

Reproductive Hormone Profiles in Sows on Estrus Synchronization using Estradiol Dipropionate and Prostaglandin F_{2α}-Analogue and the Reproductive Performance in Female Pigs on Commercial Farms

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ABSTRACT. Changes in ovarian structures and hormonal profiles in estradiol dipropionate (EDP)-induced pseudopregnant sows following PGF_{2α}-analogue (PGF_{2α}-A) administration and practicality of the estrus synchronization protocol using EDP and PGF_{2α}-A on estrus expression and reproductive performance in commercial conditions were investigated. Pseudopregnancy was defined as absence of estrus maintained for at least 20 days after EDP treatment in this study. When 4 pseudopregnant sows induced by 20 mg EDP were treated with PGF_{2α}-A as 0.175 mg cloprostenol twice at a 24-hr interval between 20 and 28 days after EDP treatment, plasma progesterone concentrations rapidly decreased after treatment. The luteinizing hormone surge and ovulation were detected in all sows. The number of ovulated follicles was 17.3 ± 1.1 (SEM). On commercial farms, 94.2% of 52 gilts and 95.2% of 21 sows received EDP became pseudopregnant. When these pseudopregnant females (48 gilts and 20 sows) were treated with PGF_{2α}-A as described above, estrus was detected in all females at 6.1 ± 0.3 days for gilts and 5.5 ± 0.2 days for sows after the first PGF_{2α}-A treatment. There were no significant differences in farrowing rate (85.0 – 100%), average total litter size (10.0 – 11.4), average born alive litter size (9.4 – 10.3) and average piglet birth weight (1.56 – 1.71 kg) between PGF_{2α}-A treated pseudopregnant female pigs that were inseminated during synchronized estrus and females inseminated during spontaneous estrus. This study indicates that estrus synchronization programs using EDP and PGF_{2α}-A are available as practical and convenient procedures for commercial pig farms.

KEY WORDS: commercial farm, estradiol dipropionate, estrus synchronization, prostaglandin F_{2α}-analogue, swine.

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Reproductive management on commercial pig farms is used to optimize convenience, economics and disease control. Batch-farrowing management of sows allows for mating and farrowing to occur at a fixed interval and leads to an all-in/all-out management. Weaning of a group of sows on the batch-farrowing system is the most effective and popular method for synchronizing estrus on commercial farms, and approximately 90% of sows come into estrus within 8 days after weaning [20, 25]. On the other hand, it is difficult for farmers to correspond sows with very short or long weaning-to-estrus intervals, and sows that fail to become pregnant at the first estrus after weaning, to the breeding schedule, and a cause of annoyance to farmers. Furthermore, in general, a swine-breeding herd has a large proportion of gilts for recruitment. The introduction of females into planned piglet production by batch farrowing requires an efficient method

of estrus synchronization. However, the sufficient method in cyclic females on commercial farms has not been established in Japan.

In female pigs, estrogen stimulation from the conceptus plays a role in maternal recognition of the conceptus for the establishment of pregnancy [26]. Pseudopregnancy was defined to maintain a functional corpus luteum (CL) in cyclic females as a substitute for these signals from the conceptus by exogenous estrogen. It has been reported that daily injection of estradiol benzoate (EB) from days 11 to 14 [9] of the estrous cycle or estradiol valerate (EV) from days 11 to 15 [27] can induce pseudopregnancy. We previously showed that a single administration of estradiol dipropionate (EDP), which exhibited prolonged estradiol-17β effects compared with EB and EV in rats [14] and humans [22], at 9 – 13 days after the onset of estrus could also induce pseudopregnancy in gilts and sows [17, 18]. Administration of EDP appears to be more effective for inducing pseudopregnancy in females in terms of effort and cost compared with the previous method of using at least 4 administrations of EB or EV [7, 9, 27].

The CL of the cycling female pig is resistant to prostaglandin F_{2α} (PGF_{2α})-induced luteolysis prior to day 12 of the estrous cycle [2, 4, 11]. By contrast, the CL of pseudopregnant females induced by the above methods regresses rapidly after PGF_{2α} administration [5, 17], and 83 – 92% of gilts [13,

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17, 27] and 100% of sows [18] exhibit estrus 5 days after $\text{PGF}_{2\alpha}$ treatment, as well as pregnant females [5, 21]. Thus, estrus synchronization protocol using pregnant or pseudopregnant female pigs can be applied for the introduction of females into planned piglet production by batch farrowing on commercial farms. However, since exogenous administration of $\text{PGF}_{2\alpha}$ to pregnant females results in abortion, a combination of estradiol and $\text{PGF}_{2\alpha}$ treatments in cyclic females may be more useful for commercial application to estrus synchronization programs in view of animal welfare.

Use of natural $\text{PGF}_{2\alpha}$ dinoprost for inducing estrus in pregnant females resulted in a wide variation of the treatment-to-estrus interval compared with that of the $\text{PGF}_{2\alpha}$ -analogue ($\text{PGF}_{2\alpha}$ -A) cloprostenol [10, 21]. However, the effect of cloprostenol on regression of the CL and estrus exhibition in pseudopregnant females is not clear.

The objects of this study were: 1) to examine changes in ovarian structures and hormonal profiles in pseudopregnant sows following cloprostenol administration; 2) to determine the efficiency of induction of pseudopregnancy by EDP treatment on commercial pig farms; and 3) to evaluate the practicality of an estrus synchronization protocol using EDP and cloprostenol on estrus expression and subsequent reproductive performance in cyclic females on commercial farms.

MATERIALS AND METHODS

Animals: The data were recorded on 2 commercial farms, on which were raised approximately 220 (Farm A in Ibaraki prefecture) and 250 (Farm B in Chiba prefecture) breeding female pigs, and the National Institute of Animal Health from August 2008 to December 2010. All animal-related procedures in this study were approved by the Institutional Care and Use Committee for Laboratory Animals of the National Institute of Animal Health. This study was conducted using 114 gilts (Landrace [L; n=67] on Farm A and Hybrid [n=47] on Farm B) and 55 sows (L [n=42] and L \times Large White [LW; n=9] on Farm A, and L [n=1] and LW [n=3] at the National Institute of Animal Health). Gilts ranged from 7 to 10 months of age and were used after showing two or three normal estrous cycles (length between 18 and 24 days) after puberty. The mean parities of sows were 1.6 ± 0.2 (mean \pm SEM), and the interval from weaning to onset of estrus in sows was within 7 days. Estrus was checked once or twice daily using a mature boar.

Experiment 1: Effects of treatment with $\text{PGF}_{2\alpha}$ -analogue on estrus synchronization and hormonal profiles in pseudopregnant pigs: Four primiparous sows (L; n=1, LW; n=3) were given 20 mg EDP (Ovahormone Depot, Aska Pharmaceutical, Tokyo, Japan) intramuscularly 9 days after ovulation for inducing pseudopregnancy as in a previously described report [18]. Animals were checked for estrus twice daily from 17 days after ovulation, and pseudopregnancy was defined as absence of estrus maintained until at least 20 days after EDP treatment, as described previously [17]. Pseudopregnant sows were treated with $\text{PGF}_{2\alpha}$ -A as 0.175 mg cloprostenol (Planate, Intervet, Osaka, Japan) intramuscularly twice over a 24-hr interval between 24 and 28 days

after EDP treatment. Each sow was fitted with an indwelling catheter in the auricular vein at 3 or 4 days before the first $\text{PGF}_{2\alpha}$ -A treatment. Blood samples were collected daily from the day of catheterization until 7 days after ovulation. Additional blood samples were collected at 8-hr intervals from 0 to 4 days after the first $\text{PGF}_{2\alpha}$ -A treatment. Thereafter, blood sampling and estrus detection were carried out at 4-hr intervals from 5 days after the first $\text{PGF}_{2\alpha}$ -A treatment to the end of the subsequent estrus. Plasma was recovered after centrifugation of blood and stored at -20°C . After sows had been deemed to be in estrus, the ovaries were monitored at 4-hr interval with transrectal ultrasonography as described previously [15] to detect the timing of ovulation and number of ovulated follicles. Ovulation was defined as the time when there was a marked reduction in the number of large follicles (≥ 6 mm in diameter) relative to that in previous observations [12].

Experiment 2: Effects of EDP on the induction of pseudopregnancy in commercial pigs: Females were given EDP at a dose of 20 mg for 52 gilts (L; n=43, Hybrid; n=9) and 30 mg for 21 sows (L; n=19, LW; n=2) once between 7 and 11 days after the end of estrus by the method described previously [18]. Animals were checked for estrus once or twice daily from 17 days after the end of estrus, and pseudopregnancy was defined as absence of estrus maintained until at least 20 days after EDP treatment, as described previously [17].

Experiment 3: Effects of EDP and $\text{PGF}_{2\alpha}$ -A on estrus synchronization and the subsequent reproductive performance on commercial farms: Pseudopregnant 48 gilts (L; n=39, Hybrid; n=9) and 20 sows (L; n=18, LW; n=2) induced by EDP treatment in experiment 2 were treated with $\text{PGF}_{2\alpha}$ -A as 0.175 mg cloprostenol intramuscularly twice at a 24-hr interval between 20 and 35 days after EDP treatment. Estrus detection was carried out once or twice daily from 3 days after the first $\text{PGF}_{2\alpha}$ -A treatment to the end of the estrus.

In the pseudopregnant group, pigs were bred by natural service (NS) and/or artificial insemination (AI) one to four times during the estrus after $\text{PGF}_{2\alpha}$ -A treatment. Non-treated 62 cyclic gilts (L; n=24, Hybrid; n=38) and 30 sows (L; n=23, LW; n=7) were inseminated by NS and/or AI one to four times during estrus, as controls.

Hormone assay: All plasma samples in experiment 1 were measured by time-resolved fluoroimmunoassay (Tr-FIA) for estradiol-17 β , progesterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations. Plasma concentrations of estradiol-17 β and progesterone were measured with Tr-FIA kits (DELFLIA Estradiol and Progesterone kits, PerkinElmer Japan, Yokohama, Japan), as previously reported [16]. The intra- and inter-assay coefficient variations (CVs) were 11.6% and 10.6% for estradiol-17 β and 9.2% and 9.6% for progesterone. Plasma concentrations of LH and FSH were determined using Tr-FIA methods described in previous reports [16, 19]. The intra- and inter-assay CVs were 11.8% and 4.8% for LH and 7.6% and 9.3% for FSH.

Statistical analysis: All data were tested for normal distribution using the UNIVARIATE procedure and analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC,

U.S.A.). Data pertaining to hormonal profiles in experiment 1 were subjected to ANOVA for repeated measures [8]. When a significant effect was detected by ANOVA, the significance of the difference between means was determined by Tukey's test. The duration of the LH surge was taken to be the time from the onset to the end of the LH surge defined by a previously described method [23]. The statistical model for gilts included the effect of breed (L on Farm A or Hybrid on Farm B) in experiment 2, but there was no effect due to breeds ($P=0.43$). In experiment 3, the statistical model for total and born alive litter sizes, and piglet birth weight included treatment (natural estrus or pseudopregnant), breed (L or Hybrid), insemination method (NS, AI or combination of NS and AI) and interaction of these factors for gilts, and treatment (natural estrus or pseudopregnant), parity (1 – 5 parities), breed (L or LW), insemination method (NS, AI or combination of NS and AI) and interactions of these factors for sows. When a significant effect was detected by ANOVA, the significant of difference between means was determined by Tukey's test. The treatment effects on the incidences of conception and parturition in experiment 3 were analyzed by logistic regression analysis. A value of $P<0.05$ was considered to be significant.

RESULTS

Ovulation and hormonal changes after PGF_{2α}-A treatment in pseudopregnant sows: Estrus after PGF_{2α}-A treatment occurred in all sows, and the interval from the first PGF_{2α}-A

treatment to the onset of estrus was 5.5 ± 0.5 days. Plasma progesterone levels decreased markedly ($P<0.05$) from 4 hr after PGF_{2α}-A treatment and decreased to less than 1 ng/ml on 1.3 ± 0.2 days after the treatment (Fig. 1a). The peak estradiol-17 β concentration was 40.8 ± 1.9 pg/ml on 5.5 ± 0.5 days after first PGF_{2α}-A treatment (Fig. 1a). Concentrations of FSH on 5.7, 8.0 and 13.0 days after the treatment increased ($P<0.05$) compared with their minimum levels 4.3 days after PGF_{2α}-A treatment (Fig. 1b). LH concentrations between 5.5 and 5.6 days after PGF_{2α} treatment were higher ($P<0.05$) than those on the day of treatment (Fig. 1b). An LH surge after PGF_{2α}-A treatment was observed in all sows, peaking at 6.0 ± 0.5 days after the treatment. Ovulation was detected in all sows at 7.3 ± 0.5 days after PGF_{2α}-A treatment, and the number of ovulated follicles was 17.3 ± 1.1 .

Induction of pseudopregnancy by EDP treatment on commercial pig farms: Forty-nine of 52 gilts (94.2%) and 20 of 21 sows (95.2%) that received a single injection of EDP became pseudopregnant. The three gilts and one sow whose pseudopregnancy was not induced by EDP had interestrus intervals of 26 – 34 days.

Estrus synchronization and the subsequent reproductive performance after PGF_{2α}-A treatment in pseudopregnant pigs on commercial farms: Estrus was observed in all pseudopregnant pigs on commercial farms within 14 days after the first PGF_{2α}-A treatment. Frequency of estrus expression after PGF_{2α}-A treatment in pseudopregnant gilts and sows is shown in Fig. 2. Interval from the first PGF_{2α}-A treatment to the onset of estrus was 6.1 ± 0.3 days in gilts and 5.5 ± 0.2

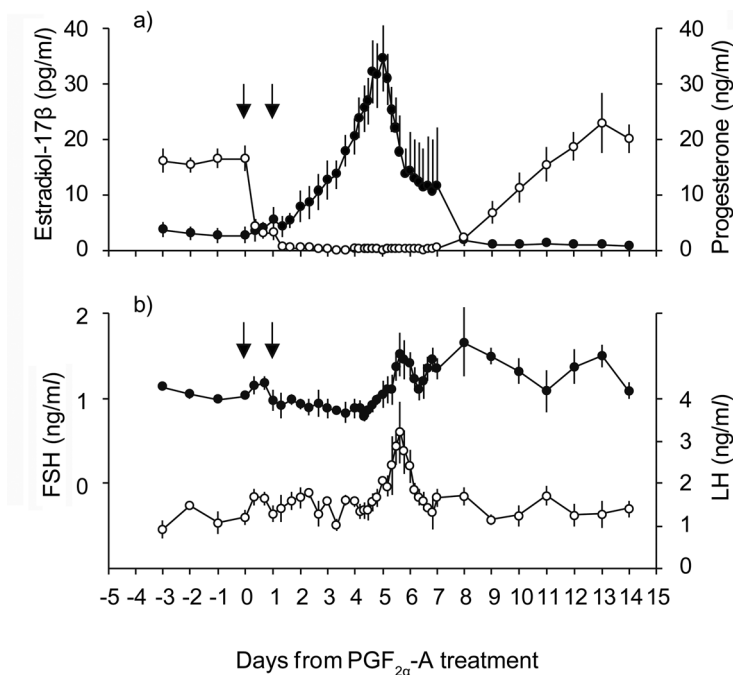


Fig. 1. Plasma concentrations of a) estradiol-17 β (closed circle) and progesterone (open circle) and b) FSH (closed circle) and LH (open circle) after PGF_{2α}-A treatment in pseudopregnant sows (n=4). Arrows in each panel indicate treatment with PGF_{2α}-A. Values are means \pm SEM.

days in sows. Thirty-one of 48 pseudopregnant gilts (64.6%) and 18 of 20 sows (90.0%) exhibited estrus 5 to 6 days after the first PGF_{2α}-A treatment.

Reproductive performance in gilts and sows treated with EDP and PGF_{2α}-A is shown in Table 1. Conception and farrowing rates in pseudopregnant gilts and sows did not differ from non-treated cyclic gilts and sows. There was no significant difference in total and born alive litter size, and piglet birth weight for gilts and sows between control and pseudopregnant groups. However, reproductive parameters in gilts were significantly different between breeds (Fig 3). In sows, piglet birth weight differed significantly among parities.

DISCUSSION

In this study, it was demonstrated that pseudopregnancy was induced by a single EDP treatment in both gilts and sows with high efficiency under commercial conditions. Estrus was observed in 72.1% of pseudopregnant pigs (64.6% for gilts and 90.0% for sows) at 5 to 6 days after PGF_{2α}-A treatment and all pseudopregnant females within 14 days. Furthermore, reproductive performance in EDP-induced pseudopregnant females following PGF_{2α}-A treatment was similar to that in natural cyclic female pigs.

The single treatment with EDP at 7 to 11 days after the end of estrus showed high efficiency (94.5%) of induction of pseudopregnancy on commercial farms. To induce pseudopregnancy by estrogen administration in female pigs, the timing of estrogen injections must match the timing of pregnancy when maternal recognition occurs [6, 7, 9]. Our previous study indicated that pseudopregnancy was induced in 80–100% sows treated once with 20 mg of EDP at 8 and 11 days after ovulation [18]. Although the duration of estrus varied from 24 to 88 hr in pigs [1, 24, 25], ovulation was assumed to take place at 72 to 85% of the duration of estrus [1, 24]. Because it was thought that the day when EDP was given between 7 to 11 days after the end of estrus corresponded to the 8 to 10 days after ovulation, the timing of EDP treatment chosen in the present study may result in a high rate of induction of pseudopregnancy under farm conditions.

Plasma progesterone concentrations in pseudopregnant sows decreased less than 1 ng/ml 30–48 hr after cloprostenol treatment. The decrease in progesterone levels to under 1 ng/ml in pseudopregnant females induced by multiple injections of EB [5] and a single injection of EDP [17, 18] occurred 21–57 hr and 24–48 hr after dinoprost treatment, respectively. In the present study, estrus was mainly observed 5 and 6 days

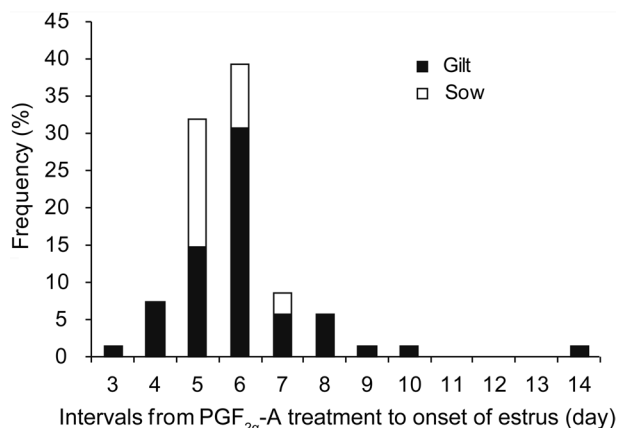


Fig. 2. Intervals from the first PGF_{2α}-A treatment to onset of estrus in pseudopregnant gilts (n=48) and sows (n=20) on commercial farms. The data are presented as frequency per total number of females.

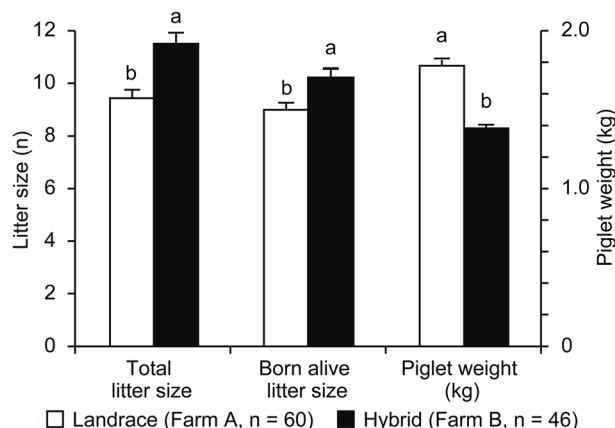


Fig. 3. Total and born alive litter sizes, and piglet weight after parturition in Landrace (n=60) or Hybrid (n=46) gilts on commercial farms. Different superscripts above bars mark significant differences between breeds ($P<0.05$).

Table 1. Reproductive performance of natural cyclic (control) and pseudopregnant female pigs on commercial farms

	No. of female pigs (n)	Conception rate (%)	Farrowing rate (%)	Total litter size ^{a)} (n)	Born alive litter size ^{a)} (n)	Piglet birth weight ^{a)} (kg)
Gilt						
Natural cyclic	62	98.4	96.8	10.6 ± 0.4	9.8 ± 0.4	1.56 ± 0.04
Pseudopregnant	48	97.9	95.8	10.0 ± 0.4	9.4 ± 0.4	1.71 ± 0.04
Sow						
Natural cyclic	30	100	100	11.4 ± 0.6	10.3 ± 0.6	1.59 ± 0.06
Pseudopregnant	20	85.0	85.0	10.8 ± 0.8	10.3 ± 0.8	1.68 ± 0.07

a) Mean ± S.E.

after cloprostenol treatment similarly to dinoprost treatment in pseudopregnant females treated with EB [9, 13] or EDP [17, 18]. The present results and previous reports indicate that cloprostenol and dinoprost have equivalent effects on luteolysis and subsequent estrus exhibition in pseudopregnant females.

There was no difference in conception and farrowing rates, total and born alive litter size, and piglet birth weight between EDP- and cloprostenol-treated and natural cyclic females on commercial farms. While three of 20 pseudopregnant sows did not become pregnant after insemination, farrowing rate of 85% in the pseudopregnant sows seems to be sufficient for practical purposes. The estrus synchronization protocol using pseudopregnant females does not seem to affect adversely reproductive performance in breeding farms from these results. However, the lack of statistical significance between groups may be due to the small sample size in this study and also due to differences in management practice, genetics and health status on each farm. Thus, additional research may be necessary to verify in detail the efficacy of this estrus synchronization protocol.

In the present study, piglet birth weight in gilts (Fig. 3) and sows were significantly different between breeds and parities, respectively, regardless of treatment or control group. It has been known that reproductive outcomes such as litter size and piglet weight are influenced by breeds, environment, genetics, nutrition and other factors [3]. On the other hand, reproductive parameters of pseudopregnant gilts treated with cloprostenol appear to be similar to those treated with dinoprost in our previous study [17]. Mean piglet birth weight (1.72 kg) for gilts treated with EDP and cloprostenol seems to be higher than that (1.37 kg) for gilts treated with EDP and dinoprost [17]. This difference between this study and the previous report is probably due to differences of breed (L and Hybrid vs. LW) and age at mating (7–10 vs. 7–8 months).

In conclusion, a single treatment of EDP at 7–11 days after the end of estrus can induce pseudopregnancy with high efficiency on commercial pig farms. Estrus was synchronized with EDP and PGF_{2α}-A treatment, and the subsequent reproductive performance of pseudopregnant females was similar to that of natural cyclic female pigs. While estrus synchronization protocol in this study leads to prolong the nonproductive period after weaning in sows, it is difficult to incorporate sows that leaved the original group due to very short or long weaning-to-estrus intervals or failure to conceive at the first estrus after weaning, into the other group. Furthermore, it would be able to mate in a more convenient period than the skip-a-heat management in which there are no costs related to hormonal treatments. This estrus synchronization program using EDP and PGF_{2α}-A can be applied for the introduction of not only gilts for recruitment but also sows into planned piglet production by batch farrowing on commercial farms.

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