

## Time and Incidence of Ovulation and Conception Rates After Incorporating Estradiol Cypionate into a Timed Artificial Insemination Protocol

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### ABSTRACT

Two experiments were conducted to determine the effect of estradiol cypionate (ECP), when incorporated into a conventional GnRH-PGF<sub>2α</sub>-GnRH timed artificial insemination protocol (Ovsynch), on systemic estradiol (E<sub>2</sub>), time and incidence of ovulation, luteal development, and conception rate in Holstein cows. Our objective was to determine if administration of 0.25 mg of ECP at the time of the second GnRH injection would effectively synchronize ovulation and increase conception rate. In Experiment 1, lactating Holstein cows (n = 23; 58.7 ± 1.2 d in milk) were synchronized with PGF<sub>2α</sub> (at d -10). Ten days later, Ovsynch was initiated with the administration of 100 µg of GnRH (d 0) followed by PGF<sub>2α</sub> on d 7. On d 9, cows were assigned randomly to be treated with either GnRH + 0.25 mg of ECP (OVS-ECP; n = 11) or GnRH and 1 mL of cottonseed oil (OVS-C; n = 12). Ovarian activity was monitored by ultrasonography on d 0, 7, and 9. To determine the time of ovulation, ultrasound examinations were conducted at 12 and 20 h posttreatment and then at least every 3 h until either 36 h posttreatment or ovulation was observed. Blood samples were collected on d 0, 7, 9, and 16 for progesterone analysis. Blood samples also were collected at the time of treatment (d 9, 0 h) and at 6, 12, 20, and 28 h for E<sub>2</sub> analysis. Incidence of ovulation did not differ between treatments. Mean ovulation time relative to the second GnRH administration was similar between treatments. Serum progesterone concentration did not differ between treatments at any time. Serum E<sub>2</sub> concentration was not different at the time of treatment (0 h); however, mean E<sub>2</sub> concentration was greater for the OVS-ECP group at 6 and 12 h after treatment compared with OVS-C. In Experiment 2, lactating dairy cows (n = 333) in 3 commercial herds were randomly assigned to OVS-ECP (n = 169) or OVS-C (n = 164). Cows were inseminated 22 to 24 h posttreatment.

Conception rates did not differ between treatments. Estradiol cypionate treatment was successful in increasing serum E<sub>2</sub> when administered at the time of the second dose of GnRH in the Ovsynch protocol. Conception rates, however, were not affected by treatment. **Key words:** timed artificial insemination, estradiol cypionate, conception rate

### INTRODUCTION

Systematic breeding programs provide an organized and efficient approach to administering AI and improving reproductive efficiency in dairy herds. To alleviate the difficulties associated with detection of estrus and to increase the AI submission rate, timed AI programs have been developed such as Ovsynch (Pursley et al., 1995). Ovsynch (d 0 GnRH, d 7 PGF<sub>2α</sub>, d 9 GnRH, timed AI 8 to 24 h) was designed to synchronize ovulation, thereby allowing timed AI of all cows without detection of estrus. Synchronization of ovulation in 84 to 100% of cows can be expected (Pursley et al., 1995; Fricke et al., 1998; Vasconcelos et al., 1999; Cartmill et al., 2001). Conception rates following timed AI associated with Ovsynch range from 22 to 42% in dairy cows (Stevenson et al., 1996; Pursley et al., 1997a,b, 1998; Fricke et al., 1998; Jobst, 1998; Stevenson et al., 1999). In practice, although AI submission rates are 100% when using Ovsynch, conception rate may be reduced with this protocol compared with cows that receive AI following detected estrus (Stevenson et al., 1999; Santos et al., 2004). Nevertheless, pregnancy rate (AI submission or estrus-detection rate × conception rate) achieved either after timed AI or after detected estrus and AI may be comparable.

Although timed AI protocols such as Ovsynch are a convenient method to increase AI submission rate, a number of studies indicate mechanisms by which induction of ovulation by administration of GnRH during proestrus can disturb normal reproductive function and negatively affect conception rates. Lucy and Stevenson (1986) found that an injection of GnRH during periestrus, and before the preovulatory LH surge, decreased serum estradiol (E<sub>2</sub>) and reduced fertility compared

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with cows having a spontaneous LH surge. These results are further supported by Kobayashi (1995) who showed that administration of GnRH after PGF<sub>2α</sub> caused a cessation of estrogen secretion by the preovulatory follicle as evidenced by a decline in blood concentrations of E<sub>2</sub>. Thatcher and Chenault (1976) reported that GnRH treatment of cattle 48 h after PGF<sub>2α</sub> reduced the frequency of estrus compared with PGF<sub>2α</sub>-induced estrus alone. Those authors suggested that this observation might be due to alterations in plasma progestins and E<sub>2</sub>. In vitro studies (Uemura et al., 1994; Takekida et al., 2000) have shown that GnRH agonists directly inhibit ovarian and granulosa cell steroidogenesis [E<sub>2</sub> and progesterone (P<sub>4</sub>)]. Lower frequency of estrus in cows treated with GnRH 48 h after PGF<sub>2α</sub> (Rodriguez et al., 1975; Thatcher and Chenault, 1976), taken together with suppressed estrual behavior following the second GnRH injection in cows subjected to Ovsynch (Pursley et al., 1995; Twagiramungu et al., 1995; Stevenson et al., 1996, 1999, and 2000; Jobst, 1998) as well as in vitro studies give further support to the premise that induction of ovulation during the follicular phase, as occurs with Ovsynch, suppresses E<sub>2</sub> secretion. Further, exogenous E<sub>2</sub> increases uterine contractions, efficiency of sperm transport, number of sperm in the oviducts, retention and adhesion of sperm to oviductal epithelium, enhances sperm capacitation and the true acrosome reaction, and increases fertilization (Hawk and Cooper, 1978; Hawk, 1983, 1987; Bathla et al., 1999; Langendijk et al., 2002). Consequently, conception rates observed following GnRH-induced ovulation and timed AI may not be optimized due, in part, to asynchronous timing of the GnRH-induced LH surge and final follicular maturation coupled with limited or brief E<sub>2</sub> secretion around the time of estrus (Lucy and Stevenson, 1986; Stevenson et al., 1999; Taponen et al., 1999). Another factor potentially limiting conception rate in cows enrolled in the Ovsynch protocol (without presynchronization) might be due to a 16 to 19% lack of synchronization of ovulation after the second GnRH (Vasconcelos et al., 1999).

Our hypothesis was that administration of 0.25 mg of estradiol cypionate (ECP) at the time of the second dose of GnRH in the Ovsynch protocol (OVS-ECP) would improve fertility compared with the conventional Ovsynch protocol (OVS-C). The objectives of these experiments were to determine the effect of 0.25 mg of ECP when incorporated into a conventional Ovsynch protocol on systemic E<sub>2</sub>, time and incidence of ovulation, luteal development, and first-service AI conception rate in dairy cows.

## MATERIALS AND METHODS

### Experiment 1

Experiment 1 was conducted at the University of Idaho Dairy Center using 23 lactating Holstein cows

(DIM = 58.7 ± 1.3 d). Mean (± SD) BW was 664 ± 92 kg and mean (± SD) BCS was 2.6 ± 0.5 (scale = 1 to 5, with 1 being emaciated and 5 being grossly over-conditioned). Average 3.5% FCM yield at the DHI test closest to the day of treatment was 41.1 ± 10.2 kg (mean ± SD). Based on transrectal palpation by the herd veterinarian, experimental cows had no abnormalities of the reproductive tract at the initiation of the experiment.

**Ovulation Synchronization and Treatment.** On d -10, estrous cycles of cows were presynchronized with an i.m. injection of 25 mg of PGF<sub>2α</sub> (Lutalyse; Pfizer Animal Health, New York, NY). On d 0, and after detection of a corpus luteum (CL) by transrectal ultrasonography (Sonovet 600, 5-MHz probe, Universal Ultrasound, Bedford Hills, NY), the Ovsynch protocol was initiated with a dose of GnRH (100 µg) administered i.m. (Cystorelin; Merial, Athens, GA). Seven days later (d 7), all cows received (i.m.) 25 mg of PGF<sub>2α</sub> to regress the CL. Forty-eight hours after PGF<sub>2α</sub> treatment (d 9), cows were assigned randomly to treatment (n = 11; OVS-ECP) or control (n = 12; OVS-C). The OVS-ECP cows received (i.m.) GnRH (100 µg) + 0.25 mg of ECP (Pharmacia Animal Health, Kalamazoo, MI), whereas the OVS-C cows received GnRH (100 µg) + cottonseed oil (1 mL). The ECP was diluted in purified cottonseed oil (Sigma-Aldrich Corp., St. Louis, MO).

**Ovarian Examination and Blood Collection.** On d 0, 7, and 9, ovaries were examined via transrectal ultrasonography and structures recorded. Time of ovulation after treatment (injection of GnRH or GnRH + ECP) was determined by ultrasonography that was conducted at 12 and 20 h after treatment and then at least every 3 h thereafter until either ovulation or 36 h, whichever occurred first. Ovulation was defined as the disappearance of any antral follicle ≥10 mm in diameter at the time of an ultrasound examination compared with the previous ultrasound examination (Kaneko et al., 1991). Time of ovulation was defined as the number of hours from the time of treatment to the midpoint of the 2 examinations between which ovulation had occurred (Walker et al., 1996). On d 16, ultrasonographic examinations of the ovaries were performed to confirm the occurrence of ovulation, as evidenced by the presence of a CL.

On d 0, 7, 9, and 16, coccygeal blood samples were collected for later measurement of P<sub>4</sub> concentrations to determine the ovarian response to the hormonal treatments. Coccygeal blood samples were obtained at 0, 6, 12, 20, and 28 h after treatment to determine whether ECP induced a significant change in serum E<sub>2</sub> compared with controls. Blood samples were immediately placed in ice and stored at 4°C for a minimum of 20 h to allow clotting. All samples were centrifuged at 4°C for 30 min

at  $2,750 \times g$ . Serum was harvested and stored at  $-20^{\circ}\text{C}$  until assayed for  $\text{E}_2$  or  $\text{P}_4$ .

**Hormone Assays.** Serum  $\text{E}_2$  concentrations were determined by radioimmunoassay as described by Perry et al. (1991) and was kindly performed by the laboratory of Matthew Lucy (University of Missouri, Columbia). The assay was conducted in nonequilibrium conditions and the standard curve and all samples were assayed in duplicate. Primary antiserum bound 35% of  $^{125}\text{I}\text{-E}_2$  in the absence of unlabeled  $\text{E}_2$ . Intra- and interassay coefficients of variation (CV) were 9.3 and 5.5%, respectively.

Serum  $\text{P}_4$  concentrations were determined using a solid-phase radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). The assay was conducted under equilibrium conditions. The standard curve ranged from 0.1 to 40 ng/mL, and all samples were assayed in duplicate. Intraassay CV was 7.8%.

### Statistical Analyses: Experiment 1

Analysis of repeated measures using the mixed procedure of SAS (Littell et al., 1998) was used to analyze serum  $\text{E}_2$  data. The statistical model included treatment, the repeated factor time, and treatment  $\times$  time interaction. Cow within treatment was designated as a random effect and pretreatment serum  $\text{E}_2$  values were used as covariates in the model.

Serum  $\text{P}_4$  data were analyzed by least squares ANOVA using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model included treatment. Separate analyses were performed for each sampling day (d 0, 7, 9, and 16) to verify that cows in both treatments had similar  $\text{P}_4$  concentrations at the initiation of OvSynch and to determine ovarian response to the first GnRH, second  $\text{PGF}_{2\alpha}$ , and second GnRH administrations.

### Experiment 2

Experiment 2 was conducted to determine the effect of OVS-ECP on first-service conception rate in artificially inseminated dairy cows. Three commercial dairies in southern Idaho participated in a field trial. Three hundred thirty-three multiparous cows (OVS-ECP:  $n = 169$ ; OVS-C:  $n = 164$ ), having fewer than 80 DIM, were submitted for the first postpartum insemination for this experiment. Hormonal treatments were similar to those in Experiment 1, except that all cows received  $\text{PGF}_{2\alpha}$  14 d before the first dose of GnRH (d 0). Before d 9, cows were assigned randomly to either OVS-ECP or OVS-C by the herd manager at each farm and without the knowledge of personnel who administered treatments. On each dairy, cows were inseminated approxi-

**Table 1.** Serum progesterone concentrations (ng/mL; least squares mean  $\pm$  SEM) on d 0, 7, 9, and 16 in lactating Holstein cows treated with OvSynch with and without estradiol cypionate (Experiment 1)

Treatment <sup>1</sup>	Day of experiment			
	0	7	9	16
OVS-ECP ( $n = 11$ )	$3.0 \pm 0.8$	$3.2 \pm 0.8$	$0.5 \pm 0.3$	$3.2 \pm 0.4$
OVS-C ( $n = 12$ )	$4.0 \pm 0.7$	$4.8 \pm 0.8$	$0.8 \pm 0.3$	$3.1 \pm 0.3$

<sup>1</sup>OVS-ECP = OvSynch was initiated with the administration of 100  $\mu\text{g}$  GnRH (d 0) followed by  $\text{PGF}_{2\alpha}$  on d 7. On d 9 cows received (i.m.) GnRH (100  $\mu\text{g}$ ) + 0.25 mg of estradiol cypionate (ECP); OVS-C = OvSynch was initiated with the administration of 100  $\mu\text{g}$  of GnRH (d 0) followed by  $\text{PGF}_{2\alpha}$  on d 7. On d 9, cows received GnRH (100  $\mu\text{g}$ ) + cottonseed oil (1 mL).

mately 22 to 24 h after treatment by a single inseminator. Cows in all 3 herds were observed for signs of estrus once daily based on tail chalk removal. Cows that exhibited estrus on or before d 9 received AI immediately and were removed from the experiment. Conception rates (number of confirmed pregnancies divided by number of cows inseminated) were calculated based upon pregnancy diagnosis by the herd veterinarian via palpation per rectum of uterine contents at 35 to 42 d after AI.

### Statistical Analyses: Experiment 2

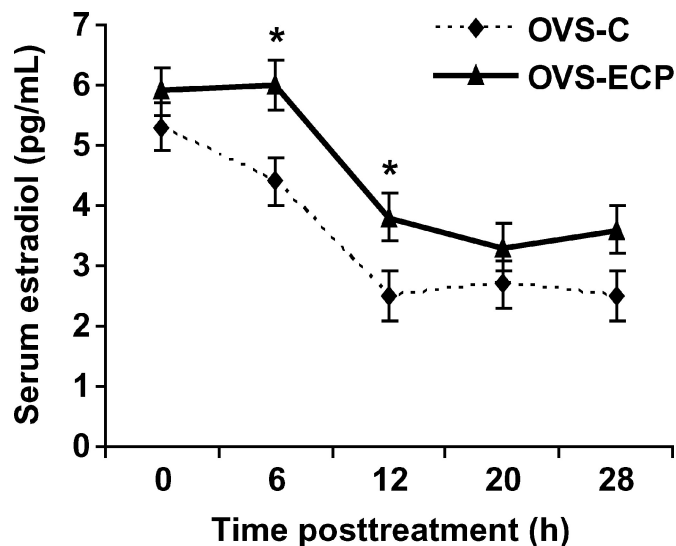
Conception rate data were analyzed using the Logistic procedure of SAS (SAS Inst.). The model included the effects of treatment, herd, and the treatment  $\times$  herd interaction. Based on the number of cows used in this experiment, a 13% difference in conception rate could be detected. This sensitivity was calculated (Agresti, 1990) for  $\alpha = 0.07$  and  $\beta = 0.20$  and an average pregnancy rate of 30%.

## RESULTS

### Experiment 1

Average BW and BCS did not differ between treatments. Mean daily 3.5% FCM production was not different between treatments. Moreover, mean serum  $\text{P}_4$  concentrations did not differ between treatments at any time (Table 1).

A time  $\times$  treatment interaction ( $P < 0.05$ ) was detected for serum  $\text{E}_2$  concentrations. At 0 h, mean serum  $\text{E}_2$  concentrations were  $5.9 \pm 0.4$  pg/mL for OVS-ECP cows and  $5.3 \pm 0.3$  pg/mL for OVS-C cows and did not differ (Figure 1). Mean serum  $\text{E}_2$  concentrations were different between treatments at 6 ( $P < 0.05$ ) and 12 h ( $P = 0.05$ ; Figure 1). At 6 h posttreatment, mean serum  $\text{E}_2$  concentration in OVS-ECP cows ( $6.0 \pm 0.4$  pg/mL) was greater ( $P < 0.05$ ) than that of OVS-C cows ( $4.4 \pm 0.3$  pg/



**Figure 1.** Mean serum concentrations of estradiol in cows treated with Ovsynch with (OVS-ECP) and without (OVS-C) estradiol cypionate injection coincident with the second GnRH injection of Ovsynch (Experiment 1). Treatment (0 h) with either GnRH or GnRH + estradiol cypionate occurred at 0 h. \*Means differed ( $P \leq 0.05$ ) between treatments at 6 and 12 h.

mL; Figure 1). Mean serum  $E_2$  concentration declined in both groups between 6 and 12 h after treatment, but remained greater ( $P = 0.05$ ) for the OVS-ECP cows than for the OVS-C cows at 12 h ( $3.77 \pm 0.36$  vs.  $2.58 \pm 0.33$  pg/mL).

Time of ovulation did not differ between treatments (Table 2). Range in time of ovulation was 16 to 32 h and 26 to 29 h for the OVS-ECP and OVS-C cows, respectively. Incidence of ovulation did not differ and was 100% for OVS-ECP and 92% for OVS-C group. Mean diameter of the ovulatory follicle, measured at 20 h after treatment, was similar between groups ( $18.3 \pm 1.2$  vs.  $18.1 \pm 1.4$  mm; Table 2). In the present study, 82% of all observed ovulations were on the right ovary (18 of 22; Table 2).

On d 16, all cows were subjected to ultrasonographic ovarian examination of the ovaries and all had a CL on the ovary where ovulation had been previously detected. Mean serum  $P_4$  concentrations on d 16 did not differ between groups and were  $3.2 \pm 0.4$  and  $3.1 \pm 0.3$  ng/mL in the OVS-ECP and OVS-C, respectively (Table 1).

### Experiment 2

Neither treatment, herd, nor treatment  $\times$  herd interaction influenced conception rate. Overall conception rate for the OVS-ECP group was 31.4% and for the OVS-C group was 36.6% (Table 3).

## DISCUSSION

### Experiment 1

In the present study, serum  $E_2$  concentrations were greater in OVS-ECP cows at 6 and 12 h after ECP treatment compared with OVS-C cows (Figure 1). Taponen et al. (1999) found that when GnRH was administered 24 h after  $PGF_{2\alpha}$  injection, serum  $E_2$  declined to basal concentrations within 1 d. Mee et al. (1993) found that serum  $E_2$  declined after estrus in both saline- and GnRH-treated cows; however, serum  $E_2$  was less in the GnRH-treated group. Serum  $E_2$  profiles in OVS-ECP cows differed from those in the OVS-C cows indicating that 0.25 mg of ECP was able to alter circulating  $E_2$  in cows during the first 12 h after treatment (Figure 1).

Lucy and Stevenson (1986) administered GnRH or saline at 72 h after  $PGF_{2\alpha}$  and found that GnRH-treated cows had a mean serum  $E_2$  concentration approximately 3 pg/mL less than that of saline-treated cows. In the present study, serum  $E_2$  in OVS-C group was 36% less at 6 h after GnRH administration compared with that in the OVS-ECP group. Serum  $E_2$  profile of the OVS-C group might suggest that the time of GnRH administration was asynchronous with the natural timing of neuroendocrine events following  $PGF_{2\alpha}$ ; thus, the

**Table 2.** Time and incidence of ovulation, and size of ovulatory follicle in lactating Holsteins treated with Ovsynch with and without estradiol cypionate (Experiment 1)

Treatment <sup>1</sup>	Time of ovulation, <sup>2</sup> h	Incidence of ovulation, %	Diameter of ovulatory follicle, <sup>2</sup> mm	Right ovary ovulation, %
OVS-ECP (n = 11)	$26 \pm 1$	100	$18.3 \pm 1.2$	82
OVS-C (n = 12)	$27 \pm 1$	92	$18.1 \pm 1.5$	83

<sup>1</sup>OVS-ECP = Ovsynch was initiated with the administration of 100  $\mu$ g GnRH (d 0) followed by  $PGF_{2\alpha}$  on d 7. On d 9 cows received (i.m.) GnRH (100  $\mu$ g) + 0.25 mg of estradiol cypionate (ECP); OVS-C = Ovsynch was initiated with the administration of 100  $\mu$ g of GnRH (d 0) followed by  $PGF_{2\alpha}$  on d 7. On d 9, cows received GnRH (100  $\mu$ g) + cottonseed oil (1 mL).

<sup>2</sup>Means  $\pm$  SE.

**Table 3.** Conception rate (number of pregnant cows/total number of cows inseminated) in lactating Holstein cows treated with Ovsynch with and without estradiol cypionate (Experiment 2)

Herd	Treatment <sup>1</sup>	
	OVS-ECP	OVS-C
	% (no.)	
1	29.4 (51)	40.4 (47)
2	33.9 (56)	38.6 (57)
3	30.6 (62)	31.7 (60)
Total	31.4 (169)	36.6 (164)

<sup>1</sup>OVS-ECP = Ovsynch was initiated with the administration of 100 µg GnRH (d 0) followed by PGF<sub>2α</sub> on d 7. On d 9 cows received (i.m.) GnRH (100 µg) + 0.25 mg of estradiol cypionate (ECP); OVS-C = Ovsynch was initiated with the administration of 100 µg of GnRH (d 0) followed by PGF<sub>2α</sub> on d 7. On d 9, cows received GnRH (100 µg) + cottonseed oil (1 mL).

preovulatory follicle may not have matured adequately before the GnRH-induced LH surge as originally hypothesized by Lucy and Stevenson (1986).

Ovulations in both groups occurred between 16 and 32 h after GnRH administration (Table 2), which is comparable to findings of other researchers (Pursley et al., 1995; Ahmadzadeh et al., 2002). Time of ovulation in our study was similar to that after spontaneous estrus (27.9 ± 5.6 h after the onset of estrus; Walker et al., 1996).

The greater E<sub>2</sub> concentration observed in the OVS-ECP compared with OVS-C group was not related to size of the ovulatory follicle, because no difference in mean diameter of the ovulatory follicle was detected between groups at 20-h posttreatment. Results for both groups are comparable to those described by Ahmadzadeh et al. (2002) in which mean diameter of the ovulatory follicle was 18.6 ± 3.2 mm. Similarly, Vasconcelos et al. (1999) measured the ovulatory follicle at the time of the second dose of GnRH in Ovsynch-treated cattle and reported the size of the ovulatory follicle to be 18.24 mm.

## Experiment 2

Despite studies that have reported that exogenous E<sub>2</sub> increases uterine contractions, efficiency of sperm transport, number of sperm in the oviducts, total number of sperm retained in the female reproductive tract, and proportion of total ova fertilized in laboratory animals and livestock (Hawk and Cooper, 1978; Hawk, 1983; Bathla et al., 1999; Orihuela et al., 1999; Langendijk et al., 2002), no difference in conception rate was detected between OVS-ECP and OVS-C groups. For 333 dairy cows in 3 different herds, the conception rates were similar (Table 3). Based on the number of cows used in this experiment, a 13 percentage-point differ-

ence in conception rate could be detected. This sensitivity was calculated (Agresti, 1990) for  $\alpha = 0.07$  and  $\beta = 0.20$  with an average pregnancy rate of 30%. The probability of detecting a smaller difference in conception rate between treatments in this experiment was limited by the number of cows enrolled.

In contrast, Cerri et al. (2004) reported that use of 1 mg of ECP to induce ovulation as part of a timed AI protocol improved conception at first postpartum insemination in dairy cows. It is possible that increased conception rates observed for cows in the Heatsynch protocol are the result of prolonged exposure to greater concentrations of estradiol during proestrus (Cerri et al., 2004). It has been proposed that estradiol during proestrus influences sperm transport and might inhibit PGF<sub>2α</sub> secretion in the subsequent estrous cycle (Mann and Lamming, 2000).

Serum E<sub>2</sub> for the OVS-ECP group was greater during the first 12 h posttreatment (Figure 1). Nevertheless, no difference in conception rate was detected between treatments in Experiment 2. One possible explanation for the similarity in conception rate between treatments was the timing of AI in relation to ECP administration and the systemic E<sub>2</sub> profile. Cows in Experiment 2 were inseminated 22 to 24 h after ECP, and not likely during the time of maximally elevated serum E<sub>2</sub> (Figure 1). Therefore, although the OVS-ECP cows were exposed to greater serum concentrations of E<sub>2</sub> than OVS-C cows during the first 12 h posttreatment, by the time of AI serum E<sub>2</sub> concentrations in most cows in the OVS-ECP group had likely declined to concentrations similar to the OVS-C group. Furthermore, it is likely that sperm capable of fertilization did not reach the ampullary-isthmus junction (site of fertilization) until 29 to 32 h posttreatment (on average), a time when the 2 groups were likely not different with regard to serum E<sub>2</sub>. In a similar experiment using beef cattle, Ahmadzadeh et al. (2003) reported that conception rate tended to improve (68 vs. 57%, for OVS-ECP compared with OVS-C groups, respectively) when AI was performed 6 to 8 h after GnRH + ECP, which corresponds to the time of maximally elevated serum estradiol in OVS-ECP cows in the current study. This may indicate that greater E<sub>2</sub> in the OVS-ECP when timed AI was performed enhanced uterine activity, sperm transport, or fertilization rates as previously suggested (Hawk and Cooper, 1978; Bathla et al., 1999; Langendijk et al., 2002).

## CONCLUSIONS

Treatment with ECP at the time of the second dose of GnRH in the Ovsynch protocol successfully increased serum E<sub>2</sub> in dairy cows. However, incorporating ECP into the Ovsynch protocol had no effect on conception

rates. More research needs to be conducted in dairy cattle to further explore the role of increased  $E_2$  during proestrus on fertility.

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