



## Effects of Standing Estrus and Concentrations of Estradiol on Uterine pH<sup>1</sup>

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#### Summary

Research has demonstrated that cows that are in estrus within 24 h of fixed-time AI had elevated concentrations of estradiol and greater pregnancy rates compared to cows that are not in estrus. Our objective was to determine if estradiol and/or estrus had an effect on uterine pH during a fixed-time AI protocol. Non-lactating beef cows (n = 20) were treated with the CO-Synch protocol (100µg GnRH on d -9; 25 mg PG on d -2; and 100µg GnRH on d 0). Half (n = 10) the cows received an injection of estradiol cypionate (ECP; 1mg) 12 h following PG. Cows detected in standing estrus within 24 h of the second GnRH injection were considered to be in standing estrus. Cows treated with ECP had greater concentrations of estradiol compared to non-treated cows ( $8.3 \pm 0.7$  and  $5.2 \pm 0.7$  PG/mL, respectively), and a treatment by time interaction influenced concentrations of estradiol. All cows had similar concentrations of estradiol at time of ECP, but ECP treated cows had elevated concentrations of estradiol following the second GnRH injection compared to control cows. Treatment, time, and treatment by estrus by time influenced uterine pH. All cows had a similar uterine pH 12 h after ECP, but control cows that did not exhibit estrus had a higher uterine pH compared to control cows that did exhibit estrus and ECP cows that exhibited estrus at time of the second GnRH injection, the time insemination would occur ( $\text{pH } 7.0 \pm 0.1$ ,  $6.7 \pm 0.1$ ,  $6.8 \pm 0.1$ , respectively). ECP cows not exhibiting estrus were intermediate ( $6.8 \pm 0.1$ ). All cows had a similar uterine pH from 24 h after time of insemination through ovulation. In

summary, ECP treatment elevated concentrations of estradiol and lowered uterine pH to a level similar to the uterine pH of control cows that exhibited estrus within 24 h of when insemination would occur.

#### Introduction

Several fixed-time insemination protocols utilize an injection of gonadotropin releasing hormone (GnRH) on d -9, an injection of Prostaglandin F<sub>2α</sub> (PG) on d -2, and a second injection of GnRH at the time of insemination (d 0). Previous research has shown animals in standing estrus within 24 h of fixed-time artificial insemination (TAI) had higher pregnancy rates compared to animals not in standing estrus (Perry et al., 2004, Perry et al., 2005). Furthermore, animals in standing estrus have elevated preovulatory concentrations of estradiol 36 h prior to when insemination would occur in a fixed time insemination protocol, and administering 1mg of Estradiol Cypionate (ECP) 12 h post PG increased concentrations of estradiol similar to animals in standing estrus (Perry and Perry, 2006). Animals in standing estrus (elevated preovulatory concentrations of estradiol) had a lower uterine pH compared to animals not in standing estrus (Elrod and Butler, 1993; Perry and Perry, 2006). Therefore, the objective of this experiment was to determine if estradiol and/or estrus had an effect on uterine pH during a fixed-time AI protocol.

#### Material and Methods

##### Experimental Design

Twenty cycling beef cows were synchronized with the CO-Synch protocol [GnRH (100 µg as 2 mL of OvaCyst i.m., IVX animal Health, St. Joseph MO) on day -9, PG (25 mg as 5 mL of ProstaMate i.m., IVX animal Health, St. Joseph, MO) on day -2, and on day 0 GnRH (100 µg, OvaCyst, i.m.)]. On day -2 an injection of 1 mg of ECP was administered to half of the animals (i.m. in 1 mL Sesame seed oil) 12 h after

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administration of PG. Control animals received a 1 mL injection of Sesame seed oil.

#### Transrectal Ultrasonography and Uterine pH

Uterine pH was determined in all animals on day -2 at time of the ECP injection, and 24, 48, 72 and 96 h after the PG injection. Uterine pH was determined by inserting a sterile, plastic infusion pipette through the cervix and into the uterus. Once the infusion pipette was in place, a flexible pH electrode (1.4 mm in diameter; Microelectrodes, Bedford, NH) was extended into the uterine body. A reference electrode was inserted into the vagina of the animals and both probes were left in place until a stable pH reading was obtained. Ovaries of all animals were examined by transrectal ultrasonography to record follicular development using an Aloka 500V ultrasound with a 7.5 MHz transrectal linear probe. All follicles ( $\geq 8$  mm) were recorded on day -2, at time of PG administration, and on day 0, at time of GnRH administration. At 48 h after the second GnRH injection, ovaries were examined to determine if ovulation had occurred. Ovulation was defined by the disappearance of a dominant follicle.

#### Blood Collection, Estrus Detection, and Radioimmunoassays

Blood samples were collected at the time uterine pH was determined on d -1, 0, 1, and 2 to determine circulating concentrations of estradiol. Blood samples were collected via venipuncture of the tail vein into 10-mL vacutainer tubes and allowed to clot at room temperature for 1 h before being placed in a 4°C refrigerator for 24 h. Samples were centrifuged ( $1200 \times g$ ) for 30

min. Serum was collected and stored at -20°C until radioimmunoassays (RIA) were performed. Circulating concentrations of estradiol were analyzed in all serum samples by RIA (Perry et al., 2004). Standing estrus was detected in all cows by visual observations or with the aid of Estrus Alert Patches (Western Point, Inc, Merrifield, MN).

#### Statistical Analysis

Differences in uterine pH and circulating concentrations of estradiol-17 $\beta$  were determined by analysis of variance for repeated measures in SAS (Proc Mixed, Littell et al., 1998).

### **Results and Discussion**

Cows treated with ECP had greater ( $P < 0.01$ ) concentrations of estradiol compared to non-treated cows ( $8.3 \pm 0.7$  and  $5.2 \pm 0.7$  pg/mL, respectively). A treatment by time interaction ( $P < 0.01$ ) influenced concentrations of estradiol (Figure 1). All cows had similar ( $P > 0.15$ ) concentrations of estradiol at time of ECP administration, but ECP treated cows had elevated ( $P < 0.02$ ) concentrations of estradiol following the second GnRH injection compared to control cows. This is similar to previous reports where administering 1 mg of ECP 36 h prior to the second GnRH injection resulted in preovulatory concentrations of estradiol similar to animals that spontaneously initiated standing estrus (Perry and Perry, 2006). In all animals concentrations of estradiol diminished after the second GnRH injection.

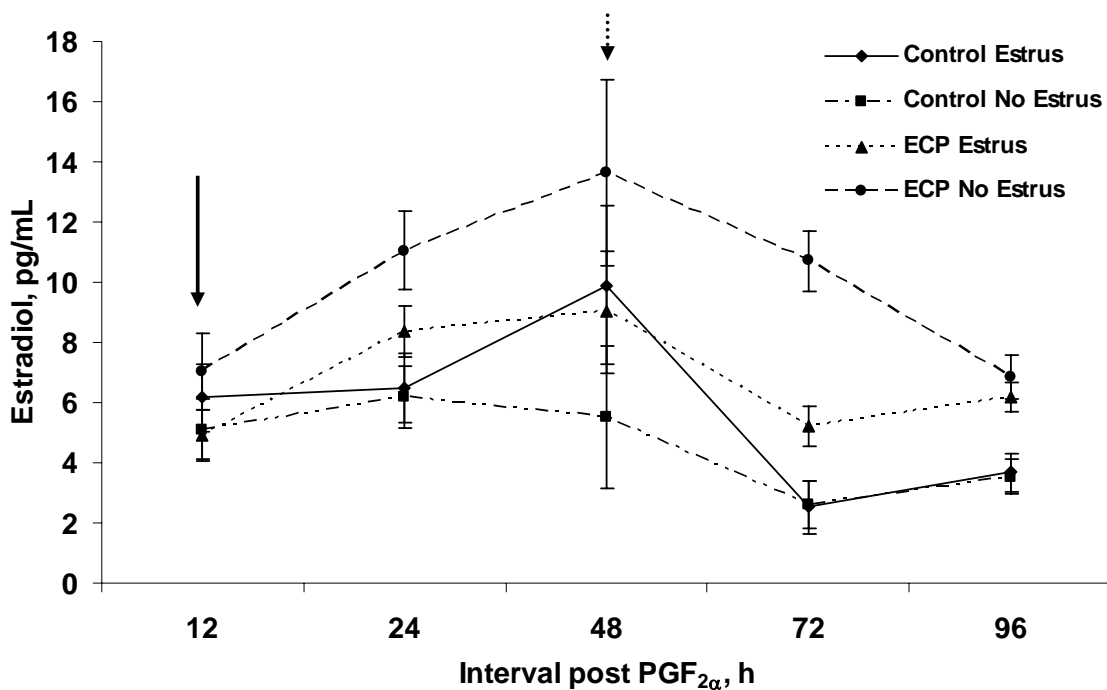


Figure 1. Circulating concentrations of estradiol for cows detected in standing estrus within 24 h of the second GnRH injection and cows administered ECP during the CO-Synch protocol. Estradiol Cypionate (solid arrow) was administered 12 h following the PG injection. Gonadotrophin Releasing Hormone (dashed arrow) was administered 48 h after the PG injection and at the time that insemination would occur.

Treatment ( $P = 0.01$ ) and time ( $P < 0.01$ ) both had a significant effect on uterine pH while treatment by estrus by time ( $P = 0.065$ ) tended to effect uterine pH (Figure 2). Control cows that did not exhibit estrus had a higher uterine pH compared to ECP cows that did not exhibit estrus ( $P = 0.03$ ) at time of ECP administration, but all cows had a similar uterine pH ( $P > 0.19$ ) 12 h after ECP. Control cows that did not exhibit estrus had a higher uterine pH compared to control cows that did exhibit estrus ( $P < 0.01$ ) and ECP cows that exhibited estrus ( $P = 0.05$ ) at time of the second GnRH injection, the time insemination would occur ( $7.0 \pm 0.1$ ,  $6.7 \pm$

$0.1$ ,  $6.8 \pm 0.1$ , respectively). ECP cows not exhibiting estrus were intermediate ( $6.8 \pm 0.1$ ). All cows tended to have a similar uterine pH beginning 24 h after the second GnRH injection through ovulation ( $P > 0.06$ ). This is consistent with previous research where uterine pH is lower in animals that are in standing estrus compared to animals not in standing estrus (Elrod and Butler, 1993; Perry and Perry 2006). However, concentrations of estradiol had no linear ( $P > 0.21$ ) or quadratic ( $P > 0.21$ ) relationship with uterine pH.

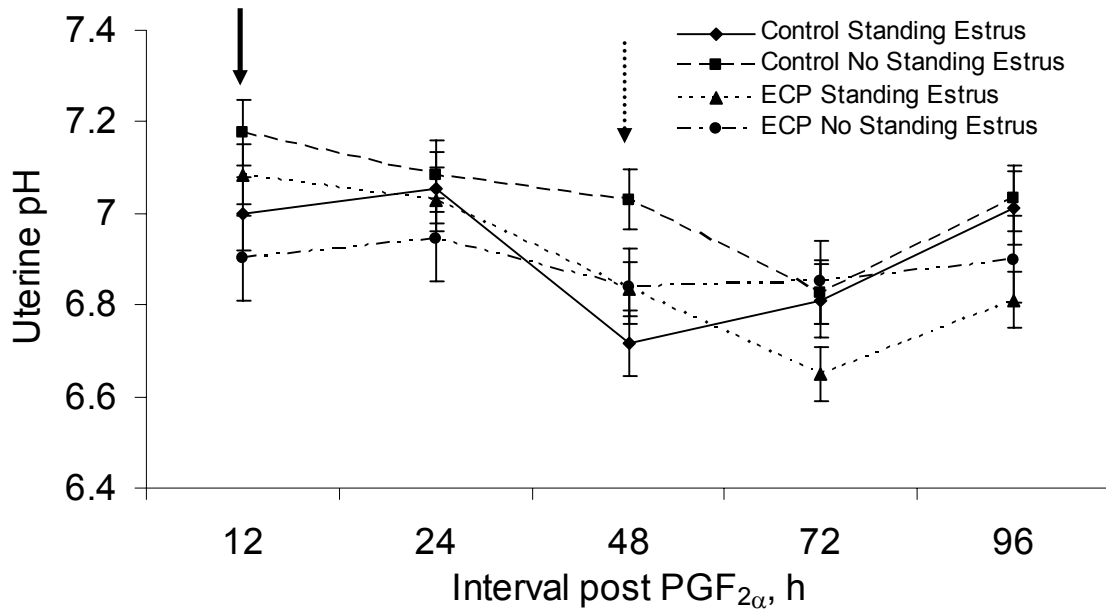


Figure 2. Effect of ECP and standing estrus on uterine pH for cows detected in standing estrus within 24 h of the second GnRH injection and cows administered ECP during the CO-Synch protocol. Estradiol Cypionate (solid arrow) was administered 12 h following the PG injection. Gonadotrophin Releasing Hormone (dashed arrow) was administered 48 h after the PG injection and at the time that insemination would occur.

In summary, ECP treatment not only elevated circulating concentrations of estradiol but also lowered uterine pH to level

similar to the uterine pH of animals who spontaneously exhibit standing estrus within 24 h of a fixed time artificial insemination.

#### Literature Cited

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