

## Oestrus synchronization in high lactating dairy cows

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### Summary

*This study was conducted to compare a recent method for oestrus synchronization in lactating dairy cows using PGF2 $\alpha$  (P), GnRH (G) and estradiol cypionate (ECP). Four treatments were performed on sixty dairy cows. In treatment 1-PP (twice prostaglandin), cows received two injections of PGF2 $\alpha$  on days 0 and 11 (n=14). In treatment 2-PGP (prostaglandin-gonadotropin-prostaglandin), cows were injected twice with PGF2 $\alpha$ , on days 0 and 11 and 100  $\mu$ g of GnRH on day 3 after the 1<sup>st</sup> injection (n=14). In treatment 3-PGPE-0 (prostaglandin-gonadotropin-prostaglandin-estradiol-0), cows were treated with PGP and 1 mg of ECP at the same time of 2<sup>nd</sup> PGF2 $\alpha$  injection (n=16). In treatment 4-PGPE-1 (prostaglandin-gonadotropin-prostaglandin-estradiol-1), cows were treated with PGP and 1 mg of ECP one day after 2<sup>nd</sup> PGF2 $\alpha$  injection (n=16). The cows were examined rectally and ultrasonographically. Every cow was blood sampled at selected intervals for progesterone and estradiol assay and inseminated at oestrus. After 1<sup>st</sup> PGF2 $\alpha$  injection, a higher percentage of ovulated cows observed with PGP than PP treated cows (64.3 % vs 50 %;  $P < 0.05$ ). The GnRH-treated cows tended to have a larger mature follicle present at 2<sup>nd</sup> PGF2 $\alpha$  injection (71.4 %) than none treated ones (50 %). Two days after 2<sup>nd</sup> PGF2 $\alpha$  injection, cows treated with PGP had a higher peak preovulatory concentrations of estradiol compared to PP treated cows ( $3.2 \pm 0.48$  vs  $2.6 \pm 0.45$  pg/mL). Additionally, cows treated with ECP (PGPE-0 and PGPE-1) had a higher peak preovulatory concentrations of estradiol ( $6.3 \pm 0.43$  and  $6.99 \pm 0.63$  pg/mL;  $P < 0.01$ ); and a higher percentage ovulation (75.0 % and 87.5 %) than the other treated groups (PP or PGP). Submission rates (number of inseminated/total  $\times 100$ ), differed statistically across treatments, especially those treated with ECP, as well as the conception and pregnancy rates observed were higher with the ECP treated cows than the other treatments. In conclusion, PGP protocol seemed to produce more mature follicles and ovulated cows. The combination of ECP and PGP (PGPE-1) enhanced oestrus*

*and increased ovulation, thus it is potentially a new method to routinely synchronize oestrus and ovulation in dairy cows.*

*Key words: oestrus, synchronization, PGF2 $\alpha$ , GnRH, estradiol cypionate, pregnancy*

### ***Introduction***

Numerous researches are focused on developing technologies to synchronize and efficiently detect oestrus (Larson and Ball, 1992; Sterry et al., 2007), as lactating dairy cows have poor reproductive performance due to low fertility and low estrus detection RATE. Recently, a protocol has been developed (ovsynch) that synchronize the time of ovulation precisely using GnRH and PGF2 $\alpha$  (Pursley et al., 1995). Ovsynch is based on the initiation of a new follicular wave with GnRH before inducing luteolysis by PGF2 $\alpha$ . The emerging dominant follicle is forced to ovulate by a second treatment with GnRH allowing timed artificial insemination (TAI). Ovsynch protocol utilizes 3 treatments; GnRH administered at a random stage of the oestrus cycle, PGF2 $\alpha$  given 7 days later, and a 2<sup>nd</sup> GnRH treatment 2 days after PGF2 $\alpha$  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 $\alpha$ , 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 oestrus in cattle (Larson and Ball, 1992; Thatcher, 2001). Prostaglandins (PGF2 $\alpha$ ) or its analogues induce luteal regression, but interval to oestrus is highly variable in randomly cycling cows (Lauderdale et al., 1974). Short-term pretreatment with P4 or multiple injections of PGF2 $\alpha$  (at 11-14 day interval) reduced the proportion of cows that are in met estrus and increases the percentage of cows in oestrus (Britt, 1979; Xu et al., 1997; Bicalho et al., 2007). A more precise expression of oestrus (after PGF2 $\alpha$ ) 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 oestrus 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 $\alpha$  protocol combined with P4 pretreatment in postpartum beef cows.

The objectives of the present study are, a) to evaluate an oestrus synchronization protocol for dairy cows based on the 7-11 system, as the interval elapsed between the GnRH and 2<sup>nd</sup> PGF2 $\alpha$  injection was prolonged to 8 days (longer than the traditional 7 days interval, b) to improve the synchrony of oestrus 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  $64.5 \pm 4.6$  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, alfalfa hay and concentrates; balanced to NRC requirements). Milk production ranged with average  $35.4 \pm 0.8$  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)**

Cows (n=14) were injected with PGF2 $\alpha$  (2 mL Estrumate, Intervet) at day 0 and day 11.

##### **Treatment 2 (PGP)**

Cows (n=14) were injected with PGF2 $\alpha$  (day 0), an injection of GnRH (100 ug; Cystorelin, Merial, Athens, GA) 3 days later and a 2<sup>nd</sup> dose of PGF2 $\alpha$  on day 11 (PGP treatment).

### **Treatment 3 (PGPE-0)**

Cows (n=16) in this group were injected as in PGP as well as with 1 mg injection of ECP (Pharmacia Animal Health, Kalamazoo, MI) at the time of 2<sup>nd</sup> PGF2 $\alpha$  injection (PGPE-0). The 0 denoted that ECP was given 0 day after 2<sup>nd</sup> PGF2 $\alpha$  injection.

### **Treatment 4 (PGPE-1)**

Cows (n=16) in this group were injected as in PGP with 1 mg of ECP given one day after the 2<sup>nd</sup> PGF2 $\alpha$  injection (PGPE-1).

### **Oestrus detection and artificial insemination**

Cows were observed for oestrus 3 times a day and artificially inseminated approximately 12 hours after the observed oestrus (a.m. - p.m. rule). 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 ( $\geq 1.5$  mm) at each observation in the 4 treatments.

### **Assay of plasma progesterone (P4) and estradiol (E2)**

Blood samples were collected on day 0 (1<sup>st</sup> PGF2 $\alpha$  injection), 3 and 5 and daily up to day 11 to ovulation or day 16 for cows that failed to ovulate after 2<sup>nd</sup> PGF2 $\alpha$  injection (examined per rectum) into EDTA-treated tubes and placed immediately on ice. Plasma was harvested (by centrifuged blood at 1500Xg for 15 min.), and assayed for progesterone and estradiol by Radioimmunoassay (RIA).

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; 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; 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 DAY 40 and confirmed by rectal palpation at day 45 after artificial insemination. The submission, conception and pregnancy rates were measured in the four groups of treated cows.

1. Submission rate = Number of cows inseminated within 4 days after 2<sup>nd</sup> PGF2 $\alpha$  injection/total number of cows.
2. Conception rate = Number of pregnant cows / number of inseminated cows.
3. Pregnancy rate = Number of pregnant cows / total number of treated cows.

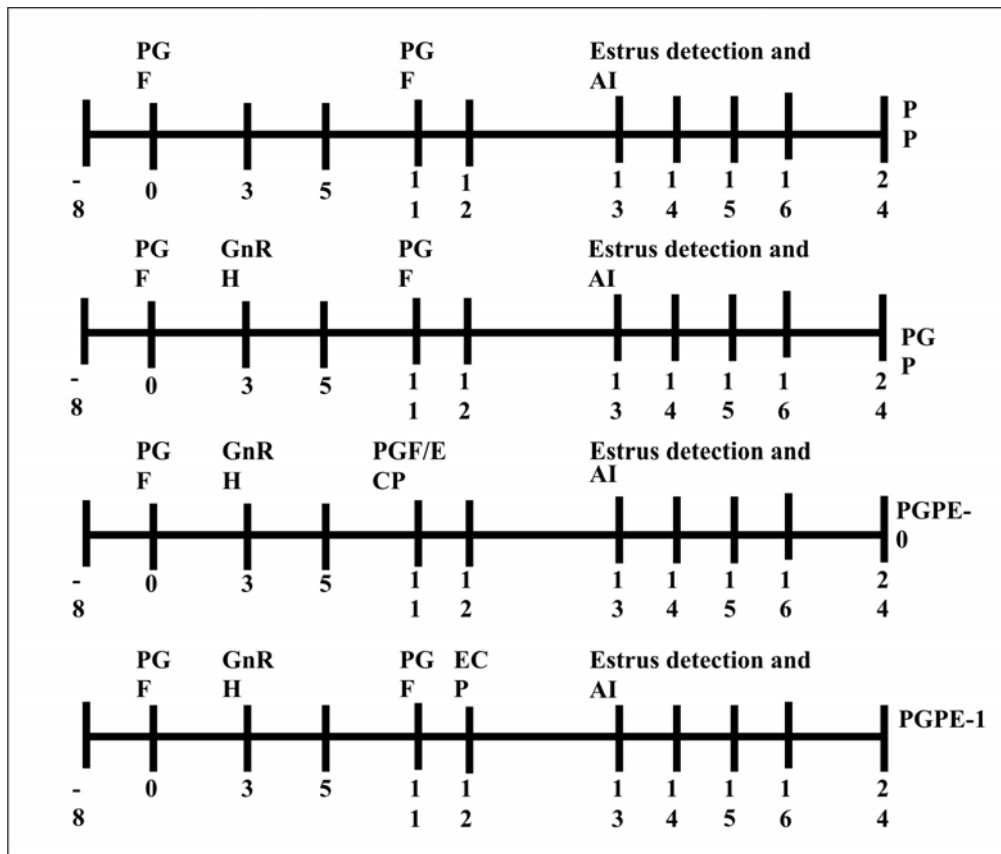


Fig. 1: Time line for treatment administration (PGF = 2 mL Estrumate; GnRH=100 ug Cystorelin; ECP=1 mg Estradiol cypionate). Estrus detection and artificial insemination for cows treated with PP, PGP, PGPE-0 and PGPE-1 protocols. Blood plasma was collected for progesterone and estradiol analysis

Slika 1: Vremenski slijed tretmana (PGF = 2 mL Estrumate; GnRH=100 ug Cystorelin; ECP=1 mg Estradiol cypionate). Otkrivanje estrusa i umjetno osjemenjivanje za krave tretirane prema PP, PGP, PGPE-0 i PGPE-1 protokolima. Uzimani su uzorci krvne plazme za utvrđivanje razine progesterona i estradiola

### 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 $\alpha$  injection was tested using a  $\chi^2$ -test.

### Results

A greater percentage of GnRH-treated cows (PGP) ovulated after 1<sup>st</sup> PGF2 $\alpha$  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<sup>nd</sup> PGF2 $\alpha$  injection for PGP vs PP treated cows. However, mature follicles were determined 2 days after 2<sup>nd</sup> PGF2 $\alpha$  injection. Although treatment with GnRH was associated with an increased ovulation percentage after 1<sup>st</sup> PGF2 $\alpha$  injection, the percentage of cows that ovulated after 2<sup>nd</sup> PGF2 $\alpha$  injection was 71.4 % versus 50 % for GnRH-treated cows than non treated ones, respectively (Table 1). The interval to oestrus ( $64 \pm 7$  h) and the interval to ovulation ( $97 \pm 9$  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 ( $3.2 \pm 0.48$  vs  $2.6 \pm 0.45$  pg/mL) following the 2<sup>nd</sup> PGF2 $\alpha$  injection (Fig. 2).

After the 2<sup>nd</sup> PGF2 $\alpha$  injection, ovulation was defined as low P4 ( $< 0.5$  ng/mL) on day 13 and high P4 ( $\geq 0.5$  ng/mL) on day 24. Not ovulated cows 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<sup>nd</sup> PGF2 $\alpha$  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 were also determined within 3 days after 2<sup>nd</sup> PGF2 $\alpha$  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 ( $55 \pm 5$  and  $91 \pm 7$  h, respectively). For cows that ovulated, the actual days of ovulation after 2<sup>nd</sup>

PGF2 $\alpha$  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<sup>nd</sup> PGF2 $\alpha$  injection (Fig. 3;  $P < 0.05$ ). Plasma estradiol concentrations were the greatest at 1<sup>st</sup> and 2<sup>nd</sup> day after 2<sup>nd</sup> PGF2 $\alpha$  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 ( $6.3 \pm 0.43$  and  $6.99 \pm 0.63$  pg/mL;  $P < 0.01$ ) following 2<sup>nd</sup> PGF2 $\alpha$  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<sup>st</sup> PGF2 $\alpha$  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<sup>nd</sup> PGF2 $\alpha$  injection and; 75 % and 87.5 %;  $P < 0.05$  for PGPE-0 and PGPE-1 vs 50 % and 71.4 % for PP and PGP treated cows, respectively.

*Table 1: Summary of ovarian response to PGF2 $\alpha$ , GnRH and Estradiol cypionate injection for cows treated with four synchronization protocols*

*Tablica 1: Sažeti prikaz reakcije jajnika na injekcije PGF2 $\alpha$ , GnRH i estradiol cipionata kod krava tretiranih prema 4 protokola sinkronizacije estrusa*

Treatment design <sup>a</sup> Plan terapije <sup>a</sup>	N	Ovarian response / Reakcija jajnika (%)			
		After 1 <sup>st</sup> PGF2 $\alpha$ injection <sup>b</sup> Nakon 1. PGF2 $\alpha$ injekcije <sup>b</sup>		After 2 <sup>nd</sup> PGF2 $\alpha$ injection <sup>c</sup> Nakon 2. PGF2 $\alpha$ injekcije <sup>c</sup>	
		Ovulating Ovulacija	None ovulating Nema ovulacije	Ovulating Ovulacija	None ovulating Nema ovulacije
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) <sup>d</sup>	2 (12.5)
Total / Ukupno	60	41 (68.3)	19 (31.7)	43 (71.7)	17 (28.3)

*a, b, c, d - significantly different at level  $P < 0.05$*

*a, b, c, d - signifikantno različiti na razini  $P < 0,05$*

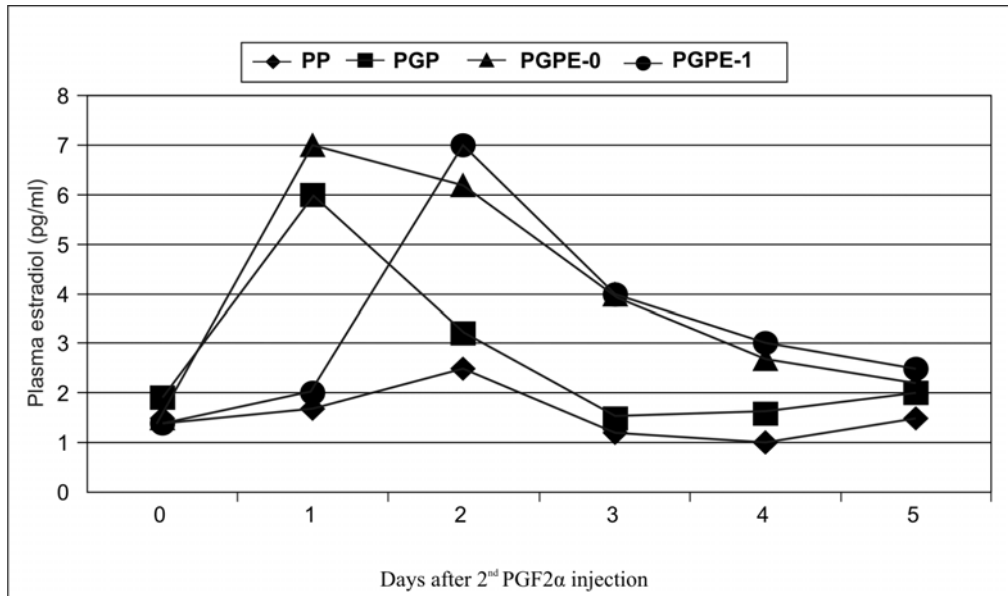


Fig. 2: Plasma estradiol concentrations (Least square means, SEM) after the 2<sup>nd</sup> PGF2 $\alpha$  injection for cows treated with PP (n=14), PGP (n=14), PGPE-0 (n=16) and PGPE-1 (n=16). There was a treatment by day interaction ( $P<0.001$ )

Slika 2: Koncentracije estradiola u plazmi (srednje vrijednosti najmanjih kvadrata, SEM) nakon 2. PGF2 $\alpha$  injekcije za krave tretirane prema protokolima PP (n=14), PGP (n=14), PGPE-0 (n=16) i PGPE-1 (n=16). Utvrđena je interakcija tretmana prema danu ( $P<0.001$ )

Plasma estradiol concentrations were similar across treatments at the time of 2<sup>nd</sup> PGF2 $\alpha$  injection. However, 2 days after 2<sup>nd</sup> PGF2 $\alpha$ , 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<sup>nd</sup> PGF2 $\alpha$  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).



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

Tablica 2: Postoci osjemenjenih krava, koncepcije i gravidnosti kod krava tretiranih različitim protokolima sinkronizacije estrusa

Treatment design <sup>a</sup> Plan terapije <sup>a</sup>	N	Reproductive parameters Reproduktivni parametri		
		Submission rate Postotak osjemenjenih (%) <sup>b</sup>	Conception rate Postotak koncepcije (%) <sup>c</sup>	Pregnancy rate Postotak gravidnosti (%) <sup>d</sup>
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  $P < 0.05$

*a, b, c, d* - signifikantno različiti na razini  $P < 0,05$

### Discussion

An oestrus 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 $\alpha$  injection followed 3 days later by a GnRH injection and another PGF2 $\alpha$  injection 8 days after the GnRH. The protocol is similar to the original PGF2 $\alpha$  protocol consisting of 2 injections of PGF2 $\alpha$  11 days apart (Lucy et al., 1986; Sterry et al., 2007; Bicalho et al., 2007), but includes an injection of GnRH 3 days after the first PGF2 $\alpha$  injection. The GnRH provides two-fold advantages. First, it forces a timely ovulation of the dominant follicle after 1<sup>st</sup> PGF2 $\alpha$  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<sup>nd</sup> PGF2 $\alpha$  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 $\alpha$ . The advantage of PGP was examined in the present experiments. In each case, the number of cows that ovulated after 1<sup>st</sup> PGF2 $\alpha$  injection was increased. For example, the percentage of cows that ovulated after 1<sup>st</sup> PGF2 $\alpha$  injection developed when GnRH was given on day 3 (PGP and PGPE-1 compared to PP).

In the present study, cows treated with PGP had mature follicles that were larger at the time of 2<sup>nd</sup> PGF2 $\alpha$  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<sup>nd</sup> PGF2 $\alpha$  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 $\alpha$  protocols. The extra day between the GnRH and PGF2 $\alpha$  injection (8 days for PGP versus 7 days for traditional protocols) may place more cows in diestrus (being responsive to PGF2 $\alpha$ ), 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<sup>nd</sup> PGF2 $\alpha$  injection (Table 1) and improved the percentage of cows in standing estrus. Both responses were associated with increased plasma estradiol concentrations after 2<sup>nd</sup> PGF2 $\alpha$  injection. Although plasma estradiol concentrations were elevated, they were within physiological ranges and there were no incidences of false oestrus (behavioral oestrus without ovulation). Addition of estradiol 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 oestrus synchronization protocol should be the implementation of timed AI. The interval to ovulation and interval to oestrus were examined in this study. The results were promising, as a higher percentage of GnRH-treated cows ovulated after 1<sup>st</sup> PGF2 $\alpha$  injection (64.3 % vs 50 %;  $P < 0.05$ ). The GnRH-treated cows tended to have a larger mature follicle present at 2<sup>nd</sup> PGF2 $\alpha$  injection. The percentage of cows that ovulated after 2<sup>nd</sup> PGF2 $\alpha$  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 ( $3.2 \pm 0.48$  vs  $2.6 \pm 0.45$  pg/mL) following 2<sup>nd</sup> PGF2 $\alpha$  injection. Cows treated with ECP (PGPE-0 and PGPE-1) had a significant higher peak preovulatory concentrations of estradiol ( $6.3 \pm 0.43$  and  $6.99 \pm 0.63$  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 $\alpha$  protocols because GnRH and PGF2 $\alpha$  are given in a series. The primary difference is that PGF2 $\alpha$  is given before 1<sup>st</sup> GnRH injection. The first injection of PGF2 $\alpha$  causes corpus luteum regression so that the most cows are on day 8 of the oestrus cycle when 2<sup>nd</sup> PGF2 $\alpha$  dose is injected. In late luteal phase, cows treated with GnRH and PGF2 $\alpha$  at 7 day interval tend to exhibit oestrus around the time of PGF2 $\alpha$  injection; i.e. before the time when the most cows are in estrus, 2-3 days after PGF2 $\alpha$  (Vasconcelos et al., 1999; Dejarnette et al., 2001). The present study did not compare PGP to GnRH and PGF2 $\alpha$  given at 7 day interval but with one day more (8 days). Nevertheless, oestrus was not observed before 2<sup>nd</sup> PGF2 $\alpha$  injection in PGP cows. The possibility that relative to GnRH and PGF2 $\alpha$ -alone, PGP increases the percentage of cows in oestrus 2-3 days after the last PGF2 $\alpha$  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<sup>nd</sup> PGF2 $\alpha$  injection but the timing of other injections would be similar (PGF2 $\alpha$  day 0, GnRH day 3, PGF2 $\alpha$  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.

At last, it can be concluded that PGP protocol increased the number of ovulated cows after 1<sup>st</sup> PGF2 $\alpha$  injection and produced more mature follicles at the time of 2<sup>nd</sup> PGF2 $\alpha$  injection. Adding ECP to PGP (especially PGPE-1) enhanced the expression of oestrus and increased ovulation percentage. Thus, a combination of PGP and ECP is potentially a new method to routinely synchronize oestrus and ovulation in dairy cows.

## *SINKRONIZACIJA ESTRUSA VISOKOMLIJEČNIH KRAVA*

### *Sažetak*

*Istraživanje je provedeno kako bismo usporedili najnovije metode sinkronizacije estrusa krava u laktaciji koristeći PGF2 $\alpha$  (P), GnRH (G) i estradiol cipionat (ECP). Koristili smo četiri tretmana na 60 mliječnih krava. U prvom tretmanu - PP (dva puta prostaglandin), kravama je dvaput injiciran PGF2 $\alpha$ , nulti i jedanaesti dan (n = 14). U drugom tretmanu -PGP (prostaglandin-gonadotropin-prostaglandin), kravama je dvaput injiciran*

PGF2 $\alpha$ , nulti i jedanaesti dan i 100 ug GnRH treći dan nakon prve injekcije (n = 14). U trećem tretmanu - PGPE-0 (prostaglandin-gonadotropin-prostaglandin-estradiol-0), krave su tretirane kao u PGP, uz 1 mg ECP u vrijeme druge injekcije PGF2 $\alpha$  (n = 16). U četvrtom tretmanu - PGPE-1 (prostaglandin-gonadotropin-prostaglandin-estradiol-1), krave su tretirane kao u PGP, uz 1 mg ECP jedan dan poslije druge injekcije PGF2 $\alpha$  (n = 16). Krave su pregledane rektalno i pomoću ultrazvuka. Svako kravi uziman je uzorak krvi u određenim razmacima, za istraživanje koncentracije progesterona i estradiola te su osjemenjene u estrusu. Nakon prve injekcije PGF2 $\alpha$  utvrdili smo veći postotak krava s ovulacijom kod PGP u odnosu na PP tretman (64,3 %, odnosno 50 %;  $P < 0,05$ ). Kod krava tretiranih s GnRH češće su utvrđeni veći zreli folikuli prilikom druge aplikacije PGF2 $\alpha$  (71,4 %) u usporedbi s netretiranim (50 %). Dva dana poslije druge aplikacije PGF2 $\alpha$ , kod krava tretiranih s PGP utvrđena je veća preovulatorna koncentracija estradiola u usporedbi s kravama u PP protokolu ( $3,2 \pm 0,48$ , odnosno  $2,6 \pm 0,45$  pg/mL). Također, kod krava tretiranih s ECP (PGPE-0 i PGPE-1) utvrđena je veća preovulatorna koncentracija estradiola ( $6,3 \pm 0,43$  i  $6,99 \pm 0,63$  pg/mL;  $P < 0,01$ ) i veći postotak ovulacija (75,0 % i 87,5 %) u odnosu na skupine s drugim tretmanima (PP ili PGP). Postotak osjemenjenih krava (broj osjemenjenih/ukupan broj krava  $\times 100$ ), statistički je bio značajno različit između protokola, pogotovo kod krava tretiranih s ECP, kao i razlike za postotak koncepcije i gravidnosti, koje su bile veće kod krava tretiranih s ECP u odnosu na ostale tretmane. Možemo zaključiti da je PGP protokol za posljedicu imao više dozrelih folikula i više krava s ovulacijom. Kombinacija ECP i PGP (PGPE-1) poboljšala je pojavu estrusa i ovulacija te je to potencijalno nova metoda za rutinsku sinkronizaciju estrusa i ovulacije kod mliječnih krava.

*Ključne riječi: estrus, sinkronizacija, PGF2 $\alpha$ , GnRH, estradiol cipionat, gravidnost*

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