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Profiles of circulating estradiol-17β after different estrogen treatments in lactating dairy cows

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Abstract

The objective of this study was to characterize the circulating concentrations of estradiol-17β (E-17β) after treatment with different types or doses of estrogens in the absence (Experiment 1) or presence (Experiment 2) of a dominant follicle in lactating cows. In Experiment 1, cows (n = 12) had all follicles > 5 mm removed by ultrasound-guided follicular aspiration every 12 h throughout the blood sampling period. Estrogen treatments started 48 h after the first follicular aspiration. Treatments were: no treatment, E-17β (0.5 mg), or estradiol benzoate (EB, 0.5 mg). Seven days after the end of the first trial, cows were then re-randomized to receive: no treatment, E-17β (1.0 mg), EB (1.0 mg), or estradiol cypionate (ECP, 1.0 mg). In Experiment 1, cows treated with E-17β had greater peak circulating concentrations of E-17β than ECP-treated cows, and EB-treated cows had intermediate concentrations. Similarly, E-17β-treated cows had the shortest intervals from treatment to peak concentrations and from peak until return to nadir; ECP-treated cows had the longest intervals, and EB-treated cows had intermediate intervals. In Experiment 2, circulating E-17β was evaluated near the time of AI (artificial insemination) in cows (n = 24) that received Ovsynch with or without E-17β supplementation 48 h after $PGF_{2\alpha}$ treatment. Treatments were: no treatment, E-17β (0.5 mg), or E-17β (1.0 mg). Cows treated with 1.0 mg E-17β had a shorter time to peak circulating E-17β concentrations and greater maximum concentrations (5.0 h; 18.5 pg/ml) than controls (9.5 h; 5.5 pg/ ml), and cows treated with 0.5 mg E-17β were intermediate (5.5 h; 10.6 pg/ ml). Thus, the presence of a dominant follicle and treatment with differing types of estrogen produce substantial differences in the circulating E-17β profile. In lactating dairy cows, a 1.0 mg dose of E-17β increased circulating E-17β concentrations during Ovsynch without disrupting the normal decline in circulating E-17β after the LH surge.

Keywords: estradiol, dairy cattle, Ovsynch.

Introduction

Reproductive efficiency is not optimal in high-producing lactating dairy cows due to multiple management and physiological factors (Lucy, 2001; Washburn *et al*., 2002). One of the physiological aspects that may affect reproductive efficiency in lactating dairy cows is the elevated metabolism of estradiol-17β (E-17β; Sangsritavong *et al*., 2002). This high E-17β metabolism appears to be due to the elevated liver blood flow that is coincident with elevated dry matter intake in lactating dairy cows (Sangsritavong *et al*., 2002). High rates of E-17β metabolism result in reduced circulating E-17β concentrations in lactating cows compared to non-lactating cows (Sartori *et al*., 2002a; 2004) and in lactating cows with high milk production compared to cows with low production (Lopez *et al*., 2004; 2005). Since E-17β is involved in many aspects of reproductive physiology, this reduction in circulating E-17β could cause numerous changes in the reproductive physiology of high producing lactating cows. For example, the duration of estrus is associated with level of milk production, and high producing cows have a shorter duration of estrus (Nebel *et al*., 1997; Lopez *et al*., 2004) than low producing cows, probably due to reduced peak E-17β concentrations (Lopez *et al*., 2004). Other changes in reproductive performance (Lucy, 2001; Washburn *et al*., 2002) and reproductive physiology (Sartori *et al*., 2002b) could also be related to reduced circulating E-17β in high-producing lactating cows. It seems logical that supplementation with E-17β at the proper time and in the correct dose may allow correction of some reproductive problems that may be caused by the high E-17β metabolism.

There are many forms of estrogens that could be used to increase circulating E-17β concentrations including: native E-17β, estradiol benzoate (EB), and estradiol cypionate (ECP; chemical structures are shown in Fig. 1). These estrogens appear to produce different profiles of circulating E-17β probably due to differences in the esterification of the molecule, which may alter its absorption and metabolism in the body. Vynckier *et al*. (1990) treated non-lactating cows with 10 mg of EB or ECP and found an earlier and greater peak in concentrations of E-17β with EB compared to ECP treatment. Other studies have also described the profile of circulating E-17β following treatment with ECP (Haughian *et al*., 2002), EB (Lammoglia *et al*., 1998; Burke *et al*., 2003; Martinez *et al*., 2005), estradiol valerate (Martinez *et al*., 2005), and native E-17β (Bo *et al*., 2000; Martinez *et al*., 2005). Unfortunately, none of these studies were performed in high-producing

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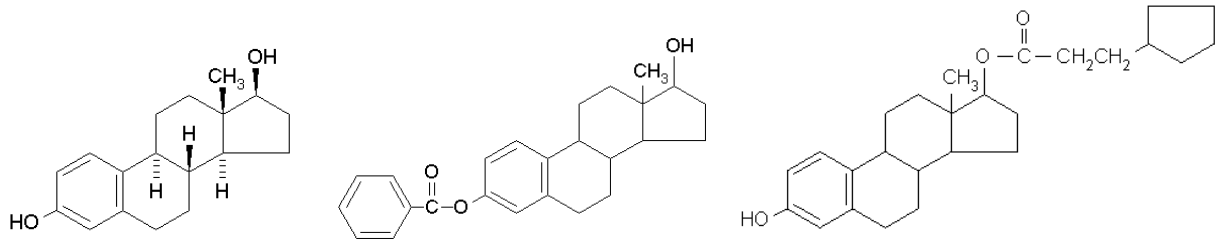
lactating dairy cows and only Haughian *et al*. (2002) examined the profile following ECP treatment in early post-partum (Day 7), lactating dairy cows. In addition, the majority of the studies characterized the E-17β profiles in the presence of endogenous E-17β produced by follicles that were present in the ovaries (Vynckier *et al*., 1990; Lammoglia *et al*., 1998; Bo *et al*., 2000; Haughian *et al*., 2002). Two studies attempted to remove the effects of endogenous E-17β production by ovariectomy (Martinez *et al*., 2005) or by aspiration of all follicles > 5 mm in diameter (Burke *et al*., 2003). In sheep, ovariectomy appears to produce a dramatic reduction in E-17β metabolism probably due to decreased hepatic enzymes involved in steroid metabolism (Freetly and Ferrell, 1994). Thus, the objective of this study was to characterize the circulating E-17β profile after treatment with low doses (0.5 or 1 mg) of E-17β, EB, or ECP in lactating dairy cows that had endogenous E-17β production removed by frequent aspiration of all growing follicles > 5mm. From these results, E-17-β was chosen for a second study to determine the E-17β profile after supplementation of lactating dairy cows with 0, 0.5, or 1 mg of E-17β during the Ovsynch protocol.



(a) (b) (c)

Figure 1. Chemical structures of (a) Estradiol-17β, (b) Estradiol Benzoate, and (c) Estradiol Cypionate.

Materials and Methods

Animals and management

Lactating Holstein cows (n = 12 in Experiment 1; n = 24 in Experiment 2) with an average BCS (Edmonson *et al*., 1989) of 2.9 ± 0.3 in Experiment 1 and 2.7 ± 0.4 in Experiment 2 were used. Milk production averaged 24.7 ± 1.5 and 40.9 ± 1.9 kg/d in Experiment 1 and 2, respectively. The average DIM (days in milk) at the start of the trial was 186.1 ± 18.3 d in Experiment 1 and 96.2 ± 7.1 d in Experiment 2. Cows were housed during December of 2003 in a stanchion/tie-stall barn at the University of Wisconsin Dairy Cattle Research Center (Madison, WI, USA; Experiment 1) and during June 2004 in a free-stall facility in Juneau,WI (Experiment 2). Animals were milked twice (Experiment 1) or thrice (Experiment 2) daily and fed a TMR that consisted of corn silage and alfalfa silage as forage with a corn and soybean meal-based concentrate. The TMR was balanced to meet or exceed minimum nutritional requirements for dairy cattle on both farms (National Research Council, 2001). Pregnancy exams were performed by rectal palpation at 35 to 41 d after AI (Experiment 2). All animal procedures were approved by College of Agriculture and Life Sciences Animal Care Committee of the University of Wisconsin-Madison.

Materials

Intravaginal progesterone (P4) implants (Eazi-Breed CIDR containing 1.38 g of P4), estradiol cypionate, and prostaglandin $F_{2\alpha}$ (Lutalyse) were from Pfizer Animal Health (Kalamazoo, MI, USA). The GnRH (Cystorelin) was from Merial Limited (Athens, GA, USA). Heat mount detectors (Kamar) were from Kamar Inc. (Steamboat Springs, CO, USA). Sesame oil, EB, and E-17β were from Sigma Chemical Co. (St. Louis, MO, USA). Benzyl alcohol was from EM Science (Cherry Hill, NJ, USA). Estradiol solutions were prepared as follows: EB or E-17β were weighed into a glass vial, and benzyl alcohol was added to make a 5 mg/ml solution. Sesame oil was then added to the preparation in order to yield a final solution of 0.5 mg/ml.

Experiment 1

Cows (n = 12) were treated (Day 0) with a CIDR for 7 days. All the animals received two treatments of $PGF_{2\alpha}$ (25 mg, i.m.; Lutalyse) 12 h apart on the day of CIDR removal (CIDR removal and the last $PGF_{2\alpha}$ treatment were simultaneous). After CIDR removal, all follicles > 5 mm were aspirated with an ultrasound-guided transvaginal approach using a 17-gauge by 55 cm needle and a 7.5 MHz convex-array transducer (Aloka SSD-900V; Aloka Co., Wallingford, CT, USA) fitted to a plastic extension. Starting on the day of CIDR removal until the end of the trial, ultrasound examinations occurred every 12 h, and the follicle aspiration procedure was performed whenever a follicle of ≥ 6 mm was detected (experimental design – Fig. 2). These procedures were designed to minimize endogenous E-17β production. Animals were randomly assigned (Experiment 1a) to be treated (i.m.) as follows: 1) control (no treatment, n = 4); 2) E-17β (0.5 mg, n = 4);

or 3) EB (0.5 mg, n = 4). Blood samples were collected every 4 h starting just before the first estrogen treatment until 52 h and every 8 h until 72 h after treatment. At 7 days after this experiment, the cows were re-randomized (Experiment 1b) to receive: 1) control (no treatment, n = 3); 2) E-17β (1.0 mg, n = 3); 3) EB (1.0 mg, n = 3); or 4) ECP (1.0 mg, n = 3). Blood samples were collected on the same schedule as in Experiment 1a. In both experiments, observations for signs of behavioral estrus were performed every 8 h for 30 min until the end of the trial. During the estrus observation period, cows were grouped outdoors.

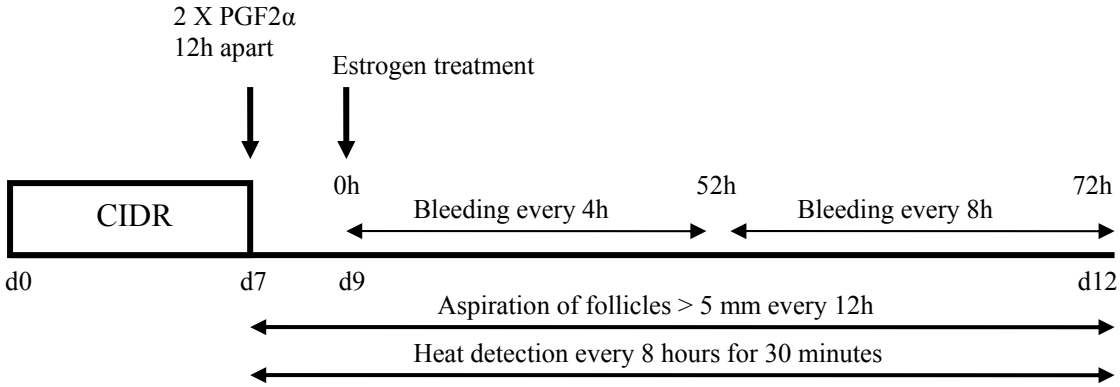


Figure 2. Experimental procedures used in both parts of Experiment 1.

Experiment 2

All cows (n = 24) underwent the Ovsynch protocol (GnRH treatment [100 µg, i.m.] followed by $PGF_{2\alpha}$ treatment [25 mg, i.m.] 7 d later and a second GnRH [100 µg, i.m.] injection 56 h after $PGF_{2\alpha}$), and AI was performed 16 h after the last GnRH treatment. At 48 h after $PGF_{2\alpha}$ treatment (8 h before the second GnRH injection), cows were randomly assigned to be treated (i.m.) as follows: 1) no estrogen supplementation (n = 8); 2) E-17β (0.5 mg, n = 8); or 3) E-17β (1.0 mg, n = 8). Blood samples were taken at 0, 4, 8, 12, and 24 h after treatments. A pressure-activated heat mount detector (Kamar) was placed on the tail head of all cows 48 h after $PGF_{2\alpha}$ and just before E-17β treatments. Activation of the Kamar was evaluated on all cows at the time of AI (24 h after E-17β treatment; Fig. 3).

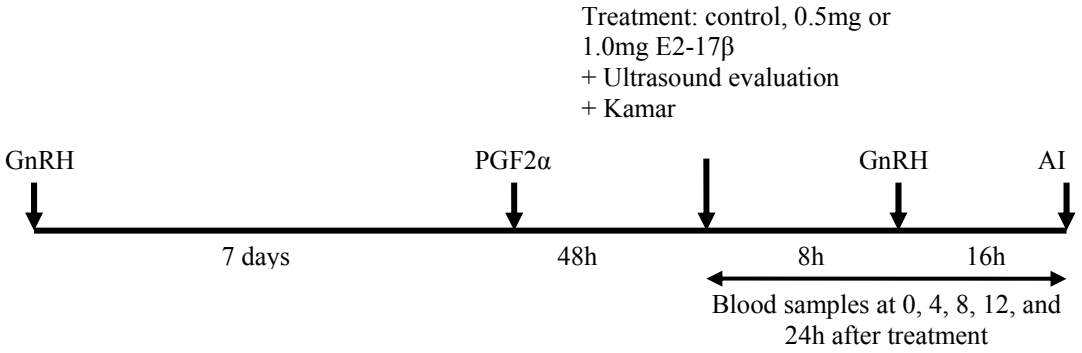


Figure 3. Experimental procedures used in Experiment 2.

Hormone assays

Blood samples were centrifuged at 1600 x g for 15 min, and serum samples were stored at - 20°C until assayed. For analysis of E-17β, samples were extracted twice with diethyl ether and serum concentrations of E-17β were measured using modifications of a commercial RIA kit for E-17β (Third generation Estradiol Assay kit; Diagnostics System Laboratories Inc., Webster, TX, USA) previously validated for use in cattle (Kulick *et al*., 1999). The intra-assay CV values were 10.6% and 9.8% for Experiments 1 and 2, respectively.

Statistical analyses

A normal distribution was assumed for the dependent variable of E-17β concentration, and the analyses were performed using the MIXED procedure of SAS (Littell *et al*., 1996). The model included the effects of treatment (0 control, 0.5 mg EB, 0.5 mg E-17β, 1.0 mg EB, 1.0 mg E-17β, or 1.0mg ECP), time (0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 60, and 72 h after treatment), the interaction between treatment and time, and cow, which was treated as a random effect and was the subject of repeated measures anaylsis. The area

under the curve for all treatments, calculated by the trapezoid method, was analyzed using the MIXED procedure of SAS. A probability of $P < 0.05$ was considered to be significant, and probabilities between 0.05 and 0.10 were discussed as tendencies.

Results

Experiment I

None of the cows in Experiment 1 displayed standing behavioral estrus regardless of type or dose of estrogen. Two cows treated with 1.0 mg of E-17β, 3 cows treated with 1.0 mg of EB, and 1 cow treated with 1.0 mg of ECP showed secondary signs of estrus such as mounting behavior and hyperactivity at 12 to 20, 20 to 44, and 28 to 36 h after estrogen administration, respectively.

At hour 0, all cows had low (< 2 pg/ml) circulating E-17β concentrations (Fig. 4 and 5). The control groups from Experiment 1a and 1b were not different at any time point; therefore, the data were combined. The area under the curve for control cows (75.7 ± 14.9 pg^2) was less ($P < 0.05$) than for all other groups (Table 1).

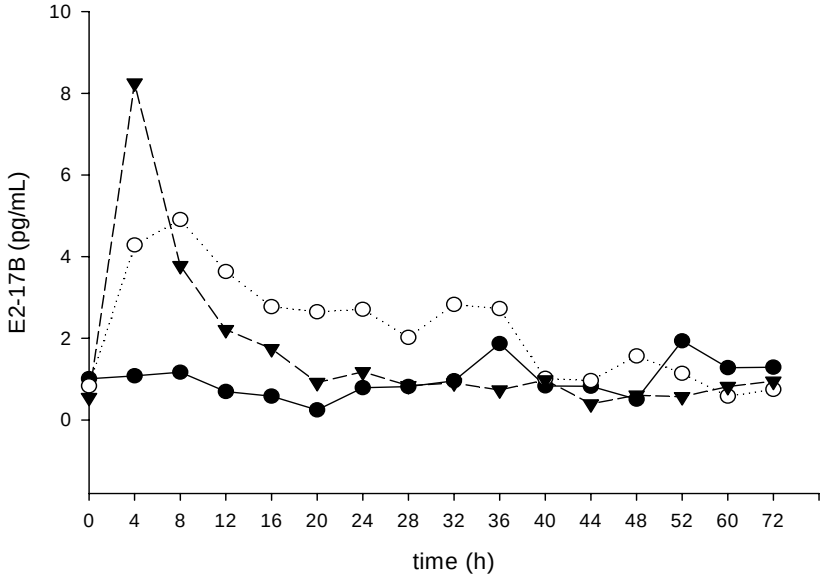


Figure 4. Experiment 1A - Mean concentrations of circulating estradiol-17β (E-17β) in cows in the absence of follicles > 5mm for control (●), 0.5mg of E-17β (▼), and 0.5 mg of EB (○). Hour 0 is the time of estrogen treatment.

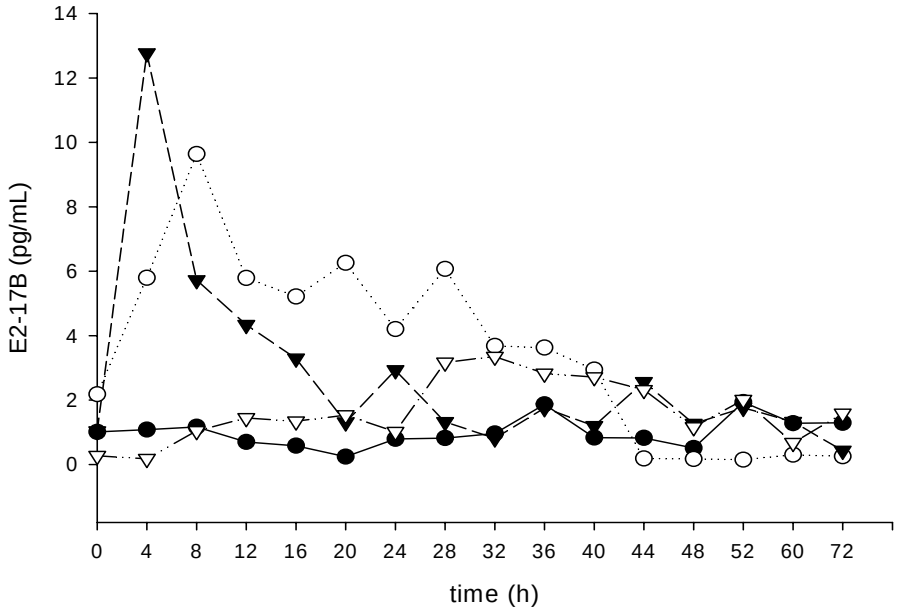


Figure 5. Experiment 1B - Mean concentrations of circulating estradiol-17β (E-17β) in cows in the absence of follicles > 5 mm for control (●), 1.0 mg of E-17β (▼), 1.0 mg of EB (○), and 1.0 mg ECP (▽). Hour 0 is the time of estrogen treatment.

Treatment with either 0.5 mg or 1 mg of E-17β produced a rapid increase in circulating E-17β, and maximum concentrations were reached 4 h after treatment. Circulating E-17β concentrations returned to basal levels in less than 24 h after either dose of E-17β (Fig. 4 and 5). After treatment with 1 mg of E-17β, circulating E-17β was greater ($P < 0.05$) than control cows at 4, 8, and 12 h. After treatment with 0.5 mg of E-17β, circulating E-17β was greater ($P < 0.05$) than control cows only at 4 and 8 h.

Cows treated with EB tended to reach peak circulating E-17β concentrations later than E-17β-treated cows but sooner than ECP-treated cows (Table 1). Cows that received 1.0 mg of EB had elevated ($P < 0.05$) concentrations of E-17β from 4 to 36 h after treatment compared to controls. Similarly, cows treated with 0.5 mg of EB differed ($P < 0.05$) from controls in circulating E-17β concentrations at 4, 8, and 12 h and tended to differ from 16 to 36 h after treatment. The temporal profiles for the 2 doses of E-17β or EB were similar; however, there were approximately twice the circulating E-17β concentrations in the 1 mg than the 0.5 mg groups (Fig. 4 and 5).

Cows treated with ECP had the lowest peak E-17β concentrations among all estrogen treatments (Table 1). Moreover, the intervals from treatment until peak concentration and treatment until return to nadir were both greater or tended to be greater for ECP-treated cows than for cows treated with other estrogens (Table 1). Surprisingly, circulating E-17β concentrations in ECP-treated cows were similar to controls at all times and only approached a tendency ($P = 0.11$) to be greater than control cows at 28 h. The area under the curve for ECP-treated cows was greater ($P < 0.05$) than controls, however, lower than all other estrogen-treated groups (Table 1).

A comparison of 0.5 mg E-17β vs. 0.5 mg EB treatments is shown in Fig. 4. The circulating E-17β concentrations were greater ($P < 0.05$) after treatment with 0.5 mg E-17β than 0.5 mg EB at 4 h after treatment. In addition, cows that received 0.5 mg E-17β tended to have a significantly shorter time from treatment to peak E-17β concentration (4 vs. 15 h), greater peak concentrations of E-17β (8.3 vs. 4.9 pg/mL; $P < 0.05$), and a shorter interval from treatment until return to nadir concentrations of E-17β (16 vs. 34 h; $P < 0.05$; Table 1) than cows that received 0.5 mg EB. However, the area under the curve did not differ between these two groups.

Comparisons of circulating E-17β profiles following treatment with 1 mg E-17β, 1 mg EB, and 1 mg ECP are shown in Fig. 5. Circulating E-17β concentrations were greater in cows treated with E-17β from 4 to 8 h in comparison to EB-treated cows ($P < 0.05$) and from 4 to 12 h in comparison to ECP-treated cows (Fig. 5). Cows treated with EB had greater ($P < 0.05$) circulating E-17β concentratons than ECP-treated cows from 4 to 28 h after treatment. Cows treated with E-17β had greater ($P < 0.05$) E-17β peak concentrations and a shorter interval from treatment to peak and treatment to return to nadir concentrations than ECP-treated cows; EB-treated cows had intermediate values (Table 1).

Table 1. Characteristics (mean ± S.E.M.) of the circulating estradiol-17β (E-17β) profile in lactating cows treated with different forms and doses of estrogens in the absence of follicles > 5 mm. Comparisons were made only within either Experiment 1A or 1B.

End point	Experiment 1A		Experiment 1B		
	0.5 mg E-17β	0.5 mg EB	1.0 mg E-17β	1.0 mg EB	1.0 mg ECP
Animals (n	4	4	3	3	3
Treatment to maximum (h)	4.0 ± 0.0 ^{B}	15.0 ± 6.2 ^{A}	4.0 ± 0.0 ^{Yy}	16.0 ± 6.1 ^{Xxy}	30.7 ± 3.5 ^{x}
Treatment to end (h)	16.0 ± 3.7 ^{b}	34.0 ± 4.8 ^{a}	22.7 ± 4.8 ^{y}	30.7 ± 7.1 ^{xy}	50.7 ± 4.8 ^{x}
Maximum concentration (pg/ml)	8.3 ± 1.0 ^{a}	4.9 ± 1.6 ^{b}	12.8 ± 4.0 ^{x}	9.6 ± 3.5 ^{y}	3.4 ± 0.2 ^{z}
Area under curve (pg^2)	110.6 ± 19.7 ^{a}	151.6 ± 13.6 ^{a}	187.9 ± 31.2 ^{x}	222.1 ± 51.3 ^{x}	118.7 ± 34.8 ^{y}

$^{a, b}$ Means within a row with different superscripts are different for Experiment 1A ($P < 0.05$).
^{x,y,z} Means within a row with different superscripts are different for Experiment 1B ($P < 0.05$).
^{A,B} Indicates tendency for a difference between means within a row for Experiment 1A ($P < 0.10$).
^{X,Y} Indicates tendency for a difference between means within a row for Experiment 1B ($P < 0.10$).

Experiment 2

In contrast to Experiment 1, all cows treated with 0.5 mg E-17β (8/8) and the majority cows treated with 1.0 mg E-17β (7/8) displayed standing estrus during the 24 h following treatment while only a few of the cows from the control group were observed in estrus (3/8). In spite of the differences in estrous behavior, the conception rate was similar among groups (control = 2/8; 0.5 mg E-17β = 2/8; and 1.0 mg E-17β = 2/8).

Prior to treatment (0 h), there were no differences among groups in serum E-17β concentrations or size of the ovulatory follicle. Cows treated with 0.5 mg E-17β had greater ($P < 0.02$) serum E-17β concentrations compared to control cows only at 4 h after treatment (Fig. 6). Cows that received 1.0 mg E-17β had elevated serum E-17β at 4 ($P < 0.01$) and 8 h ($P < 0.02$) after E-17β treatment compared to control cows. In addition, cows treated with 1.0 mg E-17β had greater circulating concentrations of E-17β than cows treated with 0.5 mg E-17β at 4 h ($P < 0.01$) and at 8 h ($P = 0.05$) after treatment. The peak E-17β concentrations (Table 2) were greater in

cows treated with 1 mg of E-17β (18.5 pg/ml), intermediate in cows treated with 0.5 mg E-17β (10.6 pg/ ml), and lowest in control cows (5.5 pg/ml). The area under the curve was greater (P < 0.05) for cows treated with 1 mg E-17β than control cows and tended (P < 0.10) to be greater than cows treated with 0.5 mg E-17β. Cows treated with 0.5 mg of E-17β tended (P < 0.10) to have a greater area under the curve than control cows.

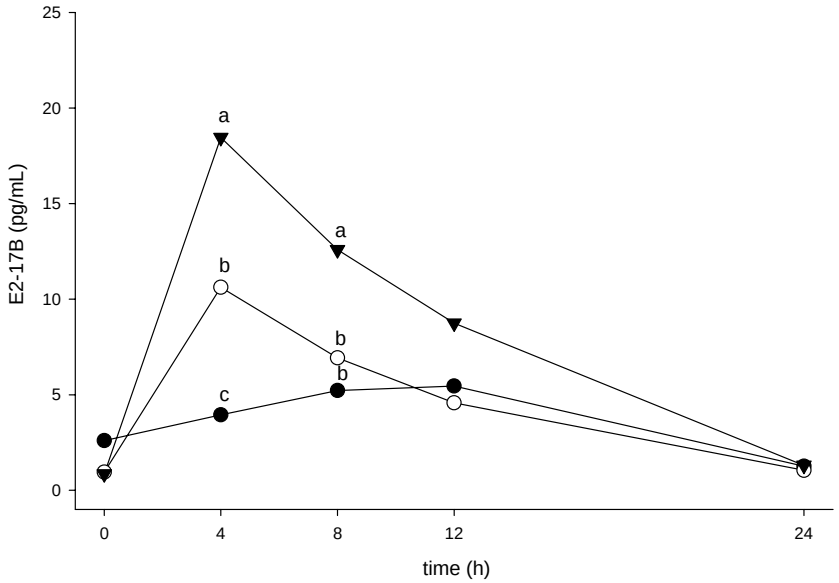


Figure 6. Experiment 2 - Mean circulating estradiol-17β (E-17β) concentrations in cows with ovulatory follicles for control (●), 0.5 mg of E-17β (○), and 1.0 mg E-17β (▼). Hour 0 is the time of estrogen treatment. Different letters within each time are different (P < 0.05).

Table 2. Characteristics (mean ± S.E.M.) of the serum estradiol-17β (E-17β) profile in lactating cows treated with two doses of E-17β in the presence of a dominant follicle (Experiment 2).

End point	E-17β (1.0mg)	E-17β (0.5mg)	Control
Animals (n)	8	8	8
Size of ovulatory follicle (mm)	16.6 ± 1.1 ^{a}	17.1 ± 1.6 ^{a}	16.1 ± 1.2 ^{a}
Treatment to maximum (h)	5.0 ± 0.6 ^{a}	5.5 ± 1.0 ^{Aa}	9.5 ± 1.0 ^{Bb}
Maximum concentration (pg/ml)	18.5 ± 4.6 ^{a}	10.6 ± 2.2 ^{Ab}	5.46 ± 1.6 ^{Bb}
LSM* - Area under curve (pg^2)	202.7 ^{Aa}	113.7 ^{B}	93.1 ^{Cb}

$^{a, b}$ Means within a row with different superscripts are different (P < 0.05).
^{A,B,C} Indicates a tendency for a difference between means (P < 0.10).
* Least Square Means

Discussion

To our knowledge, this is the first experiment to characterize the estradiol-17β profile following treatments with different dosages of commonly used estrogens in high-producing dairy cows. In experiment 1, the influence of endogenous E-17β on the circulating concentrations of E-17β was minimized because of frequent follicular aspirations. This allowed characterization of the changes in circulating E-17β concentrations due solely to the estrogen treatment. Experiment 2 was designed (based on the information from experiment 1) to evaluate whether E-17β supplementation during Ovsynch, a commonly used timed AI protocol, could produce physiological concentrations of E-17β in order to prepare for future field studies to determine the effect of E-17β supplementation on the reproductive efficiency of lactating dairy cows.

Clearly, the circulating E-17β profiles following these three estrogen treatments are very different. Treatment with E-17β resulted in a rapid increase in E-17β concentrations with a short interval from treatment to peak concentrations. Furthermore, treatment with E-17β resulted in a more rapid decrease in E-17β after peak concentrations, reaching basal concentrations before 24 h after treatment. In contrast, EB treatment resulted in reduced peak concentrations and a more prolonged elevation in circulating E-17β until about 36 h after treatment. Surprisingly, we detected very little change in circulating E-17β following treatment with 1 mg of ECP; however, there

were some numerical increases between 28 and 40 h after treatment although not significant. The temporal differences in circulating E-17β following treatment with these three different types of estrogen (Experiment 1) are consistent with other published results (Vynckier *et al*., 1990; Lammoglia *et al*., 1998; Bo *et al*., 2000; Haughian *et al*., 2002; Burke *et al*., 2003; Martinez *et al*., 2005) although the absolute elevation in circulating E-17β and the duration of the elevation were greatly reduced in our experiments compared to previous experiments. For example, in the present study, treatment with 1 mg of EB elevated circulating E-17β to ~10 pg/ml whereas 0.5 mg elevated circulating E-17β to ~5 pg/ml. In contrast, Vynckier *et al*. (1990) reported an increase from 2 to 175 pg/ml following treatment of non-lactating cows with 10 mg of EB or an elevation of ~17 pg/ml for every 1 mg of EB. Even more dramatic differences were reported in post-partum beef cows with an elevation to ~40 pg/ml (Lammoglia *et al*., 1998) or ~30 pg/ml (Burke *et al*., 2003) following treatment with 1 mg of EB. Martinez *et al*. (2005) reported an elevation of ~100 pg/ml following 5 mg of EB or ~20 pg/ml for every mg of EB in ovariectomized beef cows. It is reasonable to suggest that the decreased concentrations of circulating E-17β in the present study were due to greater steroid metabolism of high-producing dairy cows (Sangsritavong *et al*., 2002). Nevertheless, the maximum peak concentration in the E-17β-treated group might have been higher if our first sample was taken earlier than 4 h post treatment. In addition, differences in assays or other technical aspects among the cited studies and the present study cannot be ruled out because direct comparisons of estrogen treatments in cows in different physiological states have not been reported.

Differences in molecular weight (kDa) between these different estrogen products (E-17β = 272, EB = 376, and ECP = 397) need to be considered when examining the comparisons between estrogens in this experiment and other experiments in the literature. These molecular weight differences result in a product that has 28% less estrogen in EB than E-17β and about 32% less estrogen in ECP than E-17β. The distinct differences in temporal patterns between the different estrogens are unlikely to be altered by these differences in absolute quantity of estrogen; however, the magnitude of the peak concentration would be expected to be somewhat lower in EB and ECP compared to E-17β treatment due to this difference in absolute quantity of estrogen in each mg of these estrogens.

Differences in the molecule polarity between these different estrogens are import factors that may determine its profile in the blood due to differences in the absorption and metabolism rates in the body. The half-life of a particular estrogen in the bloodstream also depends on its molecular structure and is mostly influenced by the polarity of the molecule. Polarity of a molecule depends mostly on the size of the molecule (bigger molecules tend to be less polar), symmetry of the polar covalent bonds (more symmetrical molecules are generally less polar), and the presence of aromatic rings (if present, the molecule is less polar). For instance, if esterification of the C-17 hydroxyl group occurs, the final molecule will be less polar (less hydrophilic) and will generally remain in the body for a greater time period because the metabolism of estrogen involves conversion to a more water-soluble compound for elimination in the urine and feces.

The reduction in detection of estrus, either due to management constraints (Lucy, 2001) or physiologically-related decreases in the duration of estrus (Lopez *et al*., 2004), has led to extensive use of timed AI protocols in lactating dairy cows. The original Ovsynch protocol (Pursley *et al*., 1995) induced synchronized ovulation of a dominant follicle using treatment with GnRH after synchronized luteolysis. During Ovsynch, circulating E-17β does not reach sufficient concentrations prior to the second GnRH injection to induce a GnRH/LH surge or estrus in most lactating dairy cows. For example, only ~20 % of lactating cows show estrus during the Ovsynch protocol (Pancarci *et al*., 2002). An alternative timed AI protocol, Heatsynch, has utilized ECP instead of GnRH to synchronize time of ovulation. Heatsynch produced similar (Pancarci *et al*., 2002; Kasimanickam *et al*., 2005) conception rates compared to Ovsynch in lactating dairy cows, even though many more cows are detected in estrus following Heatsynch (Pancarci *et al*., 2002; Kasimanickam *et al*., 2005). In our experiment, we focused on finding an estrogen treatment and dose that might be expected to produce a more physiological pattern of circulating E-17β during timed AI protocols. Even during normal estrus and ovulation, peak E-17β concentrations are reduced in lactating compared to non-lactating dairy cows (Sartori *et al*., 2002a; Lopez *et al*., 2004; Wolfenson *et al*., 2004). This problem of insufficient peak E-17β concentrations is magnified during the Ovsynch protocol because of the premature induction of the LH surge with exogenous GnRH. In Experiment 2, the control cows had peak circulating E-17β concentrations that were very low compared to reports from non-lactating cows but were similar to previous reports with lactating cows (Sartori *et al*., 2002a; Lopez *et al*., 2004). Thus, it seems logical to attempt to improve fertility during the Ovsynch protocol by supplementing with an optimal treatment of E-17β. Previous results in sheep have shown positive (Hawk and Cooper, 1975), negative (Langford *et al*., 1980), or no effects (Hawk and Cooper, 1975) of E-17β on fertility depending on dose of E-17β and type of semen used, making it imperative that the correct type and dose of estrogen be chosen.

An initial expectation is that the estrogen treatment should produce a rapid, physiological increase in circulating E-17β in order to potentially produce a positive effect on fertility by inhibiting premature $PGF_{2\alpha}$

release from the endometrium and thus maintaining a normal CL lifespan following a timed AI protocol (Mann and Lamming, 2000). Moreover, supplementation with either 0.5 or 1 mg of E-17β at 8 h prior to GnRH produced an increase in E-17β that was similar to or greater than peak E-17β concentrations in non-lactating cows (Vynckier *et al*., 1990; Sartori *et al*., 2002a; Wolfenson *et al*., 2004). This greater peak E-17β concentration might be expected to produce greater motility of the uterus and oviduct (Hawk, 1975), increased uterine blood flow (Krzymowski *et al*., 2004), and increased phagocytosis competence (Frank *et al*., 1983). These changes might improve the uterine environment and may result in positive effects on fertility.

During the normal cycle, peak E-17β concentrations induce estrus and the GnRH/LH surge. After that, there is a rapid decrease in circulating E-17β with basal concentrations being reached by 8 to 10 h after the beginning of the GnRH/LH surge (Komar *et al*., 2001; Haughian *et al*., 2004). Thus, a rapid return to basal circulating E-17β concentrations might be important to reduce any potential negative effects of high circulating E-17β concentrations on the uterine tract during sperm transport, ovulation, fertilization, or early embryo development. In Experiment 2, circulating E-17β returned to basal concentrations by the expected time of AI (24 h after E-17β treatment or 16 h after the LH surge). Thus, even though the current trial was not designed to evaluate the effects of E-17β treatment on fertility, supplementation with either 0.5 or 1 mg of E-17β produced a circulating profile that appears to mimic expected physiological concentrations in the absence of lactation and could be used in field trials designed to determine whether increasing circulating E-17β to normal concentrations prior to ovulation will produce improvements in fertility in timed AI protocols such as Ovsynch.

Contrasting responses in expression of estrus between the two trials is intriguing. Previous reports described the importance of sufficient concentrations of circulating E-17β for expression of estrus (Lammoglia *et al*., 1998). A recent study (Lyimo *et al*., 2000) reported a high correlation between serum E-17β concentration and behavioral estrus in lactating Holstein cows. None of the estrogen treatments alone, in the absence of estrogen produced by a dominant follicle, produced an E-17β pattern that was sufficient to produce standing estrus in cows in Experiment 1. In contrast, almost all (15/16) of the cows supplemented with 0.5 or 1 mg of E-17β displayed standing estrus when a dominant follicle was present. This result emphasizes the importance of endogenous E-17β in producing behavioral estrus during an E-17β-supplemented Ovsynch protocol. Any cows not displaying signs of estrus after estrogen treatment might be expected to have insufficient endogenous E-17β (lack of a dominant follicle) or excessive circulating progesterone (lack of CL regression) and this might be used to detect cows that were not correctly synchronized by the Ovsynch protocol. This may also explain the lower conception rates in ECP-treated cows (during Heatsynch) that did not show estrus (Pancarci *et al*., 2002; Cerri *et al*., 2004).

In conclusion, the circulating E-17β profiles after a single injection of estrogen depends on the type and dose of estrogen used. Moreover, these data suggest that 1.0 mg of E-17β may be successfully used to increase circulating E-17β during timed AI protocols without disrupting the normal decline in E-17β concentrations following the LH surge. Further studies are required to confirm this theory.

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