Theriogenology 89 (2017) 122–130

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

In vitro embryo production in wood bison (*Bison bison athabascae*) using *in vivo* matured cumulus-oocyte complexes

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ARTICLE INFO

Article history:
Received 23 September 2016
Received in revised form 14 October 2016
Accepted 16 October 2016

Keywords:
Blastocyst
Oocyte
In vitro fertilization
In vitro
In vivo maturation

ABSTRACT

Experiments were conducted in wood bison to determine the effect of additional maturation time on embryo development of *in vivo* matured oocytes. In experiment 1, cumulus-oocyte complexes (COC) were collected 30 hours after hCG treatment in superstimulated wood bison, and expanded COC were fertilized immediately or after 4 hours of additional *in vitro* maturation. Embryo development was assessed on Days 3, 7, and 8 (Day 0 = day of fertilization). No difference in cleavage rate was detected (55.3% vs. 60.5%, P = 0.82), but the Day 8 blastocyst rate was higher after an additional 4 hours of *in vitro* maturation time (44.7 vs. 18.4%, P = 0.03). In experiment 2, COC were collected at either 30 hours or 34 hours after hCG treatment. Expanded COC from the 30 hours group were fertilized after 4 hours of *in vitro* maturation, whereas those from the 34 hours group were fertilized immediately. A higher cleavage rate (74.3 vs. 57.0%) and blastocyst rate (54.1 vs. 37.2%) were found in the 34 hours group versus the 30 hours group (P < 0.05). In conclusion, an additional short period of *in vitro* maturation, or an extended period of *in vivo* maturation are beneficial for *in vitro* embryo production in wood bison.

1. Introduction

Endemic diseases (i.e., brucellosis and tuberculosis) have infected wild wood bison (*Bison bison athabascae*) herds in Canada, and represent a risk to remaining healthy wood bison populations and neighboring domestic livestock [1]. Eradication of infected bison has been suggested [2], but carries with it the risk of noncompensable loss of genetic diversity in this threatened population. In a recent report on the threat posed by the loss of genetic diversity, the use of reproductive technologies was recommended as an effective strategy to preserve the genetic material (gametes and embryos) of wood bison [3].

In vitro production of embryos is one such technique for rescuing the genetics of wood bison. The technique has been developed successfully in cattle largely through the use of abattoir-derived ovaries [4]. However, bison oocytes available for research are scarce, consequently few studies on *in vitro* embryo production using abattoir-derived ovaries have been reported, and have resulted in low blastocyst

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0093-691X/$ – see front matter
http://dx.doi.org/10.1016/j.theriogenology.2016.10.017

production [5–7]. In cattle, collection of cumulus-oocyte complexes (COC) in live animals by transvaginal follicular aspiration has become an important source of genetic material for *in vitro* embryo production [8]. Since *in vitro* culture conditions do not yet faithfully mimic the intrafollicular environment [9], we developed a transvaginal collection technique in live wood bison as a method of harvesting oocytes for *in vitro* embryo production [10–12]. By using live wood bison as a source of oocytes, we now have an opportunity to optimize oocyte competence by inducing maturation *in vivo*.

In a recent study [13], we found that nuclear maturation occurred more rapidly *in vitro* versus *in vivo*, as has been reported in pigs [14], but was associated with lesser cumulus expansion than *in vivo* maturation. *In vivo* oocyte maturation was more complete at 30 hours than 24 hours after treatment of superstimulated wood bison with hCG; more than one-third of oocytes collected 30 hours post-hCG were at the MII stage and had fully expanded cumulus cells. Another third of the oocytes had fully expanded cumulus cells suggesting that nuclear maturation to the MII stage was imminent. The results provided rationale for the hypothesis that additional maturation time is required for expanded wood bison COC at the MI stage to complete maturation. Whether expanded COC collected 30 hours after hCG administration can be used for immediate *in vitro* fertilization (IVF), or whether an additional period of maturation is beneficial for fertilization and embryo development, has not been investigated in wood bison.

The objectives of the present study were to determine the effect of an additional 4 hours of *in vitro* maturation on embryo development of *in vivo* matured oocytes collected 30 hours after hCG treatment (experiment 1), and to determine if extending the interval between hCG treatment and COC collection from 30 to 34 hours will improve *in vitro* embryo production (experiment 2). The study also provided the opportunity to compare the effect of season (ovulatory vs. anovulatory) on the number of follicles ≥5 mm available for aspiration at the time of COC collection, the number of expanded COC collected after inducing *in vivo* maturation, and on the production of wood bison embryos *in vitro*.

2. Materials and methods

2.1. Animals

The study was performed with mature (6–11 year old), nonlactating female wood bison during the ovulatory season (September to November, experiment 1; n = 24) and anovulatory season (April to May, experiment 2; n = 28). The bison were part of the research herd maintained on pasture at the Native Hoofstock Centre, University of Saskatchewan. For the period extending from 10 days before the experiments to the end of the experiments, the bison were confined to corrals with free access to fresh water and alfalfa-bromegrass hay to maintain an average body condition score of 3.5 (scale of 1–5; [15]). The experimental protocol was approved by the University of Saskatchewan's Animal Research Ethics Board, and done in accordance with the guidelines of the Canadian Council on Animal Care.

2.2. Experiment 1: additional in vitro maturation

Ovarian synchronization was induced among bison (n = 24) using an intramuscular dose of 500 μg cloprostenol (Estrumate, Merck Animal Health, Kirkland, Quebec, Canada) followed 8 days later by transvaginal ultrasound-guided aspiration of all follicles ≥5 mm in diameter (follicular ablation), as described previously [11]. Briefly, for follicular aspiration, bison were restrained in a squeeze chute and caudal epidural anesthesia was induced by administration of 3–5 mL of 2% lignocaine hydrochloride (Bimeda-MTC, Animal Health Inc., Cambridge, Ontario, Canada) between the first intercoccygeal joint. The vulva was washed with detergent and disinfectant before the transvaginal probe was introduced into the vagina and placed in the fornix. Follicular ablation was performed using a 5-MHz transvaginal probe (ALOKA SSD-900, Tokyo, Japan) equipped with a disposable 18-ga × 1 ½″ needle (Vacutainer, BD, Mississauga, Ontario, Canada) attached to a 6-mL syringe by silicon tubing 60 cm long × 1.14 mm internal diameter. On the day after ablation (i.e., expected day of follicular wave emergence; Day 0), bison were treated intramuscularly with 300 mg of pFSH (Folltropin-V, Vetoquinol NA Inc., Lavaltrie, Québec, Canada) diluted in 0.5% hyaluronan (5 mg/mL, MAP-5, Vetoquinol NA Inc.) and an additional 100 mg pFSH in hyaluronan 2 days later, as described previously [10]. A luteolytic dose of 500 μg cloprostenol (Estrumate, Merck Animal Health, Kirkland, Quebec, Canada) was given on Day 3 and an intramuscular dose of 2500 IU of hCG (Chorulon, Merck Animal Health, Summit, NJ, USA) was administered on Day 4 to induce oocyte maturation *in vivo* (Fig. 1).

At 30 hours after hCG treatment, COC were collected by transvaginal ultrasound-guided aspiration of all follicles ≥5 mm in diameter, as described [10,12]. The COC were collected using a disposable 18-ga × 2″ short-bevel needle (Misawa Medical Industry Ltd., Edogawa-Ku, Tokyo, Japan) connected to a 50-mL conical Falcon tube *via* silastic tubing (internal diameter 1.14 mm; Cole Palmer, Montreal, Quebec, Canada), and a regulated vacuum pump set at a flow-rate of 20 mL/min. The collection medium consisted of Dulbecco's phosphate buffered saline (D-PBS), 0.15% (v:v) ET Surfactant (Vetoquinol NA Inc.), and 200 IU/L of heparin (heparin sodium injection USP, Sandoz, Boucherville, Quebec, Canada). The follicular aspirate was poured through an embryo filter (Emcon filter; Agtech, Manhattan, KS, USA), and the COC were rinsed from the filter into a 90-mm Petri dish using collection medium without surfactant. The COC were located under a stereomicroscopy at × 10 magnification, washed three times in holding medium (D-PBS + 5% calf serum), and morphologically classified according to the number of cumulus cell layers and the appearance of the oocyte cytoplasm. Compact COC were those with three or more layers of granulosa cells tightly surrounding the oocyte, expanded COC were those with expanded or partially dissociated cumulus cells, and denuded or degenerated oocytes were those without