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Periparturient behavior and physiology: further insight into the farrowing process for primiparous and multiparous sows

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Abstract

Giving birth is a critical time for many species and is often the most painful event ever experienced by females. In domestic species, like the pig, pain associated with parturition represents a potential welfare concern, and the consequences of pain can cause economic losses (e.g. by indirectly contributing to piglet mortality as pain could slow post-farrowing recovery, reduce food and water intake, reducing milk let-down). This study investigated pain assessment and its management in primiparous (gilts) and multiparous (sows) breeding pigs, including the provision of a non-steroidal anti-inflammatory drug (NSAID) post-parturition. Individuals were randomly allocated to receive the NSAID ketoprofen (3mg/kg bodyweight) (n = 11 gilts, 16 sows) or the equivalent volume of saline (n = 13 gilts, 16 sows) by intramuscular injection 1.5 hours after the birth of the last piglet. Data collected included putative behavioral indicators of pain (back leg forward, tremble, back arch), salivary cortisol concentrations pre-farrowing and up to 7 days post-injection. In addition, post-partum biomarkers of inflammation, including the acute phase protein C-reactive protein (CRP) and 3 porcine cytokines (interleukin-1 β (IL1 β), interleukin-6 (IL6) and tumor necrosis factor α (TNF α)) were measured in plasma collected 6 hours following the injection. Behaviors were analyzed using generalized linear mixed models, and physiological variables with linear mixed models. No difference in putative pain behaviors, salivary cortisol, CRP or cytokines were found between individuals treated with ketoprofen or those administered the saline control. However, there were some differences between gilts and sows, as sows exhibited more putative pain behavior than gilts, had higher salivary cortisol on the day of farrowing and had higher plasma TNF α . Conversely, gilts had higher salivary cortisol than sows on day 3 post farrowing and had higher CRP. This indicates that, like human females, multiparous sows experience more pain from uterine activity following birth than primiparas. This study provides useful information for developing management practices relating to post-farrowing care for breeding pigs.

Keywords: behavior, parturition stress, physiology, pain assessment, sow, welfare

50 1. Introduction

51
52 For human females, giving birth is often the most painful event ever experienced,
53 with a high percentage of women reporting severe or extremely severe pain (Melzack, 1984).
54 It is now widely accepted that many animals are capable of experiencing pain (Sneddon et al.,
55 2014) and pain associated with parturition in domestic pigs has been discussed in a recent
56 review (Mainau and Manteca, 2011).

57
58 Farrowing is a critical time in pig production, as success at this stage of the system
59 means more piglets are weaned and ultimately sold. The breeding sow is essential for
60 successful production around farrowing, and good health and welfare of the sow is reflected
61 in her ability to produce a healthy litter of piglets. Any farrowing difficulties or reduced milk
62 production can result in higher pre-weaning piglet losses (Peltoniemi and Oliviero, 2015).
63 Parturition is likely to be painful in the pig as it is in other species as pain is likely to
64 originate from uterine contractions, piglet expulsion and inflammation of the uterine tract
65 from delivering a litter of piglets (Mainau and Manteca, 2011). After birth, pain in human
66 females can be present from intermittent uterine contractions during the process of
67 involution, when the uterus returns to its normal size and from tissue damage associated with
68 a natural birth (Deussen et al., 2011). Post-birth pain experienced by the mother has
69 implications for her ability to recover in order to return normal activities and feed and care
70 for her baby (Deussen et al., 2011). It has been suggested that pain experienced by the sow
71 post-farrowing, could affect her ability to feed and care for piglets (Mainau and Manteca,
72 2011; Peltoniemi and Oliviero, 2015), which is a welfare concern for the sow and piglets and
73 also an economic one for the farmer. This has resulted in recent research administering non-
74 steroidal anti-inflammatory drugs (NSAIDs) post-farrowing and measuring the benefits to
75 health, welfare and productivity (Homedes et al., 2014; Mainau et al., 2012; Sabaté et al.,
76 2012; Tenbergen et al., 2014; Viitasaari et al., 2013, 2014).

77
78 Benefits of the post-farrowing administration of NSAIDs include a reduction in piglet
79 mortality with the use of ketoprofen (Homedes et al., 2014; Sabaté et al., 2012), and an
80 increased average daily weight gain of low birth weight piglets (< 1180 g) with the use of
81 intramuscular meloxicam (Mainau et al., 2012). A study in which oral meloxicam was
82 administered as soon as possible after the onset of farrowing showed improved piglet
83 weaning weight, average daily gain and evidence for improved immunoglobulin-G (IgG)
84 transfer (Mainau et al., 2016). Sows treated with intramuscular meloxicam spent less time
85 lying on the third day following parturition (Mainau et al., 2012) and younger sows treated
86 with ketoprofen were more active than those given a placebo (Viitasaari et al., 2014), which
87 may indicate improved recovery post-farrowing. Other sow health and welfare benefits of
88 ketoprofen included a reduced loss in back-fat and body condition score, a lower incidence of
89 constipation and less severe shoulder sores (Viitasaari et al., 2013). The administration of
90 NSAIDs, in addition to antibiotics, has also been shown to aid in treatment of infectious
91 causes of post-partum dysgalactia syndrome (PPDS) (e.g. (Hirsch et al., 2003; Tummaruk
92 and Sang-Gassanee, 2013)) and, on farms with a high incidence of subclinical forms of the
93 condition, piglet mortality was reduced (Sabaté et al., 2012).

94
95 Ketoprofen is an NSAID with anti-inflammatory, analgesia and antipyretic properties,
96 with a number of brands available and licensed to treat conditions involving pain,
97 inflammation and fever in pigs in the UK (VMD, 2011). It is absorbed well, reaching
98 maximum levels after approximately 1 hour following intra-muscular (IM) injection
99 (Raekallio et al., 2008). Nociceptive thresholds were reduced in piglets with kaolin-induced

100 inflammation, when administered ketoprofen compared with a placebo up to 24 hours
101 following IM injection (Fosse et al., 2011). It is the second most popular NSAID in the UK,
102 after meloxicam; reported to be used or prescribed by 50% of pig veterinarians (Ison and
103 Rutherford, 2014). Ketoprofen is licensed for use in pigs in the UK to be used along with
104 antimicrobials to treat respiratory disease, mastitis and metritis (or PPDS) at a dose rate of 3
105 mg per kg bodyweight (VMD, 2011). Scientific research has demonstrated the efficacy of
106 ketoprofen in experimentally infected pigs (Mustonen et al., 2012; Swinkels et al., 1994), to
107 treat respiratory disease (Salichs et al., 2013) and non-infectious lameness (Mustonen et al.,
108 2011). As previously mentioned, the post-farrowing administration of ketoprofen has shown
109 health, welfare and production benefits (Homedes et al., 2014; Viitasaari et al., 2013, 2014),
110 especially on farms with a high incidence of PPDS (Sabaté et al., 2012).

111

112 This study investigated the assessment and management of pain in primiparous
113 (hereafter referred to as gilts) and multiparous (hereafter referred to as sows) breeding pigs,
114 including the administration of the NSAID ketoprofen after farrowing. A previous
115 publication, as part of the same study, presented the effect of post-farrowing ketoprofen on
116 gilt and sow feed intake, nursing behavior and piglet performance (Ison et al., 2017). This
117 article focuses on behavioral and physiological measurements taken from gilts and sows as
118 indicators of stress and pain, including putative behavioral indicators of pain identified
119 previously (Ison et al., 2016b). Putative behavioral indicators of pain were also measured in
120 the hours preceding farrowing, to investigate the onset of potentially painful uterine
121 contractions as farrowing approached.

122

123 **2. Materials and Methods**

124

125 This experiment was carried out under UK Home Office License, in compliance with
126 EU Directive 2010/63/EU, and following approval from the SRUC Animal Welfare and
127 Ethical Review Body (AWERB).

128

129 **2.1. Gilt and sow housing and husbandry**

130

131 Thirty-two Large White \times Landrace multiparous (sows; mean parity 4.63 ± 0.43
132 (SEM)) and 24 primiparous (gilts) breeding pigs were used in this study. The gilts in this
133 study were acquired directly from a breeding company (The Camborough®, PIC, UK),
134 whereas the sows were home bred from an older genetic line of the same breed. Experimental
135 procedures were carried out at the SRUC pig research farm (Midlothian, UK), with gilts and
136 sows farrowing in 9 batches between February and October 2014. Approximately 4 days
137 before the expected farrowing date, gilts and sows were moved into individual conventional
138 farrowing crates (1.8×0.5 m), with solid concrete flooring (1.8×1.5 m), a small slatted area
139 at the back (0.5×0.5 m) and a water and feed trough at the front. A heated creep area ($1.5 \times$
140 0.65 m) was accessible to piglets, positioned in front of the water and feed trough. Individuals
141 were fed a standard pelleted lactation diet (16.4% crude protein, 6.8 % crude oils and fats,
142 4.0% crude fiber, 5.8% crude ash, 13.8% moisture, 0.8% calcium, 0.94% lysine, 0.25%
143 methionine, 0.51% phosphorus and 0.22% sodium) twice a day at 0745 and 1530 and had
144 continuous access to fresh water. Gilts and sows were fed based on a feed chart, which was
145 adjusted slightly according to the size, body condition and appetite of the individual (e.g. gilts
146 were fed slightly less than sows and a lower body condition score was given slightly more
147 feed) and increased gradually from the day of farrowing to weaning. Gilts and sows were
148 cleaned out daily at the morning feed, when they were provided with 2 handfuls of fresh,
149 long-stemmed straw. Additional straw was added and any manure removed at the afternoon

150 feed in the days preceding farrowing, to provide adequate nest-building material and maintain
151 hygiene for the newborn piglets. Lights were switched on immediately before the morning
152 feed, turned off at 1630 and an additional night-light was provided in the center of each room
153 of crates.

154

155 Cross-fostering was conducted where necessary to even up litter sizes, in order to
156 maximize piglet survival as per normal farm practice. Cross fostering was conducted
157 independently of experimental treatments and was conducted only after the 6 hour post-
158 injection data collection point. When litter sizes were uneven, the largest piglet or piglets
159 were removed and placed on a gilt or sow with a smaller litter. Piglets were given an
160 intramuscular injection of iron on day 3 post-farrowing, as per normal farm practice. On the
161 4th week after farrowing (mean piglet age 26.39 ± 0.20 (SEM)), weaning took place, which
162 involved moving gilts and sows out of the crates, followed by ear tagging and vaccination
163 (CircoFLEX) of the piglets, which is normal farm practice.

164

165 **2.2. Blinding and drug treatments**

166

167 This study was a randomized, blinded, placebo controlled trial, where gilts and sows
168 were randomly allocated to receive a single intra-muscular (IM) injection of ketoprofen
169 (Ketofen; Merial Animal Health Limited, Harlow, Essex, UK) or the equivalent volume of
170 saline, 90 minutes following the birth of the last piglet. Ketoprofen-treated gilts and sows
171 received 3 mg per kg bodyweight or 1 ml per 33 kg bodyweight rounded down to the nearest
172 0.5 ml (treated), and those that received the saline as a placebo control were given the
173 equivalent volume (control). The 56 individuals were balanced as much as possible across the
174 different batches and for parity between the 2 treatment groups (gilts: treated, $n = 11$, control,
175 $n = 13$; sows: parity 2 to 4; treated, $n = 9$, control, $n = 8$; parity 5 to 7; treated, $n = 5$, control,
176 $n = 6$; parity 8+; treated, $n = 2$, control, $n = 2$). After the first experimenter had allocated
177 individuals to the 2 treatments groups, a second experimenter added the ketoprofen or saline
178 dose to individual brown medicine bottles, sealed with rubber stoppers (Adelphi Healthcare
179 Packaging, Haywards Heath, West Sussex, UK), which were labelled only with the individual
180 gilt or sow ear tag identification number. Ketofen contains the active ingredient ketoprofen at
181 100 mg/ml contained in a solution of 1 arginine, benzyl alcohol (10 mg/ml), citric acid
182 monohydrate, and water. It is a clear colorless solution, with low viscosity, making it
183 indistinguishable from the saline placebo to the third experimenter who administered the
184 injection, and collected the data, who was blind to the treatments.

185

186 In the days leading up to farrowing, individuals were closely monitored for signs of
187 farrowing, which included observation at twice daily feeding, and through remote monitoring
188 using a digital surveillance system. Once the piglet expulsion phase began, the time of each
189 piglet birth was recorded and when a 90 minute gap from the last piglet birth and the gilt or
190 sow appeared to have finished farrowing, the ketoprofen or saline treatment was
191 administered. Ketoprofen was injected into the neck muscle, just behind the ear using an 18
192 gauge, 1.5 inch needle attached to a PVC extension tube and using a 10 or 20 ml syringe
193 (Henry Schein Animal Health, Dumfries, Dumfries and Galloway, UK). Individuals were
194 then left undisturbed for 6 hours, before sampling took place (see below). In case of any
195 adverse effects of the drug being administered, and if any additional treatments were needed
196 within 24 hours (e.g. if gilts/sows showed signs of PPDS), the farm manager was given a list
197 of the pig ear tags and treatments and was responsible for any additional treatments that were
198 required, to avoid double dosing with NSAIDs.

199

200 **2.3. Gilt and sow behavior**

201

202 Closed-circuit television (CCTV) cameras (LL20, infra-red cameras, FR concepts,
203 Ireland) were mounted above each farrowing crate, and were connected to a computer to
204 record behavior using GeoVision Digital Surveillance System software (ezCCTV ltd, Herts,
205 UK). This surveillance system enabled remote monitoring. Digital video footage was
206 collected and stored to be observed later using The Observer XT 11.0 (Noldus Information
207 Technology, Wageningen, The Netherlands). Continuous behavioral observations were made
208 for 5 minutes every hour for 24 hours before the first piglet birth. Behavior recorded included
209 gilt and sow posture and a set of other spontaneous putative pain behaviors described in
210 Table 1. After farrowing, individuals were observed for 15 minutes every 1.5 hours, starting 1
211 hour after the last piglet was born for a total of 8 observations. After these 8 observations, 3
212 more 15 minute observations were made at 3 hour intervals. Therefore, the first 15 minute
213 observation was 0.5 hours before the injection (Pre -0.5) and the remaining observations
214 afterwards (Post 1.0, 2.5, 4.0, 5.5, 7.0, 8.5, 10.0, 13.0, 16.0, 19.0). Posture and putative pain
215 behaviors were also observed during these post-farrowing observations (Table 1).

216

217 **2.4. Gilt and sow physiology**

218

219 **2.4.1. Salivary cortisol**

220

221 Gilts and sows were saliva sampled after the morning (between 0845 and 0915) and
222 afternoon (between 1545 and 1615) feed pre-farrowing, on the day of farrowing, including an
223 additional sample 6 hours after the injection, then up to 7 days after farrowing. Individuals
224 were offered 2 large cotton buds (Millpledge Veterinary, Clarborough, Nottinghamshire, UK)
225 on which to chew for approximately 30 seconds or until saturated with saliva. The cotton
226 buds were placed into pre-labelled Salivette tubes (SARSTEDT AG & Co., Nümbrecht,
227 Germany), which were sealed and centrifuged for 5 minutes at 1400 g. The supernatant was
228 pipetted into pre-labelled 1.5 ml tubes and stored at -20°C for assay at a later date. For each
229 gilt and sow, samples from 2 days pre-farrowing, the day of farrowing, and days 1, 2, 3, 5,
230 and 7 post farrowing were assayed.

231

232 On the day of assay, saliva samples were removed from the freezer to thaw. All
233 samples were centrifuged at 2300 g for 5 minutes and the supernatant pipetted into a clean,
234 pre-labelled 1.5 ml tube to remove any particulate matter in the sample. Using Coat-A-Count
235 cortisol radioimmunoassay kits (Siemens Healthcare Diagnostics Ltd., Camberley, UK), 200
236 µl of standards, quality controls (QCs), and undiluted unknown saliva samples were pipetted
237 in duplicate into the antibody coated assay tubes, followed by 1 ml of ¹²⁵I tracer. A standard
238 curve was placed at the beginning and end of the assay, followed by 3 QCs (low, medium and
239 high) and another set of QCs were added to the middle of the assay. Assay tubes were stored
240 at 4 °C overnight for incubation. On the following day, the tubes were emptied (apart from
241 the total count tubes), counted with a gamma counter (LKB-Wallac 1261), and concentrations
242 calculated using AssayZap software (Biosoft, Cambridge, UK). Salivary cortisol
243 concentrations were reported as nanograms per milliliter (ng/ml) and the range of the assay
244 was 0.125 to 50 ng/ml.

245

246 The lower and upper detectable limits for the salivary cortisol assays were 0.434 and
247 54.423 ng/ml respectively. The average intra-assay coefficient of variation (CV) across 7
248 assay runs was 5.92% (7.06%, 6.67%, 5.79%, 4.96%, 6.07%, 5.43% and 5.44% for assay

249 runs 1 to 7 respectively). The inter-assay CV, based on sets of 3 (low, medium and high)
250 samples per assay run of known cortisol concentration was 10.56%.

251

252 **2.4.2. C-reactive protein (CRP) and cytokines (IL1 β , IL6, and TNF α)**

253

254 At 7.5 hours after the injection, a blood sample was collected from the dams. Due to a
255 previous study, showing greater putative pain behavior in the first 6 hours after the last piglet
256 birth (Ison et al., 2016b), all individuals were left as undisturbed as possible during this time.
257 Therefore, at 6 hours after the treatment/control injection, each gilt or sow had her tail and ear
258 cleaned with antiseptic wipes. A topical local anesthetic cream (EMLA®) was then applied to
259 the tail and ear, and cling film was placed around the tail over the cream and held in place
260 with micro-pore tape, to form an occlusive dressing. To allow the anesthetic cream to take
261 effect, all other samples and measurements were taken, and blood sampling took place
262 approximately 1.5 hours after the cream was applied. The tail vein was first attempted and if
263 unsuccessful, the ear vein was used; it was possible to sample from the tail for 17 gilts and 27
264 sows and the ear vein was used for the remaining 7 gilts and 5 sows (gilts were tail docked,
265 sows had intact tails). Firstly the tail vein was attempted, by holding the tail up, away from
266 the body, feeling for the last moveable tail joint. At this point, on the midline of the tail, a 20
267 gauge, 1 inch vacutainer needle, attached to a vacutainer needle holder (Henry Schein Animal
268 Health, Dumfries, Dumfries and Galloway, UK) was inserted at approximately a 45° angle. A
269 6 ml EDTA vacutainer (Henry Schein Animal Health, Dumfries, Dumfries and Galloway,
270 UK) was pushed towards the needle to fill with blood and if the vein was not found, the
271 needle was gently moved around until it was. If the tail vein was not successful after 3
272 attempts, the ear vein was used. Polypropylene tubing was wrapped around the ear and held
273 in place using locking forceps, to allow the ear veins to become prominent. Once raised, a 21
274 gauge 0.75 inch winged vacutainer needle (Henry Schein Animal Health, Dumfries, Dumfries
275 and Galloway, UK) attached to a vacutainer needle holder was inserted into the most
276 prominent raised vein. The forceps were then removed and a 6 ml EDTA vacutainer was
277 pushed onto the needle to collect blood, again, if blood was not drawn, the needle was gently
278 moved until blood appeared in the vacutainer. Blood was immediately placed on ice, then
279 moved straight into a refrigerated centrifuge (at 4 °C) and centrifuged for 15 minutes at 1400
280 g. Plasma was pipetted into 4 1.5 ml pre-labelled tubes and frozen at -80 °C to be assayed at a
281 later date.

282

283 Plasma samples were assayed for C-reactive protein (CRP) using a commercially
284 available ELISA kit (Alpco ®, Salem, New Hampshire, USA), extensively used previously to
285 measure levels of CRP in studies of pigs (e.g. (Che et al., 2011)). Samples were removed
286 from the -80°C freezer the evening before the assay and placed at 4°C overnight to gradually
287 defrost. On the day of the assay, samples were placed at room temperature for 30 minutes and
288 were centrifuged for 1 minute at 865 g before any further preparation took place. An initial
289 test plate was run to establish the best dilution for the samples, with a 1:5000 dilution chosen.
290 Dilution buffer was prepared according to the instructions and samples were diluted using
291 plain 96-well V-bottomed plates. A plate layout was created with standards, blanks and
292 samples. According to the kit instructions, the CRP calibrator was reconstituted and the 6
293 standards created using a 2-fold serial dilution. Assay buffer was used for a zero standard and
294 blanks. According to the plate layout, 130 μ l of standards, samples and blanks were then
295 pipetted into a plain V-bottomed plate, before 100 μ l was pipetted into the antibody coated
296 plate supplied with the kit. The assay was then conducted according to the manufacturer's
297 instructions. Samples were assayed across 2 plates, with treatments and gilts and sows
298 balanced across plates as much as possible and 3 samples and a reference serum that came

299 with the kit, were run on both plates to act as quality controls (QCs) to calculate inter-plate
300 variation. The plate was read using a MultiskanTM FC Microplate Photometer plate reader and
301 results calculated using a 5 point logistic regression curve using Thermo Scientific SkanItTM
302 for MultiskanTM FC software (version 2.5.1) (Thermo Fisher Scientific Inc, Waltham,
303 Massachusetts, USA). The assay range was 6.25 to 200 ng/ml and sample results with a CV%
304 of greater than 10% were repeated.

305

306 Also using the gilt and sow plasma, simultaneous quantification of 3 porcine
307 cytokines (interleukin-1 β (IL1 β), interleukin-6 (IL6) and tumor necrosis factor α (TNF α))
308 in a single plasma sample was conducted using multiplex fluorescent microsphere
309 immunoassays (FMIA) with the BioPlex[®] 200 system (Bio-Rad Laboratories Ltd., Hemel
310 Hempstead, UK). Details of the development, including optimization of this multiplex assay
311 for porcine samples is currently in press (Hall and Zanella) and further details for running the
312 assay have also been published previously (Hall et al., 2015). Briefly, capture antibodies for
313 each cytokine were coupled to microspheres (beads) using the Bio-Plex amine coupling kit
314 following the manufacturer's instructions (Bio-Rad Laboratories Ltd., Hemel Hempstead,
315 UK). Samples were removed from the -80 °C freezer on the morning of the assay and put on
316 ice before further processing. Samples were undiluted for the assay. Standards were created
317 using a 2.5 fold serial dilution to create 7 standards from 10,000 to 40.96 pg/ml for each
318 cytokine, Table 2 (adapted from Hall and Zanella) shows the source of the recombinant
319 protein for the standards and the capture and detection antibodies. To run the FMIA, firstly a
320 mastermix solution of at least 3600 beads per region (cytokine) per well was produced, then
321 50 μ l was added to each well of a black flat bottomed 96-well plate (BioRad catalogue
322 #171025001). The plate was then washed twice with phosphate buffered saline (PBS) using a
323 Bio-Plex Pro II wash station (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK), with the
324 magnetic plate washing carrier installed. Then, 50 μ l of standards, unknown samples and
325 blanks were added to the plate, which was incubated in the dark for 1.5 hours at room
326 temperature, with shaking at 700 rpm. Following this, the plate was washed 3 times (as
327 before) and 75 μ l of detection antibody was added and the plate was incubated for 40 minutes
328 as before. After another 3 washes, 50 μ l of Streptavidin-PE solution (Bio-Rad Laboratories
329 Ltd., Hemel Hempstead, UK) was added and the plate was incubated for a further 20 minutes.
330 The plate was then washed 3 times, 125 μ l of assay buffer was added and the plate was
331 incubated with shaking for 5 minutes. The reaction was measured using the BioPlex[®] 200
332 instrument and analyzed using the Bio-Plex manager software (version 6.1), using a 5 point
333 logistic regression to calculate a standard curve for each cytokine. For each well, mean
334 fluorescent intensity was analyzed for 100 beads for each cytokine. Samples were run across
335 3 plates and CV% of greater than 20% for any of the cytokines were repeated and if the CV
336 failed to fall below 20%, the result was treated as a missing value.

337

338 The lower and upper detectable differences for the cytokine multiplex assay was 4.61
339 and 817.54 pg/ml for IL1 β , 0.23 and 1461.22 pg/ml for IL6, and 0.42 and 162.02 pg/ml for
340 TNF α . The intra-assay CV% for IL1 β , IL6 and TNF α was 0.89%, 2.89% and 6.13%
341 respectively and the inter-assay CV% was 6.40%, 5.25% and 6.42% for IL1 β , IL6 and TNF
342 α respectively. For C-reactive protein, the intra-assay CV% was 1.77%.

343

344 **2.5. Data analysis**

345

346 Unless stated at the start of each results section, data were available for all
347 individuals. There were 11 gilts and 16 sows in the ketoprofen treated group (treated) and 13
348 gilts and 16 sows in the saline control group (control). An additional factor in this study was

349 that 13 individuals, 5 gilts (4 treated and 1 control) and 8 sows (4 treated and 4 controls)
350 required additional treatment in the days after farrowing for PPDS. Therefore, data were
351 analyzed by treatment (treatment vs. control), parity group at the level of gilt vs. sow and
352 whether additional treatment was needed (yes vs. no). All data were analyzed and descriptive
353 statistics calculated using R version 3.3.3 (R core team, 2013). Data manipulation and
354 summaries were conducted using the ‘dplyr’ package and plotted using the ‘ggplot2’
355 package. Results were considered statistically significant at $P < 0.05$ and tendencies will be
356 discussed at $P < 0.1$.

357

358 Behavioral variables, including pre-farrowing and post-injection behaviors (Table 1)
359 were analyzed using generalized linear mixed modeling, with the glmmPQL function in the
360 ‘MASS’ package. All models included farrowing batch and dam ID as random variables. Pre-
361 farrowing behavior models included gilt or sow and time and their interactions as fixed
362 factors. Post-injection behavior models included time, gilt or sow, additional treatment (yes
363 or no), and treatment (treated or control) and their interactions as fixed factors. All models
364 analyzing count data used the negative binomial family, and for duration variables, a two-step
365 hurdle model was used (Min and Agresti, 2005). This was conducted by first analyzing the
366 variables as a binomial outcome (if the behavior occurred or not: 1,0), then for the instances
367 where the behavior did occur, the durations were analyzed with a gamma distribution and log
368 link function.

369

370 Physiological variables were analyzed with linear mixed models, using the lmer
371 function in the ‘lme4’ package, with gilt/sow, treatment, additional treatment and their
372 interactions as fixed factors. Cytokines and CRP were analyzed with batch, and salivary
373 cortisol with batch and dam ID in the random model. The salivary cortisol concentrations
374 were log transformed to improve the model fit, and also included sampling time as a fixed
375 factor, and post-hoc comparisons between fixed factors with day were made using the
376 lsmeans function in the ‘lsmeans’ package.

377

378 **3. Results**

379

380 **3.1. Pre-farrowing behavior**

381

382 Pre-farrowing behavior was observed for 55 of the 56 individuals; data were missing
383 for 1 sow in the control treatment group. Tremble and tail flick were not analyzed pre-
384 farrowing as these behaviours were rare. Figure 1 shows gilt and sow postures (A), and
385 putative pain indicators (B) by hour pre-farrowing. All behaviors apart from sit differed by
386 hour pre-farrowing (Table 3). Lying lateral decreased from 12 hours pre-farrowing, whilst all
387 other postures and the frequency of posture changes increased. In the last few hours before
388 the onset of farrowing, lying lateral increased again, while other postures decreased, but the
389 frequency of posture changes remained high, indicating restlessness (Figure 1 A). In line with
390 this restlessness, all putative pain behaviors followed a similar pattern: increasing in the last
391 few hours before the first piglet birth (Figure 1 B). Table 3 also contains the pre-farrowing
392 behaviors observed by parity group (gilt or sow), along with the gilt vs. sow and gilt/sow \times
393 hour pre-farrowing interactions. Lie lateral, back arch and the frequency of posture changes
394 differed overall between gilts and sows (Table 3), with greater lateral lying and more back
395 arches observed in sows compared with gilts, and more posture changes in gilts than sows.
396 There was a different pattern of lying lateral, back arch and posture changes between gilts

397 and sows across the 24 hours pre-farrowing, with significant gilt/sow \times time pre-farrowing
398 interactions (Table 3).

399

400 **3.2. Post injection behavior**

401

402 Post injection behavior was observed for 54 of the 56 individuals; data were excluded
403 for animals (1 control sow and 1 treated sow) who had more piglets after the injection was
404 given. Figure 2 shows the frequency or duration of putative pain behaviors, and the frequency
405 of posture changes observed by hour post injection. As shown, back arch, tremble and back
406 leg forward were lowest 7 hours after the injection, as posture changes were highest, which
407 coincided with the physiological data collection and piglet processing. Posture changes, paw
408 and tail flick were not analyzed post injection as these behaviors were infrequent. Back arch
409 ($t = -2.9$, $P = 0.004$) differed by time, decreasing by hour post-injection, whereas back leg
410 forward ($t = 1.7$, $P = 0.09$) and tremble ($t = -0.7$, $P = 0.5$) did not differ. Back leg forward,
411 back arch and tremble did not differ by treatment, additional treatment or their interactions
412 with time (Table 4). However, back leg forward and back arching differed between gilts and
413 sows, along with significant gilt/sow \times time interactions (Table 4; Figure 3). Gilts exhibited
414 fewer back arches and less back leg forward behavior than sows, who began with high
415 values, decreasing with hour post-injection, whereas gilts remained more stable across the
416 observations.

417

418 **3.3. Gilt and sow physiology**

419

420 **3.3.1. Salivary cortisol**

421

422 One sow was not saliva sampled due to aggression towards the sampler, so data
423 presented are for 24 gilts and 31 sows. Salivary cortisol concentrations differed by day, with
424 the lowest levels detected 2 days before farrowing (-2), rising significantly on day -1 and
425 even more on the day of farrowing (Figure 4 A). Cortisol levels remained high up to day 7
426 post farrowing, with the highest levels measured on day 3 post farrowing. Cortisol did not
427 differ overall by treatment ($t = 0.8$, $P = 0.4$) or between control and treated individuals on any
428 sampling day (Figure 4 B). Gilts and sows did not differ overall ($t = 1.6$, $P = 0.1$), but
429 differed in salivary cortisol on day 0 ($t = -2.3$, $P = 0.02$) with higher concentrations for sows
430 than gilts, and on day 3 with higher concentrations for gilts than sows ($t = 2.4$, $P = 0.02$;
431 Figure 4 C). Individual requiring additional treatment or not post-farrowing due to PPDS did
432 not differ overall ($t = -0.3$, $P = 0.8$) by those that did not, but those requiring treatment
433 showed significantly higher cortisol on days 1 ($t = -3.0$, $P = 0.003$) and 2 ($t = -2.5$, $P = 0.01$)
434 post-farrowing (Figure 3 D).

435

436 **3.3.2. C-reactive protein (CRP) and cytokines (IL1 β , IL6, and TNF α)**

437

438 Due to a CV% of greater than 20% data are missing for interleukin-1 β (IL1 β) for 6
439 individuals, interleukin-6 (IL6) for 9 individuals and tumor necrosis factor α (TNF α) for 11
440 individuals. There were no significant differences for treatment, or by additional treatment for
441 any of the cytokines or CRP (Table 5), however, TNF α was higher in sows than gilts ($t =$
442 2.2 , $P = 0.04$), and CRP higher in gilts than sows ($t = -4.5$, $P < 0.001$).

443

444 **4. Discussion**

445

446 Non-steroidal anti-inflammatory drugs reduce pain and inflammation by reducing
447 prostaglandin synthesis through the inhibition of cyclo-oxygenase (COX) enzymes that
448 increase following cell damage (Hudson et al., 2008). Prostaglandins, however, are involved
449 in the process of parturition, as well as inflammation, and prostaglandins have been shown to
450 increase towards the onset of parturition in pigs, peaking at the time of birth (Gilbert, 2001).
451 Due to the inhibition of prostaglandins, NSAIDs have been used in human medicine to delay
452 parturition in cases of preterm birth (Olson, 2005). However, as these drugs cross the
453 placenta, NSAID use can result in detrimental side effects for the fetus, where prostaglandins
454 play an important role in developmental and physiological regulation (Bloor and Paech,
455 2013). This has led to concern over using these drugs in livestock during parturition or
456 immediately after fetal delivery when placental tissue is being passed. However, in the
457 current study, there were no apparent adverse effects following, to the piglets or dams, of the
458 administration of ketoprofen 1.5 hours after the last piglet was born. Two sows had more
459 piglets after the injection was given, 1 in the ketoprofen and 1 in the control group. In a
460 previous study in cattle, where the NSAID ketoprofen was administered immediately
461 following calving, treated dams tended to be less likely to retain fetal membranes than
462 untreated controls (Richards et al., 2009), and a recent study administering oral meloxicam at
463 the onset of farrowing reported no adverse effects and enhanced benefits (Mainau et al.,
464 2016). Therefore, it could be beneficial to administer an NSAID earlier, without detrimental
465 side effects and additional benefits include the provision of a therapeutic dose to piglets via
466 the sow's milk to treat pain and inflammation from routine management procedures in piglets
467 (Bates et al., 2014).

468

469 **4.1. Behavior**

470

471 Pre-farrowing behavioral observations of gilts and sows showed an increase in
472 activity, including a reduction in lying lateral, and an increase in other postures and posture
473 changes, indicative of nest building behavior (Algers and Uvnäs-Moberg, 2007). Similar to
474 previous studies, this change in activity, as shown by a reduction in lateral lying, was
475 apparent from 12 hours before the onset of farrowing (e.g. (Castrén et al., 1993; Jarvis et al.,
476 2001)), and was more pronounced for gilts, compared with multiparous sows (Jarvis et al.,
477 2001; Thodberg et al., 2002). The putative pain indicators back leg forward, back arch and
478 paw began to increase between 3 and 4 hours before the onset of farrowing. This coincides
479 with a change from nest-building activity to passivity, the increase in myometrial electrical
480 activity and increasing oxytocin concentrations before the onset of piglet expulsion (Castrén
481 et al., 1993; Taverne et al., 1979).

482

483 Overall, there were no treatment differences in behavior between ketoprofen and
484 control treated individuals post-injection. This was similar to previous studies measuring
485 posture and posture changes in the hours immediately after farrowing in relation to the
486 administration of analgesics (Hausmann et al., 1999; Mainau et al., 2012; Tenbergen et al.,
487 2014; Viitasaari et al., 2014). This means that it was not possible to validate the putative pain
488 behaviors as indicators of pain post-farrowing with the use of ketoprofen as an analgesic.
489 This drug, however, may not have provided adequate analgesia for the pain experienced in
490 the early post-farrowing period, which is likely to include visceral pain from uterine
491 contractions moving placental material along the birth canal and uterine involution as the
492 uterus returns to its normal size, as well as inflammation from the process of parturition
493 (Deussen et al., 2011). In rodent studies that measured similar putative pain behaviors in the
494 periparturient period, opioid analgesics reduced the expression of these behaviors, confirming
495 them as indicators of pain (Catheline et al., 2006; Mirza et al., 2013; Tong et al., 2008), and

496 opioid analgesics may be needed to confirm these behaviors as pain indicators in
497 periparturient sows.

498

499 The earlier administration of an NSAID, during or even before farrowing may
500 increase the effectiveness, by acting at peripheral and central sites before the onset of
501 nociceptive input (Ochroch et al., 2003). The use of analgesia administered before surgery
502 has been shown to reduce post-surgical pain, for example, the use of an NSAID administered
503 before castration in piglets (e.g. (Keita et al., 2010)). Earlier administration of oral
504 meloxicam, given as soon as possible at the onset of farrowing, improved piglet outcomes
505 (growth rate, weaning weight and IgG transfer) (Mainau et al., 2016), compared with studies
506 in which NSAIDs were given after farrowing (Ison et al., 2017; Mainau et al., 2012;
507 Viitasaari et al., 2013). This result may also be reflected in indicators of stress and pain in the
508 sow. However, the administration of NSAIDs before or during farrowing has the potential to
509 have adverse effects on the sow and piglets. As well as reducing pain and inflammation by
510 reducing prostaglandin and thromboxane through the inhibition of cyclooxygenase (COX)
511 enzymes (Hudson et al., 2008), NSAIDs could also inhibit parturition, as prostaglandin
512 increases towards the onset of farrowing and peaks at the time of birth (Gilbert, 2001). When
513 the NSAID indomethacin was administered at the onset of nest-building in sows, nest-
514 building behavior was reduced, and parturition tended to be delayed (Gilbert et al., 2002). In
515 addition, NSAIDs can cross the placenta, which can result in detrimental side effects for the
516 unborn fetus, where prostaglandins play an important role in developmental and
517 physiological regulation (Olson, 2005). As discussed, the earlier administration of oral
518 meloxicam demonstrated enhanced benefits, without detrimental side effects (Mainau et al.,
519 2016). Meloxicam is a selective COX-2 inhibitor (Engelhardt et al., 1995), which could make
520 it more suitable for the treatment of inflammation (Guerguerian et al., 1998) when given
521 earlier in the farrowing process than non-selective COX inhibitors, like ketoprofen (Fosse et
522 al., 2011). Therefore, it cannot be assumed that administering ketoprofen before or during
523 parturition would have similar benefits to meloxicam.

524

525 Gilts and sows were disturbed around the sampling and piglet processing time, around
526 7 hours after the injection, showing more posture changes and less putative pain behavior
527 around this time. This behavior then increased slightly in the remaining post injection
528 observations. There were large overall differences in the expression of putative pain behavior
529 between gilts and sows, with gilts showing less back arching and back leg forward behavior
530 post-injection than sows, and with a different profile of behavioral expression across the
531 observations. This is a paradoxical result as primiparous dams are generally considered to
532 experience more pain during the fetal delivery phase of parturition than multiparous dams
533 (Mainau and Manteca, 2011; Sheiner et al., 1998). In addition, farmers considered pain at
534 farrowing more often a problem for gilts than sows, and that a greater percentage of gilts than
535 sows have difficulty farrowing (Ison et al., 2016a). It could be that inexperienced gilts are
536 more reluctant to show signs of pain or exhibit pain in a different way to more experienced
537 sows. Alternatively, it is reported in human females that uterine contractions post-birth,
538 during the process of uterine involution as the uterus contracts and returns to its pre-gestation
539 size, is more painful for multiparous, compared with primiparous women due to the loss in
540 uterine tone (Deussen et al., 2011). These after-birth pains are enhanced during breast feeding
541 as oxytocin is released causing the uterus to contract, and the pain reported by human females
542 increases with parity, as well as an increase in the frequency and intensity of uterine
543 contractions recorded using tocodynamometry (Holdcroft et al., 2003). This could explain
544 why more back arching and back leg forward behavior was seen post-farrowing in sows
545 compared with gilts, as it is likely that these putative pain indicators are from pain due to

546 uterine contraction (Ison et al., 2016b). The authors of the human study suggested childbirth
547 could have induced central neural changes that increased the predisposition for pain during
548 the post-partum period (Holdcroft et al., 2003). Another explanation for the differences in
549 putative behavioral indicators of pain could be learned pain from previous experiences of
550 parturition. In humans, fear of pain is thought to contribute to labor-related anxiety, since
551 anxiety and pain are highly correlated (Lowe, 2002). Excessive anxiety can lead to increased
552 catecholamine secretion that may enhance nociceptive stimuli from the pelvis and increase
553 the perception of these stimuli at the cortical level (Lowe, 2002). Pain has been shown to be
554 under the constant influence of learning processes affecting the anticipation of, and responses
555 to future pain, as temporary periods of conditioned hyperalgesia were induced by both the
556 predictability and uncertainty of pain from an electric shock (Taylor et al., 2017). This has
557 interesting implications for the perception of farrowing pain in primiparous and multiparous
558 sows. The farrowing environment can be stressful, with different noises, odors and often
559 includes confinement to a farrowing crate. This could contribute to anxiety due to novelty in
560 inexperienced gilts, which could enhance the perception of pain, or be associated with a
561 painful event for experienced sows. Combining an already stressful environment, with fear of
562 pain for experienced sows could be contributing to an increase in putative behavioral
563 indicators of pain.

564

565 **4.2. Salivary cortisol**

566

567 For all gilts and sows, salivary cortisol concentrations increased from 2 days before
568 farrowing, to 1 day before farrowing and doubled on the day of farrowing. This is similar to
569 previous studies where salivary (Oliviero et al., 2008) and plasma (Jarvis et al., 1997, 2001;
570 Lawrence et al., 1994; Molokwu and Wagner, 1973) cortisol were also shown to increase in
571 the days leading up to and the day of farrowing. In this study, salivary cortisol concentration
572 remained high up to day 7 post farrowing, which is in contrast to previous studies where
573 plasma cortisol concentrations returned to pre-farrowing levels 2 days after farrowing in one
574 study (Molokwu and Wagner, 1973) and another study, but only for sows housed in free-
575 farrowing pens (Oliviero et al., 2008). However, similar to the current study, sows housed in
576 crates have previously been shown to have elevated salivary cortisol concentrations for at
577 least the 5 days post farrowing when cortisol was sampled (Oliviero et al., 2008). Therefore,
578 confining sows to a farrowing crate could be contributing to elevated salivary cortisol during
579 this time.

580

581 Gilts and sows showed different salivary cortisol profiles; in line with the behavioral
582 results, sows had higher cortisol on the day of farrowing, but gilts had higher concentrations
583 on day 3 post farrowing. This is an interesting result as previous research has shown a greater
584 increase in cortisol in the pre-farrowing nest building phase in crated compared with penned
585 gilts (Jarvis et al., 1997), but a smaller difference was seen in sows (Jarvis et al., 2001).
586 Therefore, a greater pre-farrowing and farrowing day cortisol concentration was expected for
587 the gilts, compared with the sows in this study. However, in the previous studies, samples
588 were taken in relation to farrowing, whereas in this study, samples were collected at
589 husbandry times, so may not have picked up the more detailed alterations in cortisol profiles
590 with nest building and leading up to farrowing. However, it was important to measure
591 salivary cortisol at the same time each day due to diurnal variations in the concentrations of
592 this hormone, as well as limit the behavioral disturbance caused by saliva sampling. An
593 increase in cortisol is not necessarily an indication of psychological stress, it also indicates
594 the involvement of the metabolic process, including the mobilization of energy to create
595 homeostasis (Mormède et al., 2007). Nest-building activity, farrowing and the onset of

596 lactation is physically demanding, resulting in an increased energy requirement, which could,
597 in part, explain the increase in salivary cortisol concentrations, especially considering the
598 feed restrictions imposed on the modern domestic sow. Therefore, the different salivary
599 cortisol profiles shown by gilts and sows could reflect different metabolic demands
600 associated with farrowing and lactation between these 2 groups of animals. It should be
601 noted, however, that a 24 hour feed restriction treatment on grower pigs failed to elicit a
602 salivary cortisol response (Ott et al., 2014). Therefore, another explanation for the gilt/sow
603 difference in salivary cortisol could be from individual genetics, as the gilts were obtained
604 directly from a breeding company, whereas, the sows were home-bred from older genetic
605 stock.

606
607 Higher concentrations of salivary cortisol on days 1 and 2 post-farrowing was
608 measured in individuals that required additional treatment compared to healthy animals. It is
609 possible that as these animals consumed less feed, they may have needed to mobilize energy
610 reserves and increased cortisol could be an indicator of this. In addition, it could also be that
611 being unwell is stressful and unpleasant, causing an increased cortisol concentration. In a
612 study where plasma cortisol was measured in sows experimentally inoculated with
613 *Escherichia coli* into the mammary gland on the day of farrowing, 4 out of 12 sows in the
614 inoculated group developed clinical signs of mastitis (Zhu et al., 2004). All sows showed an
615 increase in cortisol associated with farrowing, but the 4 sows that developed clinical signs of
616 mastitis had a greater cortisol increase. Another study showed greater plasma cortisol levels
617 in sows with mastitis-metritis-agalactia (MMA), compared to healthy sows on days 1, 5, 10,
618 15 and 20 post-farrowing (Mirko and Bilkei, 2004). In order to better evaluate stress
619 responses in pigs, measuring a variety of salivary biomarkers is recommended, including
620 indicators of the sympathetic adrenomedullary system, as well as the hypothalamic–pituitary–
621 adrenocortical axis (Ott et al., 2014).

622 623 **4.3. C-reactive protein (CRP) and cytokines**

624
625 To minimize disturbance in order to enhance the quality of behavioral data obtained
626 during this study, only one blood sample was taken for analysis of CRP and cytokines. It
627 should be noted that one sample, with no baseline, limits the interpretation of the CRP and
628 cytokine results in this study. In addition, the impact of the blood sampling itself on plasma
629 concentrations of these biomarkers cannot be discounted, despite efforts to minimize
630 sampling stress by sampling from the tail vein, not using additional restraint (other than
631 farrowing crate housing) and using a topical anesthetic. Despite limitations of interpretation,
632 the results will be discussed in relation to previous studies measuring APPs and pro-
633 inflammatory cytokines in the periparturient period.

634
635 Acute phase proteins (APPs) such as CRP, and pro-inflammatory cytokines are useful
636 measures of inflammation and tissue damage, used to monitor, detect and diagnose disease
637 (Petersen et al., 2004). CRP has been identified as a good marker of inflammations in pigs,
638 increasing 8-fold with experimentally induced sterile inflammation, peaking at 2 days after
639 the injection of turpentine (Eckersall et al., 1996). Previous studies have shown an increase in
640 APPs and cytokines in relation to farrowing, regardless of other treatments being studied,
641 which could be an indication of tissue damage and inflammation associated with the
642 farrowing process (Sorrells et al., 2007; Szczubiał and Urban-chmiel, 2008; Viitasaari et al.,
643 2013; Zhu et al., 2004). Neither gilt or sow CRP nor the cytokines IL1 β , IL6 or TNF α
644 differed with ketoprofen treatment, which was similar to a previous study where APPs were
645 measured in sows in relation to the administration of ketoprofen post farrowing (Viitasaari et

646 al., 2013). In this previous study, the APPs haptoglobin (Hp) and serum SAA were measured
647 on the day before, the days of, then 5 and 14 days post-farrowing, demonstrating that SAA,
648 but not Hp was elevated in response to farrowing. On day 5, SAA was more elevated in the
649 ketoprofen treated sows, compared with controls, which the authors suggested could be due
650 to tissue irritation from the repeated ketoprofen injection (Viitasaari et al., 2013), but no such
651 result was seen with CRP in the current study. Although CRP was only measured at one time
652 point in this study, due to high values obtained, in comparison to other studies (Kováč et al.,
653 2008; Oravainen et al., 2006; Sorensen et al., 2006), it is likely that CRP was elevated in
654 response to parturition, but further samples would need to be taken to confirm this. It is also
655 worth mentioning that, compared with the current study, 2 previous studies where IL6 and
656 TNF α were measured using ELISA in parturient sows obtained higher values of TNF α and
657 lower values of IL6 (Szcubiał and Urban-chmiel, 2008; Zhu et al., 2004) and another study
658 showed similar values (Papadopoulos et al., 2009).

659
660 Primiparous sows had higher plasma concentrations of CRP than multiparous sows,
661 and TNF α was greater for sows than gilts. In a previous study, parity influenced serum
662 concentrations of Hp in that primiparous sows had higher values than multiparous sows
663 (Verheyen et al., 2007). In contrast to salivary cortisol, no differences in plasma
664 concentrations of CRP, IL1 β , IL6 or TNF α were detected between sows requiring additional
665 treatment, compared with those that did not. In a study where sows were experimentally
666 inoculated with intra-mammary *E. coli*, elevated IL6 and TNF α was measured in inoculated
667 sows 24 hours post-inoculation, and was greater in sows that developed clinical signs of
668 mastitis (Zhu et al., 2004). Another study detected a difference in IL6 and TNF α in sows
669 with MMA, compared with healthy controls at sampling points pre- and post-farrowing, as
670 well as detecting an increase in these cytokines with parturition (Szcubiał and Urban-chmiel,
671 2008). It is possible that 6 hours after farrowing may have been too early to detect a
672 difference, as a previous study detected a difference at 48-72 hours, but not 12-24 hours after
673 parturition (Szcubiał and Urban-chmiel, 2008) or that the degree of PPDS in the current
674 study was not severe enough to create an acute phase response. Another study found no
675 difference in CRP or Hp in sows with vulvar discharge syndrome (VDS), compared with
676 healthy controls, although severe cases of VDS were not seen (Oravainen et al., 2006). It is
677 important to note that previous studies involved clinical examinations of sows and confirmed
678 the involvement of bacterial pathogens, which was not done in this study.

679 680 **5. Conclusion**

681
682 In line with the previous paper, reporting the production results from the same study,
683 which did not demonstrate clear benefits to the immediate post-farrowing administration of
684 ketoprofen (Ison et al., 2017), results of the current study failed to show a change in
685 indicators of pain, stress and inflammation with the use of post-farrowing ketoprofen. Further
686 investigation on the timing and types of drug administration, including different regimes for
687 primiparous and multiparous sows could enable more targeted use of drugs, with potential for
688 improved sow and piglet outcomes. However, this study did highlight interesting differences
689 between gilt and sow behavior and physiology, providing a novel insight into the farrowing
690 experience for naïve and experienced individuals. It is generally thought that the piglet
691 expulsion phase is likely to be more painful and/or problematic for gilts than sows, whereas
692 the current study showed that pain in the immediate post-farrowing period could be greater
693 for sows than gilts. This has implications for the management of farrowing sows, including
694 the timing, and type of potential management interventions, including closer observation of
695 experienced sows in the hours post-farrowing.

696

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698

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707 Hokkaido, Japan 14-17 September 2015 (Ison and Rutherford, 2015).

708

709 **Author Contributions Statement**

710

711 SI was responsible for the experimental design, data collection and analysis, and manuscript
712 preparation. KR provided significant supervision and advice at all stages of the study. CA and
713 SJ provided advice on the experimental design and manuscript preparation, including useful
714 input into the interpretation of results. SH conducted the cytokine assays and assisted with the
715 manuscript preparation.

716

717 **Conflict of interest statement**

718

719 None of the authors of this paper has a financial, personal or professional relationship with
720 other people or organizations that could inappropriately influence or bias the content of this
721 paper.

722

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946

947 Table 1. Ethogram of behaviors observed during the study. Behaviors were recorded as a
 948 duration in seconds (state) or frequencies/counts (event).
 949

Behavior	Description	State	Event
Posture	Stand	Upright, with all feet on floor	✓
	Sit	Front legs straight and back end down on the floor	✓
	Kneel	Front knees on the floor, with back legs straight	✓
	Lie lateral	Lying on one side with udder exposed	✓
	Lie ventral	Lying with the udder on the floor	✓
Spontaneous behaviors	Tremble	Visible shaking as if shivering when in a lateral lying position	✓
	Back leg forward	In a lateral lying position, the back leg is pulled forward and/or in towards the body	✓
	Back Arch	In a lateral lying position, one or both sets of legs become tense and are pushed away from the body and/or inwards towards the center, forming an arch in the back	✓
	Tail flick	Tail is moved rapidly up and down	✓
	Paw	In a lateral lying position, the front leg is scraped in a pawing motion	✓
Piglet birth	A piglet is fully expelled from the sow	✓	✓

950 Table 2. Capture and detection antibodies (Ab, with optimised concentrations), recombinant
 951 protein for standards and bead region used for each cytokine (interleukin-1 β (IL1 β),
 952 interleukin-6 (IL6) and tumour necrosis factor α (TNF α)) in the multiplex assay. Adapted
 953 from Hall and Zanella.

Analyte	Reagent	Catalogue #	Description/bead region	Optimized antibody concentration ($\mu\text{g/ml}$)/bead region	Source
IL1 β	Capture Ab	MAB6811	Mouse anti-porcine	20	R&D systems
	Detection Ab	BAF681	Biotinylated goat anti-porcine	0.5	R&D systems
	Standard	681-PI	Recombinant Porcine IL-1 β	-	R&D systems
	Bead	MC10029	Pro Magnetic COOH Beads 29	29	Bio-Rad
IL6	Capture Ab	AF686 BAF686	Goat anti-porcine	20	R&D systems
	Detection Ab	686-PI	Biotinylated goat anti-porcine	0.5	R&D systems
	Standard	MC10026	Recombinant Porcine IL-6	-	R&D systems
	Bead		Pro Magnetic COOH Beads 26	26	Bio-Rad
TNF α	Capture Ab	MAB690	Mouse anti-porcine	20	R&D systems
	Detection Ab	BAF690	Biotinylated goat anti-porcine	0.5	R&D systems
	Standard	690-PT	Recombinant Porcine TNF- α	-	R&D systems
	Bead	MC10055	Pro Magnetic COOH Beads 55	55	Bio-Rad

954 Ab: antibody, Source: R&D Systems, (Abingdon, UK), Bio-Rad, (Hemel Hempstead, UK)

955 Table 3. Pre-farrowing a) putative pain behaviors and b) postures and posture changes with
 956 mean \pm SEM for gilts and sows, and showing the statistical results for gilt vs. sow, and the
 957 gilt/sow \times hour interaction.

	Gilts vs. sow			Hour pre-farrowing	Gilt/sow \times hour
	Gilt	Sow	t, <i>P</i>	t, <i>P</i>	t, <i>P</i>
<u>a) Putative pain indicators</u>					
Back leg forward, seconds	46.17 \pm 3.61	43.68 \pm 2.80	-0.9, 0.4	2.5, 0.01	0.6, 0.6
Back arch, frequency	0.25 \pm 0.03	0.35 \pm 0.03	3.5, 0.001	3.6, <0.001	-2.6, 0.009
Paw, frequency	0.15 \pm 0.04	0.18 \pm 0.03	0.3, 0.8	4.4, <0.001	0.1, 0.9
<u>b) Posture</u>					
Stand, seconds	62.84 \pm 4.38	45.39 \pm 3.06	-1.7, 0.1	-3.3, 0.001	0.9, 0.4
Sit, seconds	22.05 \pm 2.31	27.20 \pm 2.33	0.6, 0.6	-0.7, 0.5	-0.3, 0.7
Lie lateral, seconds	150.33 \pm 5.67	174.11 \pm 4.88	2.2, 0.03	-3.3, <0.001	-2.7, 0.008
Lie ventral, seconds	62.64 \pm 4.06	50.48 \pm 3.53	1.0, 0.3	-3.4, <0.001	-1.4, 0.1
Posture changes, frequency	1.44 \pm 0.08	1.22 \pm 0.07	-3.0, 0.005	7.1, <0.001	2.7, 0.007

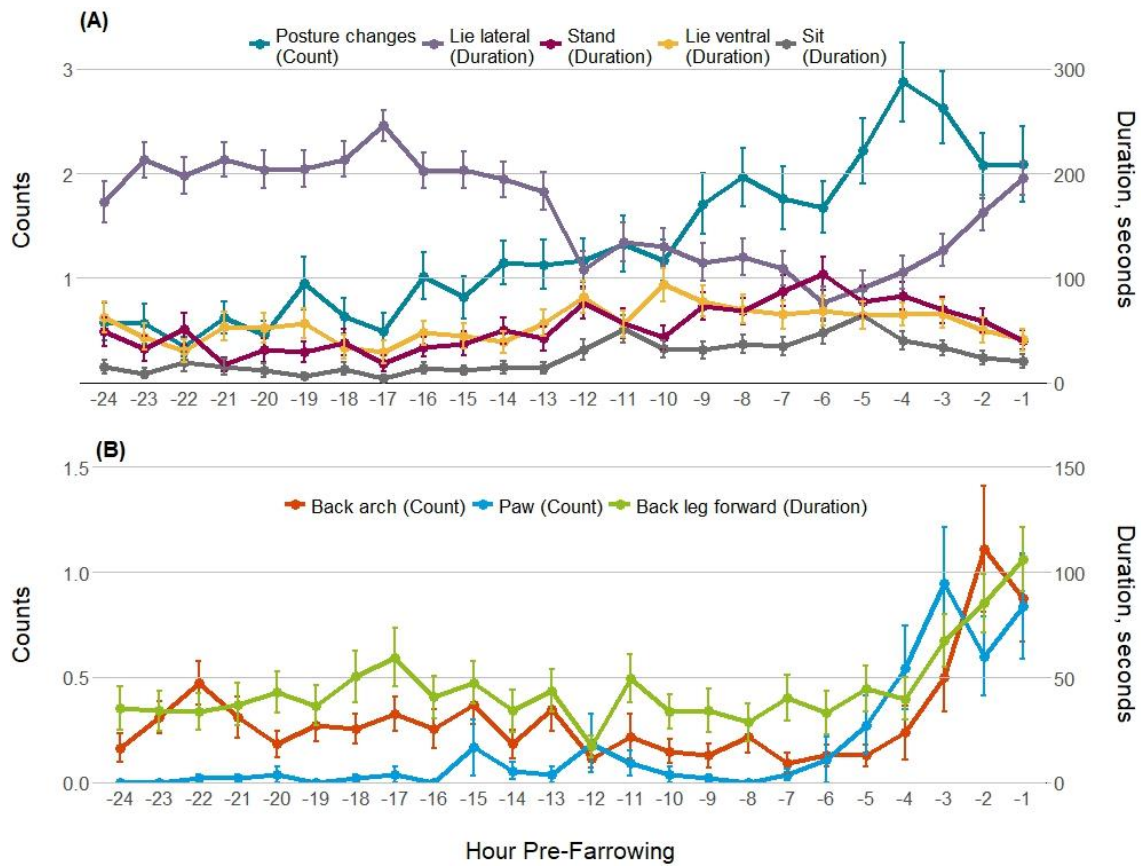
958 Table 4. Overall duration or frequencies of putative pain behaviors for 10 15 minute
 959 observations following the injection of a saline control or ketoprofen, with mean \pm SEM
 960 displayed by treatment, for gilts and sows and by whether additional treatment was needed,
 961 along with effect size (t) and *P* values overall and for the interactions with time.

Behavior	Treatment (treated vs. control)			Treated/ control \times time
	treated	control	t, <i>P</i>	t, <i>P</i>
Back leg forward, seconds	177.2 \pm 15.0	173.5 \pm 14.6	0.1, 0.9	-1.0, 0.3
Tremble, seconds	116.0 \pm 13.7	145.8 \pm 14.7	-1.6, 0.1	1.1, 0.3
Back arch, counts	2.5 \pm 0.2	2.5 \pm 0.2	0.3, 0.8	0.6, 0.5
	Gilt/sow (gilt vs. sow)			Gilt/sow \times time
	gilt	sow	t, <i>P</i>	t, <i>P</i>
Back leg forward, seconds	102.6 \pm 13.6	233.4 \pm 14.6	2.3, 0.02	-2.1, 0.04
Tremble, seconds	123.7 \pm 13.6	137.7 \pm 14.6	1.4, 0.2	-1.3, 0.2
Back arch, counts	1.5 \pm 0.1	3.3 \pm 0.2	3.6, <0.001	-2.4, 0.02
	Additional treatment (yes vs. no)			Yes/no \times time
	yes	no	t, <i>P</i>	t, <i>P</i>
Back leg forward, seconds	171.2 \pm 21.1	176.3 \pm 12.0	0.8, 0.4	0.1, 0.9
Tremble, seconds	108.0 \pm 20.5	137.5 \pm 11.6	0.4, 0.7	1.1, 0.3
Back arch, counts	2.9 \pm 0.3	2.4 \pm 0.2	0.8, 0.5	-0.5, 0.6

962 Table 5. Mean \pm SEM for interleukin-1 β (IL1 β), interleukin-6 (IL6), and tumour necrosis
 963 factor α (TNF α) and c-reactive protein (CRP) in gilt/sow plasma by treatment (control or
 964 treated), by parity group (gilt or sow) and additional treatment (yes or no). Values with
 965 different superscript letters indicate a significant difference ($P < 0.05$).

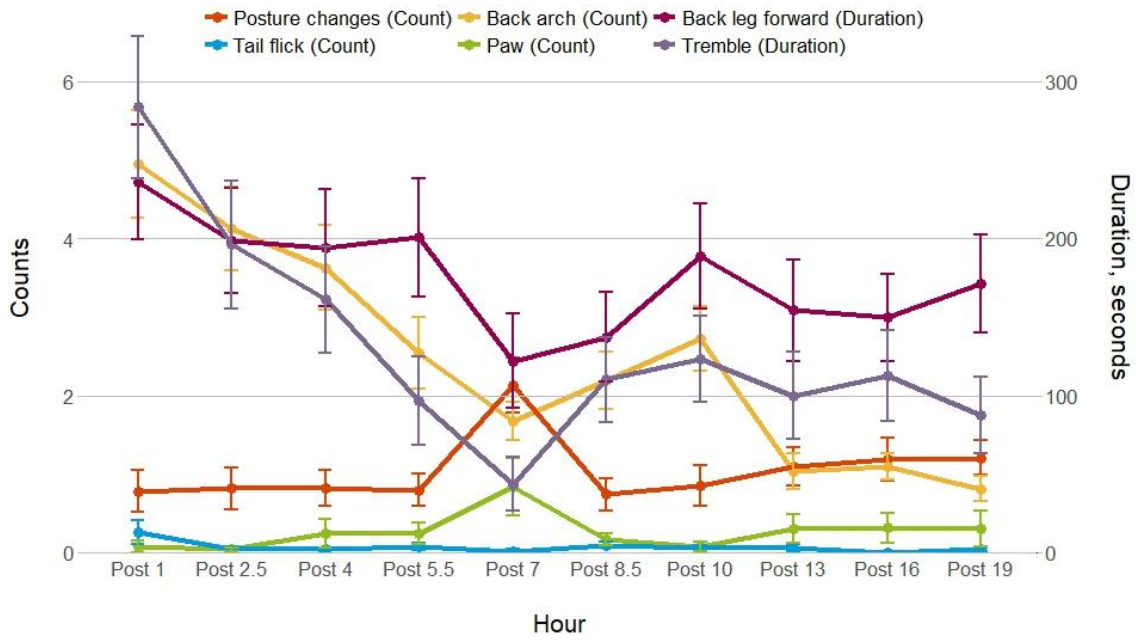
Protein	Treatment		Gilt/sow		Additional treatment	
	Control	Treated	Gilt	Sow	Yes	No
IL1 β , pg/ml	198.4 \pm 37.6	136.8 \pm 34.1	187.3 \pm 43.5	156.5 \pm 31.8	179.1 \pm 70.7	165.9 \pm 26.7
IL6, pg/ml	260.9 \pm 71.2	251.4 \pm 64.0	171.1 \pm 33.7	303.9 \pm 70.3	136.0 \pm 47.7	301.7 \pm 61.1
TNF α , pg/ml	28.1 \pm 9.2	24.2 \pm 8.1	17.8 \pm 8.3 ^a	32.0 \pm 8.4 ^b	27.4 \pm 17.3	25.7 \pm 6.3
CRP, μ g/ml	444.0 \pm 40.1	489.8 \pm 48.6	561.2 \pm 36.5 ^a	350.4 \pm 36.9 ^b	451.2 \pm 58.8	468.3 \pm 36.7

966 Figure 1. Mean \pm SEM for (A) postures/posture changes; and (B) putative pain behaviors
 967 observed for 5 minutes every hour for the 24 hours before the onset of farrowing.
 968



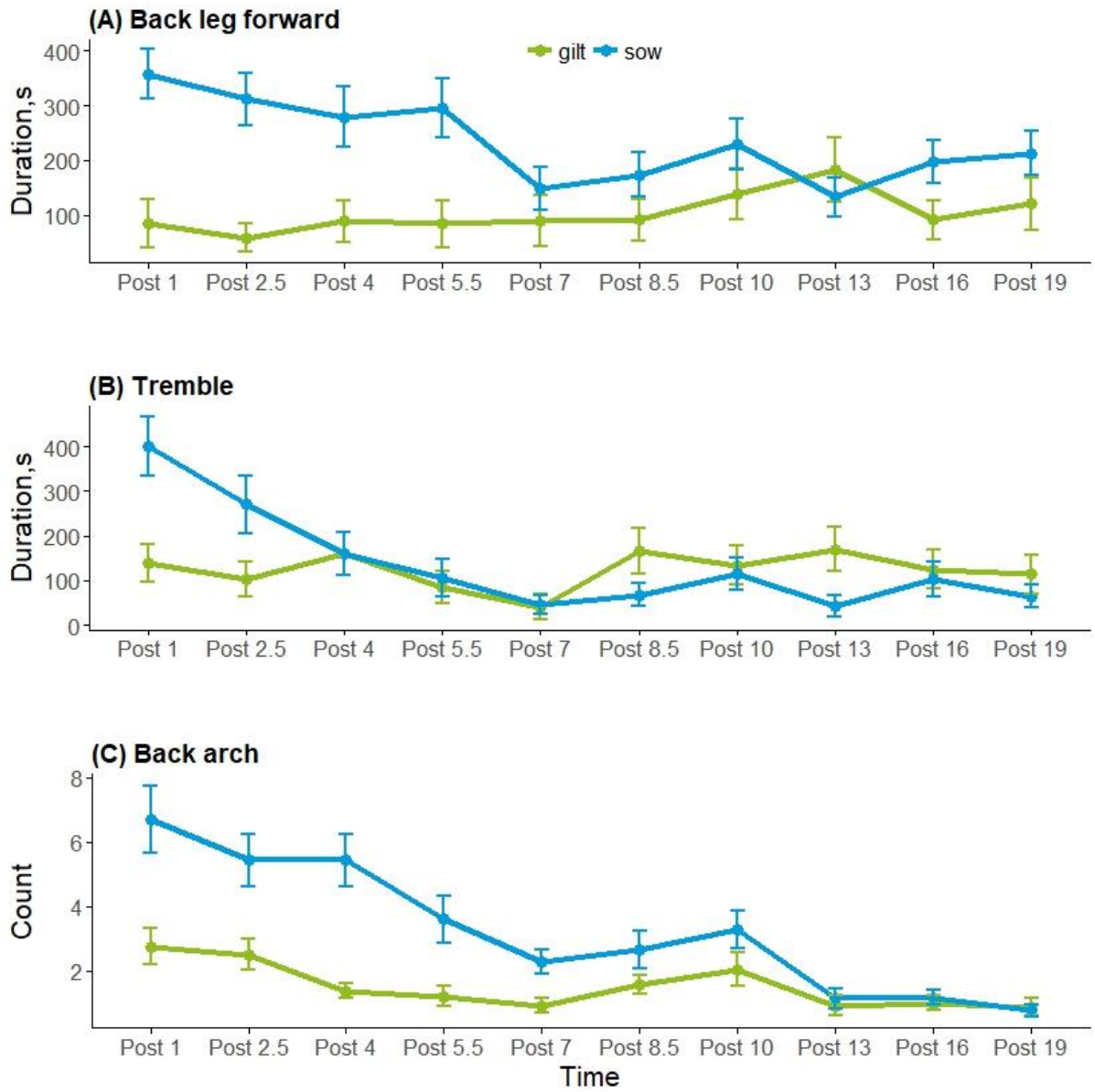
969

970 Figure 2. Mean \pm SEM for posture changes, and putative pain behaviors observed for 15
 971 minutes 1, 2.5, 4, 5.5, 7, 8.5, 10, 13, 16 and 19 hours after the ketoprofen or saline injection.
 972



973

974 Figure 3. Putative pain behaviors (mean \pm SEM) A) Back leg forward; B) Tremble; and C)
 975 Back arch for gilts (in green) and sows (in blue) by hour post injection.
 976



977

978 Figure 4. Mean \pm SEM of salivary cortisol (ng/ml) by day in relation to farrowing (-2, -1, 0, 1, 2, 3, 5, and 7) for a) all data; b) treatments
 979 (control or treated); c) gilts and sows and; d) additional treatment (no or yes). Days with different letters show a significant difference and bars
 980 with a ** indicates a difference ($P < 0.05$), and * a tendency ($P < 0.1$) by day for treatment, gilt/sow or additional treatment.

