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Effect of altering the intervals between consecutive superovulatory doses of porcine follicle-stimulating hormone on ovarian responses and embryo yields in anestrous ewes

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ABSTRACT

The effect of varying intervals between successive gonadotropin injections on the superovulatory outcomes in anestrous Rideau Arcott ewes superstimulated for ovarian follicular development with multiple doses of porcine FSH (pFSH) was evaluated in a single study. Twenty-five animals received six (1×2.5 ml and 5×1.25 ml) injections of Folltropin®-V given at 0800 and 1600 h or at 0800 and 2000 h in Group 1 ($n = 9$) or Group 2 ($n = 16$), respectively. An i.m. injection of 500 IU of equine chorionic gonadotropin (eCG; Folligon®) was given concurrently with the first pFSH dose. Time of estrus was synchronized among ewes with intravaginal sponges containing 60 mg of medroxyprogesterone acetate (Veramix®) that were left in place for 14 days; sponges were removed at the time of the 5th pFSH injection. Six days after insertion of MAP sponges, all ewes received an i.m. injection of estradiol-17β dissolved in 1 ml of sesame oil (350 μg/ewe) to synchronize follicular wave emergence. Following the last pFSH dose, all animals were given a single i.m. injection of 50 μg of gonadotropin-releasing hormone (GnRH; Cystorelin®) to induce ovulations before placing in a pen with four fertile rams for 36 h. The ovarian responses were assessed and embryos recovered surgically 7 days after GnRH injections. The mean number of corpora lutea was greater ($P < 0.05$) in Group 1 compared with Group 2 ewes (21.0 ± 2.9 compared with 10.4 ± 1.6, respectively; mean ± SEM) but there was no difference ($P > 0.05$) in the number of transferable embryos (5.4 ± 2.4 compared with 5.4 ± 1.3/ewe, respectively), and Group 1 animals had significantly more degenerated embryos than Group 2 ewes (2.6 ± 1.2 compared with 0.6 ± 0.3/ewe, respectively). A superovulatory protocol wherein pFSH injections were given at 0800 and 1600 h was more effective in terms of inducing multiple ovulations than the protocol with 12-h intervals between consecutive pFSH doses, but it was not associated with an increased production of transferable quality embryos by anestrous ewes.

1. Introduction

Intensive research efforts aimed to establish effective superovulatory protocols and to augment the application of multiple ovulation and embryo transfer (MOET)

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programs, laparoscopic ovum pick up (LOPU), and other related reproductive technologies in small ruminants have constantly been in demand (Candappa and Bartlewski, 2011; Bartlewski et al., 2016). The viability rate of *in vitro*-produced (IVP) embryos is less than that of embryos obtained using MOET procedures (Paramio, 2010) and the MOET-derived embryos have greater cryotolerance compared with IVP-derived embryos (Massip et al., 1995). Therefore, even though both technologies can be employed to maximize genetic gain in livestock species (Paramio, 2010; Candappa and Bartlewski, 2011) and in conservation programs (Souza et al., 2011), superovulatory treatments remain a primary choice to produce multiple embryos.

A majority of superovulatory protocols currently used are cumbersome and may be associated with undesirable or residual effects (e.g., ovarian hyperstimulation, ovarian cysts, luteinized unovulated follicles, etc.; El-Gayar and Holtz, 2005; Bartlewski et al., 2015, 2016). The most frustrating aspect of problems with superovulatory treatments is tremendous variability in ovulatory responses and embryo yields among individual animals (Bartlewski et al., 2016). One possible reason for such adverse effects and unpredictability of superovulatory outcomes is the dose and frequency of gonadotropin injections given to donor animals (Baldassarre and Karatzas, 2004). Previous studies in estrous cycling and seasonally anovular ewes (Duggavathi et al., 2004, 2005; Barrett et al., 2006, 2007) have shown that there exists a threshold concentration of follicle-stimulating hormone (FSH) that needs to be surpassed to stimulate the entry of small antral follicles into waves of follicular development (i.e., follicles growing synchronously to ostensibly ovulatory diameters before regression or ovulation; Bartlewski et al., 1999, 2011); typically, a follicular wave in sheep consists of 1–3 follicles. Truncation of FSH peaks effectively prevents follicle-wave emergence, but injections of physiologic concentrations of ovine FSH (oFSH) re-initiate follicle-wave emergence in ewes (Barrett et al., 2006, 2007). Two injections of exogenous oFSH given 8 h apart induced the emergence of a new follicular wave approximately 0.5 days after treatment, even in the presence of large antral follicles from the previous wave of ovarian follicular development (Duggavathi et al., 2004, 2005). During the 3- or 4-day superovulatory protocols in ewes, consecutive FSH doses are usually administered every 12 h (Bartlewski et al., 2016). Variability in responses to superovulatory treatments suggests that, at these regular intervals, the increases in serum concentrations of FSH may not be sufficiently adequate to consistently induce the emergence of multiple ovarian follicles, especially after the removal of exogenous progestin source, which results in increased FSH clearance rates (Bartlewski et al., 2008b). This problem could potentially be eliminated by increasing a dose of FSH per injection or more frequent administration of exogenous FSH. Increasing the FSH dose may result in ovarian hyperstimulation in some donor animals. Shortening the intervals between two consecutive FSH injections given on the same day, therefore, appears to be a more desirable option to attain the peak concentration of the gonadotropin capable of stimulating the emergence and growth of ovarian antral follicles.

The main objective of the present study was to compare superovulatory responses in anestrous ewes subjected to two protocols differing in the timing of exogenous FSH injections (given every 12 h or daily at 0800 and 1600 h). Anestrous sheep were chosen for this trial as the consistency for superovulatory response is poor in seasonally anovular ewes (Bartlewski et al., 2008a).

2. Materials and methods

2.1. Animals and superovulatory procedures

The present experimental procedures were in compliance with the policies and guidelines established by the Canadian Council on Animal Care (CCAC) for animal research, and had been approved by the Animal Care Committee at the University of Guelph. This study performed at mid-anestrus (May-June) utilized 25 clinically healthy multiparous Rideau Arcott ewes. Animals were kept outdoors (with an easy access to indoor facilities) at the field research station in Ponsonby near Guelph, ON, Canada (43°37' N, 80°21' W) and were fed daily maintenance diets of alfalfa pellets with hay, water and mineral licks available *ad libitum*. All ewes were fitted with intravaginal sponges containing medroxyprogesterone acetate (60 mg; Veramix®, Pfizer Animal Health, Kirkland, QC, Canada) that were kept in place for 14 days (see Fig. 1 for details of experimental design). At 6 days after insertion of MAP sponges, all ewes were given an i.m. injection of estradiol-17β (Longwing International, Oakville, ON, Canada) dissolved in 1 ml of sesame oil (350 μg/ewe) to synchronize time of follicular wave emergence. The ensuing superovulatory treatment consisted of 6 i.m. injections of porcine FSH (pFSH: Folltropin®-V, Bioniche Animal Health Canada Inc., Belleville, ON, Canada; 2.5 ml × 1 and 1.25 ml × 5), given at approximately 0800 and 1600 h (Group 1, $n = 9$) or 0800 and 2000 h (Group 2, $n = 16$). An i.m. injection of 500 IU of equine chorionic gonadotropin (eCG; Folligon®, Intervet Canada Ltd., Whitby, ON, Canada) was given concurrently with the first pFSH dose. MAP sponges were removed at the time of the 5th pFSH injection. Following the last pFSH dose and 24 h after MAP sponge withdrawal, all animals received a single i.m. injection of 50 μg of gonadotropin-releasing hormone (GnRH; Cystorelin®, Merial Canada Inc., Baie d'Urfe, QC, Canada) to synchronize the time of the preovulatory LH surge and ovulations among animals, and were relocated to a pen with Rideau Arcott rams fitted with crayon marking harnesses for the next 36 h (rams to ewes ratio of 1:4). All rams used in this experiment underwent routine breeding soundness evaluation ~3 weeks before superovulation of ewes in this study and were classified as satisfactory.

2.2. Embryo collection and grading

The number of corpora lutea (identified on the basis of projections from the surface of the ovary and the presence of the ovulatory stigmata; Bartlewski et al., 2008a,b) was recorded at laparotomy 7 days after GnRH injections. Food and water were withheld for 24 h before surgery. Surgical