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Effects of eCG and progesterone on superovulation and embryo production in wood bison (Bison bison athabascae)

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ABSTRACT

Experiments were done to determine if inclusion of eCG and progesterone in the superstimulation protocol will increase the ovarian response and embryo production in wood bison, and to provide preliminary information regarding the effect of season. In Experiment 1 (anovulatory season), bison (n = 26) were synchronized by follicular ablation (Day −1) and given FSH on Days 0 and 2, and assigned to 3 groups: Progesterone (Days 0–4), eCG (Day 3), or progesterone + eCG. On Day 5, bison were given hCG and inseminated 12 and 24 h later. Ova/embryos were collected 8 days after hCG. In Experiment 2 (ovulatory season), bison (n = 24) were synchronized and assigned randomly to two groups in which superstimulation was induced with FSH, either with or without eCG, as in Experiment 1. No differences among groups were found in ovarian response or embryo production in either experiment. The follicular count at wave emergence was positively correlated with the number of large follicles at the end of superstimulation in all groups. A significantly greater number of follicles present at wave emergence in the anovulatory vs. ovulatory season was associated with a greater number of CL at the time of embryo collection, but only half the number of freezable embryos. In conclusion, the number of transferable embryos collected (1–2/bison) was higher than in any previous report, but was not attributable to the inclusion of eCG or progesterone in the superovulatory protocol. The apparent effect of season on oocyte competence, and not superovulatory response, is worthy of further investigation.

1. Introduction

Bison herds in Wood Buffalo National Park (WBNP) in northern Alberta, Canada, are the most genetically diverse in the world (Wilson et al., 2005). However, wood bison herds in and around the park are infected with brucellosis and tuberculosis (Joly and Messier, 2001, 2004a, 2005). The interaction between the effects of these cattle-derived diseases and predation may account for the decline and stasis of the wood bison population since the early 1970s (Joly and Messier, 2004b). Further decline in the population jeopardizes the genetic diversity and long-term viability of wood bison (Wilson et al., 2005; McFarlane et al., 2006; COSEWIC, 2013). Therefore, reproductive technologies (i.e., superovulation and embryo transfer) are being developed in wood bison in an effort to preserve their genetic diversity (Toosi et al., 2013; Palomino et al., 2016).

Embryos produced through superovulation may be conserved in germplasm biobanks to provide a measure of insurance against

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the loss of genetic diversity (Wildt, 1992; Holt and Pickard, 1999; Solti et al., 2000). Conventional protocols for superovulation in cattle include administration of FSH twice daily for 4–5 days (i.e., eight treatments; Mapletoft et al., 2002). The lack of success in early superovulatory attempts in bison was attributed to the stress of multiple handling required for FSH treatments (Dorn, 1995; Othen et al., 1999). However, superstimulatory treatments in these early studies were initiated without regard to follicular wave status, which may also have contributed to the poor response in bison. By avoiding the suppressive effect of the dominant follicle on subordinate follicles within a follicular wave (Adams et al., 1993), the ovarian superstimulatory response in cattle is higher when treatment is initiated near the time of follicular wave emergence (Nasser et al., 1993; Adams et al., 1994). In this regard, induction of new wave emergence has enabled self-appointed scheduling of superstimulatory treatment to optimize the ovarian response in cattle (Bó et al., 2008).

In a recent series of studies designed to test the efficacy of less frequent superstimulatory treatment protocols in wood bison, significantly more ovulations were induced using a 2-dose vs. 4-dose protocol, and the response to a single dose of FSH diluted in 1% hyaluronan was not different from a 2-dose protocol (Toosi et al., 2013). However, the ovarian response was greater in wood bison given a 2-dose versus single dose protocol using FSH diluted in 0.5% hyaluronan (Palomino et al., 2016). Despite improvement in the superovulatory response in wood bison, the embryo collection rate remains less than 1 transferable embryo per bison, regardless of season (ovulatory or anovulatory; Palomino et al., 2016). The addition of low doses of eCG to conventional FSH superovulatory protocols has been used in Bos indicus cattle to increase the ovulatory response and embryo collection rate (Mattos et al., 2011). Equine chorionic gonadotropin is a glycoprotein secreted by the endometrial cups of the equine placenta (Allen and Moor, 1972). It has both FSH- and LH-like effects and has a long circulating half-life of 3–5 days in cattle (Murphy and Martinuk, 1991). The provision of extra LH-like activity may promote maturation and ovulatory capacity of multiple follicles induced by superstimulatory treatment.

Results of studies regarding the requirement for progesterone prior to or during superstimulatory treatment have been contradictory. Early studies in cattle suggested that circulating concentrations of progesterone at the time of the FSH treatment was positively related to the superovulatory response and number of transferable embryos (Goto et al., 1987, 1988), but treatments were initiated without regard to follicular wave status. In a later study, the ovarian response and embryo collection rate did not differ when the FSH treatment was initiated at the beginning of the first follicular wave (low progesterone) or second follicular wave (high progesterone) of the estrous cycle in cattle (Adams et al., 1994). Recently, however, a greater number of embryos were collected from superstimulated Nelore cows treated with exogenous progesterone vs no progesterone after synchronization of follicular wave emergence (Nasser et al., 2011). In wood bison, circulating concentrations of progesterone remain low (approximately 1 ng/mg) throughout the anovulatory season (Matsuda et al., 1996). Thus, we speculate that exogenous progesterone may be needed during the anovulatory season to optimize production of viable embryos in wood bison.

The objectives of the present study were to determine if a low dose of eCG at the end of the superstimulation protocol improves the ovarian response and embryo production rate in superstimulated wood bison during ovulatory and anovulatory seasons, and if exogenous progesterone improves embryo production during the anovulatory season. In addition, the study provided the opportunity to examine the effect of season on the superovulatory response and embryo production in wood bison.

2. Materials and methods

2.1. Facility and bison

The experiments were performed at the Native Hoofstock Centre, University of Saskatchewan (52°08′N, 106°38′W), Saskatoon, Canada, during the anovulatory season (Experiment 1) and the ovulatory season (Experiment 2). Wood bison (6–10 years old) with an average body condition score of 3.5 (scale of 1–5; Vervaecke et al., 2005) were used in this study. The bison had free access to supplemental hay and fresh water, and were handled according to protocols approved by the University of Saskatchewan's Animal Research Ethics Board.

2.2. Experiment 1 (anovulatory season, May–June)

To determine the effects of eCG and exogenous progesterone during ovarian superstimulation, wood bison ($n = 26$) were assigned randomly to three groups in which ovarian superstimulatory treatment was augmented by i) progesterone ($n = 8$), ii) eCG ($n = 9$), or iii) progesterone and eCG ($n = 9$; Fig. 1). Before ovarian superstimulation, follicular wave emergence (Day 0) was induced by ultrasound-guided aspiration of follicles ≥5 mm in diameter (follicular ablation, Day −1), as described previously (Palomino et al., 2014). Ovarian superstimulation was induced with pFSH (400 mg Folltropin-V diluted in 10 ml of 5 mg/ml of hyaluronan, Vetoquinol NA Inc., Lavaltrie, Québec, Canada) given intramuscularly on Day 0 (300 mg) and Day 2 (100 mg). A progesterone-releasing intravaginal device (PRID, Vetoquinol Inc.) was inserted in the vagina on the day of follicular ablation in the progesterone-treated groups. On Day 3, the eCG-treated groups were given 400 IU of eCG intramuscularly. On Day 4, the PRID was removed from respective groups and on Day 5 bison in all groups were given 3000 IU of hCG (Chorulon, Merck Animal Health, Kirkland, Quebec, Canada) intramuscularly. The bison were artificially inseminated with chilled semen (5 °C) 12 and 24 h after hCG treatment. Embryos were collected non-surgically 8 days after hCG treatment (Fig. 1).

Ovarian status and response were evaluated by transrectal ultrasonography using a 7.5 MHz linear-array transducer (MyLab Five, Esaote North America Inc., Indianapolis, IN, USA) as previously described in wood bison (McCorkell et al., 2013; Palomino et al., 2014). The ovaries were examined on Day 0 to detect wave emergence, on Days 5, 6, and 7 to detect ovulation, and on Day 13 to