—Original Article—

Estrus synchronization in microminipig using estradiol dipropionate and prostaglandin $F_{2\alpha}$

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Abstract. The induction of pseudopregnancy by the exogenous administration of estradiol dipropionate (EDP) was investigated in cyclic Microminipigs (MMpigs) and the effects of exogenous administration of prostaglandin (PG) $F_{2\alpha}$ on estrus exhibition were assessed in pseudopregnant MMpigs. In experiment 1, ovariectomized MMpigs were given a single intramuscular injection of 0.5, 1.5, or 2.5 mg of EDP. The estradiol-17β level at each of these doses was significantly higher 1 to 3 days after EDP administration than on the day of the injection. In experiment 2, animals were given 1.5 mg of EDP once at 9 to 12 days after the end of estrus (D0) and then no (1.5 mg × 1 group), one (D0 and D4; 1.5 mg × 2 group), or two (D0, D4 and D7; 1.5 mg × 3 group) additional treatments. The pseudopregnancy rate was significantly higher in the 1.5 mg × 3 than in the 1.5 mg × 1 group. In experiment 3, $PGF_{2\alpha}$ was administered twice between 26 and 28 days after EDP treatment to five pseudopregnant gilts with a 24-h interval between the two injections. Estrus after $PGF_{2\alpha}$ treatment and LH surge were observed in 100% and 80% pseudopregnant MMpigs, respectively. The interval from the day of the first $PGF_{2\alpha}$ treatment to the onset of estrus was 6.5 ± 0.2 days. These results indicate that multiple EDP treatments are required for induction of pseudopregnancy in MMpigs and estrus exhibition can be controlled in MMpigs by treatment with EDP and $PGF_{2\alpha}$.
Key words: Estradiol dipropionate, Estrus synchronization, Microminipig, Pseudopregnancy

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The miniature pig is a good model for human diseases because of its many physiological and anatomical similarities to humans [1–3] and it can be easily managed and handled as a domestic pig species. Recently, the Microminipig (MMpig) was developed as the smallest miniature pig in the world [4]. This breed has general biochemical and hematological [5–8] profiles similar to those of humans. The utility of MMpigs as experimental animals has been demonstrated in research on atherosclerosis [9–12], proarrhythmias [13], sleep conditions [14], coagulation activity [5], skin toxicity [15], and genomic sequencing [16]. MMpigs, therefore, are expected to become an important animal model for future research on human health.

As the usage of MMpigs as experimental animals grows, establishing an effective and stable MMpig breeding system will become a necessity. Synchronization of estrus is a well-known tool for effective domestic animal breeding. Artificial control of the length of the luteal phase is important for controlling the inter-estrous interval in cyclic animals. Difficulties in regressing the corpus luteum (CL) before day 12 of the estrous cycle without multiple administrations

of prostaglandin (PG) $F_{2\alpha}$ have been reported in both domestic pigs [17–19] and miniature pigs [20]. In contrast, in pregnant [21, 22] as well as pseudopregnant [22–25] pigs, the CL readily responded to single or dual administration of $PGF_{2\alpha}$, and estrus was induced promptly at around 7 days after $PGF_{2\alpha}$ treatment. Porcine conceptuses stimulate estrogen production in the gilt, and this has been reported to play a role in "maternal recognition of the conceptus for the establishment of pregnancy in pigs" [26]. Pseudopregnancy has been defined as the maintenance of a functional CL in cyclic female pigs with exogenous estrogen as a substitute for conceptus signaling. We previously showed that a single administration of estradiol dipropionate (EDP) results in the manifestation of estradiol-17β effects that last for a longer period than estradiol benzoate (EB) and estradiol valerate (EV) in rats [27] and humans [28]. We also showed that administration of EDP at 9−13 days after the onset of estrus could induce pseudopregnancy in gilts and sows [29–31]. Administration of EDP appears to be more effective for inducing pseudopregnancy in domestic pigs in terms of effort and cost than the earlier method of administering EB or EV at least 4 times [23, 32, 33]. Furthermore, estrus exhibition in EDP-induced pseudopregnant pigs can be synchronized between 5 and 8 days after the first $PGF_{2\alpha}$ treatment [29–31], without any negative effects of EDP or $PGF_{2\alpha}$ treatment on reproductive performance [29, 31]. From these reports, we concluded that estrus synchronization using EDP and $PGF_{2\alpha}$ is a practical and convenient procedure in domestic pig management.

Recently, we demonstrated that the intra-estrous cycles and

hormonal profiles of estradiol-17β, progesterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) during normal estrous cycle in MMpigs [34] were quite similar to those in domestic female pigs [35]. These results suggest the possibility of controlling estrus exhibition in MMpigs using a domestic-pig estrus synchronization protocol. Accordingly, in the present study, we aimed to 1) determine whether pseudopregnancy could be induced by treatment with EDP in cyclic MMpigs and 2) characterize the effects of exogenous administration of PGF$_{2\alpha}$ on estrus exhibition and the profiles of steroids and gonadotropins in pseudopregnant MMpigs.

Materials and Methods

Animals and estrus detection

All MMpig gilts (at least over 18 months of age because MMpig growth curve reaches plateau at approximately 18 months [6]) were purchased from Fuji Micra (Shizuoka, Japan) and maintained at Kagoshima University in a dedicated, climate-controlled room at a temperature of 18°C to 25°C and humidity of 30% to 65%, with a 12 h light/dark cycle. They were kept in individual cages with water available ad libitum and were fed a commercial diet for domestic pigs (Kodakara 73, Marubeni Nissin Feed, Tokyo, Japan) in quantities of 1.8% to 2.2% of body weight once daily. We confirmed that their body weights reached a plateau and remained unchanged by weighing once every week before each experiment. The gilts were checked for estrus twice daily, using a mature, male MMpig. Estrus was defined as a standing response for the boar. At least two estrous cycles of normal length (18–22 days) and normal duration (1.5–3.0 days) [34] were observed in each MMpig before the start of each experiment. The mean ages and weights of the experimental animals were 26.7 ± 2.1 months and 14.8 ± 0.3 kg, respectively [mean ± standard error of the mean (SEM)]. All protocols were approved by the Kagoshima University Animal Care and Experimentation Ethics Committee (VM12019 and VM14301) and the research was performed according to the Institutional Guidelines for Animal Experiments and in compliance with the Japanese Law Concerning the Protection and Control of Animals (Law No. 105 and Notification No. 6).

Experimental design

Experiment 1: We investigated the profiles of estradiol-17β after EDP (Ovahormone Depot; ASKA Pharmaceutical, Tokyo, Japan) treatment in ovariectomized MMpigs. Each MMpig was ovariectomized and fitted with a heparin-coated polyurethane catheter (CBAS-C70; Solomon Scientific, Plymouth Meeting, PA, USA) in the jugular vein at least 5 days before the treatment. Animals were given a single intramuscular injection of EDP at varying doses; 0.5 mg (0.04 ± 0.00 mg/kg, n = 4; EDP 0.5 group), 1.5 mg (0.10 ± 0.01 mg/kg, n = 4; EDP 1.5 group) or 2.5 mg (0.17 ± 0.01 mg/kg, n = 4; EDP 2.5 group); a regimen used in previous research [29, 30]. Blood samples were collected daily starting one day before EDP treatment to 14 days after the treatment. Additionally, blood samples were collected at 3-h intervals from 0 h to 12 h after treatment and 12-h intervals from 12 h to 72 h after treatment. Plasma was separated by centrifugation of the blood samples and stored at −30°C until analysis.

Experiment 2: The results of experiment 1 showed that the profiles of estradiol-17β in EDP 1.5 mg group were very similar to those in pseudopregnant domestic gilts [29]. Therefore, EDP 1.5 mg regimen was chosen for further investigation of pseudopregnancy induction in cyclic MMpigs. Thirteen animals were given a single intramuscular injection of EDP at 1.5 mg at 9 to 12 days after the end of estrus (D0 = the day of first EDP treatment). Four of these MMpigs were not given additional EDP treatment (1.5 mg × 1 group; n = 4); another four MMpigs were given one additional EDP 1.5 mg treatment on D4 (1.5 mg × 2 group; n = 4) and the remaining five MMpigs were given two additional EDP 1.5 mg treatments at D4 and D7 (1.5 mg × 3 group; n = 5). All animals were checked for estrus from 17 days after the previous onset of estrus until the end of the subsequent estrus, or until 21 days after treatment if they did not exhibit a subsequent estrus. Blood samples were collected from the jugular vein on D–3, D0, D1, D2, D7, D14 and D21. Plasma was separated by centrifugation of the blood and stored at –30°C. Pseudopregnancy was defined as the absence of estrus with plasma progesterone concentrations in excess of 5 ng/ml, maintained until 21 days after the first EDP treatment, a definition that has been used previously [29, 30].

Experiment 3: We investigated the effect of PGF$_{2\alpha}$ administration on estrus synchronization and hormonal profiles in pseudopregnant MMpigs. Five pseudopregnant MMpigs (two MMpigs from the 1.5 mg × 2 group and three MMpigs from the 1.5 mg × 3 group in experiment 2) were fitted with a catheter in the jugular vein, at least 3 days before the first PGF$_{2\alpha}$ treatment. Animals were injected twice intramuscularly with PGF$_{2\alpha}$ (1.5 mg as dinoprost; Panacelan Hi; Meiji Seika, Tokyo, Japan) with a 24-h interval between injections on D26 to D28, a regimen that has been used previously [29, 30]. Blood samples were collected daily, beginning 2 days before the first treatment with PGF$_{2\alpha}$ and continuing until 7 days after the onset of the subsequent estrus. Additionally, blood samples were collected at 12-h intervals from 0 to 3 days after the first PGF$_{2\alpha}$ treatment. Thereafter, blood sampling and estrus detection were carried out every 6 h until the end of the subsequent estrus. Plasma was separated by centrifugation of the blood samples and stored at −30°C until analysis.

Hormone assay

A time-resolved fluoroimmunoassay (Tr-FIA) was used to measure plasma concentrations of estradiol-17β in experiments 1–3, progesterone in experiments 2 and 3, and LH and FSH in experiment 3. Plasma concentrations of estradiol-17β and progesterone were measured with a Tr-FIA kit (DELFIA Estradiol and Progesterone kits; PerkinElmer Japan, Yokohama, Japan), as reported previously [36]. The respective intra-assay and inter-assay coefficient of variations (CVs) were 7.1% and 12.6% for estradiol-17β and 10.0% and 9.1% for progesterone. Plasma concentrations of LH and FSH were determined using Tr-FIA methods previously described by Noguchi *et al.* [36] and Ohnuma *et al.* [37]. The respective intra-assay and inter-assay CVs were 15.6% and 9.9% for LH and 16.0% and 8.4% for FSH.

Statistical analysis

The duration of the LH surge was regarded as the time from the onset to the end of the LH surge, as defined previously [38].

Data on hormonal profiles and intra-estrous intervals were analyzed using the General Linear Models procedure in SPSS. The statistical

model included the effects of EDP dosage, time and interaction of EDP dosage × time for experiment 1, and the effects of treatment times, time and interaction of treatment times × time for experiment 2. Hormonal profile data were subjected to analysis of variance (ANOVA) for multiple measurements. When a significant effect was detected by ANOVA, Tukey's post-hoc test was used for pairwise comparisons. Differences between the three groups in experiments 1 and 2 were tested for significance using Tukey's post-hoc test. In experiment 2, the significance of difference in inter-estrous intervals between pre and post EDP treatment was determined using a paired t–test. The effect of treatment on the incidence of pseudopregnancy in experiment 2 was analyzed using the Fisher's exact test. $P < 0.05$ was considered significant in all the above stated analyses. All values are expressed as mean ± SEM.

Results

Profiles of estradiol-17β in peripheral blood after EDP treatment in ovariectomized MMpigs

The changes in estradiol-17β profile after treatment with EDP in ovariectomized MMpigs are shown in Fig 1. Estradiol-17β levels were higher from 1 to 3 days in the EDP 0.5 and 2.5 groups and from 1 to 4 days in the EDP 1.5 group after EDP treatment ($P < 0.05$) than those on the day of each treatment. The peak value of estradiol-17β was 136.9 ± 17.0 pg/ml on 1.4 ± 0.2 days after 0.5 mg EDP treatment, 274.3 ± 27.6 pg/ml on 2.5 ± 0.3 days after 1.5 mg EDP treatment and 459.1 ± 66.4 pg/ml on 1.8 ± 0.2 days after 2.5 mg EDP treatment. There was no significant difference between groups receiving different concentrations of estradiol-17β from 1 day before EDP treatment to 0.5 days after EDP treatment. Peripheral estradiol-17β levels from 1 to 2 days after treatment in the EDP 0.5 group were lower ($P < 0.05$) than those in the EDP 2.5 group. There was significant difference in estradiol-17β concentrations at 2.5 days after EDP treatment among the three groups ($P < 0.05$). Estradiol-17β levels between 3 and 5 days after EDP treatment were lower in the EDP 0.5 group than those in the other groups. At 6 days after EDP treatment, estradiol-17β concentration was lower in the EDP 0.5 group ($P < 0.05$) than that in the EDP 2.5 group, and there was no significant difference among groups in estradiol-17β levels from 7 to 14 days after treatment.

Induction of pseudopregnancy and changes in peripheral hormones consequent to EDP treatment

The incidence of pseudopregnancy is shown for each of the treatments in Table 1. The pseudopregnancy rate in the 1.5 mg × 3 group was significantly higher than that in the 1.5 mg × 1 group ($P < 0.05$). The inter-estrous interval in MMpigs (n = 6) that were not induced into pseudopregnancy with EDP ranged from 26 to 34 days (29.3 ± 1.1 days), and it was significantly longer than that before EDP treatment; 18 to 21 days (20.2 ± 0.5 days) in the same animals ($P < 0.05$).

The plasma estradiol-17β and progesterone profiles in MMpigs after EDP treatment are shown in Fig. 2. The concentrations of plasma estradiol-17β between D1 and D2 in the 1.5 mg × 1 group and those between D1 and D7 in the 1.5 mg × 2 and 1.5 mg × 3 groups were significantly higher than the concentrations on both

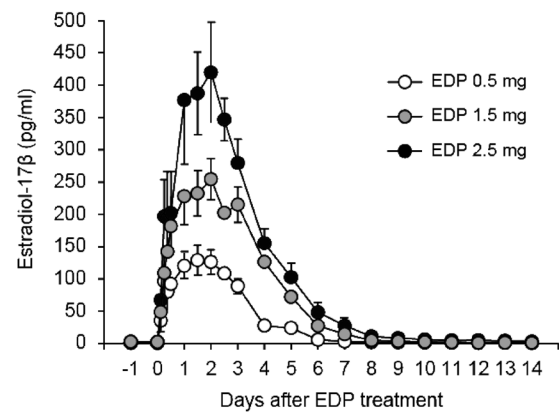**Fig. 1.** Plasma concentrations of estradiol-17β in ovariectomized MMpigs treated with 0.5 (n = 4), 1.5 (n = 4) or 2.5 mg (n = 4) of EDP. Values are means ± SEM.

Table 1. Effects of EDP treatment on the induction of pseudopregnant MMpigs

Items	1.5 mg × 1 group	1.5 mg × 2 group	1.5 mg × 3 group
No. of treatment gilts	4	4	5
No. of pseudopregnant gilts (%)*	0 (0)[a]	2 (50)[ab]	5 (100)[b]
No. of gilts that exhibited estrus (%)	4 (100)[a]	2 (50)[ab]	0 (0)[b]
Inter-estrous interval (day)	28.3 ± 0.9[†]	29 and 34	–

*Pseudopregnancy was defined as the absence of estrus with plasma progesterone concentrations above 5 ng/ml, maintained until 21 days after the first EDP treatment. [†] Values are presented as means ± SEM. [ab] Values with different superscripts within each row differ significantly ($P < 0.05$).

D–3 and D0 ($P < 0.05$). The plasma estradiol-17β levels on D7 in the 1.5 mg × 1 group were significantly lower ($P < 0.05$) than those in the other groups. The progesterone concentrations in all groups were significantly lower on D7 than on the day of first treatment ($P < 0.05$). The progesterone levels from D0 to D2 were higher in the 1.5 mg × 3 group than in the 1.5 mg × 2 group ($P < 0.05$). On D14, the progesterone concentration in the 1.5 mg × 3 group was greater than that in the other groups ($P < 0.05$).

Estrus exhibition and hormonal profile after PGF$_{2\alpha}$ treatment in pseudopregnant MMpigs

Standing estrus after PGF$_{2\alpha}$ treatment was evident in all pseudopregnant MMpigs. The interval from the first PGF$_{2\alpha}$ treatment to estrus and the duration of estrus were 6.5 ± 0.2 days and 2.5 ± 0.4 days, respectively.

The profiles of steroid hormones and gonadotropins after PGF$_{2\alpha}$ treatment in pseudopregnant MMpigs are shown in Fig. 3. The plasma progesterone concentration in the pseudopregnant MMpigs showed an abrupt decrease ($P < 0.05$) at day 0.5 (Day 0 = the day of first PGF$_{2\alpha}$ treatment) from the concentrations noted from Day –2 to Day 0 and fell below 1 ng/ml on Day 2 (Fig. 3a). The plasma
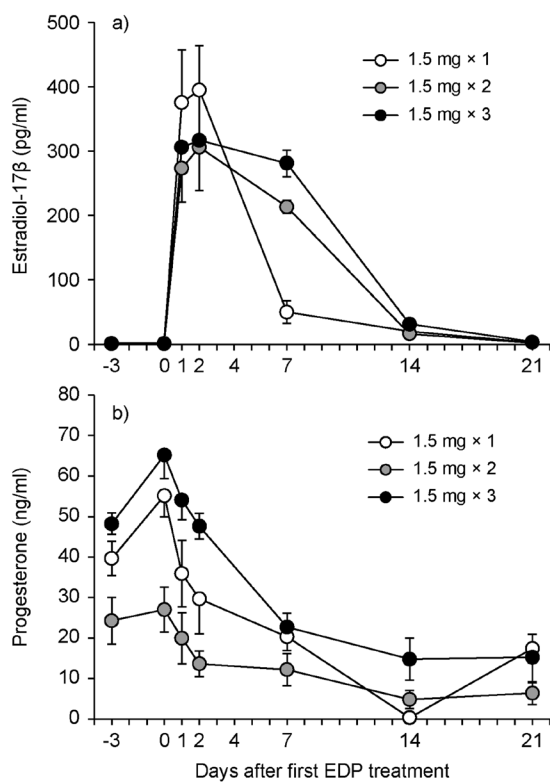
Fig. 2. Plasma concentrations of (a) estradiol-17β and (b) progesterone in MMpigs treated with 1.5 mg of EDP once at 9–12 days after the end of estrus (D0) (1.5 mg × 1 group, n = 4), twice at D0 and D4 (1.5 mg × 2 group, n = 4) or three times at D0, D4 and D7 (1.5 mg × 3 group, n = 5). Values are means ± SEM.

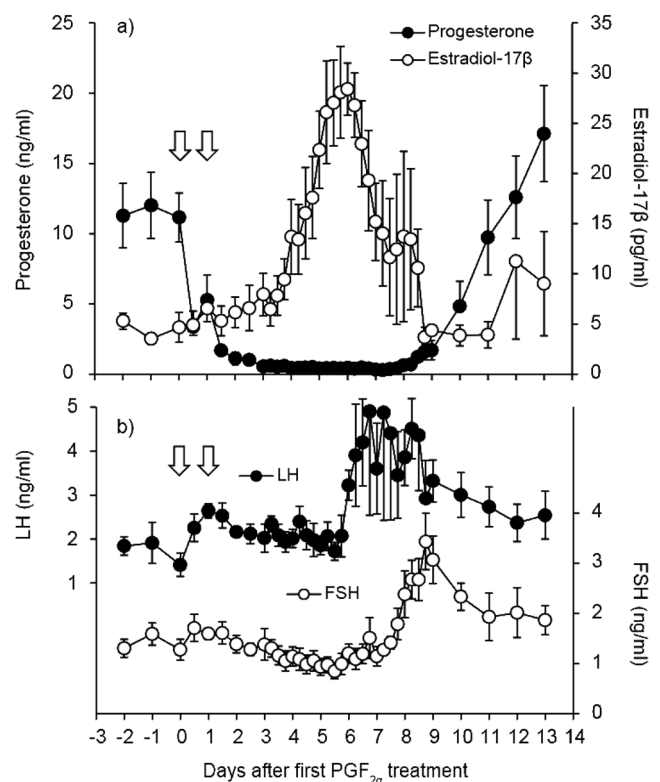**Fig. 3.** Changes in the plasma concentrations of (a) progesterone and estradiol-17β, (b) LH and FSH in pseudopregnant MMpigs (n = 5) after $PGF_{2\alpha}$ treatment. Arrows indicate treatment with $PGF_{2\alpha}$. Values are means ± SEM.

progesterone concentration in MMpigs was significantly higher (P < 0.05) between Days 11 and 13 than on Day 9. Estradiol-17β levels at Days 5.75 and 6 in pseudopregnant MMpigs were higher (P < 0.05) compared to those before $PGF_{2\alpha}$ treatment. The peak value of estradiol-17β was 39.0 ± 3.1 pg/ml on Day 6.4 ± 0.5. The estradiol-17β concentrations were lower on Day 9 than on Day 6 (P < 0.05), and did not differ significantly throughout the duration of the experiment.

An LH surge was detected in four MMpigs that exhibited estrus subsequent to $PGF_{2\alpha}$ treatment. The duration of the LH surge and the peak LH concentration in the pseudopregnant MMpigs (n = 4) were 24.0 ± 0 h and 11.5 ± 1.7 ng/ml respectively on Day 7.2 ± 0.5. The onset and end of the LH surge in the four MMpigs were found to be on Days 6.7 ± 0.5 and 7.7 ± 0.5, respectively. Maximum LH concentration in one MMpig, without an LH surge during subsequent estrus after $PGF_{2\alpha}$ treatment, was 5.2 ng/ml on Day 6.5. There was no significant difference in the concentrations of LH throughout the duration of the experiment (Fig. 3b). The plasma FSH concentrations in the pseudopregnant MMpigs between Day 8.25 and Day 9 were significantly higher (P < 0.05) than on the day of the first $PGF_{2\alpha}$ treatment (Fig. 3b).

Discussion

The results of the present study indicate that pseudopregnancy in MMpigs is readily inducible with three-time EDP treatment, but not with a single EDP treatment. The CL regression and estrus synchronization shown by pseudopregnant MMpigs following $PGF_{2\alpha}$ administration resembled that seen in pseudopregnant domestic pigs.

A high success rate for induction of pseudopregnancy was achieved in MMpigs treated three times with 1.5 mg EDP at three or four-day intervals, whereas the rates of pseudopregnancy induction in MMpigs treated once or twice with 1.5 mg EDP were low (0–50%). In pregnant commercial pigs, concentrations of estrogen in the uterine lumen have been shown to increase biphasically from 11 to 12 days and after 14 days of gestation, resulting in the first and second periods of maternal recognition [26]. Single EB injection at 9.5, 11 or 12.5 days after the onset of estrus was shown not to induce pseudopregnancy in domestic pigs [32], whereas a high success rate for induction of pseudopregnancy was achieved with pigs that received multiple treatments from days 11 to 14 or on day 15 of the estrous cycle [23, 32]. Blood estradiol-17β concentrations in domestic pigs treated with EDP at 9 to 13 days after onset of estrus were maintained at significantly high levels until 7 to 9 days after EDP treatment and 80–100% of these pigs developed pseudopregnancy [29, 30]. Previous researchers concluded that increased estrogen concentrations during

both of those periods is important for complete establishment of pseudopregnancy in cyclic domestic pigs [23, 29, 30, 32]. Although the EDP dosages relative to body weight in MMpigs and domestic pigs were similar [29, 30], peripheral blood estradiol-17β levels in ovariectomized MMpigs treated with EDP at 1.5 to 2.5 mg were maintained at significantly high concentrations for only 4 days after treatment compared to 9 days in domestic pigs. Our results of this work to develop a protocol for the establishment of pseudopregnancy in MMpigs indicate that estrogen stimulation during periods of maternal recognition is also important for pseudopregnancy induction in cyclic MMpigs.

Progesterone concentrations on D0 in EDP-treated MMpigs were significantly different between 1.5 mg × 2 group and 1.5 mg × 3 groups. There is a possibility, of the number of CLs in MMpigs being uneven among groups, because the number of functional CLs on ovaries in cyclic MMpigs ranged from 3 to 8 [34]. On the other hand, progesterone levels before the first EDP treatment in 1.5 mg × 2 group were not different between pseudopregnant MMpigs (n = 2; 23.3–27.7 ng/ml) and non-pseudopregnant MMpigs (n = 2; 25.3–26.4 ng/ml). Similar results were observed in previous work using domestic pigs (pseudopregnant pigs *vs.* non-pseudopregnant pigs = 12.7–28.9 ng/ml *vs.* 15.8–21.1 ng/ml) [29, 30]. Furthermore, high progesterone levels (above 5 ng/ml) in peripheral blood mean the presence of CLs on ovaries in both domestic pigs [35] and MMpigs [34]. These results and previous reports indicate that the pseudopregnancy rate is not affected by progesterone concentration at the time of the first EDP treatment.

The present study did not shed light on the reason for the difference in estradiol-17β peripheral blood profiles after EDP treatment between MMpigs and domestic pigs. Steroids including estradiol-17β are mainly metabolized in liver. Cytochrome P450 enzymes (CYP1A and CYP2E) and 3β-hydoroxysteroid dehydrogenase are reported to be involved in the metabolism of testicular steroids (androstenone, estradiol-17β, and testosterone) in domestic pigs [39–41]. However, mRNA expressions and activities of steroid metabolizing enzymes in the porcine liver after testicular steroid treatment are known to vary with age [42], breed [42] and gender [40, 42]. From these reports, it was surmised that the enzymes and activities involved in steroid metabolism in the liver may differ between domestic pigs and MMpigs. On the other hand, there is the possibility that estradiol-17β profiles in muscle after EDP treatment may be dependent on the body composition of pigs of different genotypes. Percentage of muscle and fat in the carcass are affected by genotypes in pigs [43]. The concentrations of androgen, a estradiol-17β precursor, in fat and estradiol-17β in peripheral blood in whole male pigs are significantly high compared to those in castrated pigs [41]. In our present study, even though the treatment dosage of EDP relative to body weight was equal in both MMpigs and domestic pigs, peak concentrations of estradiol-17β in blood after EDP treatment in MMpigs were higher than those in domestic pigs as reported earlier [29, 30]. These results from our current work and previous reports suggest that metabolism and/or transition of estrogen, after intramuscular EDP treatment, may be different in MMpigs from that of domestic pigs. Further studies are required for understanding the metabolism and transition of estrogen in MMpigs.

The plasma concentrations of progesterone in the pseudopregnant

MMpigs were reported to decrease markedly, to less than 1 ng/ml between 1.5 and 3 days after the first PGF$_{2\alpha}$ treatment, as in EDP treatment induced pseudopregnant domestic pigs [29, 30]. Interval from PGF$_{2\alpha}$ administration to spontaneous estrus (6.5 ± 0.2 days) and rate of estrus exhibition after PGF$_{2\alpha}$ administration (100%) in pseudopregnant MMpigs also corresponded with previous reports on pseudopregnant domestic pigs [29, 30]. Results of the present work demonstrated that it is possible to induce CL regression and estrus exhibition following PGF$_{2\alpha}$ treatment in pseudopregnant MMpigs, as is the case with domestic pigs [29, 30]. The profiles of steroids and gonadotropins in pseudopregnant MMpigs after PGF$_{2\alpha}$ treatment were comparable to those in pseudopregnant domestic pigs treated similarly [29–31] and to those from the late luteal phase to the follicular phase in cyclic MMpigs [34]. High blood concentrations of estradiol-17β at PGF$_{2\alpha}$ treatment in pregnant and pseudopregnant domestic pigs caused a suppression of LH surge [44]. Blood estradiol-17β concentrations at the first PGF$_{2\alpha}$ treatment in pseudopregnant MMpigs were less than 5 pg/ml, similar to those at the first PGF$_{2\alpha}$ treatment in pseudopregnant domestic pigs [29, 30]. The characteristics of spontaneous estrus and LH surge following PGF$_{2\alpha}$ treatment in pseudopregnant MMpigs and pseudopregnant domestic pigs were similar to those in the corresponding cyclic MMpig females [34, 35]. Our results and previous reports suggest that the estradiol levels, at least 19 to 21 days after the last EDP treatment in MMpigs do not affect the induction of luteolysis and hormonal changes associated with estrus exhibition subsequent to PGF$_{2\alpha}$ treatment.

In summary, we demonstrated that multiple administrations of EDP are needed to induce pseudopregnancy in cyclic MMpigs. We also demonstrated that CL can be regressed in pseudopregnant MMpigs by treatment with PGF$_{2\alpha}$ and that estrus is exhibited 5 to 7 days after the first PGF$_{2\alpha}$ treatment, as seen in pseudopregnant domestic pigs. We concluded that the combination of EDP and PGF$_{2\alpha}$ treatment for cyclic MMpigs is an effective and practical method for estrus synchronization.

Acknowledgments

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