health in terms of higher hoof lesion scores. These gilts went on to have higher numbers of piglets born dead over their first five parities, which the authors hypothesised was due to the elevated stress levels associated with impaired leg health [52]. Our finding is also in line with Pluym et al. [53], who found higher numbers of born dead and mummified piglets with an increasing incidence of claw lesions and wall cracks. Thus, it is possible that even slightly impaired leg health/locomotion could generate sufficient chronic stress levels to impair certain aspects of reproductive performance. Nevertheless, it is important to note that the fact that the study gilts had larger litter sizes in general could also explain the higher numbers of born dead and mummified piglets recorded in this study.

5. Conclusions

A detailed VAS developed in the current study was able to detect slight deviations from optimal gilt locomotion over time more effectively than a categorical scoring system. The extra information generated as a result of scoring of locomotion in terms of several locomotion components provided a greater insight into overall locomotory ability than was previously possible for sows. This should encourage the use of more detailed VAS scoring systems in the future, thus contributing to early detection and prevention of developing lameness disorders and simplifying on-farm locomotion assessment. Further work should apply the VAS developed in this study in locomotion scoring of older sows, and with multiple observers. Finally, this study pointed at the possibility of chronic stress resulting from impaired locomotion acting as a mediator for the process of reproductive performance impairment. Future research is necessary to further elucidate the mechanisms involved in the impairment of reproductive performance by slightly impaired locomotory ability, with a focus on the extent to which chronic stress associated with slightly impaired locomotion is involved in this process.

Author Contributions: Conceptualization, L.A.B., K.O. and M.E.L.; methodology, L.A.B., K.O. and M.E.L.; software, K.O. and M.E.L.; validation, L.A.B., K.O., J.M. and M.E.L.; formal analysis, K.O. and M.E.L.; investigation, M.E.L.; resources, L.A.B. and K.O.; data curation, M.E.L.; writing—original draft preparation, M.E.L.; writing—review and editing, L.A.B., K.O., J.M. and M.E.L.; visualization, M.E.L.; supervision, L.A.B., K.O. and J.M.; project administration, L.A.B., K.O. and M.E.L.; funding acquisition, L.A.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki. The research farm on which this experiment was conducted complied with Statutory Instrument number 311 of 2010 European Communities (Welfare of Farmed Animals) Regulations 2000, and experimental work was authorized by the Teagasc Animal Ethics Committee (Approval no: TAEC219-2019).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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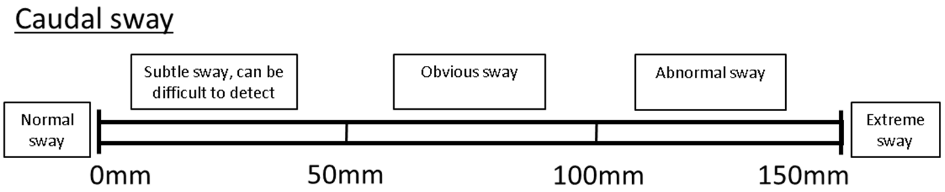
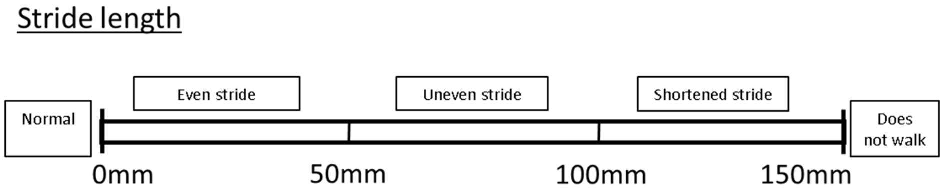
Conflicts of Interest: The authors declare no conflict of interest.

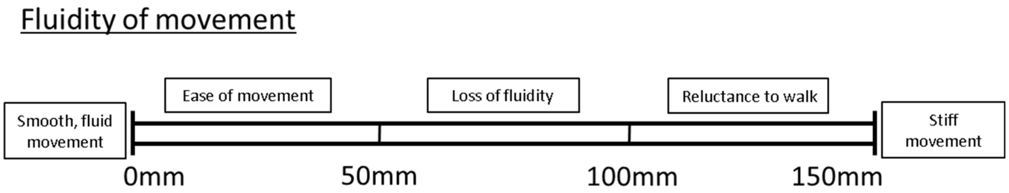
Appendix A



Caudal sway



Stride length



Fluidity of movement

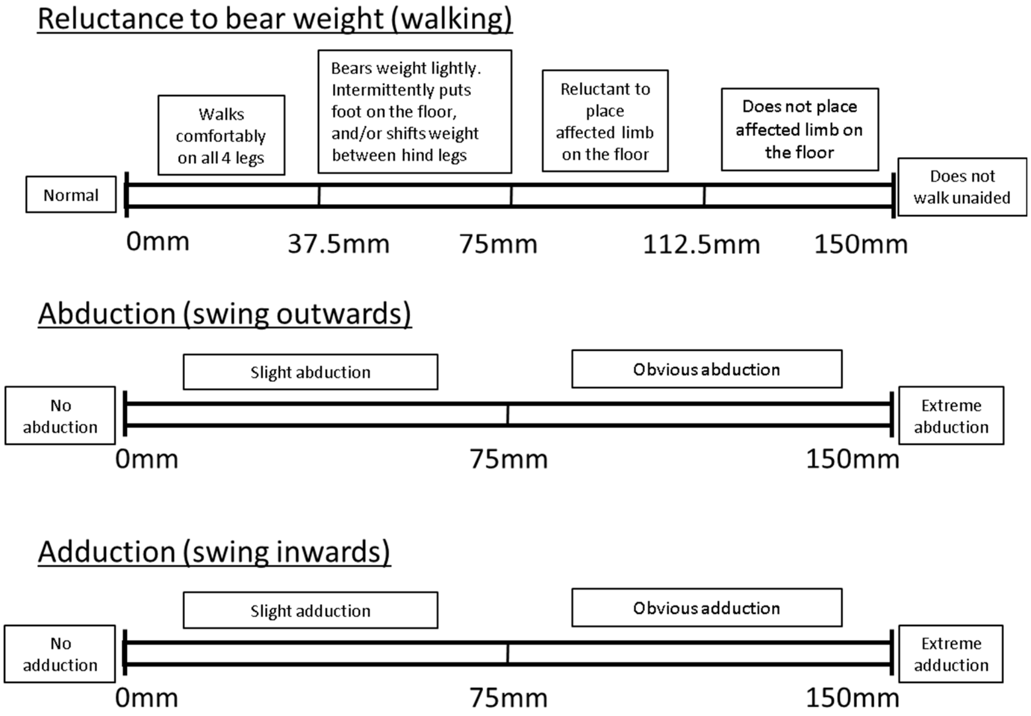
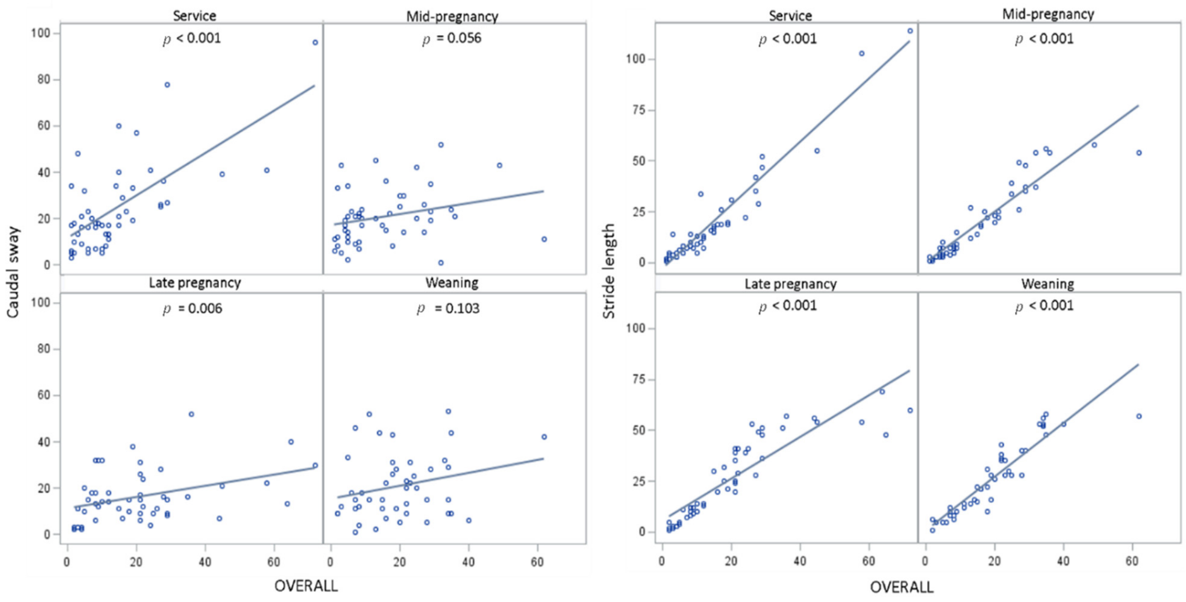


Figure A1. Individual visual analogue scale locomotion components, with descriptive sublevels based on pig locomotion assessment literature, to aid with consistency of scoring.



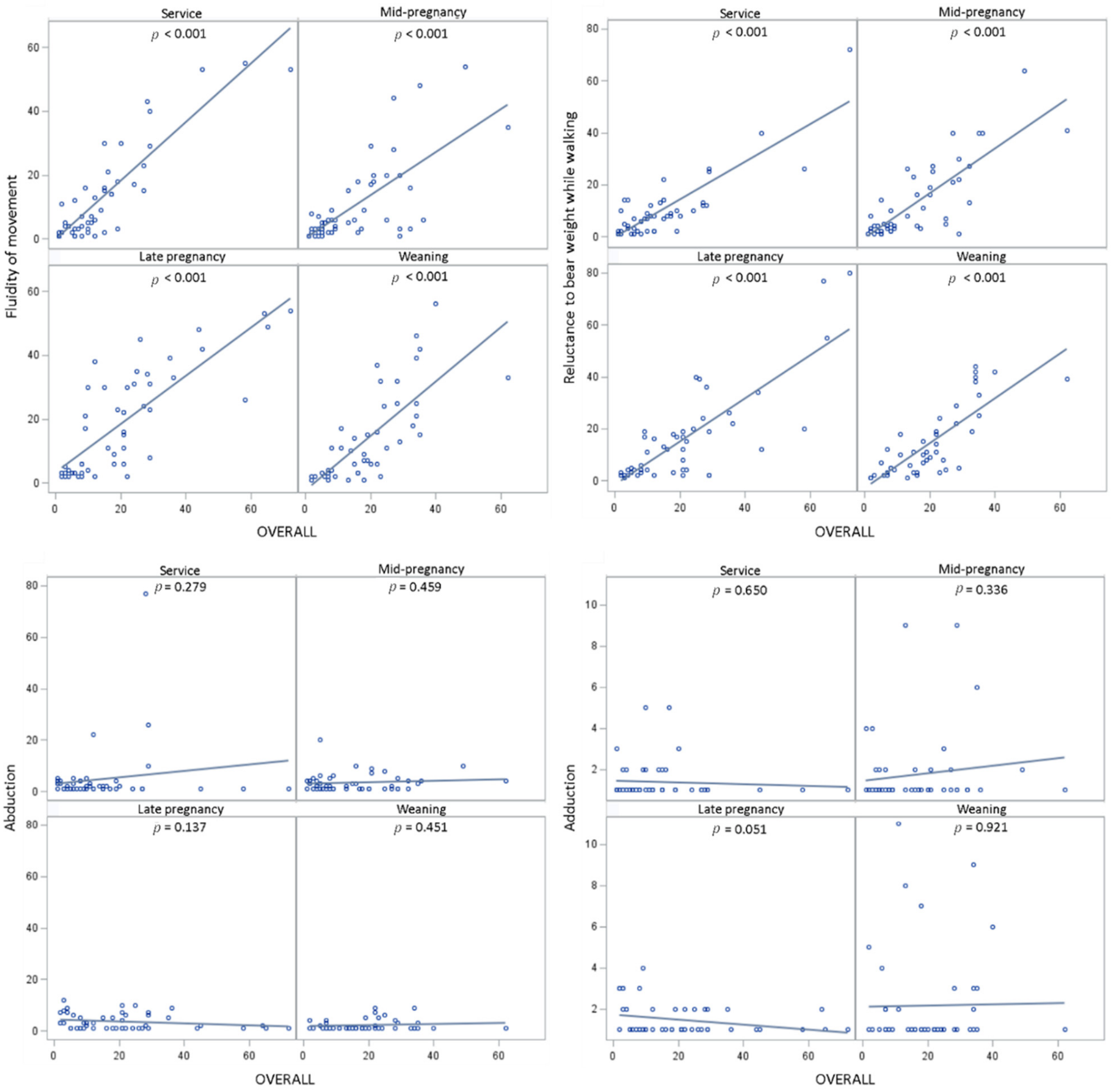


Figure A2. Graphs representing the relationship between OVERALL and locomotion components on each scoring day.

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