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Aromatase inhibitors: A new approach for controlling ovarian function in cattle

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ABSTRACT

Numerous treatments and protocols have been used to control the reproductive cycle in cattle, with varying effectiveness and many involving the administration of steroid hormones. Steroid hormones, such as estradiol, are perceived as having a negative impact on consumer health. This internationally shared opinion has led to a ban on the use of steroid hormones in food producing animals in many countries (i.e., European Union, New Zealand, and Australia). Letrozole, a non-steroidal aromatase inhibitor, inactivates the aromatase enzyme responsible for the synthesis of estrogens by reversibly binding to the "heme" group of the P450 subunit. Letrozole is approved as an adjuvant or first-line treatment for hormone-dependent breast cancer in post-menopausal women, but has been used increasingly for ovulation induction in the treatment of infertility in women. Using the bovine model to determine the effects on ovarian function, letrozole treatment was found to extend the lifespan of the dominant follicle and thereby delay emergence of the next follicle wave and/or ovulation. Letrozole treatment also had a luteotrophic effect; that is, larger CL and/or higher circulating concentrations of progesterone were detected in letrozole-treated heifers. Results of the initial studies in cattle provided the impetus for the development of aromatase inhibitor-based synchronization and fertility treatment in cattle. Biologically active concentrations of letrozole were achieved via intravenous, intramuscular or intravaginal administration, but the intravaginal route of administration is of particular interest because it permits extended and defined treatment periods, is minimally invasive, and reduces animal handling. Recent results revealed that irrespective of the stage of the cycle, a 4-day letrozole-based protocol induced ovulation in a significantly greater proportion of animals and with significantly greater synchrony than the control treatment. Evidence and reasons for the increasing use of programmed breeding and fixed-time artificial insemination are discussed in this review as a background to current development of an innovative aromatase inhibitor-based protocol as a safe and effective method of controlling the estrous cycle and ovulation in cattle.

1. Background

The 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 involving approximately 200,000 donors and 1 million recipients are produced world-wide each year by *in vivo* and vitro fertilization [2]. Assuming that synchronization treatments are used for only 10% of cows that are artificially inseminated (i.e., 2% of world population) and 50% of donors and recipients used for embryo transfer (conservative numbers), a total of 8.9 million synchronization treatments are given annually. More recent data from Brazil shows that the use of AI has increased sharply in the last 6 years to more than 10 million cows per year. More than 50% are now done by fixed-time AI (FTAI); that is, >6 million cows are synchronized per year in Brazil alone [3]. Further, the use of synchronization treatments is expected to

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expand with the current increase of *in vitro* embryo production in cattle where numbers have risen 100-fold over the last 10 years [2].

The identification of prostaglandin F2α (PGF) as the luteolysin responsible for regression of the corpus luteum (CL) in cattle provided a new means for controlling the length of the luteal phase and ovulation (reviewed in Ref. [4]). Several protocols involving different doses and intervals between doses of prostaglandins have since been designed [5,6], but the effectiveness of PGF-based synchronization is limited by two things: 1) The growing CL is refractory to PGF during the first 5–6 days after estrus [5], and 2) PGF does not influence the state of follicular readiness to respond to an LH surge. The state of maturity of the dominant follicle at the time of PGF-induced luteolysis will determine the interval to estrus and ovulation which ranges from 1 to 6 days [5,6]. Two doses of PGF 11–14 days apart is a common practice on many dairy and beef farms based on the rationale that approximately 67% of the animals (those with a CL $\geq$ 5 day-old or those experiencing natural luteal regression) should respond to the first PGF treatment and 100% should have a functional, PGF-responsive CL when the second dose is administered. The use of luteolytic doses of prostaglandin, however, still relies on estrus detection efficiency to provide acceptable outcomes. Herd heat detection rates range from 30% to 65% in high producing dairy farms [7,8] and between 50% and 70% in commercial beef farms [7,9]. Consequently, the rate of submission of animals for AI after detected estrus limits the effectiveness of the PGF protocol.

The use of FTAI can overcome the negative impact of low estrus detection efficiency, but implies effective synchrony of luteal function as well as synchronous growth and ovulation of a viable dominant follicle. Pregnancy rates obtained with FTAI may be comparable or better than those obtained after AI with estrus detection because all animals are inseminated regardless of whether they displayed estrus [10]. Gonadotropin releasing hormone (GnRH) induces pituitary release of gonadotropins (LH and FSH) and ovulation – an effect that has formed the basis of common synchronization protocols used today.

In early studies, a 10-day synchronization program involving a 6-day interval between GnRH and PGF treatment resulted in a pregnancy rate that was similar to controls, and a second GnRH treatment 36–48 h after PGF treatment improved the precision of ovulation and permitted fixed-time insemination without adversely affecting pregnancy rates [11,12]. The GnRH-PGF-GnRH protocol was coined “Ovsynch” and came into common use for FTAI in dairy [10,13] and beef cattle [14]. A limitation of GnRH-based protocols, however, is that emergence of a new follicular wave is synchronized only if GnRH treatment causes ovulation [15]. In later studies, the first GnRH treatment was found to induce ovulation in only 44–54% of dairy cows [16,17], and in only 56% of beef heifers [15] and 60% of beef cows [18]. If the first GnRH does not synchronize follicular wave emergence, ovulation following the second GnRH may be poorly synchronized [19], resulting in disappointing pregnancy rates following FTAI [20].

High progesterone concentration prior to artificial insemination in fixed-time synchronization protocols such as Ovsynch increases pregnancy rates in lactating dairy cows (5–10% improvement) [21–23]. External sources of progesterone, such as CIDR, Cue-Mate or PRID, are usually used to increase circulating concentrations of the hormone. Although the exact mechanism behind the improved pregnancy outcomes is not fully understood, it may be related to prevention of early ovulations (due to spontaneous CL regression prior to PGF treatment) [23], enhanced oocyte maturation and/or delayed PGF release from the uterus post-breeding [22,24].

The driving force behind a dramatic increase in the use of AI in Brazil was the adoption of estrogen-based protocols for controlling follicle development and synchronizing ovulation in cattle These protocols have enabled producers to control the timing of ovulation reliably, enabling efficient use of time, labour and resources by allowing pre-scheduled artificial insemination [3,25–28]. This method of synchronizing wave emergence is based on the negative feedback effect that estradiol has on FSH secretion during the luteal phase or under the influence of exogenous progesterone [25,26,29]. Exogenous progesterone suppresses LH secretion and growth of the dominant follicle in a dose-dependent manner [30]. Demise of the primary source of FSH suppression (i.e., the extant dominant follicle) then allows FSH to surge again which in-turn elicits the emergence of a new wave of follicles beginning about 4 days after estradiol and progesterone treatment [25,31].

While steroid-based synchronization protocols are effective, increasing consumer sensitivity to the possible deleterious effects of estrogens in food and in the environment [32] has led to new regulations about the use of estrogenic products in livestock. The European Union has already banned the use of estrogenic products in food producing animals [33–36]. In United States [37] and Canada [38], estrogens cannot be used for synchronization of estrus except by prescription and custom-compounding. In 2007, New Zealand and Australia banned use of estrogens in lactating dairy animals [36]. The ban of the use of estrogens in livestock and lack of commercially availability of estrogenic preparations negatively impacts the implementation of reproductive biotechnologies in cattle production systems, limiting potential reproductive efficiency and genetic improvement provided by the use of AI or multiple ovulation and embryo transfer [36].

2. Aromatase inhibitors

The aromatase enzyme, $P450_{arom}$, belongs to the super-family of P450 proteins which includes more than 480 members divided in 74 different families. $P450_{arom}$ is a unique member of family 19 [39], and is located in the endoplasmic reticulum of mammalian cells that express the CYP19 gene, such as adipose tissue, brain, adrenal glands, gonads, liver and placenta [40–42]. $P450_{arom}$ contains a heme group in its structure and is functionally associated with another member of P450 cytochrome family, NADPH reductase, which acts as a donor of reductive equivalents [39]. It is responsible for catalyzing the final, rate-limiting step in the production of estrogens (estrone and estradiol) from C_{19} substrate (androstenedione and testosterone).

Aromatase inhibitors have been classified as Type I or Type II based on their chemical structure and mechanism of action. Steroidal aromatase inhibitors (Type I) are compounds derived from androstenedione. These compounds bind irreversibly to the active site of $P450_{arom}$ inducing a covalent change on the structure of the enzyme which results in lasting and selective inhibition. Because of their irreversible effects, these compounds are also called “suicide inactivators”. Formestane and exemestane are examples of Type I inhibitors [43]. Non-steroidal aromatase inhibitors (Type II) are compounds that have a nitrogen containing heterocyclic moiety, the triazole group, as a common characteristic of their chemical structure. These inhibitors bind to the heme group in the $P450_{arom}$, occupying part of the active binding site of the enzyme and interfering with enzyme activity in a reversible way. Examples of these Type II estrogen synthetase-specific inhibitors are letrozole, anastrozole, and fradozole [43].

Since steroidal aromatase inhibitors mimic the structure of androstenedione, they have been associated with undesired mild androgenic effects. Furthermore, the irreversible nature of the inhibitor-enzyme interaction implies that recovery of estrogen production will be delayed after treatment is stopped because it is dependent on *de novo* synthesis of $P450_{arom}$ protein [44]. Exemestane, letrozole and anastrozole are the most recently

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