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Artificial rearing affects piglets pre-weaning behaviour, welfare and growth performance

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1 **Artificial rearing affects piglets pre-weaning behaviour, welfare and growth performance**

2

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4

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12

13

14 **Abstract**

15 One strategy adopted on farms to deal with managing large litters involves removing piglets from
16 their mothers at seven days old to be reared in specialised accommodation with milk replacer.
17 Effects on piglet behaviour, growth and some aspects of welfare were evaluated in this study by
18 comparing 10 pairs of two litters (one sow-reared: SR, one artificially-reared: AR) selected at seven
19 days-old at a similar weight. Piglet behaviour was recorded for 20 min following transfer of AR
20 piglets to the artificial-rearing enclosure (D0) and for 20 min hourly between 09:00h and 17:00h (8h)
21 on D5 and D12. Hourly 5 min live observations were also undertaken. Qualitative Behavioural
22 Assessment (QBA) was conducted on D14 to evaluate piglets' emotional state. Survival and illness
23 events were recorded until weaning. On D0, D1, D8 and D15 piglets were weighed and scored for
24 tear staining, dirtiness of the face and severity of lesions on the snout, limbs, ear and tail. Survival
25 and illness rates, as well as the rates of behaviours/min were analysed using GLMMs. Weights and
26 QBA scores were analysed using GLM. Lesions, tear staining and dirtiness scores were averaged per
27 litter and analysed using GLM. When AR piglets were transferred to the artificial-rearing enclosure,
28 their behaviour was not different to SR piglets. Over the two observation days, AR piglets performed
29 more belly-nosing ($F_{1,76.53} = 42.25$; $P < 0.001$), nursing-related displacements ($F_{1,79} = 19.32$, $P < 0.001$),
30 visits to the milk cup (compared to nursing bouts; $F_{1,73.8} = 38.42$, $P < 0.001$), and oral manipulation of
31 littermates' ears ($F_{1,91.95} = 12.79$, $P < 0.001$) and tails ($F_{1,58.54} = 15.63$, $P < 0.001$) than SR piglets.
32 However, SR piglets played alone ($F_{1,88.99} = 8.29$, $P < 0.005$) and explored their environment ($F_{1,99.42} =$
33 4.52 , $P < 0.05$) more frequently than AR piglets. The QBA scores indicated a lower emotional state in
34 AR piglets ($t_{25.1} = -3.25$, $P < 0.05$). Survival rate and overall illness rate of piglets were similar between
35 the treatments. AR piglets experienced a growth check following their transfer to the artificial-
36 rearing enclosure and remained lighter than SR piglets through to weaning (6.53 ± 0.139 kg vs. 7.97
37 ± 0.168 kg, $t_{256} = 9.79$, $P < 0.001$). Overall, snout lesion scores were not different between the
38 treatments, but AR piglets had lower limb ($F_{1,10.1} = 5.89$, $P < 0.05$) and ear ($F_{1,14.5} = 24.89$, $P < 0.001$)
39 lesion scores and higher tail lesion scores ($F_{1,34.5} = 15.54$, $P < 0.001$). AR piglets were dirtier ($F_{1,17.4} =$

40 23.38, $P < 0.001$) but had lower tear staining scores ($F_{1,19.1} = 68.40$, $P < 0.001$) than SR piglets. In
41 conclusion, artificial rearing impaired piglets' behaviour, welfare and growth.

42

43 **Keywords**

44 Piglets; Artificial rearing; Welfare; Performance, Behaviour

45

46 1. Introduction

47 Artificial-rearing systems involve removing piglets from their mother at two to 14 days of age (Baxter
48 et al., 2013) and transferring them to specialised enclosures which are typically located either in a
49 separate room or above the sow's farrowing crate (e.g. Rescue Decks®). These enclosures provide
50 the piglets with warmth, milk replacer and solid food (Baxter et al., 2013), and remove the need for
51 nurse sows. They could be considered as an "intervention system" to rescue piglets that cannot be
52 reared by the sow and they are becoming more commonly used as genetic selection for large litters
53 results in more piglets than available teats, thus increasing the risk of piglet mortality. However
54 there are potentially contentious issues with these systems particularly relating to the definition of
55 weaning. If weaning is considered the removal of the piglet from its mother and its mother's milk
56 then artificial rearing is early weaning. However if weaning is considered the removal of milk then
57 artificial rearing systems would consider full weaning to occur when liquid feeding (milk replacer) is
58 stopped and piglets are moved to weaner facilities and fed solid food (at approximately 28 days of
59 age). Regardless of this discussion, it is clear that there is limited scientific evidence about the
60 welfare outcomes of artificial rearing with most studies focusing on piglet health and performance,
61 with some (Cabrera et al., 2010; van Beirendonck et al., 2015), but not all (De Vos et al., 2014)
62 claiming increases in pre-weaning growth. Reduction in pre-weaning growth could be due to a short-
63 term malfunctioning of the gut (De Vos et al., 2014; Huygelen et al., 2012), although De Vos et al.
64 (2014) did report long-term improvements to gut maturation. Where heavier weaning weights were
65 recorded in artificially-reared piglets compared to sow-reared piglets, they were found to be

66 unsustainable post-weaning (Cabrera et al., 2010; van Beirendonck et al., 2015) and artificially-
67 reared piglets had lower carcass quality at slaughter (i.e. lower loin depth and lean percentage)
68 (Cabrera et al., 2010). Benefits for growth of artificially-reared piglets are likely to come towards the
69 end of lactation, as they have access to ad libitum milk replacer whereas sow-reared piglets
70 experience a decrease in sows milking capacity (Quesnel et al., 2012). Nevertheless, results tend to
71 differ slightly among studies. This could be due to a number of factors, including differences in the
72 age of piglets at the start of artificial rearing (two to 14 days-old), milk replacer formulation (e.g.
73 inclusion or not of antibiotics or blood products), different types of enclosure (e.g. remaining in the
74 farrowing crate without the sow (Cabrera et al., 2010) vs. Rescue Decks® (Rzezniczek et al., 2015)),
75 milk delivery system (nipples (De Vos et al., 2014) vs. cups (Cabrera et al., 2010; Rzezniczek et al.,
76 2015)), and finally mixing (e.g. Rzezniczek et al., 2015) or not (e.g. De Vos et al., 2014) of the piglets
77 at transfer.

78 Artificial rearing involves piglets going through the same stressors that normally occur at weaning
79 (abrupt separation from dam, and changes in the social, physical and feeding environments) but at
80 an earlier age than usual. Thus welfare issues associated with weaning could arguably be even
81 greater for artificially-reared piglets (for more details see review by Latham and Mason, 2008).
82 Rzezniczek et al. (2015) showed that artificially-reared piglets displayed the same signs of distress
83 (i.e. vocalisations, growth impairments, development of abnormal behaviours) as early-weaned
84 piglets (e.g. Orgeur et al., 2001). In addition, piglets in artificial-rearing systems showed more
85 aggressive behaviours during the pre-weaning period than piglets reared by a sow. It was
86 hypothesised this was caused by the combination of early mixing, competition caused by the limited
87 space allowance at the milk supply, and recipients' reaction to belly-nosing (Rzezniczek et al., 2015).
88 Because of the feeding conditions and the fact that artificial-rearing enclosures usually have a lower
89 space allowance (i.e. typical space allowance: 1 m²) for piglets compared to farrowing crates (i.e.
90 space allowance: 3.6 m²; Baxter et al., 2012) the behavioural development of piglets may be affected
91 by artificial-rearing. For instance, belly-nosing is rarely observed in sow-reared piglets whereas it

92 develops routinely in early-weaned piglets (Orgeur et al., 2001; Weary et al., 1999; Worobec et al.,
93 1999) and in artificially-reared piglets in milk-cup feeding systems (Rzezniczek et al., 2015; Widowski
94 et al., 2005). Belly-nosing occurs due to redirected suckling behaviour (Widowski et al., 2008) and
95 reflects frustration caused by unfulfilled nursing-related behavioural needs (Weary et al., 1999;
96 Widowski et al., 2005). Manipulation of pen mates, which includes harmful behaviours such as ear-
97 and tail-biting, was higher in frequency and duration in artificially-reared piglets (Rzezniczek et al.,
98 2015), although the causal effects could not be determined as a consistent number of parameters
99 varied between two environments (e.g. space allowance, rooting material, quality of milk, age of
100 weaning from milk). A recent study by Frei et al. (2018) showed that the provision of dummies
101 allowing massage behaviour did not eliminate belly- and body-nosing, which suggests that piglets
102 may be missing tactile properties of the sow's udder.

103 To date, there are no studies which have adopted a holistic approach to assess the effects of
104 artificial rearing on the welfare of piglets, whereby the behaviour, health and performance of
105 artificially-reared piglets were compared to sow-reared piglets. This study seeks to fill this gap in the
106 scientific knowledge by investigating the effects of artificial rearing on piglets' pre-weaning
107 behaviour, welfare (emotional state, lesions and health) and growth.

108

109 2. Material and methods

110 2.1. Ethical approval

111 Ethical approval for this study was granted by Teagasc Animal Ethics Committee (application
112 TAEC113/2016). The experiment was carried out in accordance with the Irish legislation (SI no.
113 543/2012) and the EU Directive 2010/63/EU for animal experiments.

114

115 2.2. Animals and experimental design

116 This experiment was conducted on a commercial farm in County Laois, Ireland, and involved a total
117 of 233 piglets from 20 litters. The genetic background of the piglets was Large White x Hampshire or

118 Landrace x Hampshire (balanced between treatments). All piglets were born in a conventional
119 farrowing crate (pen: 2.13 x 1.71 m, sow crate: 1.90 x 0.64 m, stocking density: 0.27 m²/piglet,
120 plastic slatted floor) from sows that were induced (2 cc. of Platane[®], MSD) at 114 d of gestation.
121 Three handfuls of shredded paper were added to help dry the piglets at birth. Piglets were teeth-
122 clipped and tail-docked (under veterinary advice) at 2 days-old and received an iron injection at 4
123 days-old. Piglets were vaccinated against porcine mycoplasma hyopneumoniae bacterin (M+PAC[®])
124 at 8 and 25 days-old, and against porcine circovirus disease at 25 days-old (Ingelvac CircoFLEX[®]).
125 Each week two litters of 7 days-old piglets, matched for piglet weight and litter size ($n = 11.7 \pm 0.2$)
126 were selected for inclusion in the study and randomly assigned to one of the two treatments. One
127 litter remained with the sow until weaning (Sow-reared, SR; $n = 10$ litters, $n = 116$ piglets) and the
128 other was transferred to an artificial-rearing enclosure (Rescue Deck[®], S&R Resources LLC) (Figure 1)
129 and fed milk replacer (Opticare Milk, SwiNco BV, The Netherlands) until weaning (Artificially-reared,
130 AR; $n = 10$ litters, $n = 117$ piglets). At transfer to the artificial-rearing enclosure (D0), the heat lamp
131 and the milk cups were already activated and creep feed (Opticare Meal, SwiNco BV, The
132 Netherlands) was available in the trough. Creep feed was also made available to SR piglets in the
133 farrowing pen.

134 For ethical reasons piglets that did not thrive during lactation (i.e. showed signs of starvation) were
135 removed from the experiment to a non-experimental sow or to another artificial-rearing enclosure
136 for greater attention (i.e. treatment). Records of these removals were used in the analysis of the
137 mortality rate in each system.

138 In this experiment, weaning was defined as the removal of milk feeding and movement of the piglets
139 to weaner facilities. Because of normal farm practices and needs, there was an age difference at
140 weaning (AR: 26 ± 0.4 d, SR: 29 ± 0.4 d; $F_{1,201} = 109.6$, $P < 0.001$). Therefore data were collected only
141 until the week preceding weaning and where weaning weights are presented they are adjusted for
142 weaning age to allow a valid comparison.

143

144 2.3. Nutrition

145 Details of all diets can be found in supplementary material (Table S1). All sows diets were home-
146 milled. The milk replacer contained 21.5 % crude protein and 9% fat, and dried porcine plasma
147 powder. Milk replacer powder was mixed with hot water (i.e. 150 g/l of water at approximately
148 55°C) in a tank which was refilled once or twice daily, depending on daily consumption. All the pipe
149 lines transporting the milk from the tank to the milk cups were flushed once daily with hot water
150 and once weekly with a liquid acid cleaner (Acidsan, Agroserve, GEA Ireland Ltd., Naas, Ireland).
151 During lactation, sows were fed a diet containing 15.5% crude protein, 4.36% crude fat and 3.95%
152 crude fibre for a metabolisable energy of 13.01 MJ/kg and a net energy of 9.4 MJ/kg. Feed allowance
153 to sows increased gradually during lactation, starting with 3 kg/day four days before farrowing and
154 finishing with 8 kg/day at weaning.
155 The creep feed provided from 7 to 22 days-old contained 19.24% crude protein, 9.54% crude fat and
156 1.53% crude fibre. Thereafter piglets were given pellets from 22 days-old until weaning, which
157 contained 17.46% crude protein, 6.88% crude fat and 2.67% crude fibre.

158

159 2.4. Housing

160 Farrowing pens were equipped with a heat pad (1.55 x 0.37 m, maintained at 30°C), a bowl water
161 drinker, and a trough was provided for solid feed from 7 days-old. Artificial-rearing enclosures (1.40
162 x 0.71 m, stocking density: 0.08 m²/piglet; fully slatted, plastic-coated expanded metal slats) were
163 equipped with a heat lamp (250 W, that maintained temperature at approximately 30°C), two milk
164 cups (11 cm diameter), a water cup, and a trough for the solid feed. A canopy covered two thirds of
165 the enclosure area, to prevent heat loss. The farrowing house temperature was maintained around
166 23°C, but the temperature in the room with the artificial enclosures was not controlled.

167

168 2.5. Measurements

169 2.5.1. Behaviour

170 Piglet behaviour in both treatments was simultaneously video recorded with a digital camcorder
171 (Panasonic HC-250EB-K, Panasonic®; fixed on a tripod) for 20 min after the transfer of AR piglets
172 (approx. 13:00 h), and 20 min per hour between 09:00 h and 17:00 h, on D5 and D12 (8 videos per
173 day). Thus in total, the behaviour of each litter was recorded by video for a total of 320 min. Hourly 5
174 min live observations of piglets were also undertaken on the same days by a single observer. Groups
175 of pigs were observed when they were not being video recorded every hour between 09:00 h and
176 17:00 h.

177 The same ethogram (Table 1) was used for both video and live observations and all occurrence
178 continuous sampling was used (Martin et al., 1993). Additionally, the behaviour “attempts to
179 escape” was recorded only on D0, when a piglet tried to climb up or jump above the walls of the
180 enclosure, as well as the behaviour “naso-naso contacts”, i.e. voluntary (gentle) touch of a piglet’s
181 snout against another’s snout. Video data were analysed by a single observer (intra-observer
182 reliability = 97%) using the software package The Observer® XT (Noldus, Wageningen, The
183 Netherlands).

184

185 2.5.2. Qualitative Behavioural Assessment

186 Qualitative Behavioural Assessment (QBA) was performed as described in the Welfare Quality®
187 assessment protocol for pigs (Welfare Quality®, 2009). Pigs were assessed at 21 days-old (D14)
188 between 12:00 h and 14:00 h, which corresponded to the period with least interference from the
189 staff (no sow feeding, milk tank filling...). Each litter of piglets, randomly selected, was directly
190 observed by a single observer (intra-observer reliability = 90%) for 20 min after which the
191 experimenter scored the 20 fixed descriptors on a 125 mm horizontal valence scale. Each descriptor
192 is associated with a weight (pre-determined in the Welfare Quality® (2009) protocol for pigs) applied
193 to the descriptor’s score to allow the calculation of the QBA score. The QBA score provides an
194 assessment of the subjects’ emotional state.

195

196 2.5.3. Mortality, removal and illness

197 Piglet deaths were recorded from D0 to weaning. Piglets which were removed for ethical reasons
198 allowed additional investigation of the risk of being moved to a non-experimental sow or another
199 artificial-rearing enclosure before weaning, depending on the availability of sows and the type of
200 illness. The occurrence, nature and duration of treatment of piglets for health problems were
201 recorded.

202

203 2.5.4. Weights

204 Piglets were weighed individually on the transfer day (designated as D0), the following day (D1), D8,
205 D15 and at weaning. Average daily gain (ADG) was calculated between each of these time points.

206

207 2.5.5. Lesions

208 The severity of lesions on piglets' snout, knees, tail and ears was scored when they were weighed.
209 The number of scratches on the ventral or lateral aspects of the piglet's snout was scored using a 4
210 point scale (0 = no lesions to 3 = snout covered by lesions) developed by Fraser (1975) and modified
211 by Hansson and Lundeheim (2012). Abrasion (presence = 1, absence = 0) and inflammation
212 (presence = 1, absence = 0) on both piglets' front knees were scored using the scale developed by
213 Westin (2013), and overall limb lesion score was calculated per piglet by summing the scores for
214 each knee (score ranging 0-4). The tail lesion scoring system of Harley et al. (2012) was modified:
215 intact tails were scored 0, tails were scored 1 if a puncture wound or swelling (evidence of chewing
216 or biting) was observed and scored 2 if there was a partial or total loss of the tail. Finally the ear
217 lesion scoring system of Diana et al. (2017) was also adapted: intact ears were scored 0, ears with
218 wounds were scored 1, and ears with partial or total loss were scored 2. Each ear was scored
219 separately and the overall score was the sum for each piglet.

220

221 2.5.6. Tear staining and dirtiness scores

222 During weighing, the stained area under the eye was scored according to its size relative to the eye's
223 area (DeBoer et al., 2015). Since the scoring system is relative to the pig's eye size, it can be applied
224 to animals of all age on a farm: score 0 was attributed to clean eyes (no sign of staining), score 1 was
225 attributed to barely detectable staining, scores 2 to 4 to eyes where the stained area represented,
226 respectively, <50%, between 50% and 100%, and >100% of the eye area. Both eyes were scored and
227 the average score for the two eyes was analysed. The percentage of face surface covered with dirt
228 (e.g. dried milk, dust, manure...) was scored from 0 for a stainless face to 4 for a face covered at
229 more than 75% with dirt (Minvielle and Le Roux, 2009).

230

231 2.6. Statistical analyses

232 Data were analysed using SAS 9.4 (SAS Inst. Inc., Cary, NC). The experimental unit for the analyses of
233 growth performance, survival and health was the pig within litter; and the experimental unit for the
234 analysis of behaviour, emotional state, lesions and coefficient of variation of weights was the litter.

235 The distribution of the data was checked using a univariate model (PROC UNIVARIATE), and data
236 were considered normal if the Shapiro-Wilk and [Kolmogorov–Smirnov tests](#) were not significant.

237 General Linear Models (GLM) and Generalized Linear Mixed Models (GLMM) were fitted using the
238 Residual Pseudo Likelihood approximation method. Statistically significant terms were determined
239 when alpha was below 0.05 and tendencies were determined when alpha was between 0.05 and
240 0.1. Results are reported as means S.E., F-values and t-values, and corresponding degree of freedom
241 (DF, subscript) are reported for overall effects of treatment and pair-wise comparisons, respectively.

242 Replicate was included as a random effect in all models.

243 For the analysis of behaviour, the 20-min videos and the 5-min live observations were pooled. Rates
244 of behaviours per minute were calculated and analysed using GLMMs (PROC GLIMMIX) with a
245 Poisson distribution and a log link function. All models accounted for the repeated effect of
246 observation within day and the random effect of number of pigs. When the interaction between
247 treatment and day was not significant, due to non-significant differences intra-treatment, treatment

248 differences were considered within each day. This was done using the “slice” statement in the PROC
249 GLIMMIX, which gave the reported F-value and P-value for treatment differences within day.
250 The QBA scores were analysed using GLM (PROC MIXED). Descriptors’ scores were not normally
251 distributed and analysed using GLMM (PROC GLIMMIX) with a gamma distribution and a logit
252 function. Each model included the random effect of pen. Principal Component Analysis (PCA) was
253 used to compute the descriptor scores into principal components, which explain the variability in
254 QBA score between litters. The first two principal components with eigenvalues above 1.0 were
255 retained to produce a two-dimensional word chart, where the 20 descriptors’ eigenvector values
256 (i.e. quantification of the weight of the descriptor) were plotted on the two principal components
257 axes. This word chart was then used to interpret the first two principal components and thus, how
258 the pigs were perceived. Each litter of AR and SR piglets received a score on each of the two main
259 principal components, which allowed defining clusters. Survival, removal and health data were
260 binary, thus these variables were analysed using GLMMs with a binary distribution and logit link
261 function.
262 Weights, average daily gains (ADG) and coefficient of variation (CV) of weights were normally
263 distributed with regards to their residuals and analysed using GLMs. For analysis of weight the initial
264 (i.e. D0) weight was used as a covariate. Day was included as a repeated effect in analysis of weights
265 and CV. For weaning weight the age of the pig was used as a covariate, as there were differences in
266 weaning age.
267 All lesion, dirtiness and tear staining scores were averaged per litter and analysed using GLMs (PROC
268 MIXED), accounting for repeated effect of day.

269

270 3. Results

271 3.1. Behaviour

272 All descriptive data (number of observations, minimum, maximum, mean, standard deviation and
273 standard error) are presented in supplementary material (Tables S2 and S3).

274

275 3.1.1. Behaviour at transfer

276 Due to technical failure (malfunctioning of the camera), one replicate could not be observed. Belly-
277 nosing, play-fighting and naso-naso contacts were not observed in either treatment during the 20
278 minutes following assignment to treatments (i.e. transfer of AR piglets to the artificial-rearing
279 enclosure and SR piglets remaining with their mothers). There was only one AR piglet which
280 attempted to escape after transfer of the litter to the artificial-rearing enclosure, thus these data
281 were not analysed. There were no other differences in the behaviours observed between AR and SR
282 piglets at the time of transfer (Figure 2).

283

284 3.1.2. Routine behavioural observations

285 Table 2 summarises the results of routine behavioural observations.

286 Over the two observation days, the rate per minute of belly-nosing was higher in AR piglets than in
287 SR piglets (0.72 ± 0.100 vs. 0.01 ± 0.006 , respectively; $F_{1,76.53} = 42.25$, $P < 0.001$). Visits to the milk cup
288 in AR piglets were more frequent than nursing bouts in SR piglets (0.35 ± 0.273 vs. 0.16 ± 0.124 ,
289 respectively; $F_{1,73.8} = 38.42$, $P < 0.001$) and AR piglets performed more nursing-related displacements
290 per minute than SR piglets (0.27 ± 0.028 vs. 0.12 ± 0.019 , respectively; $F_{1,79} = 19.32$, $P < 0.001$).

291 Although play-fighting behaviour frequency did not differ between AR and SR piglets (1.00 ± 0.120
292 vs. 1.20 ± 0.132 , respectively; $F_{1,80.09} = 1.23$, $P > 0.1$), SR piglets played alone more frequently than AR
293 piglets (0.25 ± 0.044 vs. 0.10 ± 0.027 , $F_{1,88.99} = 8.29$, $P < 0.005$). Oral manipulation of the tails ($0.08 \pm$
294 0.016 vs. 0.02 ± 0.006 , respectively; $F_{1,58.54} = 15.63$, $P < 0.001$) and ears (0.23 ± 0.025 vs. 0.12 ± 0.018 ,
295 respectively; $F_{1,91.95} = 12.79$, $P < 0.001$) were more frequent in AR piglets than in SR piglets. The rate of
296 exploration behaviour per minute was higher in SR piglets than in AR piglets (0.48 ± 0.053 vs. $0.34 \pm$
297 0.044 , respectively; $F_{1,99.42} = 4.52$, $P < 0.05$).

298

299 3.2. Qualitative Behavioural Assessment

300 The AR piglets had a lower QBA score than SR piglets (43.1 ± 6.21 vs. 77.8 ± 6.21 ; $F_{1,9} = 2.42$,
301 $P < 0.005$), which indicates a poorer emotional state. In particular, AR piglets scored lower than SR
302 piglets on positive terms (associated with positive weights) such as active ($P < 0.05$), content ($P < 0.05$),
303 enjoying ($P < 0.001$), playful ($P < 0.005$), positively occupied ($P < 0.05$), lively ($P < 0.01$) and happy
304 ($P < 0.01$); and scored higher on negative terms (associated with negative weights) such as bored
305 ($P < 0.05$), irritable ($P < 0.001$) and aimless ($P < 0.001$). However, SR piglets had a higher score for the
306 term fearful compared to AR piglets ($P < 0.005$) (Table 4). From the PCA, two principal components
307 (PC) were retained, explaining 33% and 15% of the total variation in QBA score (Figure 3a; Table 4).
308 PC1 was mostly characterised by descriptors related to positive feelings: enjoying (0.37), playful
309 (0.36), happy (0.35), lively (0.29), content (0.29), active (0.28), and positively occupied (0.26) (Table
310 4). PC2 was mostly characterised by descriptors related to low arousal: calm (0.49), indifferent (-
311 0.46), listless (0.30), bored (0.30) and fearful (-0.29) (Table 4). The AR and SR litters clustered clearly
312 (Figure 3b), and they mostly differed by their loadings on PC1. Indeed most of the AR litters had
313 lower loadings on PC1 (-1.71 to 0.08) compared to SR piglets (-0.11 to 1.56), meaning that they were
314 perceived as less enjoying, active, content, happy, playful, lively and positively occupied. The
315 clustering of litters on PC2 axis was less clear, but most (8/10) AR litters had a loading above 0 while
316 most (6/10) SR litters had a loading below 0. This would suggest that AR piglets were perceived as
317 more calm, bored and listless and less indifferent and fearful than SR piglets.

318

319 3.3. Survival, removal and illness

320 Only one piglet died in each treatment. Very few piglets had to be removed from the experiment for
321 ethical reasons and there was no difference between treatments (SR: 5.9 ± 2.32 %, AR: 7.2 ± 2.66 %).
322 Finally, over the whole experiment, 27 and 18 illness events were recorded in AR piglets and SR
323 piglets, respectively. There was large variation in the percentage of piglets treated for illness or
324 injury in the different treatment groups, but no significant differences were found (SR: 11.86 ± 6.5 %, AR:
325 16.95 ± 8.8 %, $F_{1,16.65} = 0.22$, $P > 0.6$). However, AR litters had a higher percentage of piglets

326 suffering from diarrhoea (SR: $2.7 \pm 1.97\%$, AR: $13.7 \pm 7.84\%$, $F_{1,232}=12.2$, $P<0.001$) and a lower
327 percentage of lame piglets (SR: $7.1 \pm 3.41\%$, AR: $0.7 \pm 0.7\%$, $F_{1,232}=5.33$, $P<0.05$), compared to SR
328 litters.

329

330 3.4. Weights and growth

331 AR piglets tended to be heavier than SR piglets before transfer to the artificial-rearing enclosure, but
332 even after adjusting for initial weight in the models, from D1 and until weaning AR piglets were
333 lighter than SR piglets (Table 3). AR pigs had a lower average daily gain (ADG) during the pre-
334 weaning period (0.24 ± 0.005 kg/d vs. 0.27 ± 0.005 kg/d; $F_{1,199} = 12.1$, $P<0.001$) (Table 3). In fact, AR
335 pigs' ADG was reduced during the 24 h following their transfer to the artificial-rearing enclosure
336 (Table 3). During the remainder of the lactation period, the difference in ADG of AR compared to SR
337 piglets decreased (Table 3). The coefficient of variation (CV) of weight did not differ between SR and
338 AR litters (Table 3).

339

340 3.5. Lesions

341 On the day of transfer (D0), AR and SR piglets did not differ in lesion scores for the snout, ear, tail
342 and limbs. Overall (D0, D1, D8 and D15 taken together), AR piglets had lower lesion scores for the
343 limbs (0.2 ± 0.05 vs. 0.3 ± 0.05 , respectively; $F_{1,10.1} = 5.89$, $P<0.05$) and the ears (0.1 ± 0.04 vs. $0.3 \pm$
344 0.04 ; $F_{1,14.5}=24.89$, $P<0.001$), but higher tail lesion scores (0.3 ± 0.04 vs. 0.1 ± 0.04 ; $F_{1,34.5} = 15.54$,
345 $P<0.001$) compared to SR piglets. Snout lesions did not differ (0.06 ± 0.023 vs. 0.10 ± 0.023 ,
346 respectively; $F_{1,24.6} = 1.59$, $P>0.1$). Details of treatment differences per day can be found in
347 Supplementary Material (Table S4).

348

349 3.6. Tear staining score and dirtiness score

350 On D0, AR and SR piglets had similar tear staining scores for both eyes ($t_{18} = 0.51$ and $t_{11.7} = -0.40$,
351 $P>0.05$). Overall (D0, D1, D8 and D15 taken together), AR piglets had lower tear staining scores (0.43

352 ± 0.046 vs. 0.97 ± 0.046 ; $F_{1,19.1} = 68.4$, $P < 0.001$) and higher dirtiness scores (1.18 ± 0.098 vs. $0.69 \pm$
353 0.098 ; $F_{1,17.4} = 23.38$, $P < 0.001$) than SR piglets. Details of treatment differences per day can be found
354 in Supplementary Material (Table S5).

355

356 4. Discussion

357 This study demonstrated that artificial rearing had a negative impact on piglets' behaviour, growth
358 and some aspects of welfare during lactation, compared to piglets reared with their sow.

359 With the exception of one AR piglet which attempted to jump out of the artificial-rearing enclosure
360 several times, once transferred to the enclosure, the behaviour of AR piglets was not different to SR
361 piglets (that stayed with their mother). As expected, AR piglets performed belly-nosing more
362 frequently than SR piglets (Frei et al., 2018; Rzezniczek et al., 2015). In agreement with the study of
363 Rzezniczek et al. (2015), higher occurrences of negative behaviours (e.g. ear- and tail oral
364 manipulation) were observed in AR piglets. They also performed more nursing-related
365 displacements which, together with belly-nosing, disturbed the feeding episodes of receiving piglets.
366 They played alone less than SR piglets, probably because of the lower space allowance which did not
367 facilitate running. The AR piglets performed play-fighting as much as SR piglets, which disagrees with
368 the results of Rzezniczek et al. (2015) who observed more play-fighting in sow-reared piglets than in
369 artificially-reared piglets. However, Rzezniczek et al. (2015) had seven piglets per artificial-rearing
370 enclosure and approximately 11 piglets per sow, and thus the number of partners (i.e. opportunities
371 to play) was greater in SR litters. Typical play-invite behaviours involve play behaviours clearly
372 directed at a recipient, and rejection involves turning away and not-engaging with the "actor"
373 (Martin et al., 2015). In the present study, the stocking density of the artificial-rearing enclosure was
374 higher than in the study of Rzezniczek et al. (i.e. $0.08 \text{ m}^2/\text{piglet}$ vs. $0.15 \text{ m}^2/\text{piglet}$). This likely
375 increased the number of unavoidable encounters leading to play-fights as play rejections may have
376 been hampered by the lack of space to avoid engagement. Finally the piglets in the study of
377 Rzezniczek et al. (2015) were younger (i.e. 2-6 days-old) than in the present study when transferred

378 to the artificial-rearing enclosure, which could have had a greater impact on their behavioural
379 development.

380 AR piglets experienced a growth check directly following transfer to the artificial-rearing enclosure,
381 had a lower growth rate until D15 than SR piglets, and consequently, a lower weaning weight. The
382 initial growth check experienced by the piglets is likely due to the change in diet and delivery
383 method, similarly to what is experienced by all piglets at weaning. In addition, the higher occurrence
384 of diarrhoea in artificially-reared piglets, the higher frequency of belly-nosing, and displacements at
385 the feeder, may have prevented the compensatory growth, which has been previously observed in
386 artificially-reared piglets. Belly-nosing alters the feeding and drinking patterns of both performers
387 and recipients (Torrey and Widowski, 2006; Widowski et al., 2008). In the present study, belly-nosing
388 was usually performed during feeding episodes and, indeed, disrupted feeding bouts of recipients
389 (personal observation). This could partly explain the surprising discrepancy between lower growth
390 rates of AR piglets, and their higher frequency of visits to the milk cup. In addition, the milk cups
391 could only facilitate feeding for up to 6 pigs simultaneously (3 per cup), which increased competition
392 during feeding episodes in AR piglets, as supported by the higher frequency of displacements
393 observed at the milk cup. These displacements resulted in milk projections which, together with
394 higher levels of diarrhoea, are likely to have contributed to the higher levels of dirtiness of AR
395 piglets.

396 Tear-staining is a non-invasive technique meant to assess stress level of pigs (DeBoer et al., 2015).
397 Our tear staining result indicates AR piglets were less stressed than SR piglets (as indicated by lower
398 tear-staining scores), which is not in agreement with our other welfare and performance results.
399 Nevertheless, our tear-staining results should be interpreted with caution, as this is the first study
400 using tear-staining to assess stress in piglets, and differences in environmental conditions (e.g.
401 humidity, stocking density...) could have influenced the staining. More validation work is needed to
402 determine whether this method to be considered a reliable indicator of welfare status (DeBoer et
403 al., 2015; Telkänranta et al., 2015).

404 Tail lesions were greater in AR piglets, probably due to the higher frequency of oral manipulation of
405 tail in these litters. However, surprisingly, AR piglets had lower ear lesion scores even though oral
406 manipulation of the ears was more frequently performed in this treatment. Limb lesions were lower
407 in AR piglets, compared to SR piglets, until weaning. This could be due to AR piglets not needing to
408 kneel to suckle at the udder, which leads to knee abrasion (Boyle et al., 2000).

409 The emotional state of AR piglets, as assessed by QBA, was poorer than SR piglets. Piglets mainly
410 differed in descriptors related to positive feelings (i.e. happy, content, positively occupied, enjoying,
411 playful, lively), although the loadings were rather low probably due to the low sample size.

412 Nevertheless, the results are in alignment with those from the time budget observations; lower
413 positive feelings could be due to, or reflective of, a higher occurrence of the negative behaviours
414 (e.g. oral manipulation of tails and ears, belly-nosing) observed, or disturbance of feeding episodes
415 and poorer digestive health, as evinced by higher diarrhoea levels, in AR piglets. Obviously the lower
416 space allowance could influence piglet welfare (Cornale et al., 2015), but it can also be speculated
417 that the absence of maternal care, although limited in the pig, could be a causal factor of AR piglets'
418 lower emotional state. Unfortunately, there is no scientific study on the sow-piglet bond and the
419 importance of maternal care for piglets, beyond a nutritional point of view. However naso-naso
420 contacts between the sow and the piglets have been reported as a form of social bond (Blackshaw
421 and Hagelsø, 1990) or play (Horback, 2014). This deserves more attention, especially when
422 evaluating the use of artificial-rearing systems.

423 Although the percentage of piglets with a health issue was not affected by treatment, there were
424 treatment differences with regard to specific disorders health events: a greater percentage of AR
425 piglets suffered from diarrhoea and a lower percentage of AR piglets were lame, compared to SR
426 piglets. The incidence of lameness in SR piglets can be explained by the risk of being stepped on by
427 the sow and exposure to the sow slats which can be injurious to piglets (Lewis et al., 2005). The
428 higher incidence of diarrhoea in AR piglets could have been initiated by the stress caused by
429 separation from their mother at 7 days of age, exacerbated by the change of environment and by

430 adaptation to the milk replacer. Chronic stress after separation from their mother could lead to
431 intestinal mucosal barrier dysfunction in AR piglets, as observed in early-weaned piglets (Smith et al.,
432 2009). In addition, the higher stocking density and higher humidity level (personal observation) in
433 the artificial-rearing enclosure may promote bacterial growth (Banhazi et al., 2009; Roque et al.,
434 2016). Milk replacer formulations differ and may include immune components (e.g. from porcine
435 plasma) to help protect piglets' health. Because AR piglets in the present study had higher
436 occurrence of diarrhoea, it can be speculated that the immune components of the milk replacer may
437 not be as efficiently absorbed by the piglets as compounds of sow milk (Hurley, 2015), possibly
438 because of the origin of the immune material (i.e. plasma instead of milk).

439 The analytical composition (e.g. protein level, addition of blood plasma or immune material) of the
440 milk replacer used could be a major cause of inconsistency in results from studies on artificial
441 rearing. In particular, porcine plasma in the milk replacer can act as a growth promotor but may also
442 influence the occurrence of diarrhoea in AR piglets (Van Dijk et al., 2001). Supporting the latter
443 hypothesis, Touchette et al. (2002) found that pigs fed a diet containing 7% of spray-dried plasma for
444 one week post-weaning (i.e. 14 to 21 days-old) had a depressed immunity compared to pigs fed a
445 normal diet. The occurrence of diarrhoea and the number of medications administered to piglets
446 was not measured in other studies, and deserves more attention when evaluating the effects of
447 artificial rearing. It is also worth noting that plasma products are not legal in all countries, because of
448 the threat they pose to biosecurity (Van Dijk et al., 2001) and because of the ethical concern about
449 feeding animal products to animals, despite the omnivorous characteristic of pigs.

450 Artificially-reared piglets (i.e. from 3 days-old) performed belly-nosing routinely if fed by a cup
451 system, less if fed by a nipple drinker system, but not if fed by an artificial udder (i.e. baby-bottle
452 nipples mounted in front of a water-filled bag) (Widowski et al., 2005). In addition, piglets with a
453 nipple drinker displayed stereotypic snout rubbing on the wall behind the drinkers, possibly showing
454 their motivation to perform massage behaviour as a part of natural nursing behaviour (Widowski et
455 al., 2005). This suggests that synchronous feeding is an important feature of nutrition in piglets and

456 that asynchronous feeding could lead to development of abnormal behaviours. The artificial udder
457 seemed to better permit the behaviours related to feeding (suckling, massaging and nosing), and
458 may illicit suckling through its tactile properties (Welch and Baxter, 1986). However, feeding systems
459 using nipples may require more human intervention to help the piglets to learn how to use them
460 compared to cup systems (Widowski et al., 2005), increasing time and labour costs, and may
461 therefore be impractical. Rzezniczek et al. (2015) trained artificially-reared piglets to drink from the
462 milk cups, which may have promoted piglets' growth (unpublished data, Weber et al. (2015)).
463 Therefore the feeding systems used in AR studies could have influenced results, and studies to
464 address systems allowing synchronous feeding and providing an imitation udder are warranted.

465

466 5. Conclusion

467 Artificial rearing has detrimental effects on piglets' behaviour, welfare and growth. Artificially-reared
468 piglets performed more agonistic behaviours such as oral manipulative behaviours (ear and tails)
469 and belly-nosing. The emotional state of artificially reared-piglets was lower than that of SR piglets.
470 They also had a lower growth rate and a higher incidence of diarrhoea, compared to sow-reared
471 piglets. Together, our results suggest that artificial-rearing systems need to be improved to promote
472 appropriate/natural behavioural development of piglets and improve their welfare and
473 performance.

474

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481

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604

605 **Table 1** Description of behaviours observed on D0, D5 and D12.

Behaviour	Description
Belly-nosing	A rhythmic up-and-down movement with the snout on the belly or soft tissue of another piglet (Widowski et al., 2005), especially performed on the skin behind the ear and on the abdomen between the front and the hind limbs (Rzezniczek et al., 2015). Does not include belly-sucking.
Displace	Piglet pushes another one to gain access to a milk cup (AR piglets) or teat (SR piglets)
Milk	Piglet has its snout in the milk cup/suckle a teat at milk let-down
Oral manipulation of ears and tail	Having another piglet's ear or tail in the mouth (Widowski et al., 2005). This behaviour would thus include any chewing, nibbling or biting of ears or tail of a pen-mate.
Explore	Snout touching or rooting on floor and walls, or chewing on fixtures of the environment (Melotti et al., 2011)
Play-fighting	Nudge, chase, push, push-overs (Blackshaw et al., 1997; Martin et al., 2015).
Play alone	Spring/leap, pivot, toss head, run, rolling (Blackshaw et al., 1997; Martin et al., 2015)

606

607

608

609 **Table 2** Means (\pm S.E.) occurrence of behaviour per minute of observations (20-min video and 5-min
610 live observations pooled) of sow-reared piglets (SR) or artificially-reared piglets (AR) at 12 days-old
611 (D5) and 19 days-old (D12). AR piglets were removed from their mother at 7 days-old and fed milk
612 replacer in a Rescue Deck[®] until weaning. SR piglets remained with their mother until weaning.

	Sow-Reared	Artificially-Reared	F-value	P-value
D5				
Belly-nosing ¹	0.00 (\pm 0.005)	0.63 (\pm 0.099)	F(1,87.98) = 17.27	<0.001
Displace ²	0.12 (\pm 0.026)	0.25 (\pm 0.037)	F(1,93.37) = 7.22	<0.01
Ear oral manipulation ³	0.12 (\pm 0.025)	0.33 (\pm 0.041)	F(1,105.3) = 17.1	<0.001
Explore ⁴	0.39 (\pm 0.066)	0.37 (\pm 0.064)	F(1,109.8) = 0.06	NS
Milk cup visits/nursing ⁵	0.16 (\pm 0.125)	0.33 (\pm 0.255)	F(1,87.66) = 16.11	<0.001
Play-fighting ⁶	1.07 (\pm 0.173)	1.07 (\pm 0.173)	F(1,93.96) = 0	NS
Play alone ⁷	0.27 (\pm 0.064)	0.10 (\pm 0.038)	F(1,99.06) = 4.73	<0.05
Tail oral manipulation ⁸	0.01 (\pm 0.007)	0.10 (\pm 0.023)	F(1,75.83) = 12.97	<0.001
D12				
Belly-nosing	0.00 (\pm 0.010)	0.86 (\pm 0.129)	F(1,87.98) = 39.05	<0.001
Displace	0.12 (\pm 0.026)	0.30 (\pm 0.041)	F(1,93.37) = 13.24	<0.001
Ear oral manipulation	0.12 (\pm 0.024)	0.16 (\pm 0.029)	F(1,105.3) = 1.45	NS
Explore	0.61 (\pm 0.082)	0.31 (\pm 0.059)	F(1,109.8) = 8.05	<0.01
Milk cup visits/nursing	0.16 (\pm 0.126)	0.38 (\pm 0.294)	F(1,87.66) = 24.12	<0.001
Play-fighting	1.34 (\pm 0.194)	1.07 (\pm 0.173)	F(1,93.96) = 2.55	NS
Play alone	0.23 (\pm 0.059)	0.10 (\pm 0.038)	F(1,99.06) = 3.64	0.06
Tail oral manipulation	0.02 (\pm 0.009)	0.06 (\pm 0.017)	F(1,75.83) = 4.73	<0.05

613 ¹ Effect of treatment x day on belly nosing: $F_{(1,102.8)}=0.59$, $P>0.1$

614 ² Effect of treatment x day on displace: $F_{(1,112.9)}=0.41$, $P>0.1$

615 ³ Effect of treatment x day on ear oral manipulation: $F_{(1,121.9)}=3.27$, $P=0.07$

616 ⁴ Effect of treatment x day on explore: $F_{(1,121.6)}=3.21$, $P=0.08$

617 ⁵ Effect of treatment x day on milk cup visits/nursing: $F_{(1,106.7)}=0.32$, $P>0.1$

618 ⁶ Effect of treatment x day on play-fighting: $F_{(1,112.4)}=1.28$, $P>0.1$

619 ⁷ Effect of treatment x day on play alone: $F_{(1,110.8)}=0.02$, $P>0.1$

620 ⁸ Effect of treatment x day on tail oral manipulation: $F_{(1,107.3)}=1.52$, $P>0.1$

621 **Table 3** Weight, Average Daily Gain and Coefficient of Variation of litter weight artificially-reared
622 (AR) and sow-reared (SR) piglets. AR piglets were removed from their mother at 7 days-old and fed
623 milk replacer in the artificial-rearing enclosure until weaning, while SR piglets remained with their
624 mother. D0 is the day of transfer in the artificial-rearing enclosure for AR piglets, at 7 days-old.
625 Weaning was at 26 ± 0.4 days-old for AR piglets and 29 ± 0.4 days-old for SR piglets and was
626 accounted for in the analysis.

	SR	AR	S.E.	DF	t-value	P-value
Weight (kg)						
D0	2.86	2.73	0.100	269	-3.08	N.S.
D1	3.32	3.13	0.126	211	8.51	<0.001
D8	4.91	4.33	0.060	257	8.17	<0.001
D15	6.60	5.85	0.108	217	5.98	<0.001
Weaning	7.97	6.53	0.153	256	9.79	<0.001
Average Daily Gain (kg/day)						
D0-D1	0.23	0.05	0.014	231	9.26	<.0001
D1-D8	0.28	0.22	0.014	224	5.68	<.0001
D8-D15	0.29	0.26	0.019	195	2.02	<0.05
D15-Weaning	0.27	0.29	0.010	167	-1.43	N.S.
D0-Weaning	0.27	0.24	0.005	199	3.48	<0.001
Coefficient of variation						
D0	0.12	0.10	0.007	16.1	2.39	N.S.
D1	0.13	0.11	0.009	19.9	1.66	N.S.
D8	0.14	0.14	0.014	16.6	-0.06	N.S.
D15	0.14	0.13	0.015	19	0.4	N.S.
Weaning	0.14	0.09	0.013	15.5	2.97	N.S.

627 **Table 4** Mean (\pm S.E.) scores of the descriptors used in the Qualitative Behavioural Assessment (QBA)
628 of artificially-reared (fed milk replacer away from their mother, from 7 days of age until weaning)
629 and sow-reared piglets (normal lactation with mother); and eigenvectors values of the descriptors
630 on the principal components (PC) retained from the computation of the QBA terms scores (PCA
631 analysis).

632

			Eigenvalue		6.33	3.89
			% of variance explained		33%	15%
	Sow-reared	Artificially-reared	F-value	P-value	PC1 "Positive feelings"	PC2 "Low arousal"
Active	66.9 \pm 12.55	32.9 \pm 6.17	F(1,9) = 7.98	<0.05	0.28	-0.20
Relaxed	62.2 \pm 7.82	62.1 \pm 7.81	F(1,9) = 0	N.S.	0.05	0.04
Fearful	36.7 \pm 7.5	12.6 \pm 2.58	F(1,9) = 19.1	<0.005	0.23	-0.29
Agitated	9.3 \pm 5.21	2.1 \pm 1.18	F(1,9) = 3.53	0.09	0.21	-0.08
Calm	71.8 \pm 10.34	80.4 \pm 11.57	F(1,9) = 0.37	N.S.	0.02	0.49
Content	78.3 \pm 5.24	62.3 \pm 4.17	F(1,9) = 5.84	<0.05	0.29	0.20
Tense	0.0 \pm 0.02	0.0 \pm 0.02	F(1,9) = 0.05	N.S.	0.05	-0.15
Enjoying	84.0 \pm 7.59	40.5 \pm 3.66	F(1,9) = 32.62	<0.001	0.37	0.06
Frustrated	7.8 \pm 2.18	12.4 \pm 3.46	F(1,9) = 1.38	N.S.	-0.14	-0.21
Sociable	84.2 \pm 5.73	74.4 \pm 5.06	F(1,9) = 1.93	N.S.	0.24	0.05
Bored	5.2 \pm 1.81	22.4 \pm 7.81	F(1,9) = 8.77	<0.05	-0.27	0.30
Playful	83.5 \pm 18.57	22.6 \pm 5.03	F(1,9) = 17.26	<0.005	0.36	-0.08
Positively-occupied	76.0 \pm 5.77	56.7 \pm 4.30	F(1,9) = 7.45	<0.05	0.26	0.06
Listless	0.0 \pm 0.00	0.0 \pm 0.00	F(1,9) = 1	N.S.	0.10	0.30
Lively	85.8 \pm 8.25	53.7 \pm 5.16	F(1,9) = 12.83	<0.01	0.30	-0.09
Indifferent	18.4 \pm 10.28	13.4 \pm 7.48	F(1,9) = 0.16	N.S.	-0.07	-0.46
Irritable	0.0 \pm 0.00	4.3 \pm 6.02	F(1,9) = 401.95	<0.001	-0.13	0.13
Aimless	0.0 \pm 0.00	4.7 \pm 1.94	F(1,9) = 210.3	<0.001	-0.11	-0.22
Happy	80.7 \pm 7.28	52.7 \pm 4.76	F(1,9) = 12.33	<0.01	0.35	0.18
Distressed	0.0 \pm 0.00	0.0 \pm 0.00	F(1,9) = 1	N.S.	0.00	0.00

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634

635 **Figure 1** Schematic representation (a) and picture (b) of an artificial-rearing enclosure (Rescue
636 Deck®, S&R Resources LLC). *TResearch* magazine, Teagasc. Graphic prepared by ThinkMedia.

637

638 **Figure 2** Mean (\pm S.E.) occurrence of behaviours per minute, during the 20 min following transfer of
639 artificially-reared (AR) piglets in the artificial-rearing enclosure. Sow-reared (SR) piglets remained
640 with their mother.

641

642 **Figure 3** Graphical representation of Principal Component Analysis (PCA) outcomes for Qualitative
643 Behavioural Assessment (QBA) of artificially-reared (AR) and sow-reared (SR) piglets. QBA was done
644 on D14, when piglets were 21 days-old. AR piglets were removed from their mother at 7 days-old
645 and fed milk replacer in the artificial-rearing enclosure until weaning, while SR piglets remained with
646 their mother.

647 a) Eigenvector values of each descriptor on the two principal components retained from the PCA.

648 Principal component 1 represented 33% of the total variation of QBA score, and principal
649 component 2 represented 15% of the total variation of the QBA score.

650 b) Loadings of the AR and SR litters along the two principal components retained from the PCA.

651