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**Assessing behavioural and physiological implications of intermittent suckling on primiparous sows and their litters**

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23 **Abstract.** The objective of the current study was to determine the implications of  
24 intermittent suckling (IS) on primiparous sows and their litters. Behavioural  
25 observations, udder scores, return to nursing and salivary cortisol  
26 concentrations were measured. An intermittent suckling protocol was applied to  
27 22 primiparous sows and their litters. At three weeks of age piglets were  
28 separated for eight hours a day, for seven days prior to weaning. Control litters  
29 had the sow continuously present until weaning. Video data was collected; one  
30 hour before and after separation and one hour before and after rejoining on days  
31 one, three and six. Cortisol samples were collected at 06:00 h on day one and  
32 then at 12:00 h on day two, four and seven. Udder scoring was carried out at the  
33 start of separation, day two of separation and at weaning. Return to suckling  
34 bouts were recorded on days one, three and six for two hours after rejoining. The  
35 study found that during periods of separation the IS piglets spent significantly  
36 more time resting in comparison to control litters (postsep  $86.9 \pm 1.6\%$  vs  
37  $76.5 \pm 2.3\%$ ; prejoin  $79.1 \pm 2.0\%$  vs  $64.2 \pm 2.2\%$ ;  $P < 0.05$ ). Once rejoined to the sow,  
38 piglets activity significantly increased ( $P < 0.05$ ), but then gradually declined over  
39 the one hour postjoin period. On day two, postsep cortisol concentrations for IS  
40 piglets were significantly greater than base levels seen pre-separation on day  
41 one ( $P < 0.05$ ). However, day four and seven cortisol concentrations were similar  
42 to the base concentration, suggesting that piglets had adapted to the separation.  
43 Greater activity around the udder during the rejoining period, determined by  
44 increased time spent massaging the udder and nursing ( $P < 0.05$ ) and a greater  
45 numbers of suckling bouts in IS litters ( $P < 0.05$ ), did not lead to increased  
46 physical damage of the udder. Sow behavior was altered during the rejoining  
47 period, with IS sows spending more time standing ( $26.2 \pm 2.8\%$  vs  $8.5 \pm 2.2\%$ ;

48 P<0.05). However, cortisol concentrations for sows were not significantly  
49 different between control and IS treatments (P=0.34). Therefore, based on the  
50 minimal changes in behavior and cortisol concentrations of piglets and sows  
51 during separation, eight hours separation over seven days does not appear to  
52 adversely affect piglet or sow welfare.

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54 **Key words:** weaning, separation, cortisol, udder score

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70 **Introduction**

71 Traditionally within the Australian pig industry, piglets are weaned from sows at  
72 three to four weeks of age (Langendijk *et al.* 2007a,b). This may raise welfare  
73 concerns for the piglets, as the abrupt change to a solid weaning diet and mixing  
74 of unfamiliar litters leads to stress and reduced growth (Kuller *et al.* 2004).  
75 When lactation length is shortened to between four and 21 days post farrowing,  
76 reduced reproductive performance is observed (Cole *et al.* 1975; Varley and Cole  
77 1978; Gaustad-Aas *et al.* 2004), with lower ovulation rates, lower conception  
78 rates, smaller litter sizes at the subsequent farrowing, and a greater chance of  
79 the sow developing cystic ovaries (Peters *et al.* 1969; Cole *et al.* 1975). In spite of  
80 this, a reduction in lactation length results in faster reproductive turnaround and  
81 therefore more litters per sow each year, which in turn supports increased  
82 profitability (Petchey and Jolly 1979). While early weaning has obvious  
83 economic benefits, an increased lactation length assists in the growth and  
84 development of the piglets, yet this will come at a cost to sow lifetime  
85 reproductive performance (Berkeveld *et al.* 2007a,b; Hoshino and Koketsu  
86 2008). The need for economically viable production which promotes both sow  
87 fertility and piglet growth has led to considerable research into methods to  
88 reduce the farrowing to oestrus interval or non-productive days of the sow  
89 without negatively impacting upon the welfare of sows or piglets (Kirkwood and  
90 Thacker 1989; Zak *et al.* 2008). Currently, the most successful strategy has been  
91 to induce oestrus during lactation, which also allows for an increased weaning  
92 age, improving the welfare of piglets at weaning (Langendijk *et al.* 2007a;  
93 Gerritsen *et al.* 2008).

94 Suckling inhibition is a major factor suppressing ovarian follicle development in  
95 lactating sows and needs to be limited if sows are to display oestrus and ovulate  
96 during lactation (Newtown *et al.* 1987). Intermittent suckling has been identified  
97 as a method to alleviate the restraints placed on ovulation due to the suckling  
98 suppression (Kuller *et al.* 2004). This process involves separating the sow and  
99 piglets for a period of time each day after day 14 of lactation. However, a  
100 difference in the response to separation has been identified between  
101 primiparous and multiparous sows (Hulten *et al.* 2006). During lactation,  
102 primiparous sows have been recognised as forming stronger bonds with their  
103 litters (Hulten *et al.* 2006). Without separation, primiparous sows are more  
104 likely to remain anoestrous during lactation, while multiparous sows are more  
105 likely to spontaneously ovulate (Kunavongkrit *et al.* 1982; Newtown *et al.* 1987).  
106 While separating sows and their piglets prior to weaning can stimulate sows into  
107 a lactational oestrus, the consequences of this separation on the welfare of both  
108 the sow and piglets have yet to be explored.

109 The welfare of an animal is its state in regards to its attempts to cope with its  
110 environment. When animals are faced with conditions in which they cannot  
111 cope, their individual fitness may be reduced as a consequence of physiological  
112 and behavioural responses. Attempts to cope include the functioning of the body  
113 repair system, physiological stress responses, immunological defenses and  
114 behavioural changes (Broom 1986).

115 Using the definition of Broom (1986), welfare risks can be studied at two levels:  
116 the behavioural and physiological responses of the animal to the challenge, and  
117 secondly the consequent biological cost to the animal (i.e. reduced biological

118 fitness such as reduced growth rate). The behavioural and physiological changes  
119 by the animal to the environment are the response that the animal uses in an  
120 attempt to re-establish homeostasis. Substantial and prolonged behavioural and  
121 physiological responses can have effects on biological fitness by affecting growth  
122 performance, reproduction, injury, health and survival (Broom and Jonson 1993;  
123 Barnett and Hemsworth, 2010).

124 Therefore, the overall objective of this experiment was to identify the  
125 implications of subjecting primiparous sows and their litters to an intermittent  
126 suckling regime and to assess the behaviour and physiological stress responses  
127 of the sow and her piglets. The hypothesis was that separation would not cause  
128 alterations in the behaviour or physiology of the sows and piglets that indicated  
129 the animals were distressed.

130

## 131 **Materials and Method**

132 All animal procedures were conducted with prior institutional ethical approval  
133 under the requirements of the NSW Prevention of Cruelty to Animals Act 1985,  
134 in accordance with the National Health and Medical Research  
135 Council/Commonwealth Scientific and Industrial Research  
136 Organisation/Australian Animal Commission *Code of Practice for the Care and*  
137 *Use of Animals for Scientific Purposes.*

138

### 139 ***Animals and accommodation***

140 Research was conducted at the Research and Innovation unit of Rivalea  
141 Australia, a large commercial piggery in Corowa NSW, during June and July of

142 2013. The study described in this paper was associated with a larger study,  
143 which focused on the sow's return to oestrus during lactation. A total of 22 Large  
144 White x Landrace primiparous sows and their litters were used for the  
145 experiment over three consecutive weekly batch farrowings. Each shed  
146 (farrowing unit) was comprised of 64 farrowing crates (1.67 wide x 2.25 m long).  
147 Crates had slatted floors with rubber mats beneath a single overhanging heat  
148 lamp provided for the piglets (Diagram 1). Sows were fed three times a day (*ad*  
149 *libitum*) and had unlimited access to water. Piglets could move freely around the  
150 farrowing crate and had unlimited access to drinking water. In addition, they had  
151 access to creep feed *ad libitum* from the start of the separation period. All piglets  
152 were tail docked at day one of age and no teeth clipping was performed. Males  
153 were left intact. Gilts were randomly assigned pens based on the order they  
154 entered the farrowing shed. Treatment and control groups were housed on  
155 opposing sides of the shed to minimize the effects of boar exposure, which was  
156 part of the larger study; on control sows. The piglets were weaned at  
157 approximately 28 days of age.

158

### 159 ***Treatments***

160 The two treatments were:

161 Control group (C) – A conventional lactation in which litters remained with the  
162 sow throughout until weaning.

163 Intermittent suckled groups (IS) – Seven days prior to weaning (denoted as day  
164 one), piglets were separated from the sow for eight hours each day (from 07:00  
165 to 15:00 h). A board was positioned between the sow and her litter within the  
166 crate. Separated piglets were then given access to both water and creep feed

167 during separation (Diagram 2). Approximately 30 minutes prior to rejoining the  
168 IS piglets were provided with *ad libitum* supplemental milk as an attempt to  
169 reduce the suckling intensity when they rejoined the sow.

170

171 ***Behavioural observations***

172 Digital video cameras (Signet; Model OV-3063) and recorders (Electrus  
173 Distribution, Rydalmere, NSW, Australia) were set up above each farrowing  
174 crate. Video surveillance of sows and piglets in individual crates was conducted  
175 from 06:00 until 18:00 h on days one, three and six after the start of separation.  
176 Four piglets per litter were selected as focal animals for cortisol sampling and  
177 behavioural observations. Each sow and four piglets within the litter had their  
178 posture and activities recorded in the following time frames; one hour before  
179 separation (06:00 to 07:00 h) 'presep'; and one hour after separation (09:00 to  
180 10:00 h) 'postsep'; then one hour before rejoining (13:00 to 14:00 h) 'prejoin';  
181 and one hour after rejoining (15:00 to 16:00 h) 'postjoin'. The piglets assigned to  
182 cortisol sampling were also those used for behavioural observations; with a  
183 single observer analysing all video data. For each hour of observation, the sow's  
184 and piglets' behaviour was recorded continuously for one minute every five  
185 minutes (12 minutes in total; Diagram 3). The postures and activities recorded  
186 for each sow and piglets are described in tables 1 and 2. In each pen, the light  
187 from the heat lamp obstructed the view of piglets, therefore while piglets were  
188 under the heat lamp they were classified as resting.

189 There were periods where equipment failure limited the number of successful  
190 recordings in each time frame. The rate of successful recordings were presep  
191 85%, postsep 78%, prejoin 83%, postjoin 90%. On day one, for replication one, a



192 power outage occurred at 12 noon restricting the number of postsep recordings  
193 and gave the greatest proportion of failures.

194

195 ***Saliva sampling***

196 Saliva samples were collected on day one before separation (06:00 h), and then  
197 at 12:00 to 12:30 h on days two, four and seven after separation started. Saliva  
198 samples were taken in each replicate and were performed on each sow and the  
199 four focal piglets in each litter. In order to obtain the greatest volume of saliva,  
200 the two largest females and two largest males were selected within each litter.  
201 Males were labeled A and B and females were labeled C and D. Saliva was  
202 collected using a saliva collection tube (Salivettes, Sarstedt Australia, South  
203 Australia, Australia) for centrifugation. Two people collected samples in order of  
204 pen number (Rodate *et al.* 2010). For sows, 500mm cable ties were used to hold  
205 the cotton swab, which was then placed in the sow's mouth. Sows were given one  
206 minute to chew on the cotton swab. Piglets had 200mm cable ties attached to  
207 one end of the cotton swab, which was coated in icing sugar. Piglets were placed  
208 on each handler's lap and the cotton swab was inserted into their mouth for two  
209 minutes (Escribano *et al.* 2012). Piglet sampling occurred subsequently to sow  
210 sampling in order to minimize sow stress and collection occurred in two rounds.  
211 The first samples were collected from piglets A and C and the second samples  
212 were collected from piglets B and D. After centrifugation (3000 RPM for 6  
213 minutes at 4°C), cotton swabs were removed and saliva was stored at -20°C until  
214 required. Cortisol was then analysed using the Caymans cortisol EIA kit (No.  
215 500360, Cayman Chemical Company, MI, USA). Sufficient saliva was obtained  
216 from all sows and 85% of the piglets.

217

218 ***Udder scoring***

219 Udder scoring of both control and treatment sows was carried out at the start of  
220 piglet separation, on day two of separation and at the time of weaning. The same  
221 observer was used at all times. Udder scoring consisted of counting the number  
222 of functional teats and identifying wounds. There were five possible wound  
223 categories which included A, fresh scratch or cut; B, old scratch or cut; C,  
224 bruising/swelling; D, damaged teat (still functional); and E, damaged teat (non  
225 functional; severely damaged).

226

227 ***Suckling bouts after joining***

228 During the two hour period after rejoining, the number of lactational suckling  
229 bouts was recorded. Suckling bouts were only recorded when the sow was lying  
230 and presented her udder to the piglets. No record was made of suckling bouts  
231 while the sow was standing due to the difficulty of determining if piglets were  
232 nursing or not. Data was collected on day one, three and six of the study.

233

234 ***Statistical analysis***

235 ***Behavioural analysis.*** Data was collected from 22 sows and four piglets  
236 within each litter. Statistical analysis was carried out using a Generalised Linear  
237 Mixed Model (GLMM) in Genstat 15<sup>th</sup> Edition (2012). For all behaviour models  
238 the random terms were, repetition, repetition x pen. The fixed terms in the final  
239 model were treatment, time frame (presep, postsep, prejoin, postjoin), treatment  
240 x time frame, time frame x minute, treatment x time frame x minute. For day data  
241 the fix term was treatment x day and the random model was, repetition,

242 repetition x pen. Terms found to be non significant were dropped from the  
243 model (Table 3 and 4).

244

245 Standard significance levels were used with a P value <0.05 indicating a  
246 significant effect. Because it was considered that there would be a significant  
247 correlation between time points, whereby one time point affected the result  
248 observed at the next time point, a covariant was created for each parameter and  
249 included in the model to account for this effect. All results from the GLMM  
250 behavioural analysis are given as a probability of the sow or piglet engaging in  
251 the behaviour or not (binomial). Where effects were found to be significant,  
252 individual comparisons were made using Fisher's LSD (calculated to be 2 X SED).

253

254 ***Suckling bouts after rejoining analysis.*** Data from the 22 sows was  
255 analysed using a Linear Mixed Model in Genstat 15<sup>th</sup> Edition. For this model, the  
256 random terms were shed and pen and the fixed model was treatment plus day.  
257 Terms found to be non significant were dropped from the model and standard  
258 significant levels were used, where a P value <0.05 indicated a significant effect.

259

260 ***Udder score analysis.*** An ordinal regression using weighted values was  
261 used to analyse the udder score data and was conducted in Minitab version 16  
262 (2013). Ordinal regression was used, as scores were categorized A-E in terms of  
263 severity. Counts on the number of wounds were used as the weighted values.

264

265            **Cortisol analysis.** Cortisol data was analysed using a Linear mixed model  
266 in Genstat 15th Edition (2012). For this model, the random terms were  
267 replication and pen and the fixed model terms were the treatment and day.

268

## 269 **Results**

### 270 ***Video observations of sow and piglet behaviour***

#### 271 ***Sow behaviour***

272 The proportions of time spent displaying each posture and behavior for sows is  
273 given in table 3.

274            **Posture.** The total proportion of time spent lying and standing was  
275 similar between the control and IS sows in all time frames with the exception of  
276 the postjoin period. During this time the control sows spent significantly less  
277 time standing in comparison to IS sows ( $P<0.05$ ;  $8.5\pm 2.2\%$  vs  $26.3\pm 2.8\%$ ; see  
278 Figure 1). As such, control sows spent a significantly greater proportion of time  
279 lying during this time frame ( $P<0.05$ ;  $91.3\pm 2.4\%$  vs  $76.0\pm 3.1\%$ ).

280

281            **Behaviour.** Behaviour of the IS sows was similar to control sows during  
282 the hour before separation. Once separation began, changes between the  
283 treatments were seen, with IS sows spending a significantly greater proportion  
284 of their time eating and drinking than did control sows during the prejoin period  
285 ( $P<0.05$ ;  $24.7\pm 3.3$  vs  $19.2\pm 3.3\%$ ). This difference continued through into the  
286 postjoin period, with IS sows spending significantly more time eating and  
287 drinking ( $P <0.05$ ;  $24.1\pm 3.2\%$  vs  $11.9\pm 2.7\%$ ). In addition to the significant  
288 increase in feeding activity of IS sows, behaviours including watching and  
289 playing with piglets ( $P<0.05$ ;  $31.0\pm 2.6\%$  vs  $11.0\pm 2.9\%$ ) and nursing ( $17.4\pm 2.0\%$

290 vs  $11.5 \pm 2.0\%$ ) similarly increased significantly post join. As expected, because of  
291 this increased activity, the time spent resting postjoin was significantly lower in  
292 IS sows ( $P < 0.05$ ;  $42.7 \pm 3.6\%$  vs  $67.7 \pm 3.9\%$ ; Figure 2).

293

#### 294 ***Piglet behaviour***

295 The proportions of time spent displaying each posture and behavior for piglets is  
296 given in table 4.

297 ***Posture.*** Piglet posture remained similar between control and IS litters  
298 before separation. Once separated, there were differences in posture between  
299 the treatments. Postsep, IS piglets stood significantly ( $P < 0.05$ ) less time than  
300 control piglets ( $13.7 \pm 1.2\%$  vs  $26.5 \pm 1.8\%$ ). In addition IS piglets stood  
301 significantly less often during postsep in comparison to other time frames  
302 (presep  $29.1 \pm 1.8\%$ ; prejoin  $25.8 \pm 1.6\%$ ; postjoin  $57.5 \pm 1.6\%$ ). The IS piglets spent  
303 similar proportions of time standing in the presep and prejoin periods  
304 ( $29.1 \pm 1.8\%$  vs  $25.8 \pm 1.6\%$ ), but both differed significantly ( $P < 0.05$ ), to the  
305 postsep ( $13.7 \pm 1.2\%$ ) and postjoin ( $57.5 \pm 1.6\%$ ) periods. This reduction in  
306 standing frequency led to a significantly greater proportion of time lying in IS  
307 litters during periods of separation (postsep  $86.1 \pm 2.0\%$ ; prejoin  $78.0 \pm 2.7\%$ ;  
308 Figure 3). Lying without sow contact dropped significantly once IS piglets  
309 rejoined the sows in comparison to control litters ( $30.3 \pm 3.4\%$  vs  $60.5 \pm 3.5\%$ )  
310 Within this time frame IS piglets then stood significantly more than control  
311 litters ( $P < 0.05$ ;  $57.5 \pm 1.6\%$  vs  $19.2 \pm 1.6\%$ ). Separated litters showed a gradual  
312 decline in the proportion of time spent standing postjoin, but this never reached  
313 a similar base level seen in control litters (Figure 4).

314

315            **Behaviour.** During the experiment no aggressive behavior was observed  
316 and casual observation of piglets showed no signs of injuries which may have  
317 indicated aggressive behaviours. All behaviours were similar between IS and  
318 control piglets prior to separation ( $P>0.05$ ). During the postsep period  
319 differences between treatments were observed. The IS piglets spent significantly  
320 more time inactive, with increased time spent resting compared to controls  
321 ( $P<0.05$ ;  $86.9\pm 1.6\%$  vs  $76.5\pm 2.3\%$ ). This increase in resting led to a significant  
322 ( $P<0.05$ ) decline in the proportion of time IS piglets spent walking and running  
323 ( $2.0\pm 0.4\%$  vs  $3.9\pm 0.7\%$ ), being idle ( $6.3\pm 0.8\%$  vs  $9.9\pm 1.2\%$ ) and playing  
324 ( $2.3\pm 0.6\%$  vs  $5.4\pm 1.2\%$ ). The proportion of time spent resting and inactive  
325 continued into the prejoining time frame. Once piglets were rejoined with the  
326 sow, activity levels increased. The IS piglets spent significantly less time resting  
327 postjoining in comparison to control litters ( $P<0.05$ ;  $36.1\pm 2.3\%$  vs  $74.2\pm 2.6\%$ ).  
328 Postjoin there was increased activity at the udder, with IS piglets massaging the  
329 udder significantly more often than control ( $P<0.05$ ;  $50.8\pm 2.1\%$  vs  $25.0\pm 2.6\%$ ).  
330 In addition, a significantly greater proportion of time was spent playing ( $P<0.05$ ;  
331  $11.7\pm 1.2\%$  vs  $1.7\pm 1.2\%$ ), walking and running ( $P<0.05$ ;  $12.5\pm 0.7\%$  vs  $3.5\pm 0.8\%$ ),  
332 idle ( $P<0.05$ ;  $22.4\pm 1.0\%$  vs  $6.8\pm 1.2\%$ ) and nudging ( $P<0.05$ ;  $8.6\pm 0.4\%$  vs  
333  $1.2\pm 0.4\%$ ). Each of these behaviours peaked at the start of rejoining but  
334 gradually declined over the one hour time frame (see Figures 5). Over all time  
335 frames with the exception of presep, eating and drinking was significantly higher  
336 ( $P<0.05$ ) in IS litters compared to control litters (postsep  $6.6\pm 1.0\%$  vs  $3.2\pm 0.7\%$ ;  
337 prejoin  $10.8\pm 1.3\%$  vs  $3.6\pm 0.7\%$ ; postjoin  $8.4\pm 0.5\%$  vs  $1.4\pm 0.6\%$ ).

338

339

340 ***Changes across days***

341 Changes to sow behaviours and postures were examined across days one, three  
342 and six. In sows no significant effect of treatment and day was found for standing  
343 ( $P=0.63$ ), sitting ( $P=0.39$ ), lying ( $P=0.80$ ), resting ( $P=0.07$ ), eating and drinking  
344 ( $P=0.08$ ), watching piglets play ( $P=0.74$ ), and rubbing ( $P=0.86$ ). The time sows  
345 spent watching piglets was marginally non-significant ( $P=0.051$ ), with control  
346 sows on day two spending proportionally more time watching piglets than on  
347 other days.

348

349 For IS sows the time spent nursing was similar on days one and three but higher  
350 on day six ( $P<0.05$ ; d1  $15.4\pm 3.0\%$ ; d3  $14.6\pm 3.2\%$ ; d6  $24.4\pm 3.8\%$ ). For control  
351 sows nursing time was higher on day three than other days ( $P<0.05$ ; d1  
352  $10.1\pm 3.3\%$ ; d3  $17.6\pm 4.1\%$ ; d6  $9.0\pm 3.1\%$ ).

353

354 Some changes in piglet behaviours and postures were seen across the days. The  
355 proportion of time spent sitting ( $P=0.15$ ), being idle ( $P=0.07$ ), walking and  
356 running ( $P=0.17$ ), playing ( $P=0.34$ ), nudging ( $P=0.59$ ) and watching piglets  
357 ( $P=0.93$ ) were not different across days. The proportion of time spent lying with  
358 (d1  $20.5\pm 7.4\%$ ; d6  $8.1\pm 3.4\%$ ) and without (d1  $70.9\pm 3.1\%$ ; d6  $60.3\pm 3.5\%$ ) sow  
359 contact declined significantly ( $P<0.05$ ) over the course of the experiment for IS  
360 piglets, while time lying without sow contact for control litters remained similar  
361 across days ( $P>0.05$ ; d1  $64.5\pm 3.5\%$ ; d3  $65.1\pm 3.0\%$ ; d6  $63.4\pm 3.5\%$ ). This  
362 correlated with a significant reduction in IS piglets proportion of time spent  
363 resting over the course of the trial ( $P<0.05$ ; d1  $74.4\pm 2.1\%$ ; d6  $62.3\pm 2.4\%$ ).

364

365 Significant variations across days were found for standing, eating and drinking  
366 and the time spent massaging the udder. A significant reduction in standing was  
367 seen on day three for IS litters compared to other days ( $P<0.05$ ; d1  $31.2\pm 1.5\%$ ;  
368 d3  $23.8\pm 1.3\%$ ; d6  $33.7\pm 1.5\%$ ). The proportion of time spent eating and drinking  
369 was significantly different between day one and day three while day six was  
370 similar to both days for IS litters ( $P<0.05$ ; d1  $7.4\pm 1.0\%$ ; d3  $4.8\pm 0.7$ ; d3  $6.4\pm 0.9$ ),  
371 while control litters had a significant increase in time spent eating and drinking  
372 on day six compared to other days ( $P<0.05$ ; d1  $1.7\pm 0.4\%$ ; d3  $1.5\pm 0.4\%$ ; d6  
373  $4.1\pm 0.7\%$ ). Overall, across all days the proportion of time spent eating and  
374 drinking was always significantly greater in IS litters compared to control  
375 ( $P<0.05$ ). The time spent massaging the udder did not vary across days for IS  
376 piglets (d1  $55.1\pm 5.5\%$ ; d3  $48.5\pm 5.6\%$ ; d6  $59.4\pm 5.4\%$ ), while control litters had a  
377 significant increase on days three and six compared to day one ( $P<0.05$ ; d1  
378  $19.5\pm 4.0\%$ ; d3  $32.7\pm 5.2\%$ ; d6  $28.7\pm 4.9\%$ ).

379

### 380 *Salivary cortisol*

381 Piglet sex had no effect on cortisol concentrations ( $P=0.633$ ). There was no  
382 treatment x day interaction for sow cortisol concentrations ( $P=0.340$ ).  
383 Treatment for piglets had a significant effect on cortisol concentrations but this  
384 changed with time as the interaction between treatment and time was significant  
385 ( $P=0.009$ ; Figure 6). The IS piglets showed a gradual decline in the concentration  
386 of cortisol during periods of separation (Figure 6). On day two of IS treatment,  
387 piglets' cortisol concentration was significantly greater than the concentration  
388 on day one, which was taken prior to separation ( $P<0.05$ ). Samples on day four  
389 and seven showed a gradual decline from day two concentrations and were not



390 significantly different from the base concentrations taken on one day presep  
391 ( $P>0.05$ ). Control piglets were similar to IS piglets in all time frames except day  
392 seven where control piglets had a significantly greater salivary cortisol  
393 concentration ( $P<0.05$ ).

394

#### 395 ***Udder score***

396 No significance difference in udder injury was found between treatment and  
397 control sows ( $P=0.909$ ). In addition no significant differences were found  
398 between treatments in each of the sampling time frames; Start of separation  
399 ( $P=0.212$ ), separation day 2 ( $P=0.767$ ) and weaning ( $P=0.524$ ).

400

#### 401 ***Suckling behaviour post joining***

402 The IS sows had more suckling bouts during the rejoining period ( $P<0.01$ ). Day  
403 had no effect on the number of suckling bouts post joining ( $P=0.771$ ).

404

#### 405 **Discussion**

406 The key aim of this study was to determine the behavioural and physiological  
407 implications of IS on primiparous sows and their litters. This is the first  
408 comprehensive investigation of the welfare effects of IS on primiparous sows  
409 and their litters. Based on the minimal changes in behaviour and cortisol  
410 concentrations of piglets and sows, eight hours separation over seven days does  
411 not appear to adversely impact piglet or sow welfare, as per the original  
412 hypothesis.

413

414 The results of this study indicate changes between the behaviours of IS sows and  
415 piglets compared to control sows and piglets. Piglets subjected to IS had a  
416 decreased level of activity during periods of separation, seen as an increase in  
417 resting behaviour. High periods of inactivity have been associated with  
418 symptoms of stress (Colson *et al.* 2006). In the current study, the higher level of  
419 inactivity was most likely due to a decrease in behaviours such as nursing and  
420 may be seen as an indicator of piglets' comfort in their environment, rather than  
421 a sign of distress (Berkeveld *et al.* 2007a). This interpretation is based on the  
422 salivary cortisol concentrations, which were similar between IS and control  
423 piglets during the course of the experiment.

424

425 In this study, cortisol measurements were taken as physiological indicators of  
426 stress. Obtaining saliva has been shown to be a noninvasive procedure for  
427 collecting samples for cortisol analysis (Gallagher *et al.* 2002; Escribano *et al.*  
428 2012). In addition, when working with neonates, saliva is the most accessible  
429 body fluid (Gallagher *et al.* 2002), and has been shown to have a good correlation  
430 to blood cortisol concentrations (Escribano *et al.* 2012). For the IS treated  
431 piglets, cortisol concentration on day two, was significantly higher than the base  
432 cortisol concentrations measured prior to separation on day one. This increased  
433 cortisol concentration is most likely an acute response to separation, which was  
434 also found by Kluivers-Poodt *et al.* (2010). Similar to the current study, these  
435 researchers found that after day two of separation there were no significant  
436 effects on cortisol concentrations. The present study showed that by day four  
437 cortisol concentrations in IS piglets were similar to the base values, and this may  
438 suggest piglets had adapted to the separation procedure. While cortisol was

439 higher on day two compared to its base concentration it was not significantly  
440 different to the concentrations seen in control piglets. In fact the IS and control  
441 piglets had similar cortisol concentrations across all days, except day seven  
442 where cortisol was significantly higher in the control piglets. The day seven  
443 effects could be due to husbandry procedures, as it was noted in retrospect that  
444 piglets in the experiment had been vaccinated roughly 30 minutes before saliva  
445 samples were taken on day seven. The lower response to handling, determined  
446 by a lower cortisol concentration, in IS piglets could be due to piglets being more  
447 habituated to handling during the experiment (Rodarte 2010). The handling  
448 during separation could have also reduced the fear response to human handling  
449 during the vaccination procedure (Day *et al.* 2002).

450

451 Once IS piglets were rejoined with the sow, increased levels of activity were  
452 observed. Berkeveld *et al.* (2007b) also demonstrated increased activity after  
453 rejoining piglets and sows following 12 hours of separation. There was anecdotal  
454 evidence that the greater proportion of time spent at the udder and nudging the  
455 sow in the IS treatment may cause discomfort to the sow and result in udder  
456 injury. While there was a greater level of activity around the sows once piglets  
457 were rejoined, activity gradually declined within the one hour period after  
458 rejoining. However, the activity level did not decline to the same level as the  
459 control piglets during this same time frame (Figure 5). Heightened activity of the  
460 piglets post joining, with a greater proportion of time spent playing, which is a  
461 positive welfare indicator, could support the view that the piglets were in a  
462 positive emotional state once rejoined with the sow (Nunes 2004; Cameron  
463 2008).

464

465 Sow activity remained similar between IS and control treatments with the  
466 intermittent suckling procedure leading to no significant change in cortisol  
467 concentrations and no significant variation in behaviours during the presep and  
468 postsep periods. Differences arose in the prejoin period with IS sows spending  
469 more time eating and drinking and postjoin where there were differences in  
470 postures and behaviours between the treatments. During the postjoin period IS  
471 sows displayed heightened levels of activity. Wattanakul *et al.* (1997) also found  
472 that after the separation board was removed, sows stood up and sniffed piglets  
473 more frequently. Wattanakul and colleagues (1997) continued observations for  
474 an extra hour during rejoining and found that sows tended to lie and rest at the  
475 same frequency seen before rejoining. A proposed concern with the IS separation  
476 procedure is that once the piglets are rejoined with the sow, the udder nudging  
477 and massaging would increase the amount of damage to the udder. However, no  
478 significant difference in udder damage was observed between treatment and  
479 control groups in the present study and this is supported by Bereveld *et al.*  
480 (2007a). In the majority of litters the IS piglets were provided with supplemental  
481 milk prior to rejoining and this may have reduced the suckling activity when  
482 rejoined. The problem with this consideration is that there are no details as to  
483 how many piglets actually consumed the supplemental milk replacer. Further  
484 research is required to assess whether supplemental milk provided before  
485 rejoining reduces suckling intensity post joining when IS is used.

486

487 Increased activity by the sows after rejoining has been shown to have negative  
488 effects on nursing frequency, with restless sows nursing less frequently

489 (Wallenbeck *et al.* 2008) or nursing while standing. Restless sows are more likely  
490 to terminate nursing after a shorter period of udder massage (Varlos *et al.* 2002)  
491 and sows that nurse when standing reduce the amount of milk available to their  
492 piglets, as normal stimulation of the udder is not possible (Jensen *et al.* 1991). In  
493 the present study IS sows had significantly more suckling bouts than control  
494 sows. This behavior did not change over the experimental period, suggesting that  
495 sows did not acclimate to the increased suckling attempts after rejoining by the  
496 piglets. It could be assumed that increased suckling bouts are likely to result in  
497 “unsuccessful” nursing (nursing bouts within 20 mins of each other) with effects  
498 on piglet development (Berkeveld *et al.* 2007a; Spinka *et al.* 1997). While this is  
499 possible, other reports have suggested that increased suckling attempts between  
500 milk letdown allows for greater milk intake (Spinka *et al.* 1997). This is because  
501 a similar amount of milk is let down every 35 minutes and suckling less  
502 frequently will not lead to an accumulation of milk at the udder, but a less  
503 frequent retrieval of this standard dose (Audist *et al.* 2000). Consequently higher  
504 nursing frequency has been found to positively influence piglets’ growth (Valros  
505 *et al.* 2002). Further research on the impacts of changing suckling behavior on  
506 piglet growth performance is required.

507

508 Concerns are commonly raised about the welfare implications of abrupt weaning  
509 (Weary *et al.* 2008). In the wild, piglets are weaned gradually from the sow  
510 allowing for a more gradual transition onto solid feed (Martin 1984; Jensen and  
511 Recen 1989). However, in the pig industry it is normal to abruptly wean piglets  
512 at three to four weeks of age (D’Eath 2005). This practice results in weaner ‘set-  
513 back’ because of a change in diet and the stress from the mixing with unfamiliar

514 litters (Colson *et al.* 2006; Silerova *et al.* 2010). A change from liquid to solid feed  
515 leads to low feed intake predisposing piglets to diarrhoea and consequently  
516 weight loss (Madec 1998; McCracken *et al.* 1999). By integrating IS into the  
517 production system in an attempt to induce lactational oestrus in the sow, piglets  
518 could remain on the sow for a prolonged period of time and become accustomed  
519 to a new diet, allowing for a more gradual transition to weaning (Kuller *et al.*  
520 2004; Berkeveld *et al.* 2007a). In the present study, this concept is supported by  
521 the significantly greater proportion of time that IS piglets spent eating compared  
522 to control litters. Berkeveld *et al.* (2007a) also found that shortly after the onset  
523 of IS, eating behaviour of IS piglets was greater than control piglets. Furthermore  
524 it has been found in outdoor systems that sows spend more time away from their  
525 piglets and nurse less frequently in late lactation, allowing piglets the  
526 opportunity to naturally wean onto creep feed (Pajor 2002). A similar situation  
527 is created by IS although not voluntarily. While creep feed intake was not  
528 determined in the current investigation, other studies have found increased  
529 creep feed intake with the implementation of IS (Langendijk *et al.* 2007; Gomez-  
530 Carballar *et al.* 2009). A conflicting study determined that separation for seven  
531 hours per day for two weeks prior to weaning failed to increase creep feed  
532 intake (Millet *et al.* 2007). This discrepancy may arise from the use of different  
533 separation techniques. Millet *et al.* (2007) actually removed sows from the  
534 farrowing crates and this would not only have inhibited the piglet's physical  
535 contact with the sow but also visual and auditory communication. Therefore, the  
536 piglets may have been more distressed in this instance, limiting creep feed  
537 intake.

538

539 In conclusion, IS for eight hours seven days prior to weaning in this study did not  
540 cause alternations to sow or piglet behaviour to a point that would warrant  
541 concern. In sows, behavioural changes only occurred post joining, with no  
542 behavioural or physiological differences between the treatments occurring  
543 during periods of separation. Once piglets were rejoined, they spent increased  
544 time at the udder but this did not lead to increased damage of the udder, which  
545 was a major concern within the industry. Separated piglets did have alterations  
546 in their behaviours, however, this was not to a point that would raise concern for  
547 their welfare as reflected by cortisol concentrations during periods of  
548 separation. While behavior did increase at rejoining the heightened levels of  
549 activity declined over the period, illustrating no prolonged alteration in piglet  
550 behaviour. Surprisingly separated piglets spent a proportionally greater amount  
551 of time around the creep feed area which may be advantageous as it allows for a  
552 gradual weaning process. Further research is recommended to fully understand  
553 the implication of these behavioural changes before piglet separation is  
554 recommended as an appropriate method to induce lactational oestrus in  
555 primiparous sows and a gradual transition to weaning for piglets.

556

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562

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## Intermittent Suckling to Stimulate Lactational Oestrus

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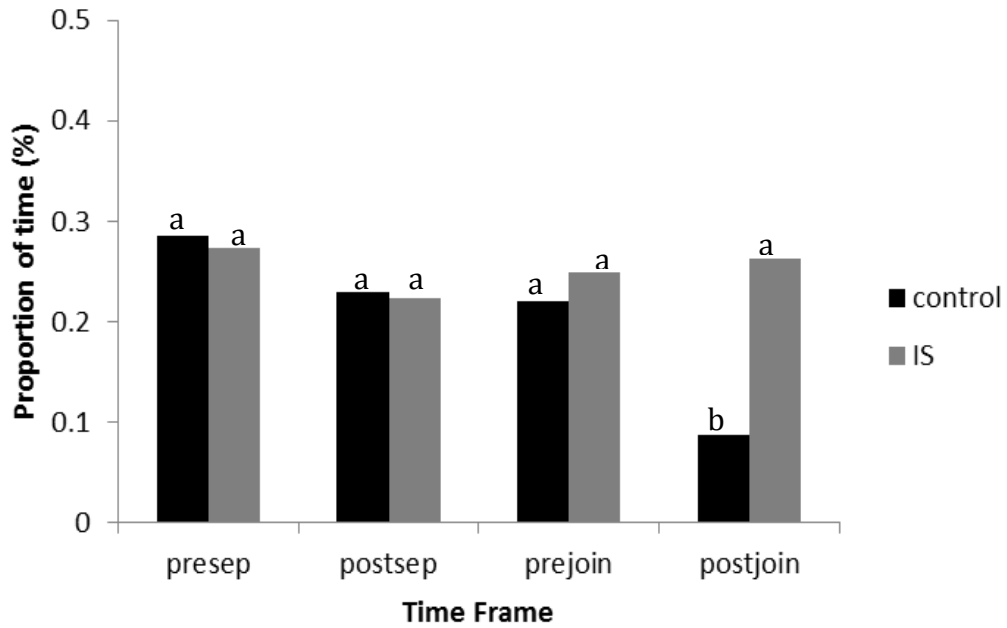
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702 **Fig. 1.** The proportion of time sows spent standing. Between and within  
703 treatments and across time frames values without common superscripts are  
704 significantly differences ( $P < 0.05$ ).

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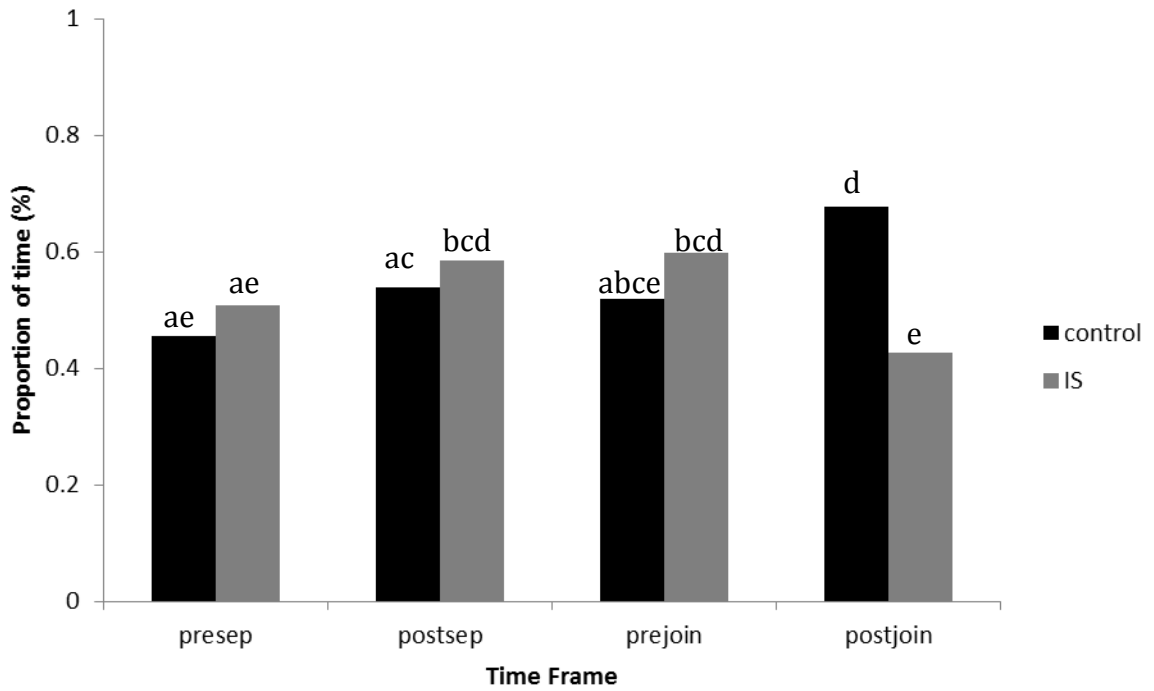
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## Intermittent Suckling to Stimulate Lactational Oestrus



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719 **Fig. 2.** The proportion of time sows time spent resting. Between and within  
720 treatments and across time frames values without common superscripts are  
721 significantly differences ( $P < 0.05$ ).

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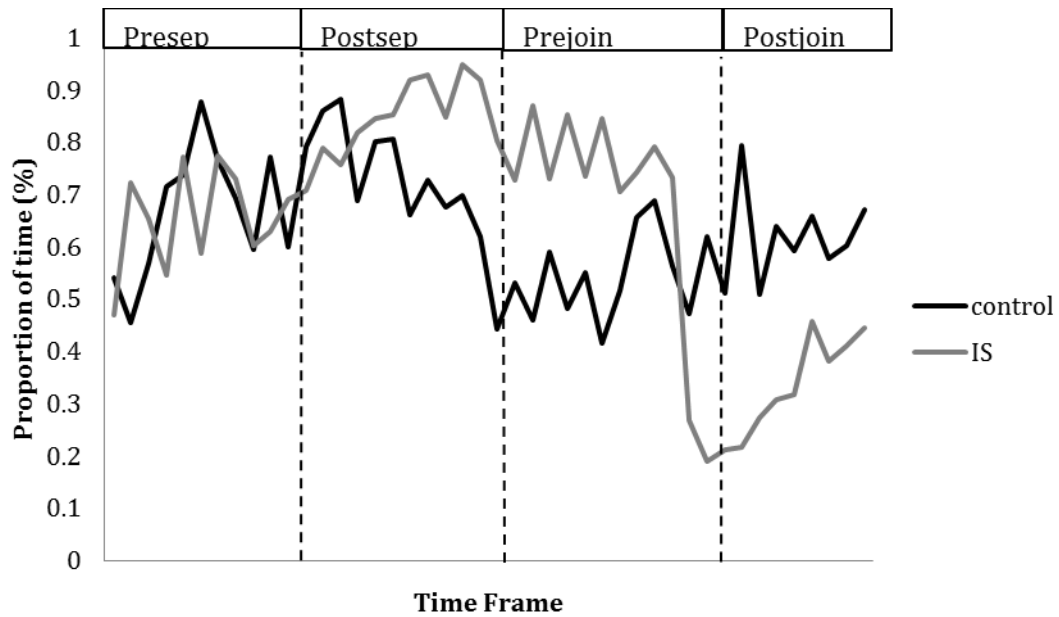
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735 **Fig. 3.** The proportion of time piglets spent lying without sow contact. Each  
 736 time frame illustrates changes over the hour before separation, one hour after  
 737 separation, one hour before rejoining and for one hour after rejoining with  
 738 observations made every five minutes.

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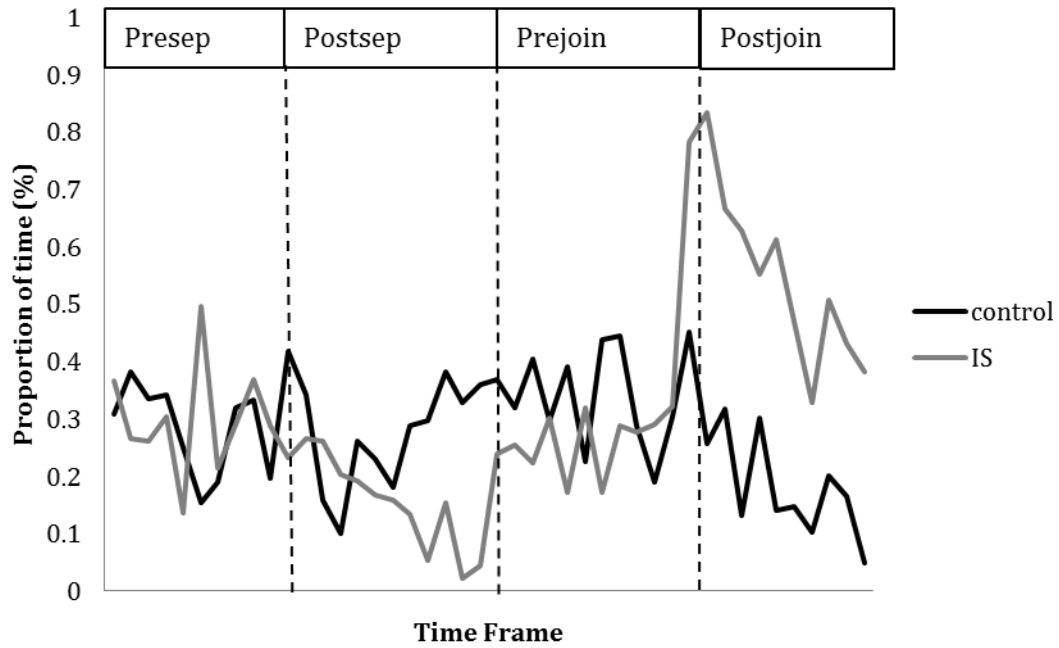
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751 **Fig. 4.** The proportion of time piglets spent standing. Each time frame  
 752 illustrates changes over the hour before separation, one hour after separation,  
 753 one hour before rejoining and for one hour after rejoining with observations  
 754 made every five minutes.

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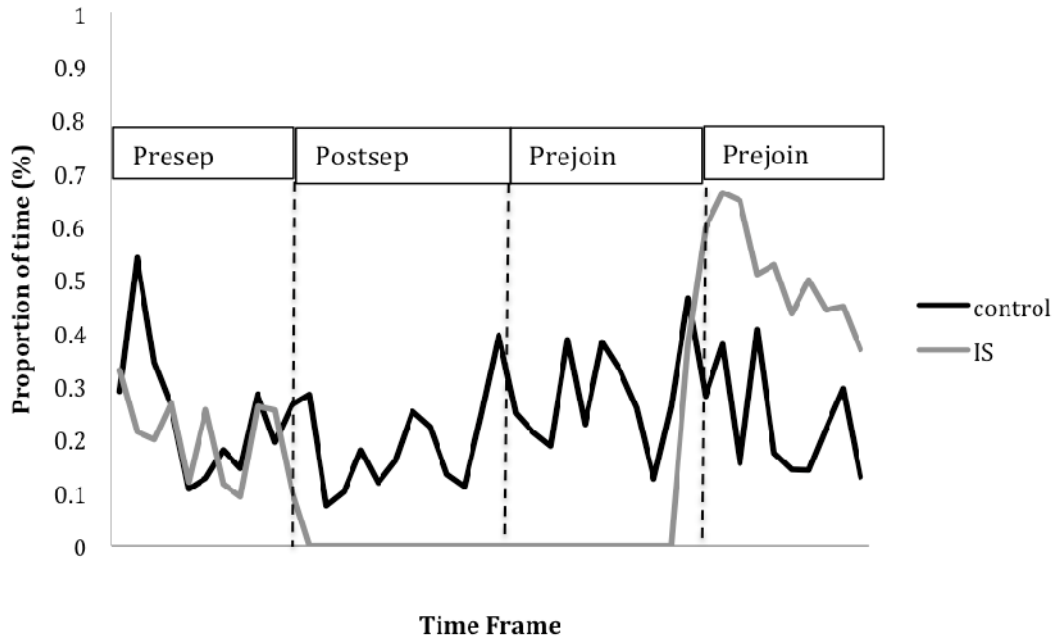
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767 **Fig. 5.** The proportion of time piglets spent massaging the udder. Each time  
 768 frame illustrates changes over the hour before separation, one hour after  
 769 separation, one hour before rejoining and for one hour after rejoining with  
 770 observations made every five minutes.

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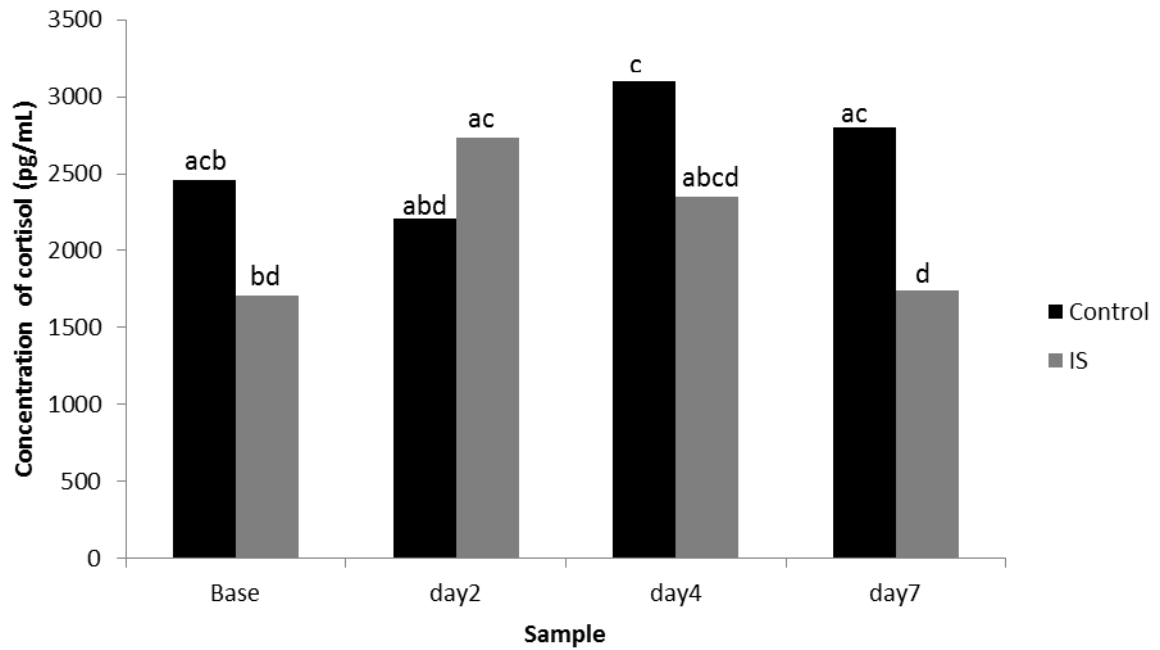
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## Intermittent Suckling to Stimulate Lactational Oestrus



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782 **Fig. 6.** The concentration of cortisol in the saliva on individual sampling days.

783 Between and within treatments values without common superscripts represent

784 significant differences ( $P < 0.05$ ).

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797 **Table 1.** Ethogram of sow behaviour recorded on days one, three and six.

<b>Behavioural Parameters</b>	<b>Definition</b>
<u>Sow</u>	
<i>Posture</i>	
Standing	Standing on all four feet
Siting	Upper body held up by forelegs and behind resting on floor
Lying	Included both sternal recumbence (lying on the udder) and lateral recumbence (lying on the side)
<i>Activity</i>	
Nursing	Lying of side with udder exposed and piglets nursing
Asleep	Head at rest with no movement by sow, can be lying either on body or on her side
Feeding/Drinking	Eating from the feed bin or drinking for the drinker
Watching	Lying, sitting or standing, showing no other state but visually awake
Looking for Piglets	May be nudging board separating sow and piglets or looking in piglets general direction (only displayed during separation)
Watching piglets / Play with sow	Making contact with piglets or moving head in the direction of piglets for periods of time

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805 **Table 2.** Ethogram of piglet behaviour recorded on days one, three and six.

<b>Behavioural Parameters</b>	<b>Definition</b>
<u>Piglet</u>	
<i>Posture</i>	
Standing	Standing on all four feet
Sitting	Behind resting on floor with upper body held up by forelegs
Lying with sow	Lying in contact with sow
Lying without sow	Lying without sow contact, generally under the lamp
<i>Activity</i>	
Idle	Standing in one place not moving. Can be associated with defecating or urination
Walk/Run	Movement around the pen
Massaging the udder / Nursing	Nursing the udder or visually massing the udder to stimulate milk release
Asleep	Lying without movement
Play/Frolick	Interaction with another piglet , themselves or an object in the pen, such as the feeder
Look for sow	When separated piglets look up at board or jumping on board to try and see the sow
Playing with sow	Piglets interacting the sow
Eating and Drinking	Eating from the provided feeder or drinking from the drinker
Nudging	Nudging the sow in an attempt to get her to nurse. Behaviour could be displayed on any part of the sow's body

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Intermittent Suckling to Stimulate Lactational Oestrus

811 **Table 3.** The proportion of time sows spent displaying various postures and  
 812 activities during each time frame. The P values for the highest level significant  
 813 interaction are given with the abbreviations being ; treatment (T), time frame  
 814 (t) and minute (m).

Parameter	Treat- ments	Timeframe				MST <sup>C</sup>	P value
		Presep	Postsep	Prejoin	Postjoin		
Sow							
Standing	Control	29.5±3.3	22.6±3.2	21.3±3.0	8.5±2.2	Txt	<0.001
	IS	26.1±3.0	22.2±2.7	24.9±2.8	26.3±2.8		
Lying	Control	70.2±3.8	80.0±3.5	79.0±3.4	91.3±2.4	Ttxtm	0.026
	IS	73.9±3.5	74.0±3.3	75.0±3.3	76.0±3.1		
Sitting	Control	7.0±1.7	6.4±1.7	6.0±1.6	4.7±1.5	Txt	0.038
	IS	6.4±1.5	8.7±1.7	5.7±1.4	4.8±1.2		
Nursing	Control	9.7±1.9	8.2±1.8	10.8±1.9	11.5±2.0	txm	0.038
	IS	8.5±1.5	*	*	17.4±2.0		
Resting	Control	45.5±4.1	54.0±4.4	52.0±4.1	67.7±3.9	Ttxtm	0.004
	IS	50.7±3.8	58.6±3.6	59.9±3.7	42.7±3.6		
Rubbing	Control	2.8±0.7	2.0±1.0	1.9±1.0	1.4±1.3	Txt	0.03
	IS	0.6±0.5	2.8±1.2	1.3±0.8	1±0.5		
Looking for piglet	Control	*	*	*	*	NA	NA
	IS	*	13.7±1.9	15.1±2.0	*		
Eating and Drinking	Control	28.1±3.8	24.5±3.8	19.2±3.3	11.9±2.7	Ttxtm	0.032
	IS	23.9±3.4	24.7±3.3	24.7±3.3	24.1±3.2		
Watching	Control	9.6±2.9	10.4±3.2	11.0±3.2	5.4±1.9	Ttxtm	0.003
	IS	12.3±3.3	12.5±3.4	9.9±2.9	4.6±1.6		
Watching/ play with piglets	Control	19.6±2.3	17.1±2.9	19.1±2.8	11.0±2.9	Txt	<0.001
	IS	19.3±3.0	*	*	31.0±2.6		

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816 <sup>B</sup> Standard Error

817 <sup>C</sup> The highest level significant interaction

818 \* Data could not be collected for these periods as behaviours could not be  
 819 determined

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Intermittent Suckling to Stimulate Lactational Oestrus

827 **Table 4.** The proportion of time piglets spent displaying various postures and  
 828 activities during each time frame. The P values for the highest level significant  
 829 interaction are given with the abbreviations being ; treatment (T), time frame  
 830 (t) and minute (m).

Parameter	Treat-ments	Timeframe (%)				MST <sup>C</sup>	P value
		Presep	Postsep	Prejoin	Postjoin		
Piglet							
Standing	Control	29.3±1.6	26.5±1.8	8	19.2±1.8	Ttxxm	<0.001
	IS	29.1±1.8	13.7±1.2	6	57.5±1.6		
Sitting	Control	0.4±0.1	0.1±0.1	0.1±0.0	0.1±0.1	t	<0.001
	IS	0.4±0.0	0.0±0.0	0.1±0.1	0.1±0.1		
Lying without the sow	Control	66.8±3.7	75.1±3.0	53.3±3.8	60.5±3.5	Ttxxm	<0.001
	IS	65.4±3.2	86.1±2.0	78.0±2.7	30.3±3.4		
Lying with Sow	Control	12.3±4.4	5.9±1.6	24.7±4.1	28.0±2.5		
	IS	14.2±4.0	*	*	25.8±2.7		
Looking for sow	Control	*	*	*	*	Txt	<0.001
	IS	*	4.0±0.7	5.9±0.8	*		
Walking/Running	Control	5.4±0.6	3.9±0.7	9.0±1.1	3.5±0.8	Ttxxm	0.003
	IS	5.1±1.3	2.0±0.4	2.1±0.4	12.5±0.7		
Playing & Frolicking	Control	5.3±0.5	5.4±1.2	9.2±1.8	1.7±1.2	Ttxxm	<0.001
	IS	5.8±2.1	2.3±0.6	5.8±1.2	11.7±1.2		
Nudging	Control	1.9±0.3	2.6±0.6	3.1±0.6	1.2±0.4	Txt	<0.001
	IS	1.8±1.1	*	*	8.6±0.4		
Idle	Control	12.5±0.9	9.9±1.2	16.5±1.4	6.8±1.2	Ttxxm	<0.001
	IS	10.5±1.5	6.3±0.8	12.6±1.1	22.4±1.0		
Massaging the udder	Control	25.0±2.6	17.1±2.1	27.5±2.7	25.0±2.6	Txt	<0.001
	IS	19.9±3.1	*	*	50.8±2.1		
Resting	Control	67.6±2.4	76.5±2.3	64.2±2.7	74.2±2.6	Ttxxm	<0.001
	IS	72.1±2.7	86.9±1.6	79.1±2.0	36.1±2.3		
Eating and Drinking	Control	2.7±0.5	3.2±0.7	3.6±0.7	1.4±0.6	Ttxxm	0.011
	IS	2.3±1.1	6.6±1.0	10.8±1.3	8.4±0.5		
Watching/Playing with sow	Control	0.7±0.3	1.9±0.6	1.7±0.5	1.0±0.3	T	<0.001
	IS	1.6±1.1	*	*	4.7±0.5		

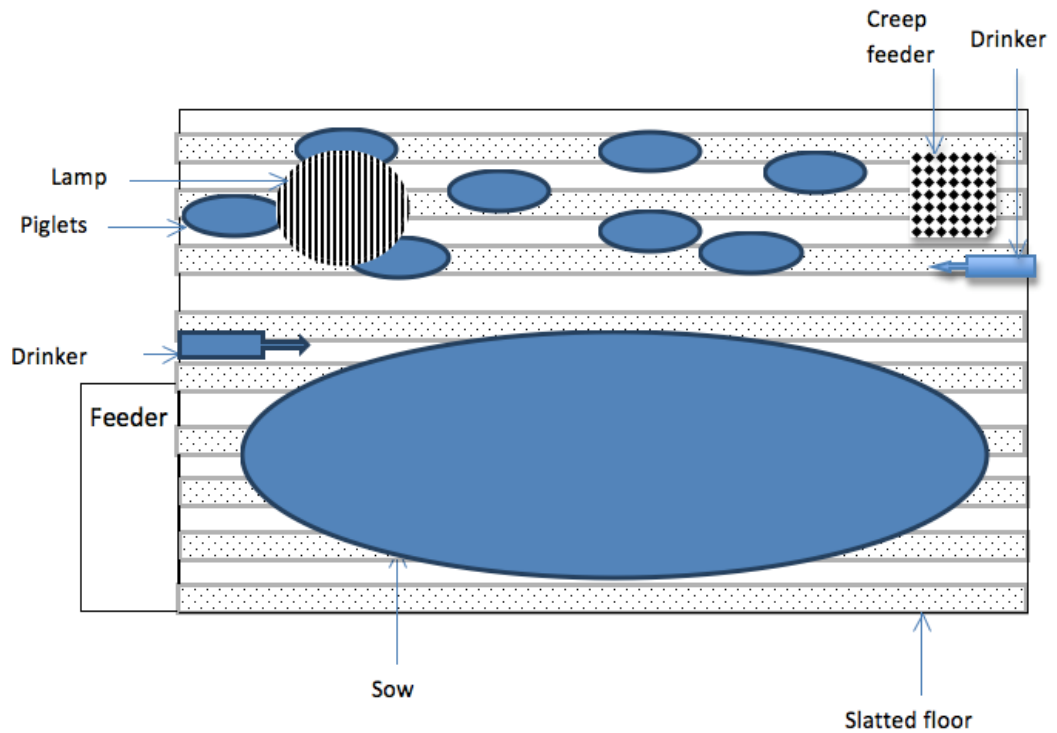
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832 <sup>B</sup> Standard Error

833 <sup>C</sup> The highest level significant interaction

834 \* Data could not be collected for these periods for these periods as behaviours could  
 835 not be determined

836 **Appendix**



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838 **Diagram.1.** Pen design for the conventional farrowing crate

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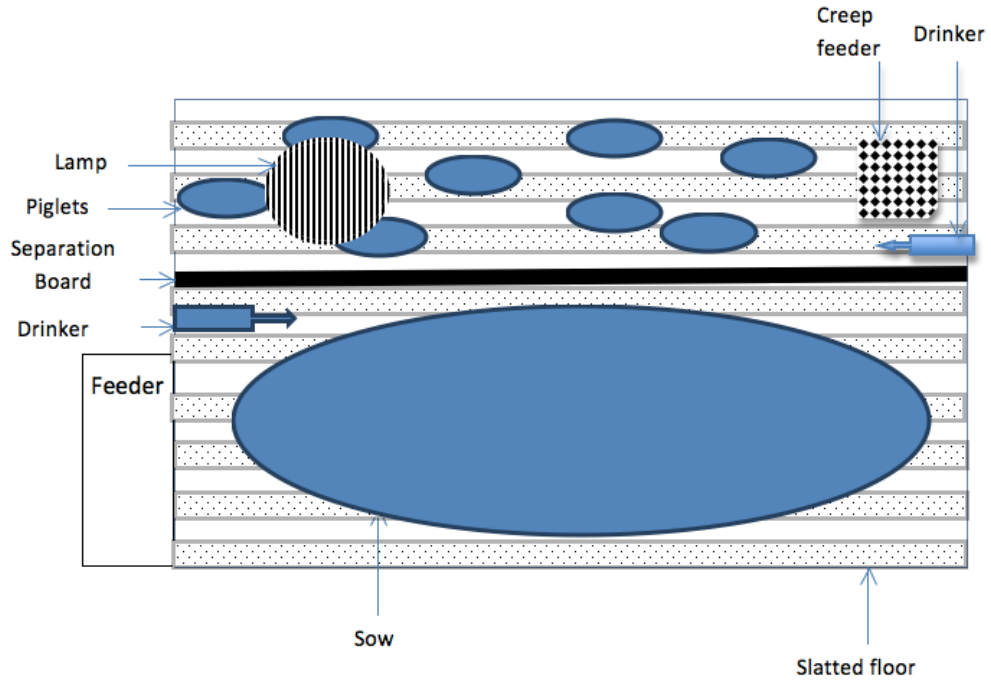
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## Intermittent Suckling to Stimulate Lactational Oestrus



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850 **Diagram.2.** Pen design during hours of separation with a board present

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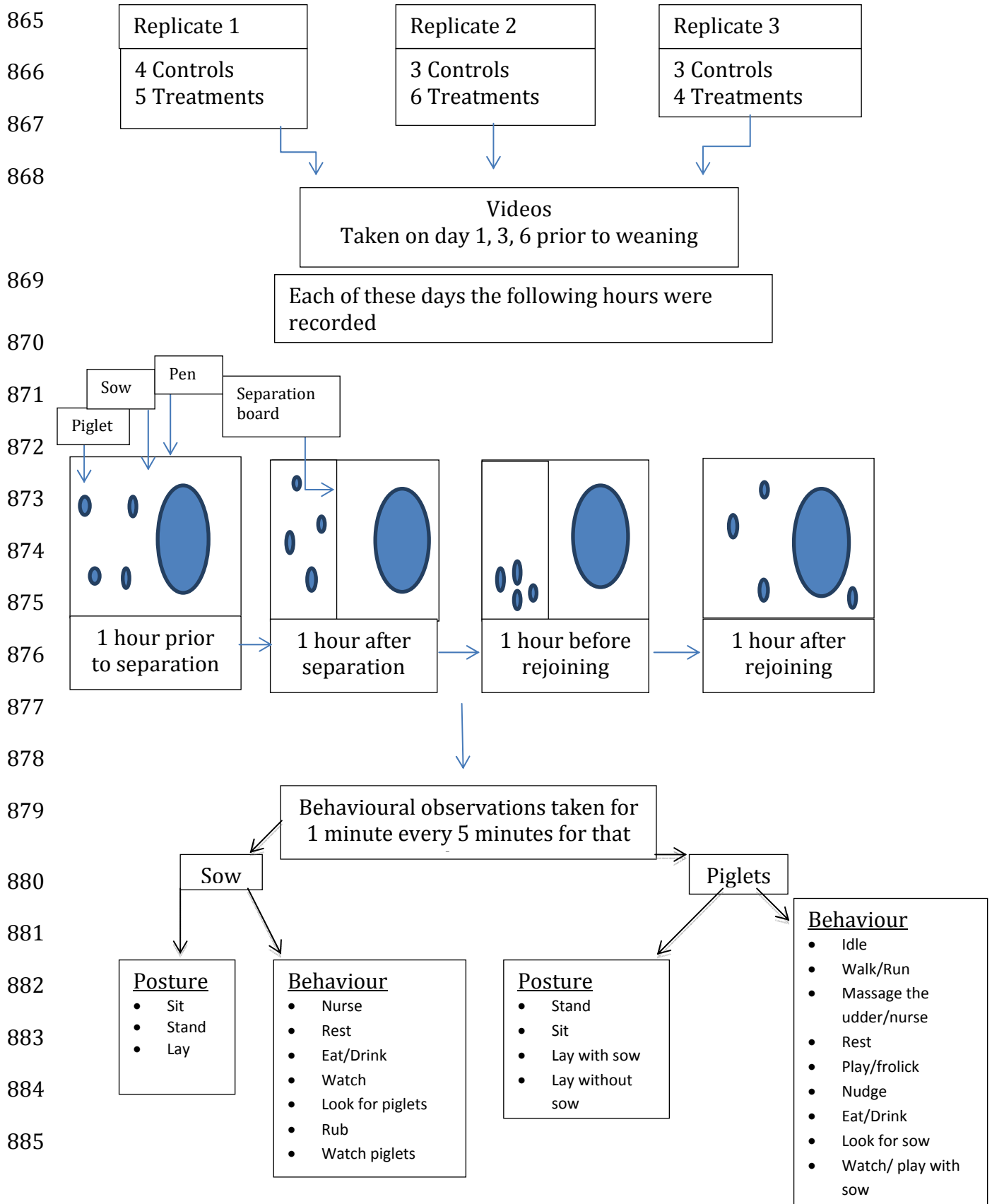
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## Intermittent Suckling to Stimulate Lactational Oestrus



886 **Diagram.3.** Video data collection times and the behaviours and activities  
 887 recorded