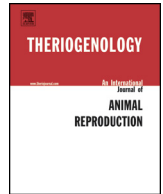




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Serial ovarian ultrasonography in wild-caught wood bison (*Bison bison athabascae*)

Robert B. McCorkell^{*,1}, Murray R. Woodbury, Gregg P. Adams

Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

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ABSTRACT

The objectives of this study were to determine the feasibility of daily examination of wild-caught wood bison and to characterize the ovarian function using serial transrectal ultrasonography and blood hormone analysis. Ten 2-year-old wood bison heifers obtained from Elk Island National Park were placed in a corral adjacent to a handling system designed for restraining bison. The handling system was left open to the corral allowing the bison to explore it freely for 2 months. Active acclimation followed for a 2-week period, during which the bison were herded daily through the handling system and rewarded with whole oats. Finally, the bison were restrained in the handling system and rewarded with whole oats upon release. Once conditioned, daily transrectal examination of the ovaries was completed in 100% of attempts for 30 days (January–February) using a B-mode scanner with a 5 to 10-MHz linear array. Follicle size and numbers were recorded, and individual follicles were identified serially. Blood samples were collected daily and the serum was analyzed for FSH concentrations. Nonrandom changes were detected in the number of follicles ≥ 4 mm in diameter per day ($P < 0.05$). Each peak in follicle numbers was associated with the development of a single dominant follicle. The interval between the emergence of successive dominant follicles was 6.8 ± 0.6 days (mean \pm SEM). The maximum diameter of the dominant follicle was 9.9 ± 0.4 mm. In conclusion, wild-caught wood bison were amenable to daily examination and blood sampling, and ovarian dynamics were characterized by wave-like development of anovulatory antral follicles. The demonstrated success of this approach to the study of ovarian function will be useful for characterizing the annual reproductive pattern in wood bison, which is necessary for the development of bison-specific protocols for controlling ovarian function for species conservation.

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1. Introduction

Bison are the largest land mammals in North America; before European settlement, they ranged widely over the continent in numbers estimated to exceed 30 million [1]. Bison were driven to near-extinction by the beginning of the twentieth century as a result of uncontrolled hunting

and loss of habitat from settlement [2]. Bison are divided into two variants: the southern plains bison (*Bison bison bison*) and the northern wood bison (*Bison bison athabascae*). With only 10,870 in designated conservation herds, wood bison remain endangered primarily because $>50\%$ reside in herds that are endemically infected with brucellosis and tuberculosis [3]. A federal environmental assessment panel concluded that the only solution to these problems is eradication of the infected bison herds [4]. In addition, the panel recommended that healthy bison be reintroduced once eradication has been accomplished and that the healthy bison be sourced from the original area

* Corresponding author. Tel.: +1 403 210 6655; fax: +1 403 239 6984.

E-mail address: robert.mccorkell@ucalgary.ca (R.B. McCorkell).

¹ Present address: Faculty of Veterinary Medicine, Comparative Biology & Experimental Medicine, University of Calgary, Alberta, Canada, T3R 1J3.

through genetic salvage operations [4]. To address this challenge, the Wood Bison Reproductive Research Group was formed to advance knowledge in the area of bison reproductive physiology and develop reproductive technologies to enable production of disease-free gametes and embryos that may be used to replace extirpated populations.

Very little information on the reproductive characteristics of bison has been published, and it is as yet unclear if bison are annually polyestrous [5] or seasonally polyestrous [6]. Bison calves are born in a synchronous pattern (April–June) in the wild, suggesting a seasonal reproductive pattern. Seasonality in bison is thought to be more the result of the effects of photoperiod or climate than the effect of predation [7]. Previous studies on bison reproductive physiology have involved the use of fecal or urinary steroid analysis, due to the difficulty in physically handling the animals [6,8,9]. However, seasonal and annual characteristics of reproductive cyclicity remain unknown.

In an initial effort to characterize the reproductive pattern of wood bison, the objectives of the present study were to determine the feasibility of daily examination of wild-caught wood bison and to characterize ovarian function using serial transrectal ultrasonography and blood hormone analysis.

2. Materials and methods

2.1. Animals

Wild wood bison were caught in Elk Island National Park in Alberta (53°41' N, 112°52' W) and transported to the Native Hoofstock Centre (NHC) near Saskatoon, Saskatchewan (52°02' N, 106°28' W). Shortly after the bison arrived at the NHC, a program of habituation and training was begun to enable frequent handling and examination without sedation (see Results). Beginning 9 months after their arrival to the NHC, 2.5-years-old female wood bison ($n = 10$) ranging from 280 to 350 kg were used during January and February to characterize day-to-day ovarian and hormonal dynamics. To facilitate daily handling, the bison were confined to a corral adjacent to the indoor handling facilities for the duration of the study. The bison had continuous access to water and were fed alfalfa brome grass hay in large round bales *ad libitum* and were given 2.0 kg of whole oats per head per day.

2.2. Handling system

The bison were handled in an indoor system composed of sorting cells, a weigh scale, and a squeeze chute. The system was designed specifically for bison (Berlinic Manufacturing, Quill Lake, SK, Canada). The bison entered through a circular alley that could be divided into four sorting cells by sliding gates (four bison per cell). The sliding gates were controlled with hydraulic motors by an operator located on an elevated platform at the center of the circle. The floor of one of the cells was equipped with a weigh scale. After entering the handling unit, the bison were sorted so that only one animal entered the final cell before the hydraulic squeeze chute. The squeeze chute was

equipped with a crash gate on the outside of the head-catch mechanism. The crash gate was designed to stop a charging animal so that the head-catch mechanism can close around the neck immediately behind the skull and prevent the animal from advancing a leg or shoulder beyond the head-catch. Once a bison was caught, the crash gate was lifted out of the way to allow access to the head and neck. The sides of the squeeze chute were adjusted to apply sufficient pressure to immobilize the animal. A door immediately behind the head-catch mechanism on each side provided safe access to the neck for jugular venipuncture. A second set of doors at the rear of the chute allowed access to the hind-quarters for transrectal examination. An operator standing to the side of the chute controlled all of the moving parts of the squeeze chute hydraulically.

2.3. Ovarian examinations

Daily examinations were done by restraining the bison, without sedation, in the hydraulic squeeze and examining the ovaries via transrectal real-time, B-mode ultrasonography (SonoSite Titan, Markham, ON, Canada) using a 5 to 10-MHz linear-array transducer (SonoSite L52 on general setting, ~7.5 MHz) mounted in a 48-cm-long rigid probe extension. Both ovaries were examined systematically, and images were recorded by carefully sketching the number, size, and relative location of all follicles ≥ 4 mm in diameter on a diagram of the ovary, similar to the method described in cattle [10] and wapiti [11]. The drawings were used to tabulate the number of follicles ≥ 4 mm within the pair of ovaries of each bison for each day of the examination period and to construct diameter profiles of individually identified follicles from their first appearance at 4 mm in diameter until they could no longer be identified (regressed to ≤ 4 mm). Follicle numbers were analyzed on an individual-animal basis by combining data from both ovaries.

Daily diameters were recorded for individually identified follicles that attained a diameter of ≥ 6 mm; the day it was first identified at a diameter of 4 mm was defined, in retrospect, as the day of its emergence. A temporal relationship between the development of the largest individually identified follicle and a subsequent decrease in the number of follicles detected was regarded as evidence of follicular dominance. The largest follicle was then referred to as the dominant follicle and all others as subordinate follicles [10,11].

The following characteristics were determined: (1) maximum diameter attained by the dominant follicle of a wave; (2) number of days between the emergence of successive dominant follicles; (3) maximum diameter of the largest subordinate follicle; (4) the day of emergence of the largest subordinate follicle relative to the dominant follicle; and (5) the day the dominant follicle diverged in its growth relative to the largest subordinate follicle (Table 1).

2.4. Hormone assays

Blood samples were collected daily via jugular venipuncture using an 18-gauge, 3.8 cm needle and a 10-mL vial without anticoagulant. Blood samples were kept chilled

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