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Synchronization Using $PGF_{2\alpha}$ and Estradiol With or Without GnRH for Timed Artificial Insemination in Dairy Cows

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Abstract. This study examined the use of $PGF_{2\alpha}$ and estradiol benzoate (EB) either with or without GnRH to synchronize estrus in dairy cows for timed artificial insemination (TAI) under field conditions. First, Holstein dairy cows with a corpus luteum (CL) received 500 µg cloprostenol and were then randomly allocated to three groups: no further treatment (control, n=236); treatment with 1 mg EB 56 h after cloprostenol (EB group, n=339); or treatment with 1 mg EB 56 h after cloprostenol followed by treatment with 100 µg gonadorelin 24 h later (EB + GnRH group, n=216). All cows received TAI 80 h after the cloprostenol injection. In a second experiment, Holstein dairy cows with a CL received 500 µg cloprostenol and were then randomly allocated to two groups: treatment with 2 mg EB 36 h later (EB group, n=284) or treatment with 2 mg EB 36 h after cloprostenol followed by 100 µg gonadorelin 24 h later (EB + GnRH group, n=229). All cows received TAI 24 h after the EB injection. Logistic analyses revealed that the odds ratio for the probability of pregnancy when 1 mg EB was administered 56 h following cloprostenol was 1.9 and 2.0 times (P<0.001) higher in the EB (39.5%) and EB + GnRH groups (40.7%), respectively, compared with the control group (25.8%). However, pregnancy rates in cows receiving 2 mg EB 24 h following cloprostenol showed no difference compared with cows treated with EB only (32.4%) or with EB + GnRH (35.8%). These results indicate that a synchronization protocol comprising $PGF_{2\alpha}$ and EB could be used for TAI in dairy herds under field conditions.

Key words: Dairy cows, Estradiol, GnRH, $PGF_{2\alpha}$, Pregnancy rate

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Reproductive management is a crucial factor in the dairy industry. One serious problem in reproductive management is the occurrence of diminished behavioral estrous symptoms as productivity increases in lactating dairy cows. Thus, diverse reproductive programs are used to synchronize estrus in dairy cows [1–3]. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is commonly used to synchronize estrus in dairy cows presenting a functional corpus luteum (CL). When $PGF_{2\alpha}$ is administered to dairy cows, approximately 50–60% of the treated cows show estrus within 2–6 days [4–7]. Consequent conception rates following artificial insemination (AI) after estrous detection have been documented as 30–42% [5, 7–10], resulting in a pregnancy rate per treated cow of approximately 20%. Thus, the wide variations in time between $PGF_{2\alpha}$ administration and estrus/ovulation and failure to detect estrus are major practical disadvantages. Therefore, a protocol designed to more closely synchronize $PGF_{2\alpha}$ treatment and estrus/ovulation is needed to improve the efficacy of timed AI (TAI).

Estradiol benzoate (EB) has been used previously to induce estrus and a luteinizing hormone (LH) peak after treatment with $PGF_{2\alpha}$ in beef [11, 12] and dairy cattle [13]. EB administered 48 h after $PGF_{2\alpha}$ treatment, compared with $PGF_{2\alpha}$ alone, resulted in more precise synchrony of estrus and the LH surge, in which the LH peak occurred 66 h after $PGF_{2\alpha}$ treatment [14]. Nevertheless improvement upon subsequent fertility by supplementation with EB has not been well established, especially for cases of TAI [13, 15]. GnRH has frequently been used in veterinary practice to promote the ovulation of preovulatory follicles in cattle. Likewise, several reports show that administration of GnRH at the time of insemination increases pregnancy rates [16–18], while others report no increase [19, 20]. The effect upon ovulation synchronization and resulting fertility of EB supplementation alone, or EB supplementation combined with GnRH when administered following $PGF_{2\alpha}$ treatment, has not been clarified in dairy cows, especially in high-yielding, modern dairy herds. We postulated that exogenous EB or/and GnRH present during proestrus post-$PGF_{2\alpha}$ treatment would result in increased pregnancy rates for TAI in dairy herds with poor estrous detection. Therefore, this study was designed to determine whether a synchronization protocol using $PGF_{2\alpha}$ and EB either with or without GnRH could be established and used for dairy herd TAI under field conditions.

In Experiment 1 (Fig. 1), the pregnancy rates were 25.8% (61/236), 39.5% (134/339) and 40.7% (88/216) in the control, EB and EB + GnRH groups, respectively. Logistic analysis revealed that the odds ratio (OR) for the probability of pregnancy was 1.9 and 2.0 times (P<0.001) higher in the EB and EB + GnRH groups, respectively, compared with that in the control group (Table 1). However, the pregnancy rates did not differ between the EB and EB + GnRH groups (P>0.05). In addition, the analysis demonstrated that cow parity (primiparous or multiparous cows), AI season or farm had no significant effects on the pregnancy rate (P>0.05). In Experiment 2 (Fig. 2), the pregnancy rates were 32.4% (92/284) and 35.8% (82/229) in the EB and EB + GnRH groups, respectively. Furthermore, the

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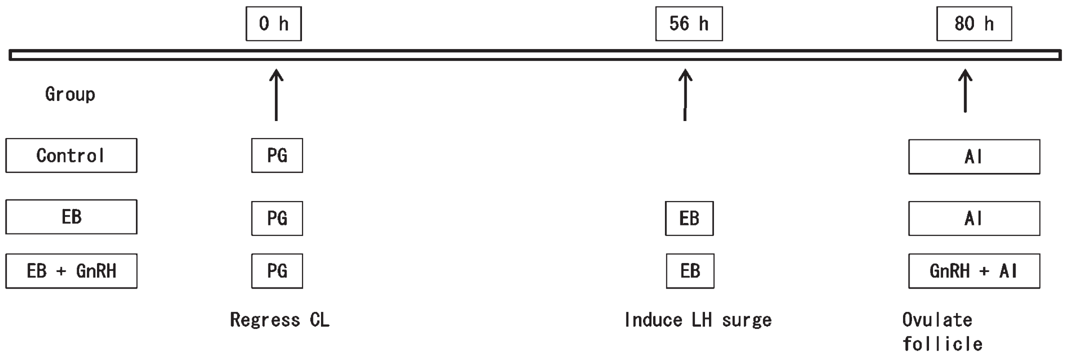

Fig. 1. Diagram showing the experimental design for the TAI regimens using $PGF_{2\alpha}$, $PGF_{2\alpha}$ + EB, or $PGF_{2\alpha}$ + EB + GnRH in dairy cows in Experiment 1. PG = 500 µg cloprostenol, EB = 1 mg estradiol benzoate, GnRH = 100 µg gonadorelin.

Table 1. Adjusted ORs of variables included in the final logistic regression model for the probability of pregnancy in dairy cows in Experiment 1

Variable	Pregnancy rate % (no. of cows)	Adjusted OR [a]	95% CI [b]	P value
Treatment group				
Control	25.8% (61/236)	Reference		
EB	39.5% (134/339)	1.91	1.32, 2.77	0.0006
EB + GnRH	40.7% (88/216)	1.95	1.30, 2.93	0.0013

[a] OR = odds ratio. [b] 95% CI = confidence interval.

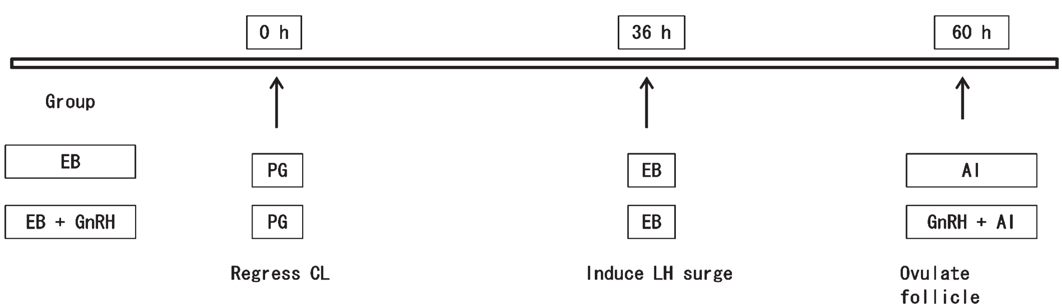

Fig. 2. Diagram showing the experimental design for the TAI regimens using $PGF_{2\alpha}$ + EB or $PGF_{2\alpha}$ + EB + GnRH in dairy cows in Experiment 2. PG = 500 µg cloprostenol, EB = 2 mg estradiol benzoate, GnRH = 100 µg gonadorelin.

pregnancy rate was not influenced by treatment group (EB or EB + GnRH), follicle size at the time of treatment (5–9 mm, 10–15 mm or 16–20 mm), cow parity, AI season or farm (Table 2).

In recent years, many dairy producers have voiced complaints about the increasing difficulty in detecting the signs of estrus, especially in cows from high milk-yielding dairy herds. Wiltbank *et al*. [21] explained that the short duration of estrus might be related to a reduction in circulating estradiol levels caused by higher metabolic clearance rates of reproductive hormones in high-producing lactating dairy cows. Therefore, a protocol for supplementation with exogenous estradiol during proestrus was developed with the aim of improving subsequent fertility. Treatment with $PGF_{2\alpha}$ (0 h) followed by supplementation with 1 mg EB 56 h later (t=56 h) and further treatment with or without GnRH at 80 h post-$PGF_{2\alpha}$ treatment doubled the OR for the probability of pregnancy compared with that in cows treated with $PGF_{2\alpha}$ alone (Experiment 1). This increase in pregnancy rate may be attributable to the tight synchrony between $PGF_{2\alpha}$ treatment and the LH surge, enabling cows to receive TAI and resulting in the acceptable pregnancy rate observed here. Dailey *et al*. [13] reported that tight synchronization on Day 3 post-$PGF_{2\alpha}$ treatment could be achieved by administering EB 40–48 h post-$PGF_{2\alpha}$ treatment. In addition, Martinez *et al*. [22] showed that when 1 mg EB was used to synchronize ovulation following a 14-day interval, administration of $PGF_{2\alpha}$ twice resulted in an acceptable pregnancy rate (45%) for TAI in cycling beef heifers. However, in a different study, no effect on subsequent pregnancy rates was observed, even

Table 2. Logistic regression for the probability of pregnancy in dairy cows in Experiment 2

Variable	DF	Chi-square	P value
Treatment group	1	0.35	0.5538
Follicle size[1]	2	2.19	0.3345
Cow parity	1	1.76	0.184
AI season	3	1.95	0.5823
Farm	11	14.16	0.2242
Treatment group* follicle size	2	3.81	0.1485

[1] Follicle size at the time of treatment.

when a higher percentage of dairy heifers showed signs of estrus following treatment with $PGF_{2\alpha}$ and EB [23]; these findings are inconsistent with our results. The pregnancy (conception) rates in cows that received EB treatment (in Experiment 1) were 39.5% and 40.7% after TAI, which were similar to the rates of 36–41% reported by Whitter *et al*. [7], Kim *et al*. [5] and Mateus *et al*. [9] and slightly higher than the rates of 32–35% reported by Drillich *et al*. [8] and Répási *et al*. [10] in AI in cows that were examined for estrous detection after $PGF_{2\alpha}$ treatment. Thus, our results may suggest a beneficial use of EB for TAI of dairy herds under field conditions.

Analysis of the data from Experiments 1 and 2 showed that additional supplementation with GnRH at the time of AI (i.e., 24 h after EB treatment) did not improve the pregnancy rate when compared with cows treated with EB alone. These findings are inconsistent with previous studies showing that injection of GnRH at the time of AI improves pregnancy rates [16, 18]. This discrepancy may be related to differences in the study protocols. In the protocol reported here, cows received supplementation with EB, while those in the other studies did not. This suggests that administration of exogenous EB following $PGF_{2\alpha}$ treatment may induce synchronization of the LH surge and ovulation, such that GnRH administration at the time of AI is unnecessary.

As mentioned above, logistic analyses of the data from Experiments 1 and/or 2 showed that the following parameters did not affect pregnancy rate: follicle size at the time of treatment (5–9 mm, 10–15 mm or 16–20 mm), cow parity, AI season and farm. Some previous studies did show that follicle size during proestrus did have an effect on subsequent conception [24, 25]. Supplementation with estradiol-17β 8 h before the final GnRH injection in the Ovsynch protocol improved pregnancy rates in cows ovulating medium-sized follicles (15 to 19 mm) but not in cows ovulating smaller or larger follicles [26]. These findings are inconsistent with our results, possibly due, in part, to a difference in the time of estradiol administration. Another report [27] showed that the size of the largest follicle, determined 48 h post-$PGF_{2\alpha}$ treatment, influenced the display of estrous behavior in cows. The presence of larger follicles resulted in increased estrous detection; however, that study did not determine subsequent fertility rates [27]. Higher fertility has been reported in primiparous cows compared with multiparous cows following synchronization [28, 29]; however, other studies found that cow parity had no effect [5, 30]. Although AI season did not affect subsequent fertility in the present study (Table 2), previous studies show that pregnancy rates following AI during the summer were lower than those following AI in the spring and/or winter [31, 32]. Probable confounding factors, including the weather, climate and environment on different continents and in different regions, may have contributed to this disagreement between studies. Moreover, Santos *et al*. [32] found that the farm on which the cattle were kept was one of the risk factors for conception rates after AI in dairy cows, a finding that is inconsistent with our own results. Differences in fertility between farms are difficult to elucidate, since conditions such as facilities, bunk space, barn structure and management systems are all different and may have different effects on individual animals.

Taken together, the results reported in the present study clearly show that EB supplementation following $PGF_{2\alpha}$ treatment increased the OR for the probability of pregnancy (by two times) compared with that of control cows not supplemented with EB. Moreover, additional administration of GnRH at the time of AI following EB administration did not increase the pregnancy rate. In conclusion, a synchronization protocol comprising administration of $PGF_{2\alpha}$ and EB should be effective for TAI in dairy herds under field conditions.

Methods

Animals and management

This study was performed using cows from 12 dairy farms located in Chungcheong Province, Republic of Korea. All cows were milked twice daily, maintained in free-stall facilities, and fed a total mixed ration. The average milk yield was 9,106 kg per year per cow.

The treated cows in Experiment 1 were characterized as follows (mean ± SD): lactation number, 2.6 ± 1.6 (range: 1–12); and postpartum interval, 143.8 ± 80.6 days (range: 52–384). The treated cows in Experiment 2 were characterized as follows: lactation number, 2.2 ± 1.1 (range: 1–8); and postpartum interval, 148.3 ± 76.7 (range: 55–399). The voluntary waiting period from calving to the first AI was 50 days for both Experiments 1 and 2. All experiments were performed with the approval of the Institutional Animal Care and Use Committee of Chungbuk National University, Republic of Korea.

Experiment 1

A total of 791 Holstein dairy cows with a functional CL >20 mm in diameter (as confirmed by ultrasonography; Tringa Linear VET ultrasound scanner fitted with a 5.0 MHz array transducer, Esaote Pie Medical, Maastricht, the Netherlands) received 500 µg of the $PGF_{2\alpha}$ analogue cloprostenol (the time of $PGF_{2\alpha}$ administration was defined as t=0 h) (Estrumate, MSD Animal Health, Seoul, Republic of Korea) and were then randomly allocated to three treatment groups (Fig. 1): no further treatment (control, n=236), injection of 1 mg EB (SY Esrone, Samyang, Seoul, Republic of Korea) 56 h post-$PGF_{2\alpha}$ treatment (EB group, n=339) or injection of 1 mg EB 56 h post-$PGF_{2\alpha}$ treatment followed by injection of 100 µg gonadorelin (a GnRH analogue; Godorel, Uni-Biotech, Yesan, Republic of Korea) at t=80 h (24 h post-EB treatment) (EB + GnRH group, n=216). The cows in each group received TAI 80 h post-$PGF_{2\alpha}$ injection, regardless of estrous detection. Likewise, the time of EB administration was set at 56 h post-$PGF_{2\alpha}$ treatment (during proestrus) based on TAI at 80 h via a timely LH surge (Fig. 1).

Experiment 2

A total of 513 Holstein dairy cows with a functional CL >20 mm in diameter received 500 µg cloprostenol (t=0 h) (Iliren Cycle BP®, Ewha Pharmtek, Seoul, Republic of Korea) and were randomly allocated to two treatment groups (Fig. 2): injection of 2 mg EB 36 h post-$PGF_{2\alpha}$ treatment (t=36 h) (EB group, n=284) or injection of 2 mg EB 36 h post-$PGF_{2\alpha}$ followed by injection of 100 µg gonadorelin 24 h post-EB (t=60 h) (EB + GnRH group, n=229). The cows in both groups received TAI 60 h post-$PGF_{2\alpha}$ injection, regardless of estrous detection. Therefore, the time of EB administration was set at 36 h post-$PGF_{2\alpha}$ treatment (during proestrus) based on TAI at 60 h via a timely LH surge (Fig. 2).

The size of the largest follicle at the time of CL evaluation by ultrasonography was also measured to determine whether it might be related to subsequent fertility and classified broadly into three categories according to the level of follicle size as 5–9 mm (small), 10–15 mm (medium) or 16–20 mm (large).

Pregnancy diagnosis

Pregnancy was determined 40 to 50 days after TAI using both ultrasonography and rectal palpation.

Statistical analysis

For statistical analysis, cow parity was categorized as primiparous or multiparous, and AI season was grouped as spring (March to May), summer (June to August), fall (September to November) and winter (December to February). Pregnancy rates in Experiments 1 and 2 were analyzed by logistic regression using the LOGISTIC procedure of SAS (SAS version 9.2, SAS Institute, Cary, NC, USA). In Experiment 1, the model for pregnancy rate included treatment group (control, EB and EB + GnRH groups), cow parity, AI season and farm as the dependent variables. In Experiment 2, the model for pregnancy rate included treatment group (EB or EB + GnRH), follicle size at the time of treatment (5–9 mm, 10–15 mm or 16–20 mm), cow parity, AI season, farm and interaction between treatment group and follicle size at the time of treatment as the dependent variables. In all models, a backward stepwise regression was used, and elimination was performed using the Wald statistic criterion when P>0.11. The adjusted OR (AOR) and 95% confidence interval (CI) were generated during the logistic regression. The results were expressed as proportions and AORs with the respective 95% CI. P<0.05 was considered significant.

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