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Comparison of the effects of pretreatment with Veramix sponge (medroxyprogesterone acetate) or CIDR (natural progesterone) in combination with an injection of estradiol-17β on ovarian activity, endocrine profiles, and embryo yields in cyclic ewes superovulated in the multiple-dose Folltropin-V (porcine FSH) regimen

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ABSTRACT

Follicular wave status at the beginning of exogenous FSH administration is an important contributor to variability in superovulatory responses in ruminants. Studies in ewes have shown a decrease in the number of ovulations when superovulation is initiated in the presence of ostensibly ovulatory-sized ovarian follicles. Hormonal ablation of large antral follicles with the progestin–estradiol (E_2-17β) treatment significantly reduces this variability in superovulated anestrous ewes, but the effects of the treatment in cycling ewes have not yet been assessed. Sixteen Rideau Arcott × Polled Dorset ewes (November–December) received either medroxyprogesterone acetate (MAP)–releasing intravaginal sponges (60 mg) or controlled internal drug release (CIDR) devices (containing 300 mg of natural progesterone) for 14 days (Days 0–14), with a single intramuscular injection of 350 μg of E_2-17β on Day 6. The superovulatory treatment consisted of six injections of porcine FSH (Folltropin-V) given twice daily, followed by a bolus GnRH injection (50 μg intramuscular) on Day 15. There were no differences ($P < 0.05$) in the ovulatory responses and embryo yields between the two groups of ewes. In both subsets of animals, the next follicular wave emerged ~2.5 days after an E_2-17β injection ($P > 0.05$). A decline in maximum follicle size after an E_2-17β injection was more abrupt in CIDR- compared with MAP-treated animals, and the ewes pretreated with exogenous progesterone had significantly more 3-mm follicles at the start of the superovulatory treatment. The metabolic clearance rate of exogenous E_2-17β appeared to be greater in MAP-treated ewes, but circulating concentrations of porcine FSH failed to increase significantly after each

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Folltropin-V injection in CIDR-treated animals. The CIDR-treated ewes exceeded ($P < 0.05$) their MAP-treated counterparts in serum E_2-17β concentrations during superovulation. In spite of differences in antral follicle numbers and endocrine profiles between MAP- and CIDR-treated cyclic ewes receiving E_2-17β before ovarian superstimulation, there were no differences in superovulatory responses.

1. Introduction

Assisted reproductive technologies are widely used in the modern agricultural industry to improve livestock genetics and enhance reproductive efficiency [1–4]. In sheep, hormonal ovarian stimulation is used in multiple ovulation and embryo transfer (MOET) programs to maximize the number of viable embryos per cycle of treatment [4]. Multiple ovulation and embryo transfer typically entails an initial progestin pretreatment to synchronize estrus and ovulations after hormonal ovarian stimulation, combined with the administration of supraphysiological doses of exogenous gonadotropins to induce the growth and ovulation of multiple antral follicles, and subsequent surgical embryo recovery from the reproductive tract of donor animals [4]. The outcome of superovulatory treatments in ewes is highly variable as ovarian responses and embryo yields are dependent on several intrinsic and extrinsic factors [5–10]. Extensive research has resulted in improved ovarian stimulation protocols [7,11–15]; however, in spite of numerous technical advancements, the variable responses still continue to limit the use of superovulation in commercial and research-allied applications.

Ovine antral follicles develop in a wave-like manner. A rise in circulating FSH concentrations triggers the simultaneous emergence of one to four small antral follicles (follicular wave) that grow to an ostensibly ovulatory size before regression or ovulation [16–18]. Three to four waves of follicular growth typically occur during the ewe's estrous cycle, with an interwave period of 3 to 5 days [16–20]. The rhythmic emergence of follicular waves continues throughout the seasonal anestrus in ewes [21]. The ovulatory response and total number of transferable embryos after superovulation are strongly affected by the size and number of antral follicles present in the ovary at the outset of the superovulatory treatment [3,5,6]. Differences in follicular wave status at the beginning of exogenous FSH administration appear to be one of the major contributors to the variability in superovulatory responses in sheep [3,5,6,8,22,23]. Several authors postulated that large ovarian follicles exerted a dominant effect in sheep by reducing FSH availability *via* estradiol and inhibin A secretion, thereby suppressing the growth of smaller, gonadotropin-dependent antral follicles [5,22–25]. Studies have shown a decrease in the number of ovulations in ewes when superovulation was begun in the presence of ovulatory-sized ovarian follicles [3,5,22,23]. A potential codominant effect of the two largest antral follicles (F1 and F2) has also been suggested as the physiological status of F1 and F2 at the outset of the superovulatory regimen in cyclic ewes has been shown to affect the ovulation rate and embryo recovery [25]. In contrast to these observations, more recent investigations into the growth dynamics of the two largest follicles detected at the start of superovulation in seasonally anovular ewes showed no difference in superovulatory outcomes regardless of follicular size or stage of the lifespan [6]. Follicular dominance in sheep remains controversial due mainly to the inconsistent evidence present in the available literature [18].

Progestin–estradiol combination treatment synchronizes follicular wave emergence in cattle and significantly reduces the variability associated with superovulatory treatments initiated at random stages of follicular wave development [26,27]. Few studies have evaluated the effects of both exogenous steroids on follicular wave kinetics in sheep. In anestrous ewes, the progestin–estradiol treatment enhanced the regression of large antral follicles and caused synchronous wave re-emergence approximately 5 days after estradiol-17β (E_2-17β) administration [27,28]. The synchronization of follicular waves with medroxyprogesterone acetate (MAP) and E_2-17β before superovulation reduced the variability in superovulatory responses without compromising embryo yields in seasonally anovular ewes [7,9]. Medroxyprogesterone acetate–releasing intravaginal sponges are made of a polyurethane material containing 60 mg of synthetic progestin and are inserted near the os cervix. Before this study could be performed in cyclic ewes, MAP-sponges were discontinued. Controlled internal drug release (CIDR) devices are an alternative progestin source commonly used for estrus synchronization in cattle. The CIDR is an elastic rubber insert containing 300 mg of natural progesterone (P_4) and sits in the mid-vagina. Controlled internal drug release devices have only recently been approved for commercial use in sheep; however, their usefulness in cattle and goats offers a promising alternative for the follicular wave synchronization with the progestin–estradiol treatment in ewes [29].

No attempt has been made to date to compare the outcomes of superovulatory treatments in cycling ewes after the MAP sponge or CIDR priming in combination with E_2-17β injections. It would be of value to assess an exogenous progestin alternative that may be used to further investigate the effect of follicular wave synchronization pretreatments on superovulatory yields in sheep. Determining an effective synchronization pretreatment and the associated ovarian and hormonal functions in cyclic ewes may also offer significant advantages to MOET programs; improved understanding as well as subsequent control and manipulation of these variables may aid in the development of an optimal superovulatory approach that will accurately and reliably produce consistent results in sheep and other species of veterinary interest. Hence, the main objective of the present study was to determine and