# New Trends For Estrus Synchronization Using A Combination Of Gonadotropins, Prostaglandin And Estradiol Cypionate In Dairy Cows

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**Citation**

H Amer. *New Trends For Estrus Synchronization Using A Combination Of Gonadotropins, Prostaglandin And Estradiol Cypionate In Dairy Cows*. The Internet Journal of Veterinary Medicine. 2006 Volume 3 Number 2.

**Abstract**

This study was conducted to through the light on a recent method for estrus synchronization in lactating dairy cows using PGF2, GnRH and estradiol cypionate (ECP). Four treatments were performed on sixty dairy cows. In treatment 1 (PP), cows received two injections of PGF2 on days 0 and 11 (n=14); while in treatment 2 (PGP), cows were injected twice PGF2 on days 0 and 11 and 100 ug of GnRH on day 3 after the 1st injection (n=14). In treatment 3 (PGPE-0), cows were treated with PGP and 1 mg of ECP at the same time of 2nd PGF2 injection (n=16). In treatment 4 (PGPE-1), cows were treated with PGP and 1 mg of ECP one day after 2nd PGF2 injection (n=16). The cows were examined rectally and ultrasonographically by B-mode Ultrasound System [Pie-Medical Scanner-240] with a 6-8 MHz linear rectal transducer. Every cow was blood sampled at selected intervals for progesterone and estradiol assay and inseminated at estrus.
The results showed a higher percentage of GnRH-treated cows (PGP) ovulated after 1st PGF2 injection than the non treated (PP) cows (64.3% vs 50%; P<0.05). The GnRH-treated cows tended to have a larger mature follicle present at 2nd PGF2 injection. Subsequently, the percentage of cows that ovulated after 2nd PGF2 injection was 71.4% vs 50% for GnRH-treated cows than none treated ones, respectively. Two days after 2nd PGF2 injection, cows treated with PGP had a higher peak preovulatory concentrations of estradiol compared to PP treated cows (3.2±0.48 vs 2.6±0.45 pg/ml). Additionally, cows treated with ECP (PGPE-0 and PGPE-1) had a higher peak preovulatory concentrations of estradiol (6.3±0.43 and 6.99±0.63 pg/ml; P<0.01); and a higher percentage ovulation (75.0% and 87.5%) than the other treated groups (PP or PGP). Thus, a higher percentage of ECP (especially PGPE-1 group) treated cows were observed in standing estrus and ovulated after 2nd PGF2 injection than the other treated groups. Submission rates (number of inseminated/totalX100), differed statistically across treatments, especially those treated with ECP, as well as; the conception and pregnancy rates were observed higher with the ECP treated cows than the other treatments. In conclusion, PGP protocol increased the number of cows that ovulated after 1st PGF2 injection and produced more mature follicles at the time of 2nd PGF2 injection. The combination of ECP and PGP (PGPE-1) enhanced the expression of estrus and increased ovulation percentage, thus it is potentially a new method to routinely synchronize estrus and ovulation in dairy cows.

## INTRODUCTION

Considerable research has focused on developing technologies to synchronize and efficiently detect estrus (Larson and Ball, 1992; Sterry et al., 2007), as lactating dairy cows have poor reproductive performance due to low fertility and low rates of estrus detection. Recently, a protocol has been developed (ovsynch) that synchronize the time of ovulation precisely using GnRH and PGF2 (Pursley et al., 1995). Ovsynch is based on the initiation of a new follicular wave with GnRH before inducing luteolysis by PGF2. The emerging dominant follicle is forced to ovulate by a second treatment with GnRH allowing for timed artificial insemination (TAI). Ovsynch protocol utilizes 3 treatments; GnRH administered at a random stage of the estrus cycle, PGF2 given 7 days later, and a 2 $^{nd}$ GnRH treatment 2 days after PGF2 injection. In lactating dairy cows, this protocol synchronized follicular development, regression of the corpus luteum, and time of ovulation allowing effective TAI without the need for detection of estrus (Burke et al., 1996; Schmitt et al., 1996; Pursley et al., 1997). Combinations of PGF2, steroids (E2 and P4) and GnRH are used to regulate the life span of the corpus luteum, control follicular wave emergence and ovulation, and synchronize estrus in cattle (Larson and Ball, 1992; Thatcher, 2001). Prostaglandins (PGF2) or its analogues induce luteal regression, but interval to estrus is highly

variable in randomly cycling cows (Lauderdale et al., 1974). Short-term pretreatment with P4 or multiple injections of PGF2 (at 11-14 day interval) reduced the proportion of cows that are in met estrus and increases the percentage of cows in estrus (Britt, 1979; Xu et al., 1997; Bicalho et al., 2007). A more precise expression of estrus (after PGF2) can be achieved by synchronizing the follicular wave that gives rise to the preovulatory follicle. Estrogens and GnRH induce atresia or ovulation of dominant follicles and synchronize the emergence of a new follicular wave (Bo et al., 1994; Burke et al., 1999). Substituting oestradiol cypionate (ECP) for the second GnRH injection synchronizes ovulation and estrus and yields similar pregnancy rates to the original ovsynch program (Lopes et al., 2000; Pancarci et al., 2002). Oestradiol cypionate is the only form of estradiol registered for use in lactating dairy cows in the USA. Kojima et al. (2000) tested a GnRH-PGF2 protocol combined with P4 pretreatment in postpartum beef cows.

The objectives of the present study are, a) to evaluate an estrus synchronization protocol for dairy cows based on the 7-11 system, as the interval elapsed between the GnRH and 2 $^{nd}$ PGF2 injection was prolonged to 8 days (longer than the traditional 7 days interval, b) to improve the synchrony of estrus and ovulation, after placement of an ECP at the end of treatment.

## MATERIAL AND METHODS

### ANIMALS

This study was conducted in a private dairy farm at Dakahlia Governorate. A total of sixty lactating Holstein cows those 64.5±4.6 days postpartum and eligible for first postpartum insemination were used. Cows were managed under routine conditions that included daily milking, free stall housing and twice daily feeding of total mixed ration (primary components are corn silage, alfa alfa hay and concentrates; balanced to NRC requirements). Milk production averaged 35.4±0.8 kg/day at the start of the experiment, and the rolling herd average was approximately 7500 kg milk per lactation period. Only healthy cows, with normal reproductive status and with history of normal parturition; were chosen for these experiments.

### TREATMENTS DESIGN

Cows were randomly assigned to each of four treatments (as described in Fig. 1):

### TREATMENT 1 (PP)

The cows (n=14) were injected with PGF2 (2 ml Estrumate, Intervet) at day 0 and day 11.

### TREATMENT 2 (PGP)

The cows (n=14) were injected with PGF2 (day 0), an injection of GnRH (100 ug; Cystorelin, Merial, Athens, GA) 3 days later and a 2 $^{nd}$ dose of PGF2 on day 11 (PGP treatment) was injected.

### TREATMENT 3 (PGPE-0)

The cows (n=16) in this design were injected as in PGP as well as a 1 mg injection of ECP (Pharmacia Animal Health, Kalamazoo, MI) at the time of 2 $^{nd}$ PGF2 injection (PGPE-0). The 0 denoted that ECP was given 0 day after 2 $^{nd}$ PGF2 injection.

### TREATMENT 4 (PGPE-1)

The cows (n=16) in this design were injected as in PGP with 1 mg of ECP given one day after the 2 $^{nd}$ PGF2 injection (PGPE-1).

### ESTRUS DETECTION AND ARTIFICIAL INSEMINATION

Cows were observed for estrus 3 times daily and artificially inseminated approximately 12 hours after the observed estrus (a.m.-p.m. rules). All the cows were examined rectally and ultrasonographically by B-mode Ultrasound System [Pie-Medical Scanner-240] with a linear rectal transducer of 6-8 MHz] to record the corpora lutea and follicles (≥1.5 mm) at each observation in the 4 treatments.

### ASSAY OF PLASMA PROGESTERONE (P4) AND ESTRADIOL (E2)

Coccygeal blood samples were collected on day 0 (1 $^{st}$ PGF2 injection), 3 and 5 and daily up to day 11 to ovulation or day 16 for cows that failed to ovulate after 2 $^{nd}$ PGF2 injection (examined per rectum) into EDTA-treated tubes and placed immediately on ice. Plasma was harvested by centrifugation (1500Xg for 15 minutes), within 4 h of collection and stored at –20°C until quantification of P4 and E2 by Radio-immunoassay (RIA). Plasma was harvested and assayed for progesterone and estradiol.

Plasma progesterone concentrations were determined using a commercial available RIA-kits (Coat-A-Count; Diagnostic Products Corporation, C.A), validated for bovine plasma (Kirby et al., 1997). Samples were analyzed across five assays and intra- and inter-assay coefficient of variation was

16.9 and 7.9%, respectively. Plasma estradiol concentrations were determined on day 11 through day 16 (in 4 treatments) with a validated RIA (Kirby et al., 1997). Samples were analyzed across 6 assays and intra- and inter-assay coefficient of variation was 7.5 and 14.7%, respectively.

## PREGNANCY CHECK

Cows were checked for pregnancy by ultrasound scanning at 40 and confirmed by rectal palpation at 45 days after artificial insemination. The submission, conception and pregnancy rates were measured in the four groups of treated cows.

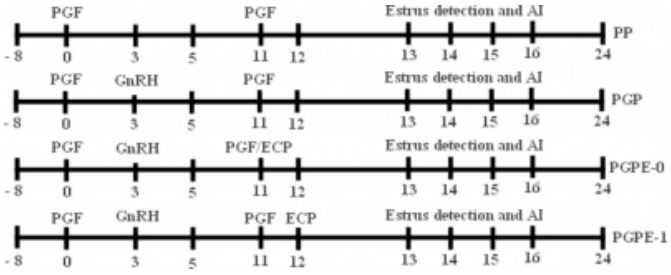
Submission rate=Number of cows inseminated within 4 days after 2 $^{nd}$ PGF2 injection/total number of cows.

Conception rate=Number of pregnant cows /number of inseminated cows.

Pregnancy rate=Number of pregnant cows /total number of treated cows.

### Figure 1

Figure 1: Time line for treatment administration (PGF=2 ml Estrumate; GnRH=100 ug Cystorelin; ECP=1 mg Estradiol cypionate). Estrus detection and artificial insemination for cows treated with PP, PGP, PGPE-0 and PGPE-1 protocols. Blood plasma was collected for progesterone and estradiol analysis.




## STATISTICAL ANALYSIS

Data were analyzed using the Statistical Analysis System (SAS, 1996). Plasma estradiol concentrations were analyzed as repeated measures using the General Linear Models Procedure (Proc. GLM). Proportion of cows ovulating after each PGF2 injection was tested using a ?2-test.

## RESULTS

A greater percentage of GnRH-treated cows (PGP) ovulated after 1 $^{st}$ PGF2 injection than the non treated (PP) cows (64.3% vs 50%; P<0.05; Table 1). The improved ovulation percentage was associated with a large follicle that tended (P<0.10) to be larger in size at the time of 2 $^{nd}$ PGF2 injection for PGP vs PP treated cows. However, mature follicles were determined by 2 days after 2 $^{nd}$ PGF2 injection. Although treatment with GnRH was associated with an increased ovulation percentage after 1 $^{st}$ PGF2 injection, the percentage of cows that ovulated after 2 $^{nd}$ PGF2 injection was 71.4% versus 50%; for GnRH-treated cows than non treated ones, respectively (Table 1). The interval to estrus (64±7 h) and the interval to ovulation (97±9 h) were also similar for both treatments.

Cows treated with PGP had higher peak preovulatory concentrations of estradiol in plasma compared to PP treated cows (3.2±0.48 vs 2.6±0.45 pg/ml) following the 2 $^{nd}$ PGF2 injection (Fig. 2).

After the 2 $^{nd}$ PGF2 injection, ovulation was defined as low P4 (<0.5 ng/ml) on day 13 and high P4 (≥0.5 ng/ml) on day 24. Cows not ovulated may had high P4 on day 13 and 24, low P4 on day 13 and 24, or high P4 on day 13 and low P4 on day 24. The development of accessory corpus luteum in the presence of a functional corpora lutea could not be determined because ovulation was based mainly on P4 concentration.

At the time of 2 $^{nd}$ PGF2 injection, the size of mature follicle was similar for all treatments after the ultrasound scanning. However, for cows treated with ECP (PGPE-0 and PGPE-1), mature follicles also determined within 3 days after 2 $^{nd}$ PGF2 injection (P<0.05). Addition of ECP to the PGP treatment increased the percentage of cows ovulating (50% and 71.4% for PP and PGP vs 75.0% and 87.5% for PGPE-0 and PGPE-1; P<0.05; Table 1). Thus, the percentage of ovulations for PGPE-0 and PGPE-1 treated cows tended to be higher compared to the other treated cows. Interval to oestrus and interval to ovulation were similar for all treatments (55±5 and 91±7 h, respectively). For those cows that ovulated, the actual days of ovulation after 2 $^{nd}$ PGF2 injection were 3, 4 and 4 days for PGP; 3, 3, 4, 4, 4 and 5 days for PGPE-0 and 3, 4, 4, 4, 4 and 4 days for PGPE-1 treatments.

Treatment with ECP also increased plasma estradiol concentration after 2 $^{nd}$ PGF2 injection (Fig. 3; P<0.05). Plasma estradiol concentrations were the greatest at 1 and 2 days after 2 $^{nd}$ PGF2 injection for PGPE-0 and PGPE-1 treated cows, respectively. In each case, the peak level of estradiol occurred one day after ECP injection. However, cows treated with ECP (PGPE-0 and PGPE-1) had a higher peak preovulatory concentrations of estradiol in plasma

(6.3±0.43 and 6.99±0.63 pg/ml; P<0.01) following 2 nd PGF2 injection.

Thirty one of sixty cows (51.6%) were classified as anoestrus at the start of the trial (plasma P4 <0.5 ng/ml on days –8, 0 and 3). The percentage of cows that developed a corpus luteum (hereafter referred to as ovulation) after 1 st PGF2 injection was greater when cows received GnRH (75% and 81.2%; for PGPE-0 and PGPE-1 versus 50% and 64.3% for PP and PGP; P<0.001). A higher percentage of ECP treated cows were observed in standing oestrus and ovulated after 2 nd PGF2 injection and; 75% and 87.5%; P<0.05 for PGPE-0 and PGPE-1 vs 50% and71.4% for PP and PGP treated cows, respectively.

Plasma estradiol concentrations were similar across treatments at the time of 2 nd PGF2 injection. However, 2 days after 2 nd PGF2, plasma estradiol concentrations were greater in cows that received ECP compared to PP or PGP (P<0.001; Fig. 2). The percentage of cows that ovulated (subsequently developed a corpus luteum) after 2 nd PGF2 injection was the greatest with ECP groups than the others (P<0.05).

Submission rates (number of inseminated cows/totalX100), the conception and pregnancy rates was observed to be higher with the ECP treated cows than the other treatments (Table 2).

### Figure 2

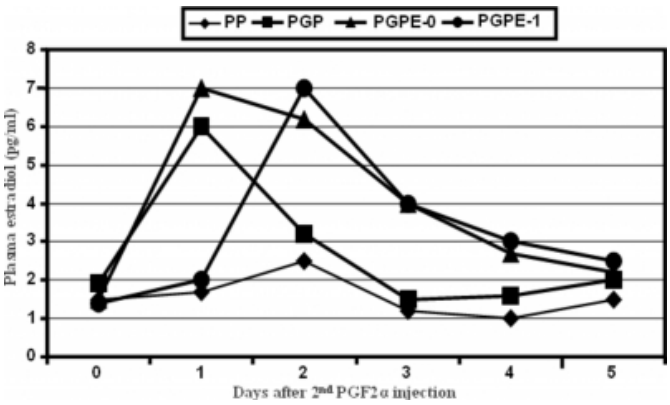
Table 1: Summary of ovarian response to PGF2, GnRH and estradiol cypionate injection for cows treated with four synchronization protocols.

| Treatment Design [a] | No. | Ovarian response (%) | | | |
|---|---|---|---|---|---|
| | | After 1st PGF2α injection [b] | | After 2nd PGF2α injection [c] | |
| | | Ovulating | None ovulating | Ovulating | None ovulating |
| PP | 14 | 7 (50) | 7 (50) | 7 (50) | 7 (50) |
| PGP | 14 | 9 (64.3) | 5 (35.7) | 10 (71.4) | 4 (28.6) |
| PGPE-0 | 16 | 12 (75) | 4 (25.0) | 12 (75) | 4 (25) |
| PGPE-1 | 16 | 13 (81.2) | 3 (18.8) | 14 (87.5) [d] | 2 (12.5) |
| Total | 60 | 41 (68.3) | 19 (31.7) | 43 (71.7) | 17 (28.3) |

a, b, c, d (significantly different at level P<0.05)
PP=2 ml Estrumate on days 0 and 11; PGP=2ml Estrumate on days 0 and 11, 100 ug GnRH on day 3.
PGP=2ml Estrumate on days 0 and 11; 100 ug GnRH on day 3
PGPE-0=2ml Estrumate on days 0 and 11, 100 ug GnRH on day 3, 1 mg ECP on day 11 (at 2nd PGF2α dose)
PGPE-1=2ml Estrumate on days 0 and 11, 100 ug GnRH on day 3, 1 mg ECP on day 12 (one day after PGF2α)

### Figure 3

Figure 2: Plasma estradiol concentrations (Least square means, SEM) after the 2 PGF2 injection for cows treated with PP (n=14), PGP (n=14), PGPE-0 (n=16) and PGPE-1 (n=16). There was a treatment by day interaction (P




### Figure 4

Table 2: Submission, conception and pregnancy rates for cows treated with four synchronization protocols.

| Treatment Design [a] | No. | Reproductive parameters | | |
|---|---|---|---|---|
| | | Submission rate (%) [b] | Conception rate (%) [c] | Pregnancy rate (%) [d] |
| PP | 14 | 9 (64.3) | 5 (35.7) | 3 (21.4) |
| PGP | 14 | 9 (64.3) | 5 (35.7 | 4 (28.6) |
| PGPE-0 | 16 | 12 (75) | 7 (43.8) | 6 (37.5) |
| PGPE-1 | 16 | 13 (81.3) | 9 (56.3) | 8 (50) |

a, b, c, d (significantly different at level P<0.05)
PP=2 ml Estrumate on days 0 and 11; PGP=2ml Estrumate on days 0 and 11, 100 ug GnRH on day 3.
PGP=2ml Estrumate on days 0 and 11; 100 ug GnRH on day 3
PGPE-0=2ml Estrumate on days 0 and 11, 100 ug GnRH on day 3, 1 mg ECP on day 11 (at 2nd PGF2α dose)
PGPE-1=2ml Estrumate on days 0 and 11, 100 ug GnRH on day 3, 1 mg ECP on day 12 (one day after PGF2α)

## DISCUSSION

An estrus synchronization protocol (termed PGP) similar to 7-11 synch (Kojima et al., 2000; Kanitz et al., 2006) but without Melongesterol acetate was developed. The protocol consists of a PGF2 injection followed 3 days later by a GnRH injection and another PGF2 injection 8 days after the GnRH. The protocol is similar to the original PGF2 protocol consisting of 2 injections of PGF2 11 days a part (Lucy et al., 1986; Sterry et al., 2007; Bicalho et al., 2007), but includes an injection of GnRH 3 days after the first PGF2 injection. The GnRH provides two-fold advantages. First, it forces a timely ovulation of the dominant follicle after 1 st PGF2 injection (Thatcher and Chenault, 1976). The timed ovulation prevents late ovulations that would potentially result in an immature and unresponsive corpus luteum at the time of 2 nd PGF2 injection. Second, the GnRH forces a percentage of anoestrus cows to ovulate (Britt et al., 1974) and induces cyclicity in cows that would otherwise not respond to PGF2. The advantage of PGP was examined in the present experiments. In each case, the number of cows

that ovulated after 1 st PGF2 injection was increased. For example, the percentage of cows that ovulated after 1 st PGF2 injection developed when GnRH was given on day 3 (PGP and PGPE-1 compared to PP).

In the present study, the cows treated with PGP had mature follicles that were larger at the time of 2 nd PGF2 injection. However, 2 days later, follicles were similar in size because the mature follicles of PP-treated cows continued to grow, whereas those of PGP-treated cows remained the same size. Treatment with GnRH appears to produce more mature follicles at the time of 2 nd PGF2 injection; supported by the fact that both PGP and PGPE-1-treated cows were in estrus before PP-treated cows. Luteal and follicular maturity may be an advantage of the PGP protocol over traditional GnRH-PGF2 protocols. The extra day between the GnRH and PGF2 injection (8 days for PGP versus 7 days for traditional protocols) may place more cows in diestrum (being responsive to PGF2), and more mature follicle may lead to a more functional CL upon ovulation (Vasconcelos et al., 2001; Kanitz et al., 2006; Sterry et al., 2007).

Addition of ECP increased the number of cows that ovulated after 2 nd PGF2 injection (Table 1) and improved the percentage of cows in standing estrus. Both responses were associated with increased plasma estradiol concentrations after 2 nd PGF2 injection. Although plasma estradiol concentrations were elevated, they were within physiological ranges and there were no incidences of false estrus (behavioral estrus without ovulation). Addition of oestradiol to PGP protocol also had the unexpected effect of increasing follicular growth. The suppressive effect of estrogens on follicular development is well documented (Bo et al., 1994; Burke et al., 2000; Martinez et al., 2002) but stimulatory effects on preovulatory follicular growth have not been reported in cattle (Burke et al., 2000). It appears that exogenous estradiol stimulated some aspects of dominant follicular growth but the exact mechanism is not known.

One major goal for any new estrus synchronization protocol should be the implementation of timed AI. The interval to ovulation and interval to estrus were examined in this study. The results were promising, as a higher percentage of GnRH-treated cows ovulated after 1 st PGF2 injection (64.3% vs 50%; $P<0.05$). The GnRH-treated cows tended to have a larger mature follicle present at 2 nd PGF2 injection. The percentage of cows that ovulated after 2 nd PGF2 injection was 71.4% vs 50% for GnRH-treated cows than none treated ones, respectively. Cows treated with PGP had higher peak preovulatory concentrations of estradiol in plasma compared to PP treated cows (3.2±0.48 vs 2.6±0.45 pg/ml; following 2 nd PGF2 injection. Cows treated with ECP (PGPE-0 and PGPE-1) had a significant higher peak preovulatory concentrations of estradiol (6.3±0.43 and 6.99±0.63 pg/ml); and a significant higher percentage ovulation (75.0% and 87.5%) than the other treated groups (PP or PGP). The PGP protocols are similar to the other GnRH-PGF2 protocols because GnRH and PGF2 are given in a series. The primary difference is that PGF2 is given before 1 st GnRH injection. The first injection of PGF2 causes corpus luteum regression so that most cows are on day 8 of the estrus cycle when 2 nd PGF2 dose is injected. In late luteal phase, cows treated with GnRH and PGF2 at a 7 day interval tend to exhibit estrus around the time of PGF2 injection; i.e. before the time when most cows are in estrus, 2-3 days after PGF2 (Vasconcelos et al., 1999; Dejarnette et al., 2001). The present study did not compare PGP to GnRH and PGF2 given at a 7 day interval but with one day more (8 days). Nevertheless, estrus was not observed before 2 nd PGF2 injection in PGP cows. The possibility that relative to GnRH and PGF2-alone, PGP increases the percentage of cows in estrus 2-3 days after the last PGF2 injection should be tested in larger experiment.

GnRH is an obvious substitution for ECP in the PGPE-1 protocol. The GnRH would be given 48 hours after 2 nd PGF2 injection but the timing of other injections would be similar (PGF2 day 0, GnRH day 3, PGF2 day 11, GnRH day 13; PGPG protocol). In a previous study, the PGPG protocol was tested in 45 postpartum dairy cows and 93% of the cows ovulated on day 14 (Sterry et al., 2007). Thus, the PGPG protocol may be an alternative to PGPE-1 that is highly suited for timed AI.

In conclusion, we can conclude that PGP protocol increased the number of ovulated cows after 1 st PGF2 injection and produced more mature follicles at the time of 2 nd PGF2 injection. Adding ECP to PGP (especially PGPE-1) enhanced the expression of estrus and increased ovulation percentage. Thus, a combination of PGP and ECP is potentially a new method to routinely synchronize estrus and ovulation in dairy cows.

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