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Superovulation in waptiti (*Cervus elaphus*) during the anovulatory season

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ABSTRACT

The objective was to evaluate the efficacy of three previously unreported ovarian superovulatory treatment protocols in wapiti. Protocols were initiated specifically at the time of ovarian follicular wave emergence, and intended to enable determination of the effects of frequency of treatment (i.e., animal handling) and LH supplementation on ovarian response. Thirteen parous wapiti hinds, 2 to 4 y of age, were used late in the anovulatory season (July). The ovaries were examined daily by transrectal ultrasonography. Hinds were given 5 mg estradiol 17-β im (day of treatment designated as Day 0) to induce a new wave of ovarian follicular development. On the expected day of wave emergence (Day 3), hinds were assigned randomly to three treatment groups and given: (1) 100 mg FSH im once a day for 4 days (N = 5); (2) 200 mg FSH sc on Day 3 and Day 5 (N = 4); or (3) 200 mg FSH plus 2.5 mg LH sc on Day 3 and Day 5 (N = 4). All hinds were given 10 mg LH im on Day 6 to induce ovulation. The mean (± SEM) number of ovulations per animal in the respective groups was 6.2 ± 2.0, 15.5 ± 5.9, and 14.8 ± 2.7. In conclusion, the technique of inducing follicular wave emergence to initiate superovulatory treatment at the time of wave emergence was effective in wapiti during the anovulatory season. The most efficient and effective method of ovarian superovulation in this study involved administration of estradiol 17-β on Day 0, followed by 200 mg FSH sc on Days 3 and 5, and induction of ovulation (10 mg of LH) on the evening of Day 6. Compared with conventional methods that require 14 days and handling the hinds six times, the protocol used herein reduced the treatment period to 8 days and the number of animal handlings to four.

1. Introduction

Artificial insemination has been similarly successful in red deer and the conspecific wapiti [1–3], but the same is not true with embryo transfer. Red deer have been reported to produce an average of three recipient pregnancies per donor [4], which is unmatched in wapiti [5,6]. Also, superovulation during the anovulatory season in wapiti has apparently not been reported.

The conventional method of ovarian superstimulation in red deer includes placement of a progesterone-releasing device (CIDR) intravaginally for 12 days to synchronize estrous cycles [7]. Superstimulatory doses of FSH are then given twice daily, beginning on the eighth day after CIDR placement and ending 12 to 24 h after CIDR removal [4]. The period of CIDR placement is intended to be sufficiently long to ensure regression of any existing CL before CIDR removal. A common addition to the protocol is eCG, which is intended to reduce the variability in response [7–9]; it was often given with the last FSH treatment, but others reported improved results when given with the first treatment of FSH [10].

Ovarian follicular wave status at initiation of superstimulatory treatment influences superstimulatory response in cattle [11–15] and sheep [16]. Ovarian response was reduced when treatment was initiated during follicular dominance, versus treatment initiated at follicular wave emergence. Treatment as little as 1 day after wave

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emergence significantly reduced the superstimulatory response compared with treatment initiated the day before or the day of wave emergence [13,14]. The influence of follicular wave status at the time of ovarian superstimulation has apparently not been evaluated in cervid species.

Ovarian follicular wave emergence has been induced in cattle by removal of follicular dominance using exogenous hormones (estrogen and progesterone) [17–20] or physical ablation by transvaginal ultrasound-guided follicle aspiration [21]. Both methods have improved the response to superstimulatory treatment in cattle [17,22,23]. One study in red deer [9] lends support to the hypothesis that the same relationship between follicular dominance and superstimulatory response exists in cervids, because ovulation rates were higher in hinds in which treatment was initiated 1 day after expected ovulation (i.e., coincident with wave emergence) [24] than in hinds treated later. Results of another study in wapiti documented the effectiveness of a method to electively induce follicular wave emergence directly, without the necessity of inducing ovulation [24].

Handling stress has been identified as suppressive to the superstimulatory response in wapiti [6]. In red deer, fallow deer, and white-tailed deer, the adrenal gland has been identified as a source of progesterone [25–27]. Release of progesterone as well as cortisol has been associated with animal handling [26]. Progesterone suppresses the growing phase of large follicles in a dose-dependent manner [28] by suppressing LH pulse frequency [29], whereas cortisol can suppress the preovulatory LH surge [30]. Another factor is the level of LH activity in superstimulatory gonadotropin preparations. Better results were obtained when wapiti were superstimulated with an FSH preparation that contained LH rather than a more purified preparation [6]. Empirically, eCG has been incorporated in conventional superstimulation protocols in cervids because of its FSH- and LH-like activity [31].

The present study was designed to evaluate the efficacy of three previously unreported ovarian superovulatory treatment protocols in wapiti during the anovulatory season. Protocols were initiated specifically at the time of follicular wave emergence, and intended to enable determination of the effects of frequency of treatment (i.e., animal handling) and LH supplementation on ovarian response.

2. Materials and methods

Thirteen parous wapiti hinds, 2 to 4 y of age, were used during July (late anovulatory season). The hinds were maintained on alfalfa pasture in a 4-hectare pen on a farm near Saskatoon, Saskatchewan, Canada (52°07′N, 106°38′W). Hinds were moved from the pen through a 300 m long alley to a handling facility, and then restrained in a squeeze chute for examination and treatment. Ovaries were imaged by transrectal ultrasonography using a 7.5 MHz linear-array transducer (Aloka SSD500, Tokyo, Japan). During examination, drawings of each ovary were made to record the size and location of all follicles ≥4 mm in diameter [32,33].

Hinds were given 5 mg estradiol 17-β dissolved in 1 mL of canola oil im [17] (treatment designated as Day 0) to induce a new wave of follicle development. On Day 3, the expected day of wave emergence [34], hinds were assigned randomly to three superstimulatory treatment groups and given: (1) 100 mg FSH (Folltropin-V, 20 mg/mL; NIH-FSH-P1, Bioniche Inc., Belleville, ON, Canada) im once a day, in the morning, for 4 days (FSH daily; N = 5); (2) 200 mg FSH sc (caudal to the midpoint of the left scapula, a location chosen to reduce variation because of body condition) on the morning of Day 3 and Day 5 (D3&5; N = 4); or (3) a treatment designed to replicate a previously commercially available less pure form of FSH that was anecdotally reported to be more successful at superstimulation in wapiti: 200 mg FSH plus 2.5 mg Armour standard of LH (Lutropin-V, Bioniche Inc.) sc (caudal to the midpoint of the left scapula) on the morning of D3&5 (FSH/LH D3&5; N = 4). All hinds were given 10 mg of LH im on the evening of Day 6 to induce ovulation. Ultrasonographic examinations of the ovaries were done once daily from Days 0 to 3 and from Days 6 to 8. Ovulation was defined as having occurred when a follicle ≥6 mm identified the previous day could no longer be identified at subsequent examinations.

Procedures used in this study were approved by the University of Saskatchewan Committee on Animal Care and Supply, in accordance with the principles outlined by the Canadian Council on Animal Care.

2.1. Data analysis

Data are presented as mean ± SEM. Comparisons among groups were made using ANOVA. When no difference was found among groups, specific groups were combined to increase numbers for statistical inference, and compared with the remaining group by Student *t* test. Statistical tests supported the assumptions that the data were normally distributed and had homogeneity of variance.

3. Results

Ovarian follicular superstimulation was evident in all hinds in the study. The number of follicles ≥6 mm and the maximum follicle diameter on Day 7 was similar among groups (Table 1). Only one hind (in the FSH D3&5 group) failed to ovulate by Day 8, despite development of 11 follicles ≥6 mm in diameter. Multiple ovulations were detected in all other hinds on Day 8. No differences among groups were detected in the number of ovulations or the number of unovulated follicles on Day 8 (Table 1). After removal of data from the single anovulatory hind, there tended to be more follicles ≥6 mm on Day 7 ($P < 0.07$) and more than twice the number of ovulations ($P < 0.05$) in the two groups treated with FSH on D3&5 (combined) than in the group treated daily (Table 1).

4. Discussion

The study was conducted in the late anovulatory season of the annual reproductive cycle [35]. This interval is advantageous because calves can be weaned from the hinds before treatment, thereby reducing animal handling problems. In addition, producing embryos before the onset of