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Synchronization of ovulation in cattle with an aromatase inhibitor–based protocol

M.J. Yapura [a,1], R.J. Mapletoft [b], R.A. Pierson [c], J. Singh [a], G.P. Adams [a,*]

[a] Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
[b] Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
[c] Department of Obstetrics, Gynecology, and Reproductive Sciences, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

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ABSTRACT

A study was designed to determine the effect of stage of the estrous cycle on the proportion of animals that ovulated and the synchrony of ovulation of heifers treated with an aromatase inhibitor–based protocol. Forty-eight heifers were treated intramuscularly with 500 μg of cloprostenol (PGF) followed by 100 μg of GnRH 24 hours later to serve as control data for comparison of the ovulatory response to a subsequent aromatase inhibitor protocol. Daily ultrasound examinations were done to determine the incidence of and interval to ovulation. At the time of ovulation (Day 0), heifers were assigned randomly to five day-groups (n = 8-11/group) and given an intravaginal device containing 3 g of letrozole for 4 days starting on Day 0, 4, 8, 12, or 16. At the time of device removal, heifers were given PGF followed by GnRH 24 hours later. Ultrasound examinations were done daily from 2 days before device insertion to 9 days after the posttreatment ovulation. The preovulatory follicle diameter after letrozole treatment was larger in the Day 4 group compared to the Day 0 and 16 groups and intermediate in the Day 8 and 12 groups ($P < 0.001$). Compared to control data, the percentage of heifers that ovulated after letrozole treatment was greater (87.1% vs. 69.4%, respectively; $P < 0.05$) as was the synchrony of ovulation (residuals: 0.24 ± 0.07 vs. 0.68 ± 0.13; $P < 0.01$). The day on which letrozole treatment was initiated did not affect the proportion of heifers that ovulated or the interval to ovulation. Plasma estradiol concentrations at the time of removal of the letrozole device in the Day 0 and 4 groups was lower ($P < 0.05$) than in the corresponding controls. Estradiol concentrations in the Day 8 and 12 groups did not differ from already low concentrations in the respective controls. Corpus luteum diameter profiles and progesterone production were not affected by day-group although reduced luteal lifespan after letrozole treatment was observed and requires further investigation. In summary, a protocol involving a letrozole-impregnated intravaginal device for 4 days, PGF treatment at device removal, and GnRH 24 later resulted in a greater ovulation rate and greater synchrony of ovulation than in heifers not given letrozole. Results suggest that the protocol may be initiated effectively at random stages of the estrous cycle and may provide impetus for further studies to assess the efficacy of a letrozole-based synchronization protocol for fixed-time insemination.

* Corresponding author. Tel.: +1 306 966 7411; fax: +1 306 966 7405.
E-mail address: gregg.adams@usask.ca (G.P. Adams).
[1] Present address: IVABS, Massey University, Tennent Drive, Palmerston North 4412, New Zealand.

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1. Introduction

The overall demand for treatments used to control the estrous cycle in cattle may be illustrated by estimates of the use of artificial insemination (AI) and embryo transfer (ET). A conservative estimate of the worldwide use of AI is 83 million cows per year, representing about 20% of the breedable cattle population [1]. In addition, approximately 1 million embryos are produced worldwide each year by *in vivo* and *in vitro* fertilization [2], involving approximately 200,000 donors and 1 million recipients. Assuming that synchronization treatments are used for only 10% of cows that are artificially inseminated and 50% of donors and recipients used for ET (conservative numbers), a total of 8.9 million synchronization treatments are given annually. Furthermore, the use of synchronization treatments may be expected to expand further with the current growth of *in vitro* embryo production in cattle where numbers have increased 100-fold over the past 10 years [2].

Estrogen-based protocols have enabled producers to control the timing of ovulation reliably, enabling efficient use of time, labor, and resources by allowing prescheduled AI. These protocols have also been used to optimize the productivity of superovulation and ET programs by effectively synchronizing follicular wave emergence before the initiation of superstimulatory treatment [3–7]. However, the use of estradiol has been banned in many countries (i.e., European Union, New Zealand, and Australia) [8,9]. This situation has created a void in treatments that efficiently control ovarian dynamics for the purpose of fixed-time insemination and ET in cattle.

Aromatase inhibitors have been investigated as a tool with which the estrous cycle in cattle might be controlled with the same precision as the older estradiol-based protocols but with the advantage of being a steroid-free alternative [10,11]. Studies conducted in cattle have focused on the use of letrozole, a nonsteroidal aromatase inhibitor that inactivates the aromatase enzyme responsible for the synthesis of estrogens by reversibly binding to the "heme" group of the P450 subunit. Letrozole is indicated as an adjuvant or the first-line treatment for hormone-dependent breast cancer in postmenopausal women [12] and has been used in assisted reproduction in women because of its potential effect on removing the negative feedback of estradiol on FSH secretion [13–15].

The intravaginal route of administration of letrozole is of particular interest because it allows for extended treatment protocols, is minimally invasive, reduces animal handling and stress, and is most likely to be accepted by practitioners and producers [16]. In an earlier study [11], we tested a prototype of an intravaginal device for providing an extended period of letrozole treatment. However, letrozole was released too rapidly and plasma levels were near baseline within 24 hours after device insertion [11]. In a subsequent experiment, we modified the vehicle formulation, and two variant intravaginal devices were tested in cattle to determine their pharmacokinetic characteristics. A wax-based vehicle with a higher melting point than the previous gel-based vehicle provided a more steady and continuous delivery of letrozole over a 5-day treatment period in heifers, and the addition of a letrozole-containing gel coating resulted in more rapid initial absorption that hastened the increase on plasma concentrations of the active ingredient [17]. This device (letrozole-containing wax-based plus gel coat) reduced plasma estradiol concentrations resulting in increased follicular growth and lifespan, without adversely affecting progesterone production in the subsequent CL [17]. We concluded that letrozole-impregnated intravaginal devices formulated with a wax base plus a gel coat vehicle was most suitable for the application of a letrozole-based protocol for the synchronization of ovulation in cattle.

The main findings of studies involving the use of letrozole in cattle were that it extended the lifespan of the dominant follicle, resulting in a delay in emergence of the next follicular wave and/or a delay in the timing of ovulation. Letrozole treatment also had a consistent luteotrophic effect (i.e., larger CL and/or more progesterone) which may be useful for enhancing embryo development and reducing embryonic loss [10,11,18,19]. Results provide rationale for continued development of aromatase inhibitor-based synchronization protocols for cattle.

The objectives of this study were to determine the effect of a letrozole-based synchronization protocol on ovulation rate and synchrony in heifers treated with letrozole at different stages of the estrous cycle. We hypothesized that the addition of a letrozole-impregnated intravaginal device to a PGF- and GnRH-based protocol, initiated at random stages of their estrous cycle, would increase the percentage and synchrony of ovulations in cattle over PGF and GnRH alone.

2. Materials and methods

2.1. Cattle

Hereford-cross beef heifers ($n = 49$), 15 to 20 months of age and weighing between 379 and 667 kg (mean of 505 $\pm$ 8.6 kg), were chosen from a group of 51 heifers maintained in outdoor pens at the University of Saskatchewan Goodale Research Farm (52° North and 106° West). Heifers were fed alfalfa/grass hay and grain to gain approximately 1.3 kg/day and had water available ad libitum during the experimental period from December to February. Heifers were initially examined by transrectal ultrasonography (MyLab5 VET; Canadian Veterinary Imaging, Georgetown, Ontario, Canada) to confirm that they were postpubertal by observing the presence of a CL [20]. Animal procedures were performed in accordance with the guidelines of the Canadian Council on Animal Care and were approved by University of Saskatchewan Protocol Review Committee.

2.2. Treatments

Heifers in which a CL was detected during the initial examination ($n = 49$) were treated intramuscularly with 500 μg of cloprostenol (PGF, Estrumate; Schering-Plough Animal Health, Pointe-Claire, Quebec, Canada) to induce luteolysis, followed by 100 μg of GnRH (Fertiline;