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# Letrozole: A Steroid-Free Estrous Synchronization Method

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### Summary and Implications

Most bovine estrous synchronization protocols utilize progesterone plus estrogen to control ovulation timing. A drug that inhibits estrogen production (Letrozole) may be an alternative, steroid-free synchronization method (not yet commercially available). However, low estrogen can negatively affect the health of follicles/oocytes and impact fertility. To determine its effects, Letrozole was administered intramuscularly while tracking follicle growth and circulating hormones. Letrozole response was variable. Two of three cows experienced delayed luteolysis/ovulation and extended progesterone production. This preliminary data indicates that Letrozole treatment allows normal follicle progression but drug response may vary and little is known about effects on oocyte quality.

### Introduction

The benefits of reliable estrous synchronization for timed artificial insemination are well known. Since estrous is controlled primarily by the cyclical nature of steroid hormones, most synchronization protocols administer steroids such as progesterone and estrogen to prevent ovulation until a desired time. However, there is a public desire to avoid hormone treatments in beef cattle including legal prohibition of estrogen use in some countries. For this reason, some researchers seek to devise new synchronization methods using pharmaceuticals that are not hormone-based but instead control the synthesis of hormones in the treated animal. Having a lower concentration of estrogen within the bloodstream prevents the sequence of events that result in ovulation (i.e. loss of the corpus luteum due to luteolysis, decreased circulating progesterone, and a surge in Luteinizing Hormone). Thus, some researchers have proposed Letrozole, an aromatase inhibitor that decreases estrogen production, as a steroid-free estrous synchronization method. These researchers have had success in controlling the timing of ovulation. However, having lower concentrations of estrogens inside the dominant ovarian follicle is associated with decreased oocyte quality and fertility. Plus, delaying ovulation with a method that changes gonadotropin pulsatility (as with altering circulating estrogen) can potentially cause the development of a persistent follicle and reduced quality of the oocyte within the follicle. To determine whether Letrozole treatment promotes persistent follicle formation, a pilot trial to test its effects on follicle development and circulating hormone concentrations was performed using a small group of beef cows.



### Procedure

All procedures were approved by the Animal Care and Use Committee at the University of Nebraska-Lincoln. Six nonlactating, composite beef cows (25% MARC III [¼ Angus, ¼ Hereford, ¼ Pinzgauer, ¼ Red Poll] and 75% Red Angus) ages 2-4 yr old from the beef physiology herd at the Eastern Nebraska Research and Extension Center (ENREC) were initially synchronized with two injections (25 mg/cow; i.m.; 12 h apart) of prostaglandin F2α (PGF2α) following by a second pair of PGF2α injections 14 d later. Transrectal ultrasound with an Aloka UST-5541-7.5 probe was conducted every other day for three weeks then daily for 50 d total, and the follicle and corpus luteum dimensions were measured along the longest axis and the perpendicular axis (the average of those two dimensions is presented). Ovulation was determined by the absence of the preovulatory follicle. Estrus was detected using Estrotect™ Heat Detector patches on the tail head. Blood samples were collected every other day for the first week then daily, and circulating progesterone was measured with radioimmunoassays performed in duplicate. For this experiment, Day 1 of the reproductive cycle was defined as the day of ovulation detection after the second set of PGF2α injections. Treatment (250µg/kg Letrozole in 8-10 mL of a 1:5 mixture of benzyl alcohol and sesame oil) or control injections (8 mL of 1:5 benzyl alcohol/sesame oil) began on Day 10 of the cycle.

### Results

When using Letrozole to inhibit aromatase activity, an important consideration is the success and degree of circulating estrogen suppression. The purpose of the drug is not to completely prevent all estrogen production but instead to prevent or delay the peak in estrogen production that occurs prior to ovulation. In the three treated cows, there was still a moderate amount of circulating estrogen during and after the treatment period. However, the peak of estrogen from the dominant follicle that must occur before ovulation occurs was delayed in two of the three treated cows (Figure 1, Treated Cows 1 and 2). This suggests the possibility that the processing of Letrozole by the liver may vary from animal to animal, thus making the effectiveness of the drug inconsistent.

The effects of Letrozole on the reproductive cycle were also variable, with the successful suppression of the estrogen peak corresponding with a delay in ovulation. Of the three control cows that received vehicle injections only, two of the cows had a two-follicular-wave cycle and one of them had a three-wave cycle (Figure 2). The intervals

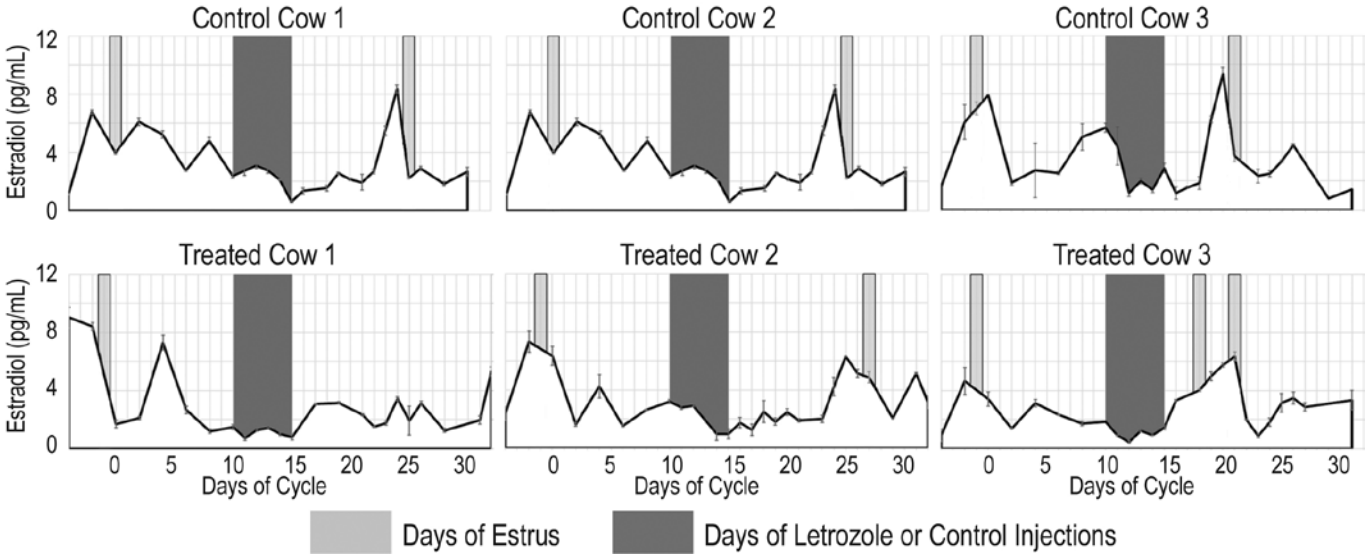


Figure 1. Letrozole aromatase inhibition delays the circulating estrogen peak that occurs prior to estrus and ovulation. The top three graphs depict the daily estradiol concentration (ng/mL) of the Control cows, and the bottom three graphs depict estradiol of the Letrozole-treated cows. The day of estrus is shown with a light-gray bar, and the six days of injections are depicted with dark-gray bars. Treated Cows 1 and 2 had delays in the pre-estrus estradiol peaks.

between ovulation for the control cows were 20 d, 25 d, and 26 d. Comparatively, one Letrozole-treated cow had a two-wave cycle with no delay in ovulation (Cow 3, 21 d), while the other two treated cows had three-wave cycles with ovulation intervals of 27 (Cow 2) and >32 (Cow 1, did not ovulate during the experimental period) (Figure 2). The ovulation delays were accompanied by an extended period of peak progesterone production and a delay in corpus luteum lysis. This suggests that the way in which Letrozole delays ovulation is by preventing/delaying luteolysis.

## Conclusions

Letrozole estrous synchronization is feasible because the decreased circulating estrogen delays luteolysis, possibly by inhibiting the animal's own PGF2α production. Commercial PGF2α can then be administered to trigger luteolysis and the subsequent ovulation event. The results of this study indicate that there is little risk to the developmental progression of the follicle from the Letrozole treatment, since the dominant follicle during the treatment period will either ovulate or undergo atresia rather than becoming a persistent or cystic follicle. However, a follicle that ovulates after being exposed to Letrozole may not contain a high quality oocyte. More research is needed to confirm that Letrozole does not adversely affect the health or capacity of the oocyte to be fertilized before it is incorporated into synchronization protocols. A controlled-release version of this drug (similar to a CIDR) has been patented, but this is not yet commercially available for timed AI purposes. This drug should not be recommended to producers until further research assures the health and quality of the oocyte within the follicle and until larger studies show that the animal- to-animal variability is acceptable.

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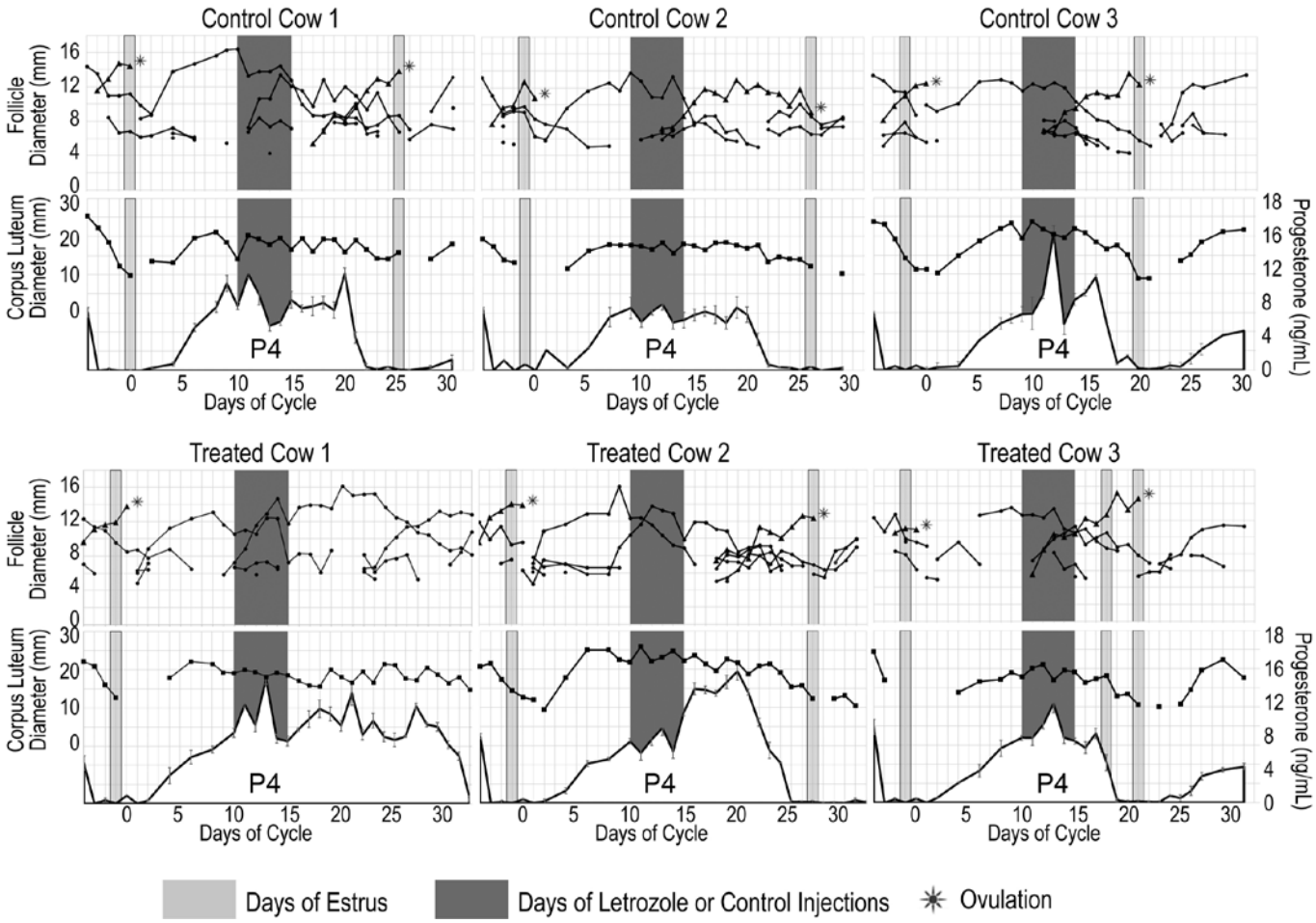


Figure 2. Letrozole-treated cows experience variable delayed ovulation and extended peak progesterone production. For the follicle diameter graphs, each point represents an individual follicle measurement and the tracked follicles are connected by lines. The top three graphs depict the ultrasound-tracked follicle measurements and ovulation (shown as a star-shaped icon) of the Control Cows, while the three graphs immediately below are the ultrasound-tracked corpus luteum diameter (primary vertical axis) and the circulating progesterone (ng/mL; secondary vertical axis) of the Control Cows. The six graphs at the bottom show the follicle measurements, corpus luteum measurements, and circulating progesterone for the Letrozole-treated cows. The day of estrus is shown with a light-gray bar, and the six days of injections are depicted with dark-gray bars. Treated Cows 1 and 2 had delayed luteolysis and extended peak progesterone concentrations.