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—Original Article—

Estrus Synchronization with Pseudopregnant Gilts Induced by a Single Treatment of Estradiol Dipropionate

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Abstract. The aims of this study were to determine whether a single treatment of estradiol dipropionate (EDP) could induce pseudopregnancy in gilts and to determine the effectiveness of $PGF_{2\alpha}$ treatment on estrus synchronization in EDP-induced pseudopregnant gilts. In experiment 1, gilts were treated with 20 mg of EDP (n=11) or vehicle (n=5) on Day 12 (Day 0=onset of estrus). Establishment of pseudopregnancy was defined as a lack of estrus and maintenance of the plasma progesterone concentration above 1 ng/ml between Days 12 and 36. Nine of 11 gilts (82%) treated with EDP became pseudopregnant. The plasma estradiol-17β level was significantly higher in the EDP-treated gilts than in the control gilts until Day 29. In experiment 2, $PGF_{2\alpha}$ was administered twice with a 24-h interval from Day 36 in pseudopregnant gilts (n=6) or Day 10 in cyclic gilts (control; n=5). Estrus after $PGF_{2\alpha}$ treatment was observed in 83% of the pseudopregnant gilts. The interval from the day of the first $PGF_{2\alpha}$ treatment to the onset of estrus and the peak of the LH surge was significantly shorter in the pseudopregnant gilts than in the control gilts. In experiment 3, six pseudopregnant gilts were bred by artificial insemination at the estrus after $PGF_{2\alpha}$ treatment. The farrowing rate and average litter size did not differ between the $PGF_{2\alpha}$-treated pseudopregnant and cyclic gilts. These results indicate that a single treatment of EDP on Day 12 of the estrous cycle can induce pseudopregnancy in pigs and that a convenient protocol for administering $PGF_{2\alpha}$ to EDP-induced pseudopregnant pigs is available for estrus synchronization programs in cyclic pigs.

Key words: Estradiol dipropionate, Estrus control, Pig, Pseudopregnancy

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It is desirable to have female pigs in estrus at certain times to provide maximum use of intensive pork production facilities. Application of estrus synchronization programs enhances economic performance in the swine industry by reducing the labor required to detect estrus, facilitating the use of artificial insemination (AI) and assisting in batch farrowing. Therefore, being able to control the time of estrus in a breeding herd results in decreased reproductive efforts and costs.

Artificially shortening or extending the luteal phase is important for control of the interestrous interval in cyclic animals. The corpus luteum (CL) of the cycling pig generally exhibits resistance to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced luteolysis prior to Day 12 of the estrous cycle [1–3]. Consequently, multiple administrations of $PGF_{2\alpha}$ in the early luteal phase are required to induce luteolysis of the functional CL and shorten the estrous cycle [2, 3]. In contrast to the CL before Day 12 of the estrous cycle, the CL of the pregnant pig easily reacts to exogenous administration of $PGF_{2\alpha}$ [4, 5], and prompt estrus can be induced between 4 and 7 days after $PGF_{2\alpha}$ treatment; however, exogenous administration of $PGF_{2\alpha}$ is associated with abortion in pregnant pigs [4, 6].

In pigs, two phases of estrogen stimulation from conceptuses are required for the establishment and maintenance of pregnancy [7]. Estrogens produced by blastocysts between 11 and 12 days of gestation provide the initial signals for maternal recognition of pregnancy in swine [8–11]. The second period of estrogen production for maintenance of pregnancy occurs between 15 and 30 days of gestation [8, 9, 11]. Luteal function can be extended in cyclic pigs by appropriately timed treatments of estradiol, mainly estradiol benzoate (EB) [12–14] or human chorionic gonadotropin for stimulation of follicular estrogen synthesis [15–17] as a substitute for the signals from conceptuses. The generally used method to extend luteal function involves daily treatments of EB on Days 11 to 15 of the estrous cycle [14]. Gilts that have been rendered pseudopregnant by 4 or 5 administrations of EB from Day 11 maintain luteal function for at least 60 days [13, 14]. The CL of pseudopregnancy induced by these methods regresses rapidly after $PGF_{2\alpha}$ administration as well as pregnancy [5]. Furthermore, the exhibited estrus [12, 18, 19] and fertility [12, 13, 18] after $PGF_{2\alpha}$ treatment in pseudopregnancy are similar to those of pregnancy.

The need for multiple administrations of estradiol to induce pseudopregnancy or $PGF_{2\alpha}$ luteolysis has made this technique impractical for commercial application to estrus synchronization programs. Furthermore, induction of abortion and frequent administrations of drugs to control estrus are problems for animal welfare. Cushman *et al*. [20] reported that a single treatment of slow-release estradiol-17β incorporated into poly (D,L-lactide) microspheres could induce pseudopregnancy in pigs and that the CL could be regressed with $PGF_{2\alpha}$. Unfortunately, this reagent has not been available as a pharmaceutical for commercial use, although a method for estrus synchronization with few treatments may outperform the traditional methods in terms of both conve-

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nience and animal welfare.

Estradiol dipropionate (EDP), which is an estradiol diester with aliphatic acid (propionate) radicals in the 3- and 17-positions, is hydrolyzed *in vivo* and obtains efficacy as free estradiol-17β. Compared with EB, EDP exhibits a low threshold value with a relatively intense and prolonged effect in rats [21] and humans [22]. However, there have been no reports of using EDP to induce pseudopregnancy in swine.

The aims of the present study were 1) to determine whether a single treatment of EDP alters luteal function and the secretions of estradiol-17β, progesterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in cyclic gilts; 2) to characterize in detail the effects of exogenous administration of $PGF_{2\alpha}$ on estrus exhibition and the secretion of ovarian steroids and gonadotropins in pseudopregnant gilts; and 3) to examine the fertility of pseudopregnant gilts after $PGF_{2\alpha}$ treatment.

Materials and Methods

Animals

Crossbred gilts (Landrace × Large White) were purchased from Cimco (Tokyo, Japan) at approximately 160 days of age and kept in individual pens. Estrus detection started at arrival and was performed twice daily with a mature boar. Estrus was defined as the period that gilts showed a standing response for the boar. For all studies, the animals were used after showing two or three normal estrous cycles (age and body weight: 7.6 ± 0.2 months and 112.3 ± 2.1 kg; mean ± SEM). All animal-related procedures employed in this study were approved by the Institutional Care and Use Committee for Laboratory Animals of the National Institute of Animal Health (Protocol No. 08-002).

Experimental design

In experiment 1, we investigated whether pseudopregnancy could be established in gilts by a single treatment of EDP (Ovahormone Depot; Aska Pharmaceutical, Tokyo, Japan). Gilts were given intramuscularly vehicle (20% [v/v] benzyl benzoate in sesame oil, 4 ml) as a control group (n=5) or EDP (20 mg in 4 ml of vehicle) as a treatment group (n=11) on Day 12 (Day 0=onset of estrus). Gilts were checked for estrus twice daily from Day 17 until the end of estrus was observed or to Day 36 if they did not exhibit a subsequent estrus. Each gilt was fitted with an indwelling catheter in the auricular vein on Day 8 or 9. Blood samples were collected daily beginning on Day 9 until 10 days after the onset of estrus or through to Day 36 if estrus was not observed. In addition, blood samples were collected at 12-h intervals from 0 to 120 h after the treatment. Plasma was recovered after centrifugation of the blood samples and stored at –20 C until analysis. Pseudopregnancy was defined as a lack of estrus and maintenance of the plasma progesterone concentration above 1 ng/ml between Days 12 and 36.

In experiment 2, the effect of combining EDP-induced pseudopregnancy and $PGF_{2\alpha}$ administration as a means of synchronizing estrus in gilts was examined. Pseudopregnancy was induced by a single treatment of EDP on Day 12 as described in experiment 1. Gilts were injected intramuscularly with $PGF_{2\alpha}$ as 15 mg dinoprost (Panacelan Hi; Meiji Seika, Tokyo, Japan) twice with a 24-h interval on Days 10 and 11 for the control group (n=6) or on Days 36 and 37 for the pseudopregnant group (n=5). Each gilt was fitted with an indwelling catheter in the auricular vein at 3 or 4 days before the day of the first $PGF_{2\alpha}$ treatment. Blood sampling and estrus detection were carried out every 6 h from the first day of $PGF_{2\alpha}$ treatment until the end of the subsequent estrus. Thereafter, blood samples were collected once daily until 10 days after the onset of estrus. Additional blood samples were collected at 3-h intervals from 0 to 48 h after the first $PGF_{2\alpha}$ treatment. Plasma was recovered after centrifugation of the blood samples and stored at –20 C until analysis.

In experiment 3, the efficiency of a novel estrus synchronization program on the fertility of gilts was investigated. Pseudopregnancy was induced as described in experiment 1, and $PGF_{2\alpha}$ treatment was carried out for luteolysis as described in experiment 2. In the pseudopregnant group (n=6), gilts were bred by AI using liquid semen (Cimco) at 12 and 24 h after the onset of the estrus after $PGF_{2\alpha}$ treatment. Control cyclic gilts (n=4) were artificially inseminated twice during natural estrus using the same protocol as the pseudopregnancy group.

Hormone assay

The plasma concentrations of estradiol-17β and progesterone were measured using time-resolved fluoroimmunoasssay (Tr-FIA) kits (DELFIA Estradiol and Progesterone Kits; PerkinElmer Japan, Yokohama, Japan) as previously reported [23]. Estradiol-17β and progesterone were extracted from the plasma samples with diethyl ether before being applied to the kits. The respective intra- and interassay coefficients of variation (CVs) were 9.6% and 9.2% for estradiol-17β and 10.6% and 11.6% for progesterone.

The concentrations of FSH or LH in the plasma of the gilts were determined by competitive immunoassays using europium (Eu)-labeled FSH [24, 25] or LH [23] as probes, respectively. In the Tr-FIA for porcine FSH, an anti-porcine FSH serum (AFP-2062096) was used as the primary antibody, and porcine FSH antigen (AFP-10640B) was used for Eu-labeling and as the reference standard. In the Tr-FIA for porcine LH, an anti-porcine LH antibody (AFP-15103194Rb) was used as the primary antibody, and porcine LH antigen (AFP-11043B) was used for Eu-labeling and as the reference standard. Porcine FSH and LH immunoassay kits were provided by Dr. AF Parlow (National Hormone and Peptide Program, Harbor-UCLA Medical Center, Torrance, CA, USA). The respective intra- and interassay CVs were 4.8% and 11.8% for FSH and 9.3% and 7.6% for LH.

Statistical analyses

Data pertaining to the hormonal profiles were subjected to analysis of variance (ANOVA) for repeated measures [26]. When a significant effect was detected with ANOVA, the significance of the difference between the means was determined using Duncan's multiple range test. Differences between two means were tested for significance using the Student's *t*-test. All data were analyzed using the General Linear Models procedure for statistical analysis systems [27]. A value of $P<0.05$ was considered to be significant. 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

Table 1. Effects of EDP treatment for the induction of pseudopregnant gilts

Items	Control (vehicle)	EDP treatment
No. of treatment gilts	5	11
No. of pseudopregnant gilts	0	9
No. of gilts with detected luteolysis*	5	2
No. of gilts that exhibited estrus	5	2
Interval from treatment to onset of estrus (day)	7.9 ± 0.5†	15 and 16

* Luteolysis was defined as a plasma progesterone concentration of < 1 ng/ml. † Values are presented as means ± SEM.

method [28]. The treatment effects on the incidence of pseudopregnancy and fertility were analyzed using the chi-square test [27].

Results

Induction of pseudopregnancy and changes in peripheral hormones by a single treatment of EDP

The effects of EDP on the incidence of pseudopregnancy are shown in Table 1. In the control group, all the gilts exhibited estrus, and the interestrous interval ranged from 18 to 21 days. Nine of 11 gilts that received a single treatment of EDP on Day 12 became pseudopregnant. The two gilts that were not induced into pseudopregnancy with EDP displayed interestrous intervals of 27 and 28 days, respectively.

The plasma estradiol-17β profiles for all the control and treatment groups in 9 pseudopregnant gilts are shown in Fig. 1a. In the nine pseudopregnant gilts injected with EDP, the concentrations of plasma estradiol-17β rapidly increased ($P<0.01$) 1 day after the EDP treatment and were maintained at significantly high levels for 9 days compared with the level at 0 h after the treatment. The plasma estradiol-17β concentrations in the pseudopregnant gilts increased to maximum levels of 180.2 ± 19.7 pg/ml from 1.5 to 3 days after the EDP treatment and then significantly decreased at 5 days after compared with 3 days after EDP treatment. Compared with the controls, the plasma estradiol-17β levels were significantly greater ($P<0.01$) in the pseudopregnant gilts beginning at 12 h and continuing for 17 days after the treatment. In two gilts that did not become pseudopregnant after EDP treatment, the plasma estradiol-17β concentrations reached the peak value (252.2 pg/ml and 75.8 pg/ml) at 1 to 2 days after EDP treatment and then decreased to 13.0 and 5.2 pg/ml at 9 days after the treatment.

The plasma progesterone concentrations in all the control gilts were decreased to less than 1 ng/ml from 3 to 5 days after vehicle treatment (Fig. 1b, Table 1). In nine of 11 gilts treated with EDP, the plasma progesterone levels remained above 5 ng/ml until 24 days after the treatment (Fig. 1b). In the remaining two gilts, the plasma concentrations of progesterone started to decline at 5 and 6 days after the treatment, and became less than 1 ng/ml at 10 days after the treatment; this was followed by the onset of estrus. The progesterone levels in the pseudopregnant gilts were significantly higher ($P<0.05$) and lower ($P<0.05$) than those in the control gilts between 3 and 10 days and between 12 and 17 days after the treatment, respectively.

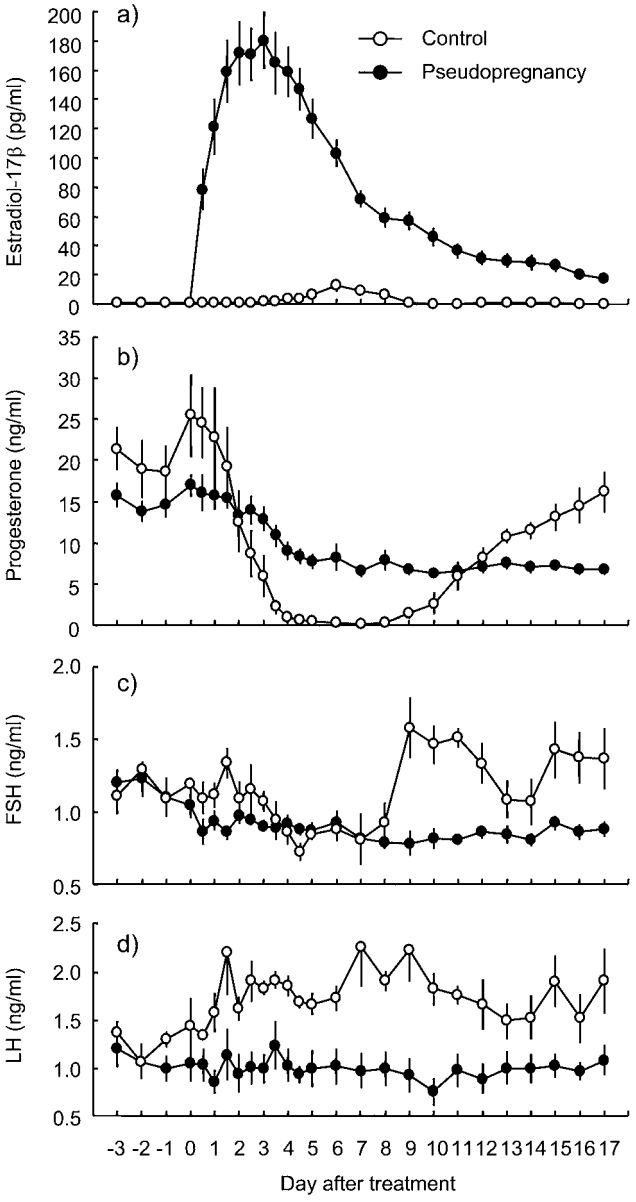

Fig. 1. Plasma concentrations of (a) estradiol-17β, (b) progesterone, (c) FSH and (d) LH in pseudopregnant gilts (n=9) treated with 20 mg of EDP and control gilts (n=5) treated with vehicle. Values are means ± SEM.

Table 2. Effects of $PGF_{2\alpha}$ on regression of the CL and hormonal profiles in pseudopregnant gilts

Items	Control	Pseudopregnant
No. of treatment gilts	5	6
No. of gilts that exhibited estrus	4	5
Interval from 1st $PGF_{2\alpha}$ treatment to (day)		
luteolysis*	7.1 ± 1.3^a	1.2 ± 0.1^b
onset of estrus	10.9 ± 1.6^a	5.7 ± 0.3^b
estradiol-17β peak	10.5 ± 1.6^a	5.2 ± 0.1^b
LH peak	11.1 ± 1.5^a	5.7 ± 0.1^b

* Luteolysis was defined as a plasma progesterone concentration of <1 ng/ml. Values are presented as means ± SEM. [a,b] Values with different superscripts within each row differ significantly from one another ($P<0.01$).

The plasma FSH levels in the pseudopregnant gilts were significantly lower ($P<0.05$) than those in the control gilts from 9 to 17 days, except on 13 days, after the treatment (Fig. 1c). Compared with the control gilts, the concentrations of plasma LH were significantly lower ($P<0.05$) in the pseudopregnant gilts from 2 to 17 days, except for 13 and 14 days, after the treatment (Fig. 1d).

Estrus exhibition and hormonal profile after $PGF_{2\alpha}$ treatment in pseudopregnant gilts

Standing estrus after $PGF_{2\alpha}$ treatment appeared in four of five gilts (80%) in the control group and five of six gilts (83%) in the pseudopregnant group. The interval from the first $PGF_{2\alpha}$ treatment to estrus was significantly shorter in the pseudopregnant gilts than in the control gilts (Table 2). The duration of estrus did not differ significantly between the control and pseudopregnant gilts (43.5 ± 13.3 and 50.4 ± 13.8 h, respectively).

The interval from the first $PGF_{2\alpha}$ treatment to the peak estradiol-17β concentration was significantly shorter in the pseudopregnant gilts than in the control gilts (Fig. 2a, Table 2). There was no difference between the peak values of estradiol-17β during the follicular phase for the control and pseudopregnant gilts (22.6 ± 6.7 and 26.6 ± 2.0 pg/ml, respectively). The concentrations of plasma estradiol-17β in the pseudopregnant gilts were significantly greater ($P<0.05$) than those in the control gilts from 18 h to 5.5 days after the first $PGF_{2\alpha}$ treatment, whereas they were significantly lower ($P<0.05$) in the pseudopregnant gilts compared with the control gilts from 6.75 to 8 days after the first treatment of $PGF_{2\alpha}$.

There was no difference in the progesterone levels before the $PGF_{2\alpha}$ treatment between the control and pseudopregnant gilts (Fig. 2b). The plasma progesterone concentrations in the pseudopregnant gilts were immediately decreased to less than 1 ng/ml (Fig. 2b, Table 2). The concentrations of plasma progesterone in the pseudopregnant gilts were significantly lower ($P<0.05$) than those in the control gilts between 6 h and 5.75 days after the first $PGF_{2\alpha}$ treatment.

The FSH levels in the pseudopregnant gilts were significantly suppressed ($P<0.05$) compared with the control gilts from 3 days before to 6 h after the first $PGF_{2\alpha}$ treatment (Fig. 2c). Furthermore, the plasma FSH concentrations of the pseudopregnant gilts were significantly lower ($P<0.05$) than those in the control gilts from 2.5 to 4.75 days after the $PGF_{2\alpha}$ treatment.

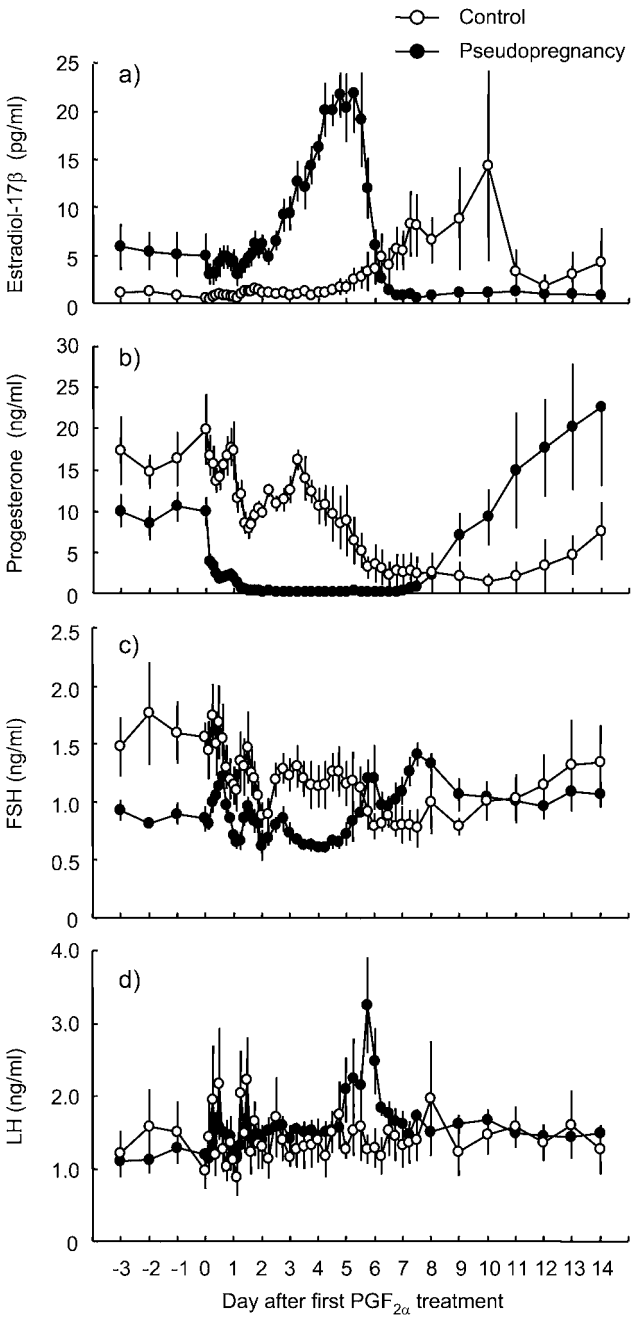

Fig. 2. Changes in the plasma concentrations of (a) estradiol-17β, (b) progesterone, (c) FSH and (d) LH in pseudopregnant (n=5) and control (n=4) gilts that exhibited estrus after $PGF_{2\alpha}$ treatment. Values are means ± SEM.

An LH surge was determined in all gilts that exhibited a subsequent estrus after the $PGF_{2\alpha}$ treatment. The interval from the first $PGF_{2\alpha}$ treatment to the peak LH concentration was significantly shorter in the pseudopregnant gilts than in the control gilts (Fig. 2d, Table 2). The duration of the LH surge (21.6 ± 2.4 h) and the peak

Table 3. Reproductive parameters in pseudopregnant gilts following synchronization with EDP and $PGF_{2\alpha}$

Items	Control	Pseudopregnant
No. of treatment gilts	4	6
Duration of estrus (day)	2.3 ± 0.3	2.3 ± 0.2
No. of pregnant gilts	4	5
No. of gilts farrowing	4	5
Litter size	10.3 ± 1.3	10.6 ± 1.6
Piglet birth weight (kg)	1.28 ± 0.03[a]	1.37 ± 0.03[b]

Values are presented as means ± SEM. [a,b] Values with different superscripts within each row differ significantly from one another ($P<0.05$).

LH concentration (3.9 ± 0.5 ng/ml) in the pseudopregnant gilts were similar to those in the control gilts (22.5 ± 1.5 h and 4.6 ± 0.7 ng/ml, respectively). There was no significant difference in the concentrations of LH between the two groups during the experimental period, except at 5.75 days after the first $PGF_{2\alpha}$ treatment (Fig. 2d).

In a pseudopregnant gilt that did not exhibit estrus after $PGF_{2\alpha}$ treatment, the progesterone concentrations rapidly decreased following $PGF_{2\alpha}$ treatment and remained less than 1 ng/ml for 20 days after the treatment. An increased endogenous estradiol-17β secretion and the LH surge were not observed until 20 days after $PGF_{2\alpha}$ treatment. In a gilt of the control group that failed to return to estrus, the plasma progesterone levels began to decrease from 5 days after $PGF_{2\alpha}$ treatment and remained less than 1 ng/ml from 8 to 20 days after the treatment. The estradiol-17β concentrations in plasma increased slightly to 6.2 pg/ml at 11 days after the treatment and then decreased to a nadir. The LH surge was not observed after $PGF_{2\alpha}$ treatment.

Fertility in pseudopregnant gilts

In the pseudopregnant group, all the gilts exhibited estrous behavior at 5.9 ± 0.5 days after the first treatment of $PGF_{2\alpha}$. The durations of estrus, pregnancy rates and farrowing rates were similar in the two groups (Table 3). The numbers of piglets were essentially the same in the two groups. The piglet birth weight was heavier ($P<0.05$) in the pseudopregnant gilts than in the control gilts.

Discussion

The results of the present study indicate that pseudopregnancy is highly induced in response to a single treatment of EDP on Day 12 of the estrous cycle in gilts and that a combination of EDP-induced pseudopregnancy followed by $PGF_{2\alpha}$ administration offers an effective means for synchronizing estrus in gilts.

A single treatment with EDP on Day 12 of the estrous cycle could induce pseudopregnancy in cyclic gilts. In pregnant pigs, the estrogen concentrations in the uterine lumen increase biphasically. Uterine luminal increases in the estrogen concentration at 11 to 12 days and after 14 days of pregnancy are necessary for complete establishment of pregnancy in pigs [9]. Gilts treated with a single treatment of EB on Days 9.5 to 12.5 have an average estrous cycle length of about 29 days [14]. However, exogenous EB administration between Days 11 and 14 or 15 of the estrous cycle could prolong CL function and the interestrous interval to more than 60 days [13, 14]. The peripheral estradiol-17β levels are elevated for 96 h after a single treatment of EB in ovariectomized pigs [29], but there is no significant difference in the plasma estradiol-17β concentrations between EB-treated and control pigs at 9 days after the treatment [30]. In the present study, the plasma concentrations of estradiol-17β in the pseudopregnant gilts after a single treatment of EDP were significantly higher until Day 29 (17 days after the EDP treatment) than those in the cyclic pigs. These findings suggest that a single treatment of EDP on Day 12 can maintain the peripheral estrogen concentrations at high levels in gilts during the two phases required for maternal recognition of pregnancy and can produce similar effects to administration of EB from Day 11 through to Day 14 or 15 [13, 14] on the induction of pseudopregnancy. Consequently, this one-shot method appears to be more effective for inducing pseudopregnancy in pigs in terms of effort and cost compared with the previous method using at least four administrations of EB [13, 14].

The plasma concentrations of progesterone in the pseudopregnant gilts decreased to markedly less than 1 ng/ml between 24 and 33 h after $PGF_{2\alpha}$ treatment. In previous reports, the progesterone levels declined to 1 ng/ml or less from 45 to 57 h following $PGF_{2\alpha}$ treatment in pregnant pigs [4, 5] or from 21 to 57 h in pseudopregnant pigs [5]. Estrus was observed 4 to 6 days after $PGF_{2\alpha}$ administration in the pseudopregnant gilts, which is in agreement with previous reports on pregnant [4, 6] and pseudopregnant [12, 18, 19] pigs. The present results demonstrated that exogenous $PGF_{2\alpha}$ was effective in regressing the CLs of pseudopregnant gilts induced by EDP and causing synchronized estrus.

In our study, all the pseudopregnant gilts were administered $PGF_{2\alpha}$ on Day 36 (24 days after EDP treatment), and 90% of the pigs exhibited a synchronized estrus. In a previous study, pregnant and pseudopregnant gilts with high estradiol-17β levels failed to return to estrus and exhibit an LH surge after $PGF_{2\alpha}$ treatment [31]. In another study of gilts given EB daily from Day 11 to 14, estrus could not be synchronized by $PGF_{2\alpha}$ treatment 1 day after the end of EB treatment, whereas most of the gilts administered $PGF_{2\alpha}$ at least 5 days after the end of EB treatment exhibited estrus 4 to 6 days after the first $PGF_{2\alpha}$ treatment [13]. This diminished capacity to return to estrus may be caused by the residual high estradiol-17β concentration in the peripheral blood at the time of the $PGF_{2\alpha}$ treatment. In the present study, there was no significant difference in the plasma estradiol-17β level between Day 12 and 22 (0 and 10 days after EDP treatment, respectively), while the plasma estradiol-17β concentrations in the pseudopregnant gilts remained significantly higher until Day 29 compared with the control gilts. The minimum dosing interval between EDP and $PGF_{2\alpha}$ to cause estrus in the pseudopregnant gilts after $PGF_{2\alpha}$ treatment is not clear at the present time. Since $PGF_{2\alpha}$ treatment before Day 36 in pseudopregnant gilts with EDP may induce luteolysis and estrus without affecting the estradiol-17β level, further studies are required to clarify the timing of $PGF_{2\alpha}$ administration before Day 36.

The fertility at estrus in the $PGF_{2\alpha}$-treated pseudopregnant gilts appeared to be normal based on comparisons with the control gilts.

However, induction of pseudopregnancy prior to breeding may cause a significant improvement in piglet birth weight. These results correspond with those in previous studies [13, 18]. The uterine capacity during pregnancy is considered to be dependent on the uterine size, nutrient supply, gaseous exchange and placental surface area [32]. The uterine length appears to be an important limiting factor for the litter size and piglet weight in swine [33]. Pope and First [34] reported that uterine elongation appeared to result from treatment with estradiol-17β on Days 12 and 13 of the estrous cycle, which is similar to the increase seen in pregnant sows. In a previous report, the number of ovulated follicles was not affected by treatment with EB followed by $PGF_{2\alpha}$ [19]. An increase in the placental weight associated with space and nutrition in the uterus results in an increase in fetal weight [35] and piglet birth weight [36]. Based on the present study and previous reports [13, 18], it seems that uterine growth induced by exogenous estradiol leads to an increase in piglet weight following placenta development. However, the follicular growth and ovulation after $PGF_{2\alpha}$ treatment in the EDP-treated pseudopregnant pigs were not evaluated in this study. Further studies are required to examine the influence of this estrus synchronization program using EDP and $PGF_{2\alpha}$ on follicular development and the numbers of ovulations in pigs.

In summary, we demonstrated that a single treatment of EDP on Day 12 of the estrous cycle can prevent luteolysis and result in maintenance of a functional CL in gilts. The CL of the pseudopregnant pig is regressed with $PGF_{2\alpha}$ and estrus occurs, followed by luteolysis. Furthermore, this study indicates that use of a combination of EDP and $PGF_{2\alpha}$ for pigs has no detrimental effects on their fertility. We conclude that utilization of EDP-treated pseudopregnant gilts is effective for estrus synchronization with convenient application methods.

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