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Superovulation in wood bison (*Bison bison athabascae*): Effects of progesterone, treatment protocol and gonadotropin preparations for the induction of ovulation

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ABSTRACT

Experiments were done to determine the ovarian response and embryo production following superstimulation of wood bison. In Experiment 1 (Anovulatory season), the efficacy of pLH vs. hCG for inducing ovulation was compared in wood bison superstimulated with a single dose of pFSH in 0.5% hyaluronan and the effect of exogenous progesterone (PRID) on superovulatory response and embryo quality was examined. In Experiment 2 (Ovulatory season), the efficacy of pLH vs. hCG for the induction of ovulation was compared in wood bison superstimulated with pFSH in a single intramuscular dose vs. a two-dose regimen 48 h apart (split dose) in 0.5% hyaluronan. In Experiment 1, the number of CL was greater ($P < 0.05$) in bison treated with hCG than pLH (6.6 ± 1.8 vs. 2.8 ± 0.8) and in those that were not given PRID (6.0 ± 1.5 vs. 2.7 ± 1.0). There was no effect of progesterone treatment on embryo quality. In Experiment 2, the number of CL was greater ($P < 0.05$) in bison treated with hCG than with pLH (6.3 ± 0.8 vs. 3.8 ± 1.2) and in bison superstimulated with split dose vs. single dose of FSH (7.1 ± 0.9 vs. 3.0 ± 0.8). The number of ova/embryos and freezable embryos did not differ among groups in either experiment. In conclusion, hCG induced a greater ovulatory response than pLH in both seasons. Two doses of FSH induced the greatest superovulatory response during the ovulatory season. Exogenous progesterone did not improve embryo quality during the anovulatory season.

1. Introduction

Wood bison (*Bison bison athabascae*) herds in the Wood Buffalo National Park represent the greatest source of genetic diversity of this threatened species (Wilson et al., 2005; COSEWIC, 2013). At only 6% of their estimated historic population of 168,000 (Joly and Messier, 2001), further loss of genetic diversity threatens the ability of extant wood bison to adapt to environmental changes or survive adverse stochastic events (Frankham 2005; McFarlane et al., 2006). Recovery of this genetic resource, however, is complicated by the existence of endemic tuberculosis and brucellosis within herds in Wood Buffalo National Park. These zoonotic diseases have been implicated in the lack of population growth of wood bison and, perhaps more importantly, resulted in regulations that prevent their

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expansion into former ranges outside the Park (Mitchell and Gates, 2002; Shury et al., 2015).

Superovulation and embryo transfer are technologies that may be used for reclamation of threatened and endangered species (Wildt, 1992; Holt and Pickard, 1999; Loskutoff et al., 1995; Solti et al., 2000). In bison, the first attempts at superovulation involved the use of bovine protocols, but results were disappointing (Dorn et al., 1990; Robison et al., 1998; Othen et al., 1999). In one study, 14 plains bison were treated with FSH to induce superovulation and only 5 transferable embryos were collected (Dorn et al., 1990). These modest results were attributed to a poor superstimulatory response, likely associated with the stress of handling (Dorn, 1995). In a later study, however, the superovulatory response did not differ in plains bison treated with multiple follicle stimulating hormone (FSH) treatments vs. a single treatment with equine chorionic gonadotropin (eCG; Othen et al., 1999). On average, one corpus luteum (CL) per cow was found in both groups ($n = 5$ bison/group), but embryo collection was not done. In another study in plains bison (Robison et al., 1998), a single dose of eCG ($n = 16$) resulted in a mean of 2 CL and 0.6 transferable embryos per animal. More recently, we found that the superstimulatory response (i.e. number of follicles >5 mm at the end of treatment) in wood bison treated with eCG was approximately half that of bison treated with FSH during the ovulatory season (5.6 vs. 12.2, respectively; Palomino et al., 2014b) or anovulatory season (8.1 vs. 14.6, respectively; Palomino et al., 2013). Consistent with the premise that the stress of multiple handling events in a wild species is deleterious to a superovulatory response (Solti et al., 2000), we found that two doses of FSH given subcutaneously induced a greater ovarian response than twice daily intramuscular treatments in wood bison (Toosi et al., 2013). Additionally, a single dose of FSH diluted in a slow release formulation (hyaluronan) resulted in a superovulatory response that did not differ from the two-dose regime (Toosi et al., 2013).

Although the ovulation-inducing effects of gonadotropin realeasing hormone (GnRH) and porcine luteinizing hormone (pLH) were not compared directly in our previous study in wood bison (Toosi et al., 2013), results of separate experiments reported therein provided rationale for the hypothesis that pLH was more effective than GnRH in inducing ovulation in superstimulated wood bison (i.e., mean of 8 vs. 3 CL per bison, respectively; Toosi et al., 2013). The use of human chorionic gonadotropin (hCG) for inducing ovulation has been examined in superstimulated beef and dairy cattle (Madill et al., 1994; Bo et al., 2006), but no reports were found on the use of hCG in bison. Interestingly, hCG was more effective than pLH for inducing ovulation in non superstimulated wood bison (Palomino et al., 2015).

Wood bison are seasonal breeders (McCorkell et al., 2013a; Palomino et al., 2013; Palomino et al., 2014b) and superovulation has been attempted only during the ovulatory season (Dorn et al., 1990; Toosi et al., 2013). Superovulation during the anovulatory season has been reported in a number of seasonal species including sheep (Barrett et al., 2004; Iida et al., 2004), goats (Baril and Vallet, 1990), and wapiti (McCorkell et al., 2013b), but has not been reported in bison yet. Studies in cattle suggest that embryo development is affected by circulating concentrations of progesterone (Goto et al., 1987; Goto et al., 1988; Nasser et al., 2011), and since endogenous progesterone is low throughout the anovulatory season in wood bison (Matsuda et al., 1996), we speculated that exogenous progesterone may be needed to obtain viable embryos in wood bison during the anovulatory season.

To improve the ovarian response and embryo production in wood bison, we evaluated novel superovulatory treatment protocols during the anovulatory and ovulatory seasons. During the anovulatory season (Experiment 1), the objectives were to determine the necessity of exogenous progesterone during ovarian superstimulation and compare the ovulation-inducing effects of pLH vs. hCG. During the ovulatory season (Experiment 2), the objectives were to compare the response to a single vs. split intramuscular dose of FSH in hyaluronan, and the ovulation-inducing effects of pLH vs. hCG.

2. Materials and methods

2.1. Animals

The experiments were done during the anovulatory season (May-June, Experiment 1) and the ovulatory season (September–October, Experiment 2) in the same year. Female wood bison (6–10 years old), with an average body condition score of 3.5 (scale of 1–5; Vervaecke et al., 2005), were maintained in pens (6–8 bison/pen) at the Native Hoofstock Centre, University of Saskatchewan. Animals were fed with alfalfa/grass hay and fresh water *ad libitum* and supplemented with approximately 1.5 kg/head/day of a mix of oats and barley. Protocols and use of bison were approved by the University of Saskatchewan's Animal Research Ethics Board, and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

2.2. Experiment 1 (anovulatory season)

A 2×2 factorial design was used to examine the effects of exogenous progesterone during ovarian superstimulation and the ovulatory response to pLH vs. hCG. (Fig. 1). Emergence of a new follicular wave was induced in 20 female wood bison by ultrasound-guided aspiration of all follicles $\geq$5 mm in diameter (follicular ablation), as described previously (Bergfelt et al., 1997; Palomino et al., 2014a). On the day after follicular ablation (i.e., expected day of wave emergence; Day 0), the bison were given a single intramuscular dose of 400 mg of pFSH (Folltropin-V, Bioniche Animal Health Canada Inc., Belleville, Ontario, Canada) diluted in 10 mL of 0.5% hyaluronan (5 mg/mL, MAP 5, Bioniche Animal Health). Half of the bison were given a progesterone-releasing intravaginal device (PRID, Vetoquinol Inc., Quebec, Canada) containing 1.55 g of progesterone in a stainless steel spiral covered with an inert silicone rubber matrix on Day 0. The PRID were removed on Day 4, and on Day 5, bison in each group (with or without progesterone) were divided into two subgroups and given either 25 mg of pLH (Lutropin-V, Bioniche Animal Health) or 3000 IU of hCG (Chorulon, Merck Animal