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In vitro-production of embryos using immature oocytes collected transvaginally from superstimulated wood bison (*Bison bison athabascae*)

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ABSTRACT

Two experiments were done to test the hypothesis that morphologic characteristics of wood bison cumulus-oocyte complexes (COC) are reflective of the ability of the oocyte to develop to an advanced embryonic stage after *in vitro* maturation, fertilization and culture, and to determine the effect of prolonging the interval from the end of superstimulation treatment to oocyte collection (FSH starvation period). Experiments were done during the anovulatory season. In Experiment 1, ovarian superstimulation was induced in 10 bison with two doses of FSH given at 48 h intervals beginning at the time of follicular wave emergence. COC were collected 3 days (72 h) after the last dose of FSH by follicular aspiration and classified as compact, expanded or denuded. The COC were matured *in vitro* for 24 h before fertilization *in vitro* (Day 0). Embryo development was assessed on Days 3, 7 and 8. The blastocyst rate was 7/34, 2/10 and 0/3 in COC classified as compact, expanded and denuded, respectively; however, only compact COC resulted in embryos that reached the expanded blastocyst stage. In Experiment 2, COC were collected at either 3 or 4 days (72 or 96 h) after the last dose of FSH (n = 16 bison/group) to determine the effect of the duration of FSH starvation on oocyte competence. The COC were classified as compact good (>3 layers of cumulus cells), compact regular (1–3 layers of cumulus cells), expanded or denuded, and then matured, fertilized and cultured *in vitro*. Although follicles were larger ($P < 0.05$) in the 4-day FSH starvation group, there was no effect of starvation period on the distribution of COC morphology; overall, 112/194 (57.7%) were compact, 29/194 (26.3%) were expanded, 39/194 (20.1%) were denuded, and 14/194 (7.2%) were degenerated ($P < 0.05$). Similarly, there was no effect of starvation period on embryo development. Compact good COC had the highest cleavage (88%) and blastocyst rates (54%; $P < 0.05$), followed by compact regular COC at 73% and 25%, respectively. Expanded and denuded COC had low cleavage (40% vs. 59%, respectively) and blastocyst rates (5% vs. 8%, respectively). We conclude that morphologic characteristics of wood bison COC are reflective of the ability of the oocyte to develop into an embryo *in vitro*. Importantly, oocytes collected from superstimulated bison during the anovulatory season were competent to develop to the blastocyst stage following *in vitro* maturation, fertilization and culture.

1. Introduction

Wood bison (*bison bison athabascae*) are the largest terrestrial mammal in North America. This species is listed as Threatened under Schedule I of the Canadian Species at Risk Act [1]. The population in Wood Buffalo National Park in Canada represents the largest, most genetically diverse reserve of wood bison in the world [2,3], but 10 of the 12 free-ranging herds have an on-going disease

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prevalence of 30–40% for brucellosis and tuberculosis [1]. To retain the genetic diversity of wood bison and to mitigate the effects of endemic disease, the use of reproductive technologies was recommended in a recent report as an effective strategy to preserve the genetic material (gametes and embryos) of wood bison [4].

In vitro production of embryos (IVP) is used worldwide in a variety of species (e.g. cattle [5], llamas [6], deer [7]) and has been proposed as a means of rescuing the genetics of wood bison (reviewed in Ref. [8]). Collection of potentially competent oocytes is the first step for successful *in vitro* embryo production [9]. Immature oocytes are matured *in vitro* after collection as a standard procedure in various species (e.g, cattle [10], human [11], river buffalo [12]). Importantly, immature oocytes may be obtained from a variety of donors including those that are pregnant [13], prepubertal [14], or that have recently died [15]). While the use of immature oocytes for *in vitro* embryo production provides an opportunity to rescue biological material for conservation purposes, fewer than 10% of immature oocytes derived wood bison ovaries obtained following slaughter developed into blastocysts in the only study reported to-date [16]. Because slaughterhouse-derived ovaries are not readily available for bison, and because ovarian status may influence oocyte competence, we developed a practical and effective method of collecting cumulus oocyte complexes (COC) from live wood bison by transvaginal ultrasound-guided follicle aspiration [17,18]. By using this approach, results of a recent study revealed that the proportion of wood bison oocytes that reached the MII stage of development was maximal after 24 h of *in vitro* maturation [8].

Results of several studies support the notion that morphology of the COC is related to the *in vitro* developmental potential of immature oocytes in different species (e.g., cattle [19–23]; goat [24]; sheep [25,26]; water buffalo [27]). In cattle, immature oocytes are routinely selected for *in vitro* embryo production based on the appearance of the ooplasm and the characteristics of cumulus cells surrounding the oocyte (i.e., compactness and number of cell layers [28]. In bison, the morphologic characteristics of immature oocytes and their relationship to *in vitro* embryo development have not been reported.

The ability of oocytes to develop into viable embryos (oocyte competence) has been associated with the physiologic status of the follicle from which it came (reviewed in Ref. [29]). For instance, the period of FSH starvation between the end of superstimulatory treatment and the time of oocyte collection in cattle (i.e. also referred to as the FSH withdrawal or coasting period) impacts the follicle diameter and maturity, and in turn, the *in vitro* developmental potential of the oocytes [30,31]. During FSH starvation, granulosa cells undergo transcriptomic changes related to post-LH surge maturation and, depending on the duration of FSH starvation, these changes may increase oocyte competence (reviewed in Refs. [29,32]). A 48-h period of FSH starvation, compared to 24 and 72 h, resulted in increased production of bovine embryos *in vitro* [33]. In another study in cattle, oocyte competence was highest after an FSH starvation period of between 44 and 68 h, and was lower after 92 h of starvation [34]. The effects of FSH starvation on the competence of wood bison oocytes has not been reported.

The objectives of the present study were to test the hypothesis that morphologic characteristics of wood bison cumulus-oocyte complexes are reflective of the ability of the oocyte to develop to an advanced embryonic stage after *in vitro* maturation, fertilization and culture (Experiments 1 and 2), and to determine the effect of prolonging the interval from the end of superstimulation treatment to oocyte collection (FSH starvation period) on follicular response, oocyte morphologic indicators, and blastocyst development (Experiment 2).

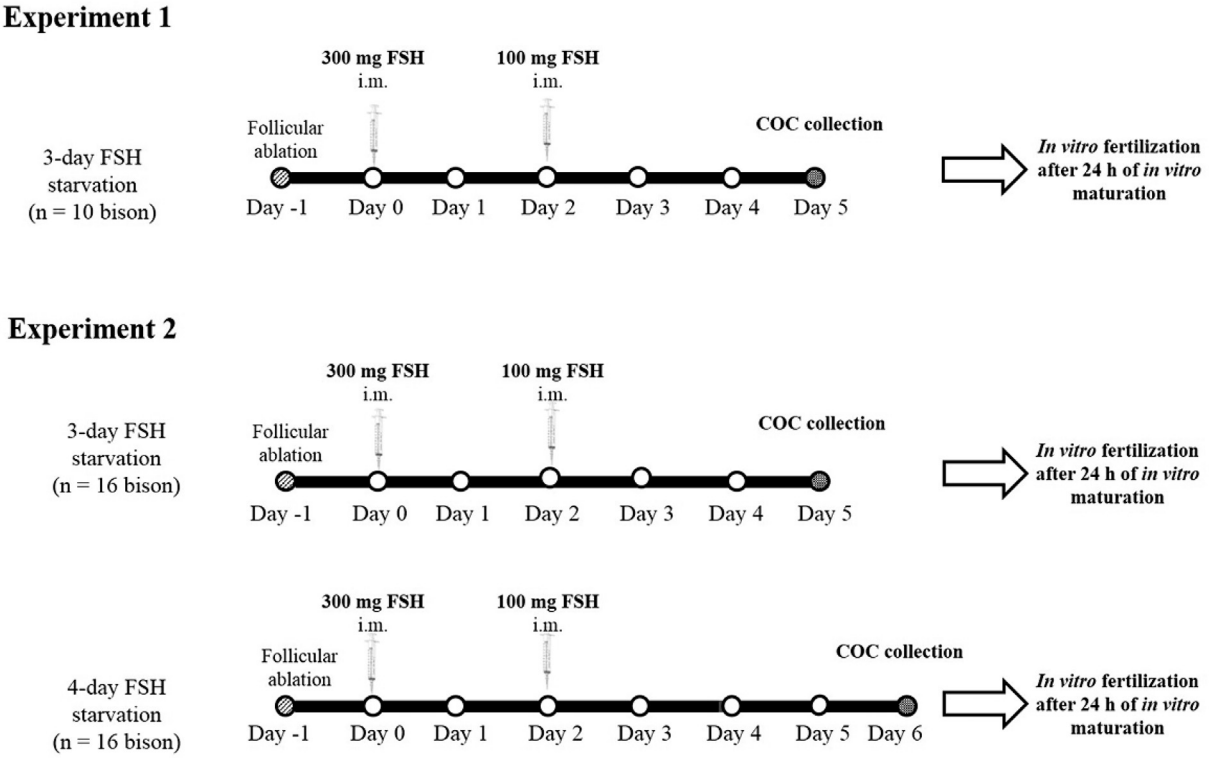

Fig. 1. Experimental design including the superstimulation protocol for the purpose of *in vitro* embryo production in wood bison to determine the developmental competence of cumulus-oocyte complexes (COC) of different morphologic categories collected by transvaginal ultrasound-guided follicular aspiration. In Experiment 2, COC collection was performed either 3 days or 4 days after the last dose of follicle stimulating hormone (FSH).