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GnRH agonist deslorelin implant alters the progesterone release pattern during early pregnancy in gilts

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Abstract

The aim of this study was to investigate the relationship of progesterone (P) and luteinizing hormone (LH) during recognition and establishment of pregnancy in the gilt. Therefore effects of eliminating episodic LH pulses on P patterns were determined during early pregnancy. To this end, a slow-release GnRH implant deslorelin was used for GnRH downregulation. A group of gilts (GnRHa, n=8) was implanted with the GnRH agonist on Day 11 of pregnancy, while a control group (C, n=5) was treated with a non-impregnated placebo implant. Blood was collected via a *vena cava caudalis* catheter at 10-minute intervals for 8 hours on Day 16 and 21 of pregnancy. As expected, the GnRH implant reduced LH secretion ($P<0.01$) and abolished LH pulses completely at Day 16 and Day 21 of pregnancy. On Day 16, there was no difference in P levels between the treatments. However, on Day 21, the GnRH agonist treatment led to significantly increased P concentrations ($P<0.01$) compared with the control gilts. Progesterone was secreted in a pulsatile manner in both treatment groups and no relationship between LH pulsatility and P pulsatility was observed.

In conclusion, abolishment of LH pulsatility did not affect the pulsatile pattern of P secretion but led to an unexpected overall increase in P on Day 21 of pregnancy; this effect was delayed and

33 occurred 10 days after commencing treatment with the GnRH depot agonist. The elevation of P on
34 Day 21 of pregnancy in the GnRH_a group suggests either a reduced negative feedback effect or an
35 increased autocrine response by the corpora lutea.

36

37 Keywords: deslorelin, early pregnancy, gilt, GnRH agonist, LH, progesterone

38

39 **Introduction**

40 The first 35 days of gestation in the pig are defined as “early pregnancy”. It is a period of profound
41 changes in the sow.

42 During pregnancy in the pig, the corpus luteum is the main source of progesterone (P). Around the
43 12th day of pregnancy, oestrogens are produced by the embryos, which is the signal needed to
44 prevent luteolysis (Ziecik 2002). A second oestrogenic signal from day 13 to day 18 is required to
45 support embryonic survival by maintaining the luteal function beyond 25 days (Pusateri *et al.*
46 1996b). This second signal can only appear if the corpora lutea produce sufficient P for embryos to
47 use as raw substance for the production of oestradiol (Pusateri *et al.* 1996a; Pusateri *et al.* 1996b).

48 Early studies using methods such as hypophysectomy (Anderson *et al.* 1967, Kraeling & Davis
49 1974) have demonstrated that a gonadotrophin of pituitary origin is important to maintain the CL
50 around Day 20 (Anderson *et al.* 1967) and Days 70, 80 and 90 (Kraeling & Davis, 1974) of
51 pregnancy in pigs. Moreover, it was shown that during the embryonic period, administration of LH
52 antiserum from Day 25 to Day 29 terminated pregnancy (Spies *et al.* 1967), confirming that LH is
53 at least one of the gonadotrophins that have a significant role in CL maintenance.

54 Subsequent studies using a GnRH agonist (Peltoniemi *et al.* 1995), immunization against GnRH
55 (Tast *et al.* 2000), or GnRH antagonist treatments (Virolainen *et al.* 2003) in early pregnant gilts
56 demonstrated that manipulation to decrease LH lasting longer than 48 hours may eventually
57 interrupt CL function and terminate pregnancy. However, this disruption effect on pregnancy may

58 occur only after a time lapse of up to 2 - 4 weeks (Peltoniemi *et al.* 1995). In all these studies, P
59 concentration was measured in peripheral circulation, mainly in the jugular vein, where P
60 concentration is supposed to be subjected to metabolization by the liver. In a later study, a clear
61 difference between the 'local' P (close to the ovary, measured in the *vena cava caudalis*) and the
62 'metabolized' P (as measured in the *vena jugularis*) was demonstrated and a direct relationship
63 between LH and P was suggested (Hoving *et al.* 2017, Virolainen *et al.* 2005). Thereafter, Brussow
64 *et al.* (2011) found effects of a short-acting GnRH agonist treatment on LH concentration
65 immediately after the treatment at Day 12 but not on 'local' P at Day 13, 15 and 17 of pregnancy
66 (Brussow *et al.* 2011). However, the exact nature of the relationship between LH and P remains
67 unclear and the chronic effect of a long-acting GnRH agonist implant on LH and local P during
68 maternal recognition, rescue of CL and implantation in the gilt is yet not understood. Short- and
69 long term factors, such as stress, may act on LH pulsatility respectively (Soede *et al.*, 2007),
70 rendering both approaches (short- and long term) relevant for research on reproductive biology.
71 Deslorelin is a GnRH analogue, and has been developed in a slow-release formulation to prevent
72 oestrus and pregnancy in the bitch and to suppress testosterone chronically (thereby sexual
73 behaviour) in the male dog (Trigg *et al.* 2001, Junaidi *et al.* 2007, Kaya *et al.* 2018, Borges *et al.*
74 2015) and the tomcat (Fontaine 2015, Camila *et al.* 2016, Romagnoli *et al.* 2017). In the male dog,
75 insertion of a deslorelin implant led to a peak in LH within an hour of treatment (Junaidi *et al.*
76 2009); the vertex was over within 3 hours and LH returned to the basal level by Day 3 after
77 treatment. The suppressive effect on LH and testosterone is effective for at least 6 to 12 months in
78 the dog, depending on the formulation and the breed (Lucas 2014). In the boar, slow-release
79 deslorelin has been used during puberty and effectively downregulated testosterone and thereby
80 male reproductive function (Kopera *et al.* 2009, Kauffold *et al.* 2010B).
81 The aim of this study was to use the commercially available GnRH agonist deslorelin for long-term
82 down-regulation of LH and thereby to explore the effect of manipulation of LH pulsatility on P

dynamics during some of the milestones of maternal recognition and pregnancy establishment in the gilt. We therefore investigated the relationship of these two hormones on Day 16 during the second conceptus' signal to maintain CL function and at day 21 when implantation is supposed to be completed. We hypothesized that abolishment of LH pulsatility via the GnRH agonist implant would suppress P pulsatility during this phase of pregnancy and therefore show the dependence of P pulsatility on LH pulsatility.

89

90 **Materials and methods**

91 *Ethical permission*

92 The study protocol was approved by the Animal Experiment Board ELLA in Finland (permission
93 ESLH-2009-06207/Ym-23).

94 *Animals and management*

95 Thirteen Landrace Large White hybrid gilts that were part of a sow pool system (Peltoniemi *et al.*
96 2009) were housed in groups and fed 3.0 kg per day [13 MJ/kg, 14.5% crude protein and 7.4 g
97 lysine of a commercial ration (Tiineyspekoni[®], Suomen Rehu, Finland)]. Gilts were selected for
98 breeding at approximately 7 months of age and 135 kg $\pm$ 8.7 kg of weight. They were introduced to
99 the boar on a daily basis until detection of standing oestrus, and were then, oestrus-synchronized by
100 oral administration of 5 mg altrenogest (20 mg; Regumate[®], MSD Animal Health, Finland) per gilt
101 per day for 18 days. At standing oestrus, gilts were artificially inseminated (AI) with 3 x 10⁹ sperm
102 cells per dose while in nose-to-nose contact with a boar. AI was repeated the following day if the
103 gilt was still receptive. Day of first AI was considered Day 0 of pregnancy (Day 0). Three days after
104 the second AI, the gilts were transported to the animal experimental unit at the University of
105 Helsinki. In the experimental unit, the gilts were kept individually on straw bedding in pens of 2 x 2
106 m, sows were able to move freely and have contact with their conspecifics. On Day 23, the animals
107 were euthanized and brought to the Department of Pathology, Faculty of Veterinary Medicine,

University of Helsinki, for standard autopsy and cross-pathology. The uteri were opened, embryos were counted and their length was measured. Ovaries were macroscopically examined and the number of CL was determined.

Treatments

The timing of the treatment was chosen so as to downregulate GnRH around the time CL is supposed to become dependent from pituitary stimulation. The purpose was also to ensure that the implant has started to work prior to that time. Therefore, Day 11 was chosen to cannulate and insert the implant. The experimental gilts (n=13) were randomly allocated to either the treatment GnRHa (n=8) or control (n=5) groups. GnRHa gilts were intramuscularly implanted with a 4.7-mg deslorelin implant (Suprelorin[®], Inc. Virbac, France) in the neck, approximately 5 cm caudal from the base of the ear. The implant contained [d-Trp6, Pro9Net] GnRH that was released slowly at a rate of approximately 20 µg/day in a mouse study (Navarro 2012). Peak level was achieved approximately 14 days after insertion of the implant and deslorelin remained measurable until Day 80 after commencing treatment in the dog (Trigg *et al.* 2001, Navarro 2012). The deslorelin implant suppresses the pituitary-gonadal axis (Trigg *et al.* 2001) in a dual-phase mechanism; the implant initially stimulates the pituitary axis for a few hours to release both follicle-stimulating hormone (FSH) and LH and subsequently activates a complex network of transduction pathways resulting in down-regulation of the GnRH receptors through the inhibition of mRNA coding for the β subunits of the gonadotropins LH and FSH (Trigg *et al.* 2001, Navarro 2012). This down-regulating effect of the deslorelin implant on LH release (and thereby steroids) has been reported to last for months or years, depending on the species, breed, and gender (Lucas 2014).

Control gilts received a nonimpregnated (placebo) implant intramuscularly in the neck, approximately 5 cm caudal from the base of the ear.

Vena cava caudalis cannulation under general anaesthesia

132 To study local P close to the ovary and before metabolization by the liver, a method for
133 catheterization of the caudal vena cava (Benoit & Dailey 1991) was used with a modification for
134 hybrid gilts (Virolainen *et al.* 2005). Anaesthesia was induced and maintained intramuscularly;
135 azaperone 4 mg/kg (Stresnil®; Janssen Animal Health, Beerse, Belgium) was injected into the neck
136 muscle caudal to the base of the ear as a premedication. After waiting for 10 minutes, anaesthesia
137 was induced with a combination of detomidine 0.08 mg/kg (Domosedan®; Orion Ltd, Espoo,
138 Finland), butorphanol 0.2 mg/kg (Torbugesic®; Scan Vet, Fredensborg, Denmark), and ketamine 10
139 mg/kg (Ketaminol®; Merck, Animal Health, Boxmeer, the Netherlands) according to a protocol
140 previously described (Heinonen *et al.* 2009).

141 Gilts were then laid on the left side, the lateral aspect of the right rear leg was shaved approximately
142 20 cm dorsal to the hock. The area was then washed with povidone iodine soap (Betadine®
143 75mg/ml, Takeda Pharma Sp. z o.o.) and disinfected with 70% ethanol. A plastic cover was then
144 used to hygienically protect the area for surgery. An incision was made through the skin
145 approximately 3 cm dorsal to the hock and 2 cm lateral to the *Achilles* tendon. The subcutaneous fat
146 (1-2 cm) was separated and the lateral saphenous vein isolated by blunt dissection. After this,
147 tweezers were placed under the vessel and two loose ligatures were inserted approximately 1 cm
148 apart. Slight traction was maintained on the distal ligature, a small transversal incision was made in
149 the vein between the ligatures and a catheter (PVC tubing, 1.0 mm i.d., 1.5 mm o.d., Sterihealth
150 Laboratory Products Pty LTD, Australia) was inserted 52 cm into the vein, assuming a good
151 localization of the tip of the catheter in the vena cava (Virolainen *et al.* 2005). After a blood sample
152 was collected to control patency, the catheter was flushed and secured with the dorsal ligature
153 around the vein. The plantar ligature was used to tie off the vein and assured an extra fixing of the
154 catheter. The incision was sutured using Coated Vicryl 0® (Ethicon, Johnson & Johnson). The free
155 end of the catheter was left (1 m) running dorsally along the caudal surface of the rear leg lateral to
156 the tail and was bent on the back of the pig. Catheters were sutured to the skin and self-made cotton

157 pouches were used to protect the free end. A bluntly truncated injection needle (18Gx40, BBraun
158 medical Inc.) was inserted tightly into the end of the tube and locked with a luer-lock cap (BBraun
159 medical Inc.). The pouches were then fixed to the animal with adhesive tape wrapped around the
160 abdomen. When ready, the legs were bandaged and catheters covered with adhesive tape
161 (Leukoplast[®], BSN medical GmbH). Immediately after surgery, the animals were treated with one
162 injection of meloxicam 0.4 mg/kg (Metacam[®], Boehringer Ingelheim Vetmedica GmbH)
163 intramuscularly and benzylpenicillin 6 mg/kg (Geopenil[®] vet, Orion Pharma eläinlääkkeet)
164 intravenously. Benzylpenicillin treatment was continued for two times a day on the following three
165 days.

166 Catheters were flushed once a day on non-sampling days with 2 ml of 10 IU/ml heparinized saline.

167 *Blood collection*

168 Frequent blood collection through the *vena cava caudalis* catheters was performed on Day 16 and
169 Day 21. Sampling took place at 10-minute intervals from 8.00 to 16.00 in all experimental animals.
170 Before each sampling, 1 ml of fluid (estimated volume of the catheter) was withdrawn and
171 discarded before collection of a 5-ml blood sample. After each sample, the catheter was flushed
172 with 2 ml of 10 IU/ml heparinized saline. Blood was collected in 5-ml lithium heparin tubes and
173 centrifuged within 10 minutes at 1800 x g for 15 min. Harvested plasma was stored at -20°C until
174 the day of laboratory analysis.

175 *Ultrasound/grey-scale analysis*

176 To determine the grey-scale value and to assess pregnancy, the uterus of each gilt was
177 transcutaneously scanned on Day 12 and 13 using a Honda Electronics HS – 1500 Vet scanning
178 device with a 5 MHz linear probe (Kauffold *et al.* 2010A). Equipment settings were standardized
179 and remained unchanged between the scanning sessions. For grey-scale analysis, a cross-section of
180 each uterine horn was imaged in a circular and in a standardized focus zone. A region of interest

181 (ROI) covering the total area of the cross-section of interest was defined and the grey-scale value
182 was determined using the software integrated into the device used. The outcome of the scan
183 (pregnant or not pregnant) was determined as described by Kauffold *et al.* (2010A).

184 *Hormone analyses*

185 *Luteinizing hormone*

186 LH analyses were performed by an enzyme-linked immunosorbent assay (LH Detect[®],
187 ReproPharm, INRA, Paris, France) developed and previously used for porcine plasma (Norrby *et al.*
188 2011). The intra-and inter-assay coefficient of variation (CV) at 0.66 ng/ml were 6.9% and 17.8%,
189 respectively. The detection limit of the assay was 0.02 mg/ml.

190 *Progesterone*

191 Blood samples were analysed for P using a direct commercial RIA (Spectria, Orion Diagnostica,
192 Turku, Finland), which was validated to measure P in pig plasma (Peltoniemi *et al.* 1995). The
193 sensitivity of the assay was 0.09 ng/ml. The intra-assay and inter-assay CVs for three reference
194 concentrations (0.82, 4.8, and 8.4 ng/ml) were less than 6%.

195 *Data and statistical analysis*

196 Data was analysed by repeated measurement analysis of variance (split-plot ANOVA, Gill & Hafs
197 1971) using the General Linear Model with repeated measures. All data were analysed by IBM[®]
198 SPSS[®] Statistics version 24. The ANOVA model for outcome variables (LH/P concentrations)
199 included treatment as the between subject fixed effect and period (day, clock time) was considered
200 as the within subject repeated factor. In the analysis, the experimental time was divided into four
201 periods of 4 hours (8.00-12.00 and 12.10-16.00 on both sampling days). As a post-testing
202 procedure, the Tukey procedure was used for pairwise comparisons and the Dunnett procedure for
203 timewise comparisons. Probabilities less than 0.05 were considered significant.

204 LH and P were analysed on Days 16 and 21, respectively. The basal LH and P concentration over
205 time was defined as the mean of three consecutive samples preceding every suspect pulse. In case

206 there was more than one possible pulse, the basal level was calculated using the basal value of the
207 pulse that gave the lowest concentration of hormone of interest. In case no pulses were detected
208 (LH/GnRHa gilts), or two pulses were seen within 20 minutes, the basal level was defined as the
209 lowest eight consecutive values in the pattern. Pulses were identified as previously described
210 (Virolainen *et al.* 2005) for gilts during early pregnancy; a pulse was defined as at least two
211 consecutive samples that exceeded the basal concentration by more than three standard deviations,
212 subsequent values above this threshold were deemed to belong to the same pulse. Amplitude was
213 the difference between the maximal pulse value minus basal concentration.
214 Data from two sows in the GnRHa group were excluded from the hormone analysis due to catheter
215 failure.

216

217 **Results**

218 *Macroscopic examination of the reproductive organs*

219 All gilts maintained in the analysis were confirmed to be pregnant by grey-scale analysis after
220 scanning on Days 12 to 13 and remained pregnant until Day 23 when they were euthanized. One
221 gilt in the GnRHa group and one gilt in the control group visibly aborted on Day 21. Both were
222 excluded from Day 21 hormone analyses and post-mortem findings. On Day 23, both GnRHa and
223 control gilts had similar CL count (17.3 ± 4.3 and 16.8 ± 3.3 , respectively; $P>0.10$) and number of
224 viable embryos (15 mm in length) (11.0 ± 2.6 and 9.8 ± 7.0 , respectively; $P>0.10$). On GnRHa
225 gilts' ovaries no follicles could be detected. Two sows in the GnRHa group each had an ovarian
226 cyst, about the same size as the CL next to it.

227

228 *Luteinizing hormone*

229 In the control group the basal LH concentration was at least twice as high as in the GnRHa group on
230 both sampling days but the apparent difference between treatments was not significant ($P>0.05$)

231 (Table1). On Days 16 and 21 of pregnancy there was no day effect on LH pulses in the control
232 group (Table 1), whereas LH pulsatility was effectively abolished in the GnRHa group on Days 16
233 and 21 of pregnancy (treatment effect: $P<0.01$) (Figure 1). Further analysis investigated the effect
234 of morning (8:00-12:00) vs afternoon (12:10-16:00) on both sampling days and revealed a
235 significant treatment * period interaction. GnRH agonist treated gilts had a significantly decreased
236 mean LH concentration compared to the controls on Day 16 (morning and afternoon) and on Day
237 21 in the afternoon but not in the morning (Figure 2).

238 *Progesterone*

239 The pattern of P concentration as measured in the *vena cava caudalis* by frequent sampling was
240 highly pulsatile and the levels observed were high and varied considerably over time. In the control
241 and the study group together, there were 1.20 ± 0.92 and 0.73 ± 0.65 P pulses detected during the 8-
242 hour sampling on Day 16 and 21, respectively ($P>0.05$) (Table 2). There was an effect of stage of
243 pregnancy on P concentration such that the overall concentration on Day 16 (22.93 ± 8.38 ng/ml)
244 was approximately twice as high as on Day 21 (11.71 ± 6.71 ; $P=0.016$) (Table 2) (Figure 2). The
245 GnRH agonist implant did not have an apparent effect on P concentration on Day 16 (Figure 2).
246 Although the GnRH agonist treatment tended to result in a higher pulse frequency, pulse amplitude
247 and basal level of progesterone on Day 21, the difference was not significant (Table 2). However, on
248 Day 21, the GnRHa group showed a significantly higher P concentration ($P<0.01$) (Figure 2).

249

250 *Relationship between P and LH pulsatility*

251 In GnRHa gilts, abolishment of LH pulsatility did not have an impact on P pulsatility. In the control
252 animals, only in two of the gilts an LH pulse was followed by a P pulse within 10 minutes on Day
253 16. Otherwise, no temporal association was observed between P and LH pulsatility (such that a LH
254 pulse would precede a P pulse).

255

256 Discussion

257 A slow-release GnRH implant (deslorelin) inserted on Day 11 of pregnancy effectively abolished
258 LH pulsatility during the establishment phase of pregnancy, both at 5 days after treatment (Day 16
259 of pregnancy) and at 10 days after the treatment (at Day 21 of pregnancy). The treatment did not
260 have any apparent effect on P pulsatility as measured by frequent blood collection close to the
261 ovary on either day of sampling. Although the GnRH-agonist treatment abolished LH pulsatility, it
262 caused an increase in overall P concentration on Day 21 of pregnancy.

263 There are two reports of GnRH depot agonist application during early pregnancy in the pig. Similar
264 to the present study, Peltoniemi *et al.* (1995) used a slow-release GnRH agonist to explore the effect
265 of LH secretion pattern on CL function. They administered Gosrelin (3.6 mg) with a release rate of
266 120 ug/day at Day 14 of pregnancy, whereas deslorelin (4.7 mg) with a release rate of 20 µg/day at
267 Day 11 of pregnancy was applied in our study. Despite these differences, the effect of the
268 treatments on LH appear to be very similar as LH pulses were effectively abolished in both studies,
269 for at least 60-66 h (Peltoniemi *et al.*, 1995) and even 5 and 10 days (present study) after the
270 implant was inserted.

271 In our study, the GnRHa treatment did not affect progesterone release patterns (frequency,
272 amplitude, basal level) as measured in the *vena cava caudalis*, neither at Day 16, nor at Day 21 of
273 pregnancy. Our hypothesis that LH pulsatility is a pacemaker for P pulsatility was therefore refuted.
274 In contrast, the results even imply that on Day 16 and Day 21, the P release pattern was independent
275 of the LH release pattern.

276 Average P levels in the caval vein were similar (20-25 ng/ml) in both groups at Day 16, by Day 21
277 P levels had declined; the descent was steeper in the control group than in the GnRHa group,
278 creating a significant difference between them (on average 8 ng/ml for the control group and 14
279 ng/ml for the GnRHa group). A decline in P levels around this time is commonly seen (Tast *et al.*,

2002) and seems related with the conceptuses' uptake rate of P to produce the oestrogen signal (Bazer *et al.*, 1983) and the lower release from the CL (Langendijk *et al.*, 2017).

The lack of effect on P profiles at Day 16 (5 days following GnRH analogue treatment) seems to corroborate earlier findings with a GnRH analogue treatment around this time. Peltoniemi *et al.* (1995) inserted a long acting GnRH agonist implant at Day 14 and, after an initial 2 day increase in P, peripheral P levels in the subsequent 8 days were not affected when compared to the control animals. The short term enhanced P concentration as seen by Peltoniemi *et al.* (1995) is expected to be a direct response to the GnRH agonist-stimulated release of LH. The short-acting GnRH analogue used by Brussow *et al.* (2011) on Day 12 of pregnancy failed to increase *vena cava* P concentrations on the day after the treatment (Day 13) and did not change P concentrations on Day 15 nor on Day 17 (similar to our results on Day 16). Also, in the study of Virolainen *et al.* (2003) a GnRH antagonist treatment that started at Day 14 of pregnancy, did not affect P patterns from Day 15 to Day 29, even though the treatment appeared to abolish episodic LH release for 3 days. Brussow *et al.* (2011) explained their lack of effect on P release by the relative autonomy of the early CL function in the pig, whereas Virolainen *et al.* (2003) considered the variation in individual responses to explain the variation in LH secretory pattern. Our results support the suggestion by Virolainen *et al.* (2003) that basal LH stimulation seems to be sufficient to support CLs' P production.

Another explanation might be that the porcine ovary has GnRH receptors as has been shown in the rat (Ramakrishnappa *et al.* 2005), and therefore a GnRH agonist could locally stimulate the ovary. In the sow, there is at least some *in vitro* evidence that GnRH may exert a direct effect on the ovary and uterus (Li *et al.* 1993, Ledwiz-Rigby 1990) and P release in the pig. In that case a GnRHanalogue providing direct and constant GnRH- stimulation, that is independent from P feedback could maintain or even enhance P production from the CL without LH stimulation. This might be an explanation for the P patterns seen in the GnRHa group, at Day 16 and Day 21.

305 At day 21 of pregnancy (10 days after the GnRHanalogue implant insertion), mean P levels were
306 higher in the GnRH group than in the control group but basal level, pulse frequency and amplitude
307 were not affected. These results seem to corroborate earlier findings of Peltoniemi *et al.* (1995) who
308 also found long term stimulatory effects of a GnRH analogue treatment. The sows in the study of
309 Peltoniemi *et al.* (1995) which were treated with a long acting GnRH analogue at Day 21 or Day 29
310 of gestation had increased peripheral P concentrations for 5 and 12 days, respectively. Peltoniemi *et*
311 *al.* (1995) discussed that these increased levels may have been due to accessory CL induced by the
312 GnRH agonist used (gosrelin). However, there was no other evidence for the induction of accessory
313 CL.

314 Accessory CL as a source of P and an explanation for a higher P level on Day 21 can be excluded in
315 the present study. The ovulation rate at autopsy on Day 23 of pregnancy was similar in the GnRH_a
316 gilts when compared with the control gilts. Furthermore, if accessory CL had been induced, an
317 elevation of P would already have been expected by Day 16 (5 days after treatment). The two cysts
318 found in the GnRH_a group might be an indication for an attempt to form accessory CL. In that case
319 the deslorelin-initiated GnRH surge led to a maturation of follicles but the GnRH agonist may have
320 suppressed the LH surge needed for an ovulation resulting in a formation of these cysts (Gerritsen
321 *et al.*, 2013, Kauffold *et al.*, 2014). However, since formation of accessory CL did not appear to
322 happen, they do not provide a possible explanation for the higher mean P at Day 21.

323 A possible explanation for the enhanced P might be found in the so-called ‘progesterone-loop’
324 effect (Yung, Y. 2014). In mares, deslorelin acetate induced higher expression of LH receptors in
325 the Corpus luteum (Maia 2016). A higher expression of LH receptors was combined with higher
326 amounts of P, which was called as the “progesterone loop” - effect (Yung,Y. 2014). Wherein the
327 ‘progesterone loop’, P induced more LH receptors in luteinized human granulosa cells *in vitro*.
328 However, until today it is unclear if P has an autocrine effect and can stimulate its own secretion in

329 mammals as has been suggested earlier (Rothschild, 1995). If that mechanism was possible in the
330 pig it could be an explanation for the higher P concentration on Day 21.

331 Thus, the reason for the observed late enhancement of P in the present study and in the publication
332 of Peltoniemi *et al.*(1995) remains unclear and might be dependent on the GnRH agonist and the
333 day of pregnancy the implant is inserted.

334 Higher peripheral and caudal caval P concentrations have been postulated to positively influence
335 embryo survival and embryo development (Athorn *et al.* 2013, Virolainen *et al.* 2005), but it is
336 unclear in how far enhanced P might affect pregnancy in the long term. Also the present study did
337 not focus on the maintenance of pregnancy. By Day 21 one abortion had occurred in both the
338 control group and the GnRHa group for unknown reasons; at euthanasia at Day 23 of pregnancy, 12
339 days after insertion of the GnRHagonist implant embryo survival and embryo development were
340 unaffected by the treatment.

341 Although the study of Peltoniemi *et al.* (1995) showed enhanced P levels using a slow-release
342 GnRHagonist, the treatment subsequently resulted in abortions in almost all animals. The gilts in
343 their study were implanted with the GnRHagonist on Day 14 and Day 21 and they were observed to
344 abort about 15 days (mean) after the beginning of the treatment (Peltoniemi *et al.* 1995). The fact
345 that the experimental animals maintained their pregnancy for 12 days after the GnRH agonist
346 implant was inserted (as in the present study) up to 15 days (as in the study of Peltoniemi *et al.*,
347 1995) only emphasizes the time span where manipulation of CL function can be tolerated without
348 an immediate response. It might be possible that only after a certain time span of suppressed LH
349 pulsatility, the CL cannot compensate and P concentration declines below the concentration needed
350 to maintain pregnancy.

351 Further research is therefore necessary to explore the influence of episodic LH release on the
352 maintenance of CL function.

353 The GnRH agonist used was a slow-release pellet (containing 4.7 mg deslorelin) and was reported
354 to have a long-term suppressive effect on reproductive steroids in dogs (Trigg *et al.* 2006, Junaidi *et*
355 *al.* 2009, Kaya *et al.* 2015, Borges *et al.* 2015) and cats (Fontaine 2015, Camila *et al.* 2016,
356 Romagnoli *et al.* 2017). These treatment properties are usually applied for time-limited sterilization
357 and contraception or for postponing oestrus, which is of high relevance in other species such as the
358 horse. The present study, however, underlines that despite the long-term effects, treatment with a
359 deslorelin implant may also have positive effects on P concentrations in the short to intermediate
360 term. This aspect could be considered valuable in species with CL insufficiency such as the horse
361 and cattle.

362 Early pregnancy in the pig can be terminated before a positive pregnancy diagnosis by traditional
363 ultrasound methods. We therefore used grey-scale scanning (Kauffold *et al.* 2010A) of the gilts to
364 determine pregnancy status as early as 12 to 13 days after AI. The method was used to verify the
365 pregnancy status in relation to the treatment on one hand and sampling for P dynamics on the other.
366 In this method, a distribution of pixels found in the area of interest in the image results in the grey-
367 scale value, which can be used to verify the pregnancy status of the animal (Kauffold *et al.* 2010A).
368 We found perfect agreement between the grey-scale determined pregnancy status, traditional
369 scanning of the uterus on Days 21 to 23, and pregnancy status as determined during autopsy. It
370 seems therefore that the grey-scale method is a valuable tool particularly for non-invasive detection
371 of early pregnancy.

372 In conclusion, the present study shows that the slow-release GnRH implant deslorelin (Suprelorin[®])
373 effectively abolishes LH pulsatility during Day 16 and Day 21 of early pregnancy in the pig. In
374 contrast to our expectations, the abolishment of LH pulsatility did not seem to affect P pulsatility or
375 level on Day 16. However, an increase in local P close to the ovary was observed another 5 days
376 later. A reduced negative feedback effect or an increased autocrine response by the corpora lutea.
377 could be the reason for the increased P.

378

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383

384 **Conflict of interest**

385 None of the authors have any conflict of interest to declare.

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405 Table 1. LH characteristics from plasma samples collected on Day 16 and 21 of pregnancy for the
 406 GnRH agonist deslorelin (treatment on Day 11) for implanted and control gilts. A sampling window
 407 of 8 hours was used with a 10-minute sampling interval.
 408
 409

Day	Group	Frequency/8 hrs	Amplitude (ng/ml)	Basal level (ng/ml)	Mean level (ng/ml)
16	Control, n=5	1.20 ± 0.45**	1.24 ± 0.53**	0.55 ± 0.59	0.62 ± 0.60**
	GnRHa, n=6	._**	._**	0.27 ± 0.21	0.30 ± 0.25**
21	Control, n=4	1.40 ± 0.55**	1.03 ± 0.73**	0.57 ± 0.98	0.73 ± 1.01**
	GnRHa, n=5	._**	._**	0.20 ± 0.14	0.30 ± 0.22**

410 **Denotes significant differences between the treatments within days ($P<0.05$).
 411

412 Table 2. P characteristics collected from plasma samples on Days 16 and 21 of pregnancy for the
 413 GnRH agonist deslorelin (treatment on Day 11) for implanted and control gilts. A sampling window
 414 of 8 hours was used with a 10-minute sampling interval.
 415

Day	Group	Frequency/8 hrs	Amplitude (ng/ml)	Basal level (ng/ml)	Mean level (ng/ml)
16	Control, n=5	1.40 ± 1.14	30.12 ± 17.13	17.88 ± 4.64	21.10 ± 5.55
	GnRHa, n=6	1.00 ± 0.71	27.84 ± 0.00	18.93 ± 7.25	24.75 ± 10.90
21	Control, n=4	0.60 ± 0.55	9.99 ± 9.38	6.98 ± 5.18	8.45 ± 4.00**
	GnRHa, n=5	0.83 ± 0.75	14.94 ± 12.85	12.16 ± 7.29	14.42 ± 7.61**

416 **Denotes significant differences between the treatments within the column ($P<0.05$).

Control day 16

GnRH agonist day 16

Figure 1. Individual profiles of P (black line, filled diamonds) and LH (grey line, filled circles) taken at 10-minute intervals from 8.00 in the morning until 16.00 in the afternoon.

Figure 2: Overall LH and P moving average values (average of five values) of deslorelin-implanted (dashed line) and control (dotted line) gilts from plasma samples collected on Days 16 and 21 of pregnancy. A sampling window of 8 hours was used with a 10-minute sampling interval. The slow-release GnRH treatment significantly increased P secretion on Day 21 of sampling ($P < 0.01$) and significantly reduced LH secretion on Day 16 from 8:00-12 and 12:10 to 16:00 and on Day 21 from 12:10-16:00 ($P < 0.01$).

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