Development and use of a genome biobank to restore the genetic diversity of North American bison

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Abstract

Perhaps more than any other North American species, bison symbolize the evolution of the continent's ecosystem and the physical and spiritual health of its human inhabitants. There is, however, a critical lack of genetic diversity among herds comprising North America's meta-population of plains and wood bison, and threats of ongoing loss as a result of endemic disease and stochastic events in small geographically isolated herds. The problem is insoluble without a method to overcome disease-related limitations to movement of breeding stock. The number of stakeholders involved underlines the importance and impact of bison in Canada and the United States, as well as the complexity of finding a remedy. One solution is the production of disease-free gametes and embryos (germ plasm) from genetically isolated and valuable herds. The use and transport of germ plasm, rather than live animals, minimizes the biosecurity risks associated with reportable diseases (e.g., brucellosis and tuberculosis), and will enable establishment of healthy seed-stock for replenishing herds threatened by genetic bottle-necks and endemic disease. Successful establishment and use of a genome biobank for bison will benefit all layers of governments by enabling more effective and efficient wildlife management, protecting the agricultural livestock sector, food inspection, and human health and safety. Bison producers will benefit by having access to new genetics, improved breeding management, expanded national and international markets for meat and breeding stock, and the ability to mitigate real and perceived threats of disease transmission to domestic livestock and humans. Disease mitigation will facilitate re-establishment of bison back into the vastness of their former range, restore the ecological balance of the grassland and boreal forest regions, and hasten environmental recovery of land disrupted by commercial activities. Revitalizing the bison species will have both symbolic and material impact particularly on communities in and around National Parks. The following discourse provides an overview of the origin of and ongoing threats to the bison species, and a review of the development and deployment of a bison genome resource biobank that has the potential to create sustainable re-expansion of genetic diversity among bison herds throughout North America.

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List of abbreviations:

AI: artificial insemination

AB: Alberta

BSA: bovine serum albumin

BC: British Columbia

BNP: Buffalo National Park:

C2S2: Conservation Centers for Species Survival

CC: cholesterol cyclodextrin

CC-TG: cholesterol-cyclodextrin tris-glycerol extender

CITES: Convention on International Trade in Endangered Species

CL: corpus luteum

COC: cumulus-oocyte complex

COSEWIC: Committee on the Status of Endangered Wildlife in Canada

CR1aa: Charles Rosenkran's aminoacid

DF: dominant follicle

DNA: deoxyribonucleic acid

E: estradiol

eCG: equine chorionic gonadotropin

EINP: Elk Island National Park

EY: egg yolk

FSH: follicle stimulating hormone

GnRH: gonadotropin-releasing hormone

h: hour

hCG: human chorionic gonadotropin

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

hpa: hectopascal

hr: hour

im: intramuscular

iSCNT: interspecies somatic cell nuclear transfer

IU: international unit

IUCN: International Union for Conservation of Nature

IVF: *in vitro* fertilization

IVP: *in vitro* production of embryos

Km: *kilometer*

LH: *luteinizing hormone*

MB: Manitoba

MBS: Mackenzie Bison Sanctuary

mg: milligram

mm: millimeter

NMO: National Museum collection in Ottawa

NT: Northwest Territories

P: progesterone

PCR: polymerase chain reaction

pFSH: porcine follicle stimulating hormone

PGF: Prostaglandin F_{2a}

pLH: porcine luteinizing hormone

PRID: progesterone-releasing intravaginal device

SARA: Species at Risk Act

sc: subcutaneous

SCNT: Somatic Cell Nuclear Transfer

SNP: single-nucleotide polymorphism

SRF: sustained-release form

TB: tuberculosis

Tes: N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid

TEYG: tris-egg yolk-glycerol extender

Tris: tromethamine

TSBH: Texas State Bison Herd

US: United States

WBNP: Wood Buffalo National Park

WE: wave emergence

YNP: Yellowstone National Park

YT: Yukon Territories

ZP: zona pellucida

Executive Summary by Section

Section 1 Introduction

- The problem, and the problem with the problem.
- New attitudes and new technology create an opportunity to develop a bison genome biobank which can be used to re-establish genetic diversity within and among small bison populations that have been disconnected by disease and distance for the last 100 years.

Section 2 A brief history

Bison evolution and taxonomy

- The North American bison population is composed of two subspecies or designatable units, the plains bison and the wood bison.
- Plains bison (*Bison bison bison*) and wood bison (*Bison bison athabascae*) are descendants of bison that crossed the Bering land bridge ~195k and again ~21k yr ago.

Historical record of bison populations in North America

- It is estimated that between the late 1600s and early 1700s there were approximately 30 million plains bison and 168,000 wood bison in North America.
- The ontogeny of the near-extinction event of the North American bison is reviewed, and is represented as a progressive escalation of over-harvesting over a period of 200 years.
- Overharvesting has been attributed primarily to a shift in the use of bison from a subsistence resource to a lucrative commodity. Factors contributing to the demise of North American bison included the re-introduction of the horse as a competitor for grassland and as tool for hunting, introduction of cattle disease during south-north cattle drives, the expansion of the railroad that resulted in a sudden expansion of trade, the number of settlers and domestic livestock, and sport hunters, the Indian wars, and the invention of a new and efficient way to tan hides for the leather industry.
- By 1888, plains bison were reduced to 85 individuals in North America while only 6 were known to be in existence in Canada, and the wood bison population plummeted to 250 by 1900.

Early conservation efforts in Canada and the United States

- Between 1907-1912, 700 plains bison were purchased from the Pablo-Allard herd (Montana) and moved to Buffalo National Park (Wainright), where the herd increased to

over 6,000 animals by the 1920s, beyond the Park's carrying capacity. Co-mingling with cattle at the Park led to the introduction of cattle diseases and genetic introgression into bison.

Section 3 Implications of the loss of genetic diversity

Genetic diversity

- Both plains and wood bison have undergone a serious loss of genetic diversity because of founder effects, population bottlenecks, genetic drift, and selection.
- For long-term viability, an effective population of ≥1,000 animals is needed to retain 90% of their allelic diversity (traits) for 200 years. In Canada, nearly all conservation herds are in isolated groups of <500 bison with no new bison genes added to its herds since the introduction of bison from the US over 110 years ago.

Conservation herds vs commercial herds

- The growing number of bison in captive livestock production herds has had a disguising effect on the conservation issue.
- Only 5% of bison in North America are in conservation herds while the remaining 95% (~470,000) are in production herds where the bison are handled as livestock and many are of mixed ancestry.

Genetic bottlenecks in the post-contraction era

- After the purchase of over 700 plains bison from the Pablo-Allard herd in Montana in the early 1900s, the Canadian plains bison population increased rapidly and peaked at over 8,000 in the 1920s. However, overcrowding and the transmission of bovine diseases resulted in closure of Buffalo National Park and the plains bison population dropped precipitously between 1930-1940 to an effective population of less than 1,500 where it remains today.
- 6,673 plains bison shipped from Buffalo National Park to Wood Buffalo National Park from 1925-1928 hybridized with the resident wood bison and introduced bovine diseases.
- "Pure" wood bison may no longer exist, though genotypic and phenotypic data distinguish populations of wood bison from plains bison.
- Bison herds in the Greater Wood Buffalo National Park region remain endemically infected with bovine brucellosis and tuberculosis.
- Founder herds of plains bison (n=50) and wood bison (n=23) at Elk Island National Park herd are the source of every conservation herd in Canada, with the exception of wood bison in the Mackenzie Bison Sanctuary which originated from the same source as those at EINP.
- The wood bison population is stagnant and remains at <6% of historical numbers.

- Population recovery in both plains and wood bison is jeopardized by genetic isolation among conservation herds, most of which are comprised of fewer than 500 individuals.

Section 4 Phenology of wild bison – general annual pattern

The male

- Bison males display breeding behaviour and produce sperm as young as 1 year of age; however, the onset of puberty in natural and commercial settings is typically considered to be 2 years of age.
- Breeding in free-ranging herds is typically done by older, larger bulls that are able to compete for dominance. Prime reproductive performance is between 8-12 years of age with a sharp decline by 15 years of age.
- Adult male bison spend much of their time in small groups of ≤20 bulls or as solitary bulls until the breeding season when they join mixed groups for access to the females. The *rut* or *rutting season* is broadly defined as the period when males display increased sexual behaviour, compete with other males and seek out females to copulate. Most breedings occur between late July and mid-September.
- Breeding outcomes in bison are dependent upon the dominance hierarchy with the majority of the breeding being done by the high-ranking males. Microsatellite analyses of distinct bison herds have shown that a single bull is the predominant breeder in each herd.

The female

- The onset of puberty in female bison has not been critically examined. Study of ovarian follicular dynamics in 2-year-old bison by daily ultrasonography during Jan. and Feb. revealed a wave-like pattern of follicle growth characterized by regular emergence of a group of follicles with subsequent regression of all but one anovulatory dominant follicle every 7 -8 days.
- While ovarian follicle development is evident in 2-year-olds and some female bison breed successfully at this time, the majority of females reach sexual maturity with ovulatory cycles and breeding at 3 years of age.
- Bison females are seasonally polyestrous with the onset of the breeding season characterized by a short 10-day estrous cycle in August, followed by longer cycles averaging 21 days in length. The end of the ovulatory season has not been clearly defined but luteal phases based on fecal progesterone analyses have been recorded up until April.
- Changes in sexual behaviour are much less obvious in females and many matings are never observed even with intensive observations. This has made the calculation of gestation length difficult, but reports suggest a mean gestation period of 266 days.

Section 5 The male: Reproductive physiology and technology

- Bison bulls attain puberty at approximately 16 months of age, however, semen quality continues to improve significantly until 24 months of age. As a result, it is important for selection of breeding bulls to be done at 2 years and not younger.
- Bison are seasonal breeders although it is less evident in males than females. Sperm production occurs year-round, but the summer season is the time when semen characteristics are optimal.

Semen collection and evaluation

- Semen/sperm collection is possible by epididymal aspiration in post-mortem samples and by electroejaculation in live animals.
- Administration of pipothiazine palmitate effectively reduced the stress response and enhanced semen collection during electroejaculation.

Chilled and cryopreserved semen

- Cryopreservation of bison sperm has been challenging due to the impact of both the extender dilution and freeze-thaw stages. Significant efforts at developing animal protein-free extenders have resulted in the incorporation of cholesterol-loaded cyclodextrin into a tris-glycerol extender without detrimental effects on post-thaw motility and retention of intact acrosome and plasma membrane compared to the standard tris-egg yolk-glycerol extender.
- Improvement in bison sperm cryopreservation has enabled collection and processing of bison semen during any season of the year for the purpose of artificial insemination.

Section 6 The female: Reproductive physiology and technology

Ovarian and endocrine dynamics during the ovulatory and anovulatory seasons

- Serial ultrasonography in bison has permitted detailed characterization of ovarian dynamics during the anovulatory and ovulatory seasons.
- Correlations between ovarian, endocrine and behavioral events provide an understanding of the relationships between the onset of ovulatory cyclicity, the expression of estrus, and the seasonal resumption of fertility.
- The first ovulation of the season for individual bison occurred between August 11 and August 28, and was not associated with behavioral signs of estrus.
- The first ovulation was followed by a single wave of follicle development and the formation of a small and short-lived corpus luteum (CL) resulting in a short interovulatory interval (cycle) of 8 days.
- The interval between the second and third ovulations was characterized by two waves of follicle development and a longer-lived CL resulting in a 20-day cycle.

- Unlike the first ovulation of the season, the second and third ovulations were preceded by estrous behavior.
- On a herd basis, none of the 2-year-old females and only 1/3rd of the mature females (3-14 years old) had entered the ovulatory season by the first week of August. By the first week of September, 44% and 92% of the 2-year-old and ≥3 year-old females, respectively, had made the transition to the ovulatory season.

Elective control of ovarian function

- Ovulation can be induced effectively during both the anovulatory and ovulatory seasons, and the ovulatory response was better with hCG than either LH or GnRH. Similar results were reported in bison undergoing ovarian superstimulation; hCG induced a greater ovulatory response than pLH in both the ovulatory and anovulatory seasons.
- Ovarian follicular wave synchronization was achieved during the anovulatory season using two different techniques. The follicle ablation technique consistently induced the earliest and most synchronous response; the estradiol + progesterone treatment technique is quick and does not require ultrasound equipment and expertise.
- Both techniques (follicle ablation and estradiol + progesterone treatment) effectively synchronized ovulation in bison during the ovulatory season and pre-scheduled (fixed-time) artificial insemination resulted in pregnancy and live births.
- Protocol refinements over three studies improved ovulation rate (96%), ovulation synchrony (≤36 hours), and pregnancy rate to fixed-time artificial insemination (45%).
- Subsequent fixed-time artificial insemination field trials resulted in pregnancy rates of 33-50% in cycling bison.
- Synchronization for fixed-time AI will be more effective when initiated after the first week of September in mature bison cows, and later in 2-year-olds after confirming the presence of a CL.

Section 7 Embryo technology

Superovulation for embryo collection and transfer

- Gonadotropin-induced multiple follicle growth (ovarian superstimulation) is used to increase the number of follicles for oocyte collection for *in vitro* fertilization or to induce multiple ovulation for *in vivo* fertilization and embryo collection.
- Superstimulation protocols have been designed to reduce the number of treatment/handling events to mitigate the effects of stress.
- Reduced-treatment/handling protocols induced multiple ovulation during both the ovulatory and anovulatory seasons, and live calves have been produced after transfer of fresh and frozen bison embryos.
- Treatment with two doses of pFSH given 2 days apart, beginning on the day of follicle wave emergence, resulted in a greater ovarian superstimulatory response than less- or more-frequent treatment, regardless of season.

- Treatment with pFSH was more effective than eCG for ovarian superstimulation in bison during both the anovulatory and ovulatory seasons, and addition of eCG to a pFSH protocol did not improve the response.
- Ovulation was induced more effectively with hCG than pLH or GnRH in both superstimulated and non-superstimulated bison, regardless of season.
- The antral follicle count at wave emergence was positively correlated with the superstimulatory response, and was significantly greater in the anovulatory vs ovulatory season.
- Despite a greater superstimulatory response in the anovulatory season, more freezable embryos (Grades 1 and 2) were obtained during the ovulatory season.
- Exogenous progesterone during superstimulatory treatment did not improve embryo quality in either the ovulatory or anovulatory season in bison.

Ovarian superstimulation for oocyte collection

- Transvaginal ultrasound-guided follicular aspiration is a practical and feasible technique for oocyte collection from live bison, with an average collection rate of 50-60% during both the anovulatory and ovulatory seasons.
- Good quality oocytes for *in vitro* fertilization and embryo production may be collected throughout the year.

In vitro fertilization and embryo production

- Initial studies involving *in vitro* maturation of bison oocytes collected post-mortem resulted in low embryo production after *in vitro* fertilization.
- The developmental competence of oocytes after *in vitro* fertilization was enhanced by a 30-34-hour period of *in vivo* maturation induced by hCG.
- Morphologic characteristics of bison cumulus-oocyte complexes (COC) were predictive of the potential of oocytes to develop into embryos after *in vitro* maturation, fertilization and culture.
- The developmental competence of *in vivo* matured bison oocytes was similar between ovulatory and anovulatory seasons. The overall efficiency of *in vitro* embryo production was better during the anovulatory season because of a greater number of follicles >5 mm in the ovary available for transvaginal ultrasound-guided aspiration, and greater COC collection efficiency.
- Recent progress has resulted in the birth of the world's first bison calves by *in vitro* embryo production.
- Further studies are needed to improve protocols for cryopreserving bison embryos for future use, as well as evaluate the potential of these embryos to complete full-term pregnancy after transfer to recipients.

Somatic cell nuclear transfer (SCNT)

- Somatic cell nuclear transfer preserves the entire genome from a valuable individual and offers an alternative strategy for embryo production when viable gametes are not attainable.
- Most SCNT attempts in wild bovids, including bison, have relied on the use of domestic cattle oocytes as recipients for wild cattle somatic cell nuclei (interspecies SCNT).
- Although interspecies SCNT overcomes the challenges of obtaining oocytes from valuable bison females, the presence of interspecific cytoplasmic organelles (e.g. domestic cattle mitochondria) may have a negative impact on embryo development
- The long-term effects of SCNT on bison health and survival require further investigation.

Section 8 Factors affecting restoration of genetic diversity in North American bison

Geographic isolation and small effective population size

- Bison occupy less than 1% of their historical range in North America and expansion is impeded by fragmented or unsuitable habitat.
- The total effective population size (<1500 for plains bison and 9000 for wood bison) is low, and remaining populations are fragmented into small, geographically and genetically isolated herds.
- Most plains and wood bison herds are derived from small founder populations primarily originating from Elk Island National Park.

Inter- and intra-species hybridization

- Intentional hybridization of plains bison with cattle during the original conservation efforts resulted in cattle introgression in most plains bison herds.
- Although there is some concern of historical mixing of wood and plains bison, distinct phenotypic and genotypic differences exist between these subspecies.
- To-date, Canada's plains and wood bison conservation herds do not show evidence of bovine introgression and have distinct subspecies traits, but hybridization remains a threat to the genetic sustainability of bison populations. Linking phenotypic and genotypic data is necessary for addressing genetic diversity.

Disease

- Disease (brucellosis, tuberculosis, and anthrax) has played an important role in the decline of plains and wood bison populations, and remains a threat today.
- Risk of disease transmission to healthy free-ranging bison herds and cattle ranches has resulted in the formation of buffer zones, which for wood bison, prevents establishment of free-ranging herds in over 40% of their original range.
- Approximately half of the remaining wood bison reside in populations affected by tuberculosis and brucellosis.

Inadvertent selection

- Inadvertent selection results in the fixation of alleles that promote survival in a specific context, which ultimately undermines efforts to increase genetic diversity and population fitness.
- Despite care to ensure unbiased selection in bison conservation programs, animals that adapt poorly to management procedures, particularly in captivity, are inevitably culled or removed from the population.
- Disease exerts a significant selection pressure on wildlife populations by decreasing population size, and thus, genetic diversity.

Solidarity – social, geo-political, commercial

- Short-term conservation objectives have focused on protecting remaining bison and minimizing the spread of disease, but do not address the root causes of the threats. The most important impediment to addressing long-term survival of bison has been the lack of solidarity among interested parties.
- The Hook Lake Wood Bison Recovery Project was an important attempt to establish a disease-free herd using genetic resources from a diseased population, and highlighted the difficulty, cost, and risk involved in transporting live animals.
- Creation of a bison genome biobank and implementation of reproductive technologies will be instrumental for the successful decontamination and distribution of genetic material from diseased populations. This approach will ensure the preservation of unique alleles and reconnection of genetically isolated herds, and presents an opportunity for local communities, private industry, and commercial bison producers to participate in the restoration of bison.

Section 9 Implementation and management of a genome resource biobank

Feasibility and long-term goals

- Previous reviews have consistently highlighted the importance of understanding the basic reproductive pattern of a species as a foundation for developing effective assisted reproductive technologies. In bison, significant progress has been made in the last decade on the entire range of reproductive technologies to warrant their use for the restoration of genetic diversity in this species.
- Important milestones (e.g. births of calves following artificial insemination and embryo transfer) demonstrate the ability to preserve genetic material year-round and transfer it between herds and institutions.
- A national bison genome resource biobank will not only support the contribution of remaining individuals in the future gene pool, but will play a critical role in re-

establishing connectivity between populations to avoid inbreeding and unintentional selection.

Biosecurity

- For a species which harbors endemic disease, sanitary measures to minimize the risk of disease transmission are essential. Significant progress has been made with wood bison embryos and sperm in the laboratory to encourage the collection of material from diseased animals in the wild.
- Embryo washing procedures (based on guidelines of the International Embryo Technology Society) successfully removed *Brucella abortus* biovar 1 from 100% of experimentally infected *in vivo* and *in vitro* derived wood bison embryos when using culture medium containing antibiotics.
- Sperm washing procedures (using BoviPure separation and purification product) successfully removed *Brucella abortus* biovar 1 from 100% of experimentally infected wood bison sperm samples when using a semen extender containing both antibiotics and trypsin.

Genetic fidelity and genetic diversity

- Quantification of genetic variation is integral to managing the genetic fitness of a herd and will be important for identifying candidate sources of germ plasm that are unrelated. Criteria used for selection of candidate donors will include historical knowledge of herd provenance, assumed geospatial isolation, phenotype and genotype.
- Development of tools for genomic selection, including the use of high-density SNP chips and full genome sequencing, is essential to help resolve the phylogenetic distinction between subspecies and establishment of markers of interspecies and intraspecies introgression.
- Genotypic and phenotypic analyses have identified herds of interest, including the Ronald Lake Herd whose value can be attributed to its disease-free status, genetically distinctive fingerprint, and characteristic wood bison phenotype.

Strategic use of a genome resource biobank

- Establishment and operation of an effective bison genome resource biobank is necessary to integrate *in situ* and *ex situ* conservation efforts.
- Large population sizes are essential for the long-term maintenance of a genetically and demographically healthy species.
- Participation of diverse stakeholders will enhance the operational and genetic sustainability of the biobank, but primary support should come from the Canadian government.

Roles of conservation herds, zoological and heritage parks, and commercial producers

- Bison conservation will benefit from the collaboration of multiple partners to maintain herds that remain interconnected via the genome resource biobank and application of reproductive technologies.
- A bison consortium will provide a broad base of support for the recruitment of funds, a larger audience for education and public outreach, and most importantly, a distribution of animals across geographical areas to protect against sudden changes in the environment.
- Our work in bison reproduction has highlighted the importance of strong partnerships across academic, conservation, and governmental institutions that provided the necessary support (i.e. access to diverse sources of animals, funding, expertise) to achieve our objectives. Inter-institutional partnerships will be especially valuable for implementing a national bison strategy that spans decades (and generations).

Appendix 1: Cost of implementing a bison biobank

- Final stage Research and Development project
- Future Bison Biobank Business Plan
- Potential Partners

1. Introduction

During an interview announcing that a Canadian zoo successfully produced a bison calf from an embryo sent from another province (https://www.canadiangeographic.ca/article/inside-toronto-zoos-bison-breakthrough), I was asked a simple question - why do you want to conserve this species? Thinking this to be self-evident, I hadn't previously attempted to articulate 'why', and I struggled to answer this simple but monumental question.

Nature underpins every person's wellbeing and ambitions – from health and happiness to prosperity and security. Everything that has built modern human society, with its benefits and luxuries, is provided by nature – and we will continue to need these natural resources to survive and thrive… As we better understand our reliance on natural systems it's clear that nature is not just 'nice to have' (WWF Living Planet Report – 2018).

The bison is the largest land mammal in the western hemisphere, and numerous examples of this iconic species on flags, coins, emblems, sports teams, businesses and academic institutions in Canada and the United States emphasize the historic, cultural, ecologic, and economic significance of the bison in North America. In particular, bison play an important role in ecosystem health (Sanderson et al., 2007), as well as in the livelihood and culture of aboriginal communities (Garret, 2007). In recognition of the importance of this species, on October 30, 2013, the U.S. Senate passed a resolution officially designating November 2 as National Bison Day (http://newswatch.nationalgeographic.com/2013/11/01/honoring-an-american-icon-with-national-bison-day/). As well, the Northern Tribes Buffalo Treaty was signed on Sept. 23, 2014 in Montana by First Nations on both sides of the Canada-United States border to establish inter-tribal alliances for the restoration of bison on reserves or co-managed lands within the U.S. and Canada (http://www.calgaryherald.com/news/Historic+Buffalo+Treaty+signed+First+Nations+bring+back+bison/10229219/story.html?federated=1).

Bison have served as an ecologic and social keystone species for millennia, but dramatic population loss brought the species to the brink of extinction at the turn of the 20th century, and genetic bottlenecks during the last century continue to threaten the genetic diversity and long-term viability of the species. The problem is clear – restore genetic diversity among extant populations of bison or risk the extinction of another of North America's megafauna. There is, of course, a problem with the problem – it's complicated. The bison dilemma is a quintessential example of a *One Health* issue; that is, a health issue that meets at the interface between the environment, humans and animals. There are many stakeholders in the bison narrative, and while there is universal agreement that bison, as a species, is worthy of conservation, it is ironic that jurisdictional quagmires during the last century have been the primary obstruction to bison recovery (reviewed in Appendix 2 of Environment and Climate Change Canada, 2016). These quagmires are real, understandable and not to be taken lightly. For example, the approach taken to prevent the spread of zoonotic diseases from wild bison reservoirs is to kill bison that wander

outside of national park boundaries. This approach, now operant for >30 years, has controlled the spread of disease outside of Wood Buffalo National Park, but continues to threaten the world's largest and most diverse population of wood bison, and the diseases remain endemic.

Times and attitudes have changed, and with the advent of new techniques, there is renewed interest in addressing the problem using new approaches, including the development and deployment of a bison genome biobank (McPhee and Adams, 2016), development of effective vaccines, and selective culling based on new and more sensitive diagnostic tests (Shury et al., 2015). It will take a *One Health* approach to address the bison dilemma – an approach that recognizes ethical, environmental, economic, legal and social aspects, and is guided by information based on peer-reviewed science.

The following is an historical overview of the rise and fall of North American bison, conservation efforts during the last century, and on-going threats to the genetic diversity and fitness of bison populations today. An in-depth review of the literature is provided on the current knowledge of bison reproductive biology, and recent progress on development of reproductive technologies designed to enable the use of a bison genome resource biobank. Successful deployment of a genome biobank will re-establish genetic diversity within and among small bison populations that have been disconnected by disease and distance for the last 100 years.

We'll never see bison roaming the entire Great Plains again. We'll never see 20 million to 30 million bison again. No one is trying to go back in time. We're trying to go forward. We're trying to restore this important animal where we can, where people want them, and to the level where they will help restore the natural balance (Jonathan Proctor in The Guardian, 2018).

2. A brief history

2.1 Bison evolution and taxonomy

Bison phylogeny is highly controversial, but it is generally agreed that bison migrated to North America in two waves during the Late Pleistocene when low sea levels exposed the land between North America and Asia ~ 195,000 years ago and again ~ 21,000 years ago (Froese et al., 2017). Bison spread across modern-day Alaska to continental North America during the last interglaciation. The oldest bison fossil found in North America to-date is a 130,000 year-old steppe bison (*Bison priscus*; Froese et al., 2017). The steppe bison is the most probable common ancestor for all Siberian and North American bison, and

gave rise to the giant long-horned bison (*Bison latifrons*) ~120,000 years ago and the smaller antique bison (*Bison antiquus;* Shapiro et al., 2004; Hardy, 2015; Froese et al., 2017). *Bison antiquus* evolved into the intermediate form of *Bison antiquus occidentalis (Bison occidentalis)* after the northern and southern bison herds were separated by an ice sheet during the last glacial maximum (Wilson et al., 2008; Markewicz, 2017). *Bison occidentalis* is the most recent extinct ancestor of modern North American bison, *Bison bison* (Austin, 2005; Markewicz, 2017) which first appeared ~ 9,500 years ago (Hardy, 2015). *Bison bison* then diverged into plains bison (*Bison bison bison)* and wood bison (*Bison bison athabascae).* The evolutionary history of the European bison (or wisent, *Bison bonasus*) remains unclear. They were thought to evolve after *Bison antiquus* migrated back across the Bering land bridge during the late Pleistocene (Pucek et al., 2002), but recent analysis of ancient mitochondrial genomes and genome-wide nuclear DNA surveys revealed that the wisent is the product of hybridization between the extinct steppe bison (*Bison priscus*) and ancestors of modern cattle (aurochs, *Bos primigenius*) over 120,000 years ago (Soubrier et al., 2016).

Taxonomy/phylogeny:

- *Bison priscus* **(extinct Steppe Bison)**
  - *Bison latifrons* **(extinct Long-horned Bison)**
  - *Bison antiquus* **(extinct Antique Bison)**
    - *Bison antiquus antiquus*
      - *Bison bonasus* **(European Bison)**
        - Bison bonasus bonasus **(European Bison or Wisent)***
        - Bison bonasus caucasicus (**extinct Caucasian Wisent-1925)**
        - Bison bonasus hungarorum **(extinct Carpathian Wisent)**
    - *Bison antiquus occidentalis*
      - *Bison bison* **(North American Bison)***
        - Subspecies: Bison bison bison **(Plains Bison)***
        - Subspecies: Bison bison athabascae **(Wood Bison)***

*Extant species (Pucek et al., 2002; Austin, 2005; Froese et al., 2017)

2.2 Historical record of bison populations in North America

The North American bison population is composed of two subspecies or designatable units, the plains bison and the wood bison (COSEWIC, 2004). The better known of the two, the smaller plains bison, once thrived on the great plains of North America, from central Saskatchewan to northwestern Mexico (List et al., 2007). The lesser known wood bison ranged from central Alberta to Alaska and the Canadian Arctic Archipelago (Gates et al., 2001). Bison are important to the ecosystems that they inhabit due to the significant impact they have on their environment. Plains bison are a keystone species in the short- and tall-grass prairies, as are wood bison in the boreal regions (Knapp et al., 1999; Gates et al., 2001;

COSEWIC, 2004). Bison create a unique, diversified landscape through the effects of grazing, dispersing seeds, wallowing (rolling) and fertilizing. These simple behaviors benefit the entire ecosystem by diversifying plant life and creating habitats for insects, birds and small mammals. In addition, bison were a main food source of predators such as wolves, grizzly bears, and indigenous peoples. With an abundance of bison, predation pressure was spread more widely among hooved prey animals. As well, their carcasses were a food source for scavengers and supplied the land itself with a nutrient source. A modern-day example of the environmental impact of bison is the Beaver Creek Bison Project run jointly by Syncrude Canada Ltd and the Fort McKay First Nation. The herd began in 1993 with the release of 30 wood bison from Elk Island National Park onto land reclaimed from Syncrude's oil sands operation near Fort MacMurray, Alberta. The herd has grown to over 300 and has resulted in the return of 65 species of birds, 15 species of mammals, frogs, and invertebrates not seen in recent times (https://emeraldfoundation.ca/aef_awards/beaver-creek-wood-bison-project/). The bison were, and still are, an essential component of the material and social livelihood of First Nations people throughout North America.

It is estimated that between the late 1600s and early 1700s there were approximately 30 million plains bison and 168,000 wood bison in North America (Gates et al., 2001; Montoya, 2001; Cunfer and Waiser, 2016; Environment and Climate Change Canada, 2016). Recent critical analysis of historical data, including estimated hunting pressure and yearly reproductive rates has largely dispelled early population estimates of 60 to 90 million plains bison, particularly in view of the estimated carrying capacity of the plains at approximately 29 million (Cunfer and Waiser, 2016). By the turn of the 20th century, plains and wood bison populations were reduced to near-extinction after a series of events that resulted in a contraction to less than 1% of their historic numbers and geographic range (Freese et al., 2007; Sanderson et al., 2008).

The ontogeny of the near-extinction event of the North American bison has been the subject of numerous reviews (Roe, 1951; Isenberg, 2001; Brower, 2008; Cunfer and Waiser, 2016) and may more accurately be represented as a 'perfect storm' of environmental and social factors that continue to threaten the species today. *"The near extermination of the American buffalo did not happen overnight, nor was one generation of human beings fully responsible for clearing the plains and the prairies of this most noble animal. The process was slow. It started with the Indians. Then came the white man, and as he developed the fur trade west of the Mississippi, the momentum of the killing increased" (from Dary, 1974).* The population decline began in the 1700s and became more precipitous during the 1800s, ending with the slaughter of over 10,000 plains bison in a single hunt in Dakota Territory in 1883 (Roe, 1951; Cunfer and Waiser, 2016). The decline has been attributed primarily to a shift in the use of bison from a subsistence resource to a lucrative commodity.

It is an evolutionary irony that horses, a species that evolved in North America but did not survive the continent's megafauna extinctions of the Pleistocene, played a prominent role in the near-extinction of bison, a species that did survive the North American ice-age. After their reintroduction into North

America beginning in 1690, horses thrived in both captive and feral populations (Flores, 2008). It is estimated that some tribal herds had as many as 6 horses per person, which not only competed for available grazing space, but vastly increased the efficiency of harvesting bison; i.e., the native hunter was no longer confined to pursuing bison on foot (Brower, 2008). The historical record suggests that First Nations people had been over-hunting bison since at least 1790. It is estimated that subsistence requirements consumed ~60,000 bison per year in the southern herd in the United States while the natural net increase was only ~42,000 (Cunfer and Waiser, 2016). The Native peoples also hunted bison for items to trade with other tribes initially, and later for use in the Euro-American market. The pressure on bison populations increased further as a result of inter-tribal agreements to allow hunting on each other's land as a way to maintain military alliances. Within 60 years the southern herd had been overhunted by approximately 2 million bison (Cunfer and Waiser, 2016).

The *great contraction* that brought the bison species to the brink of extinction (Fig. 2.2.1) gained momentum during the mid-1800s as a consequence of a number of converging factors. The expansion of the railroad in the United States in the mid- to late-1800s resulted in a sudden expansion of trade, increase in the number of settlers and domestic livestock, and sport hunters, and effectively split the Great Plains bison herd into northern and southern herds. The southern herd was decimated between 1870 - 1874 and the northern herd between 1876 - 1883 (Roe, 1951). The arrival of domestic cattle brought diseases to which wild bovids of the continent were naive. It has been suggested that Texas tick fever, a tick-borne protozoal disease which made Texas longhorns the "pariah of the plains" during the cattle drives of the 1800s (Byrns, 2017), was responsible for epidemic outbreaks that spread northward causing mortality rates of 70-90% in previously unexposed cattle herds as well as bison herds (Koucky, 1983). In 1867, Charles Goodnight discovered 25 miles of dead bison along the Concho River Valley that had not been killed by humans (Cunfer and Waiser, 2016). Earlier epidemics through eastern Nebraska in 1825 and 1858 killed all of the bison in this region, consequently starving the local Native tribes (Koucky, 1983). It has been estimated the 4 million bison deaths were not accounted for in deaths caused by hunters, suggesting that disease may have had an important impact on the decline of the bison during the 1800s.

The discovery in Europe of a new and efficient way to tan hides for the leather industry in 1870 was another major player in the *great contraction* (Taylor, 2011). The tanning process was not developed in the United States until almost 10 years later (Feir et al., 2017; Taylor, 2011), but by then this innovation, along with free trade with Europe, created an unquenchable demand for bison hides. Previously, the meat and fur trade was restricted mostly to the winter months because of the lack of refrigeration and thickness of the fur coat, but Europe's tanning innovation led to expansion of American hide hunters who could now market flint hides (furless hides) all year long. Although US government quota regulations existed for several animal species during the late 1800s, including the seal trade in Alaska (Taylor, 2011), no attempt was made to restrict the bison trade. To the contrary, a law that permitted only Indian hunters to harvest female bison was vetoed by President Grant in 1874 (Roe, 1951). Without regulation, both Euro-American and Native American hunters were undeniably wasteful, killing hundreds in a single day while taking only the tongue or the hide, or nothing in the case of sport hunting (Roe, 1951).

The US military on the Great Plains at the time were involved in the Indian Wars and although the military did not engage in systematic eradication of the bison, neither did it do anything to prevent it (Isenberg, 2001). General Philip Sheridan declared, *Let them kill, skin, and sell until the buffalo is exterminated, as it is the only way to bring lasting peace and allow civilization to advance* (Isenberg, 2001). The military advantage of turning a blind eye to the bison slaughter had a weakening effect on the Native populations that depended heavily on bison for subsistence. Peak prices for hides occurred in 1882, but by 1884, hide hunting as a business ended because there were no more bison (Dary, 1974).

Plains bison in Canada were virtually eliminated by 1879 (Roe, 1951; Cunfer and Waiser, 2016). Because of the Hudson's Bay Company's *deliberate and consistent opposition to settlement in western Canada*, few white settlers were on the Canadian plains during the 1870s (Dary, 1974). Unlike the southern railways in the United States, the Canadian Pacific Railroad played no part in the bison slaughter because it was built after the bison had been exterminated. Overhunting by Canadian Metis (individuals with mixed European and Native American ancestry) and Native populations has been ascribed as a major reason for the decline of the bison in Canada (Roe, 1951; Dobak, 1996; Brower, 2008; Taylor, 2011; Cunfer and Waiser, 2016). Unlike the southern market, the main bison market in Canada was not for robes or hides, but for pemmican – a concentrated mixture of fat and protein that was resistant to spoilage and used as a critical provisional item for the Hudson's Bay Company boatmen (Cunfer and Waiser, 2016). The Hudson's Bay Company controlled the pemmican market by introducing quotas and lowering the purchase price of bison, thus creating an increasing need to harvest more bison to make up for the loss of income (Cunfer and Waiser, 2016).

Unlike the plains bison, specific documentation of the historic decline of the wood bison population is sparse, perhaps because of its more remote habitat and smaller starting numbers. The near-extinction of wood bison coincided with that of the plains bison, and has been attributed to much the same factors. Exploitation for trade, changes in habitat, and severe winters reduced their historic numbers of over 160,000 to less than 250 by the turn of the 20th century, surviving in scattered herds around Grand Detour, Pine Lake and Lake Claire (present day WBNP; see Section 3.2; Environment and Climate Change Canada, 2016; Gates et al., 2001; Larter et al., 2000).

2.3. Early conservation efforts in Canada and the United States

Near the end of the "great contraction", a few individuals in Canada and the United States made an effort to capture wild calves between the late 1860s and 1870s (Lothian, 1981; Brower, 2008). Charles Goodnight was one of the first to capture calves and started his own herd in Texas (U.S. Fish and Wildlife Service; Lothian, 1981). Samuel Walking Coyote, a Pend d'Oreille Indian in Montana captured calves after hunting the last herd of bison in the area, saved the calves and raised them (U.S. Fish and Wildlife Service; Lothian, 1981). Other influential ranchers included Frederick Dupree of South Dakota, Charles Alloway and James MacKay of Manitoba, and Charles Jones of Kansas who earned the moniker

"Buffalo" Jones by capturing the greatest number of bison calves (n=56; Brower, 2008). These small private herds grew slowly and were traded back and forth among cattle ranchers as the wild herd plummeted to non-existence.

Although difficult to substantiate, the anthropogenic causes listed above were apparently coincident with periods of drought and harsh winters on the plains which added to bison mortalities from disease, starvation and exposure (Isenberg, 2001; Kolipinski et al., 2014). The decline of the bison led quickly to widespread starvation and death of First Nations people (Roe, 1951). By 1888, plains bison were reduced to 85 in North America while only 6 were known to be in existence in Canada (Fig. 2.2.1; Roe, 1951; Brower, 2008; Hedrick, 2009; Government of Canada - Species at Risk Public Registry, 2018).

In 1906, in what was perhaps one of the greatest wildlife preservation efforts in history, the Canadian Government purchased the largest and last free-ranging plains bison herd in existence from Montana rancher Michel Pablo in an effort to bring bison back from the brink of extinction (Lothian, 1981; Gates et al., 2001; Brower, 2008). Starting in 1907, 410 bison from Pablo-Allard herd were brought by rail in two shipments from Ravalli, Montana to Lamont, Alberta near the northern boundary of Elk Island Park (what is now Elk Island National Park; EINP). This location was intended to be a temporary home for the bison while Buffalo National Park near Wainwright, Alberta was being fenced. When the fencing of Buffalo National Park was completed in 1909, 325 plains bison were transported from Elk Island Park to Buffalo National Park (Brower, 2008). Between the years of 1909 to 1912, over 300 more bison were brought in 6 shipments from the Pablo-Allard herd in Montana directly to Buffalo National Park in Wainwright, Alberta. Thirty bison from the C.E. Conrad herd in Montana were also purchased and brought to Wainwright (Lothian, 1981; Brower, 2008). The Conrad herd was thought to represent new genetics, but Conrad had purchased his herd from Allard several years earlier (Lothian, 1981). In 1909 and 1914, 30 bison were shipped to Buffalo National Park from the exhibition herd in the Banff Zoo which had existed from 1897 to 1937 (Lothian, 1981; Brower, 2008). The Banff exhibition herd originated from a small group of animals from Texas and 13 hand-raised calves that had been captured southwest of Battleford, Saskatchewan in 1873 and near the Canada - US border in 1874 (Lothian, 1981). The Banff herd had grown to over 100 animals in 1909 when they started distributing the herd elsewhere. The Canadian bison conservation effort was so successful that by 1920 the herd at Buffalo National Park had grown to over 6,000 animals and exceeded the Park's carrying capacity. Unfortunately, major issues due to poor management arose in Buffalo National Park that resulted in overcrowding, the spread of zoonotic diseases from cattle, and cattle/bison hybridization. These issues led to ill-considered translocations and extensive culling between 1923-1940 (Brower, 2008; see Section 3).

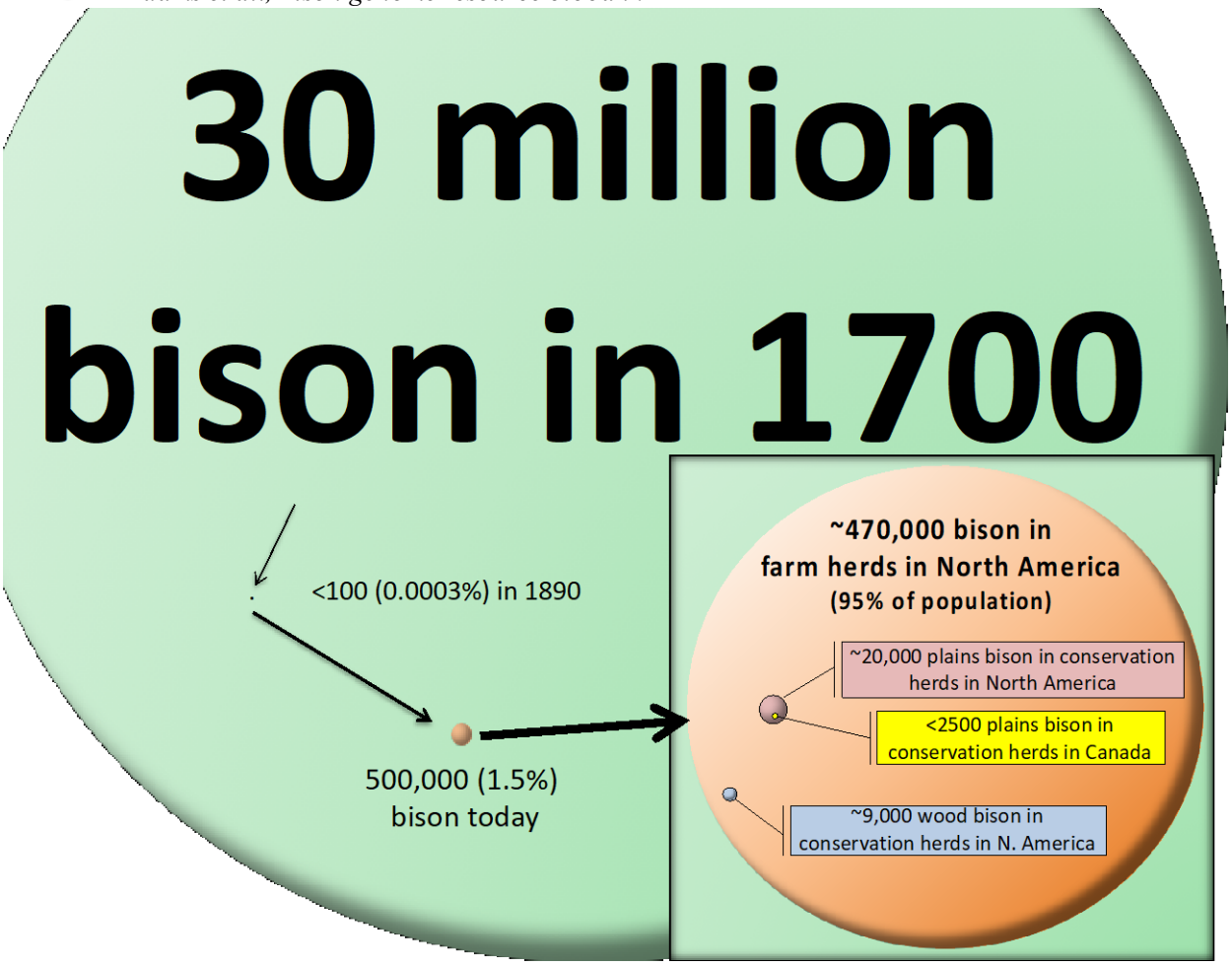

Figure 2.2.1. Near-extinction of North American bison since 1700.

During the early conservation effort, several ranchers in the United States and Canada initiated attempts to create cattle x bison hybrids, known as cattalo. The Canadian government became interested in Mossom Boyd's experiments regarding cattalo, and after his death the Boyd herd was moved from Ontario to Wainwright. The hybridization experiments continued at Wainwright from 1916 to 1935 in a concerted effort to introduce bison traits such as meat quality and quantity, hardiness, feed efficiency, and disease resistance into domestic cattle (Deakin et al., 1943; Derr et al., 2012; Hedrick, 2009). Other influential cattalo breeders included Robert Wickliffe of Kentucky who started his trials in 1815, "Buffalo" Jones of Kansas, Charles Goodnight of Texas, and Colonel Samuel Bedson of Manitoba. (Brower, 2008). Even Michel Pablo and Charles Allard, the previous owners of the Pablo-Allard herd, had a cattalo herd numbering between 150-200 head (Brower, 2008). Introgression of domestic cattle genes persists in most plains bison herds in existence today, and remains a complicating issue in on-going conservation efforts (see Sections 3 and 8).

3. Implications of the loss of genetic diversity

The Canadian Government Species at Risk Act (SARA) classifies the wood bison as Schedule 1 *threatened*, and plains bison have no designated status (Government of Canada, 2018). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) classified wood bison as *endangered* in 1978, *threatened* in 2000 and as a *special concern* since 2013 (COSEWIC, 2013); plains bison have been listed as *threatened* since 2004 (COSEWIC, 2013).

Both plains and wood bison remain classified as a *near threatened species* according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Aune et al., 2017). Importantly, the IUCN states that bison are a conservation-dependent species; i.e., without conservation efforts, bison as a distinct species would cease to exist. In 1997, the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) down-listed wood bison from Appendix I (species threatened with extinction and trade of specimens is prohibited-except in special circumstances) to Appendix II (species not necessarily threatened with extinction and trade of specimens is controlled; Environment and Climate Change Canada, 2016). Currently, neither plains nor wood bison are listed by CITES, therefore trade is not monitored. Both Canadian and American governments have designated national parks specifically to preserve bison and their natural habitat. Privately funded conservation herds can also be found on reserves such as the American Prairie Reserve in Montana and the Tallgrass Prairie Reserve in Oklahoma.

3.1 Genetic diversity

Genetic diversity is crucial for the survival of a species as it provides the capacity for a species to adapt to changing circumstances within its environment. Although the relationship between genetic diversity and species survival is complex, a species with high variation in alleles has an increased probability of successfully responding to a new stressor, including changes in climate, food availability, and pathogens. In contrast, low allelic variation may impact a species' ability to cope with these changes thereby reducing overall fitness, and threatening long-term viability. As well, genetic diversity within a species has a direct impact on *biodiversity*; the diversity of species within a biome. Changes in genetic diversity from the loss of a species leads to a loss of biological diversity within the ecosystem (Lankau, 2007). Both plains and wood bison have undergone a serious loss of genetic diversity because of founder effects, population bottlenecks, genetic drift, and selection (Wilson and Strobeck, 1999; McFarlane et al., 2006).

Random *allelic mutations* are a natural part of an evolving species and serve to increase genetic diversity, but they occur at a very slow rate. Conversely, *genetic drift* is a natural random process involving the loss of alleles or traits. In small populations, the rate of genetic drift is greater than that of mutations, resulting in *genetic fixation* – the loss of alternative trait characteristics from the gene pool. The factor that has the greatest impact on genetic diversity is the *effective population* size (McFarlane et al., 2006); that is, the number of animals of breeding age (adults). It is estimated that only 1/3rd of a bison population are of effective breeding age (McFarlane et al., 2006). For long-term viability, an effective population of ≥1,000 animals is needed to retain 90% of their allelic diversity (traits) for 200 years (Gross and Wang, 2005; Freese et al., 2007; Hedrick, 2009). In Canada, nearly all conservation herds consist of isolated groups of <500 bison with no new bison genes added to these herds since the introduction of bison from the US over 110 years ago. Therefore, the question arises: How do we genetically re-connect formerly roaming herds to conserve bison for generations to come?

An example of the effects of lost genetic diversity is the Texas State Bison Herd (TSBH). The herd was composed of 40 plains bison that were direct descendants of the Charles Goodnight herd in the 1880s without subsequent introduction of new genetics. The herd was closely monitored for 6 years to evaluate the *inbreeding depression,* which was found to be equivalent to that of 2 generations of full sibling matings (Halbert et al., 2005). During the 6 years of observation, the herd had a mortality rate that was 12.5 times higher than other herds, and a natality rate that was 67% lower than other herds, resulting in a stagnant population size of 31-40 animals (Hedrick, 2009). Analyses showed a 99% chance of population extinction within the following 41 to 51 years if no new genetics were added (Halbert et al., 2005). However, addition of new male bison genes into the herd would increase its chance of survival for 100 years up to 100%. Importing new bison genetics into small populations *will increase genetic variation, improve population fitness, decrease levels of inbreeding, increase adaptive response and perhaps most significantly, [provide] a substantially higher probability of population survival* (Halbert et al., 2005).

3.2 Conservation herds vs commercial herds

Paradoxically, the growing number of bison in livestock production herds has had a disguising effect on the conservation issue. There are an estimated 500,000 bison in North America today (Hedrick, 2009). While the number of commercial (farmed) bison has increased dramatically since 1970, the number of bison in conservation herds has remained stagnant since it peaked in the 1930s (Freese et al., 2007). Only 5% of bison (~20,000 plains bison and 9,000 wood bison) in North America are in conservation herds (Fig. 3.2.1) while the remaining 95% (~470,000) are in production herds where bison are handled as livestock and are of mixed ancestry (Fig. 2.2.1; Hedrick, 2009; COSEWIC, 2013). For the purposes of restoring genetic diversity, the effective population size includes only disease- and bovine gene-free bison (i.e., those in conservation herds). Of the 20,000 bison in conservation herds in North America, 13% are outside of the plains bison's historical range (Kohl et al., 2013). Strictly speaking, a true conservation herd must satisfy 4 criteria to be considered *wild by nature* (COSEWIC, 2013): 1) non-manipulated breeding competition and natural or naturally imitated culls, 2) interventions are not made to alter or

increase fitness, 3) allowed to roam free on more than 200 km^2 of land that allows for seasonal movements, and 4) natural predation occurs (COSEWIC, 2013).

3.3 Genetic bottlenecks in the post-contraction era

Plains bison

From 1907 to 1912 the Canadian government purchased and imported over 700 plains bison from Michel Pablo in Montana most of which were placed in the newly established Buffalo National Park, a 159 square-mile area near Wainwright, Alberta (Markewicz, 2017). While this effort played an important role in saving the plains bison from extinction, government records of events from 1912 to 1925 document the first appearance and subsequent prevalence of bovine tuberculosis in the herd at Wainwright (reviewed in Fuller, 2002; Brower, 2008). By 1920, the number of bison in the herd exceeded the carrying capacity of Buffalo National Park and the mounting pressure of starvation and disease forced a difficult decision: slaughter the entire herd and start over with disease-free stock, or translocate the herd elsewhere. Unfortunately, a definitive decision was not made. From 1925-1928, 6,673 plains bison were transported by rail to waterways, and then via specially constructed barges down the Athabasca and Peace Rivers to Wood Buffalo National Park. The plains bison then proceeded to breed with the resident wood bison resulting in hybridization (Wilson and Strobeck, 1999; COSEWIC, 2013). From 1923-40, over 19,000 plains bison were slaughtered as a result of annual culling with final depopulation of the Wainwright herd. Buffalo National Park was finally closed and turned over to the military in 1940 (Brower, 2008; Fuller, 2002) leaving the only remaining herd of plains bison in Canada at Elk Island National Park (Brower, 2008), with the exception of 20 bison that had been moved from Buffalo National Park to Manitoba (Riding Mountain/Lake Audy herd). The population dynamics of plains bison in Canadian conservation herds are shown in Fig. 3.3.1.

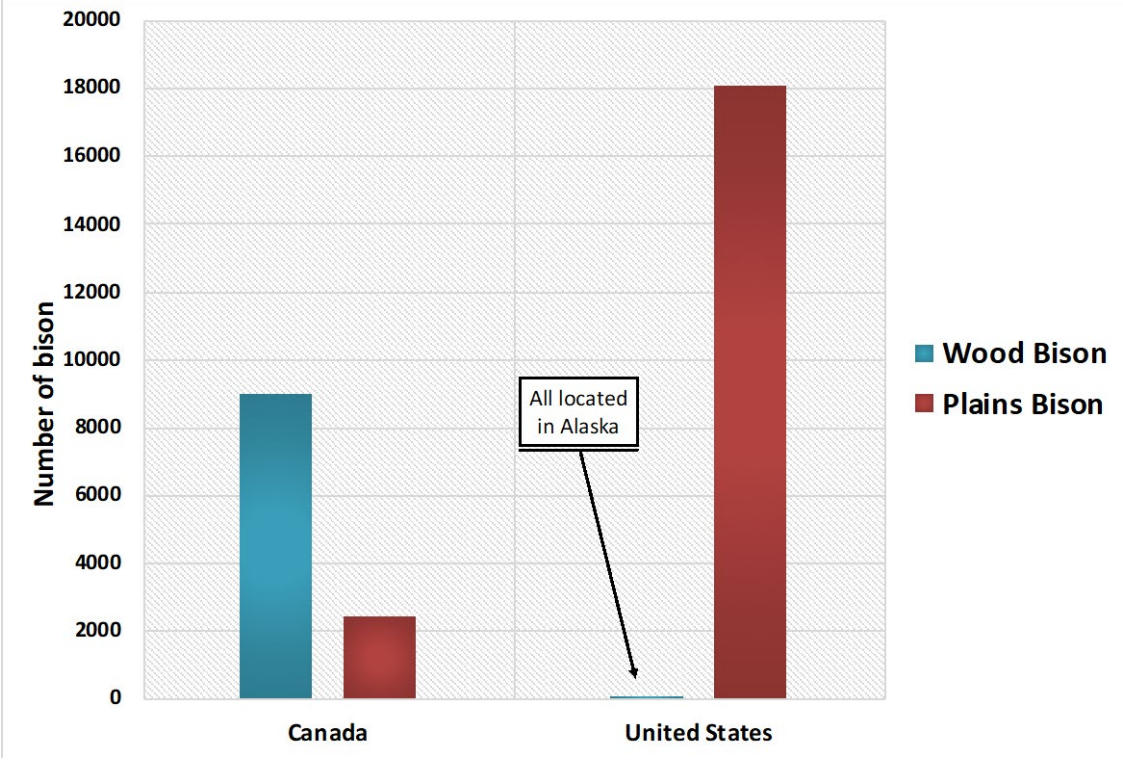

Figure 3.2.1. Distribution of wood and plains bison located in wild or conservation herds in North America (Adapted from Gates et al., 2010).

During the 1909 translocation of plains bison from their temporary home at Elk Island to Buffalo National Park, approximately 50 plains bison evaded capture and remained at Elk Island. These 50 escapees are the founding animals for the plains bison herd at Elk Island National Park, as well as every present-day plains bison conservation herd in Canada (Markewicz, 2017). The *effective population size* of plains bison in Canada's plains bison conservation herds (i.e., bison of known genetic provenance) is estimated at less than 1500, distributed among 5 herds (Fig. 3.3.2.); again, all five conservation herds were derived from a single founder population of 50 bison that originated at Elk Island National Park. Canada's largest conservation herd of plains bison (Pink Mountain) (Rowe, 2006) has been maintained outside of historic plains bison range since the 1970s, and 2 of the 4 other herds have fewer than 200 animals (Province of British Columbia, 2000, COSEWIC, 2013). There are also 4 other plains bison display herds in Canada that are not considered conservation herds; Buffalo Pound Provincial Park, Canadian Forces Base-Wainwright, Riding Mountain National Park and Waterton Lakes National Park.

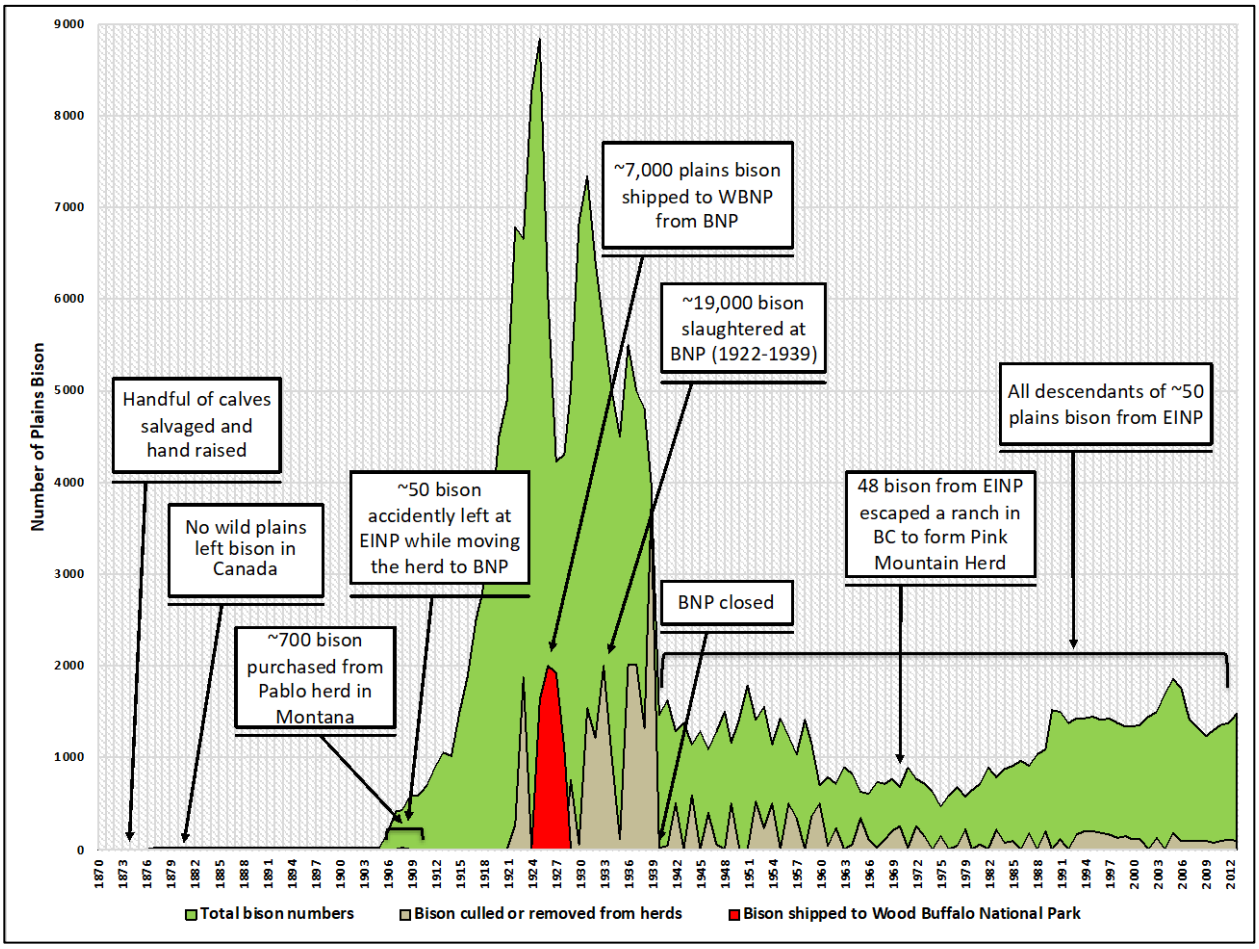

Figure 3.3.1. Population dynamics of plains bison in Canada's conservation herds since ~1870. (EINP=Elk Island National Park, BNP=Buffalo National Park, WBNP=Wood Buffalo National Park, (Lothian, 1981; Rowe, 2006; Brower, 2008; Markewicz, 2017; Parks Canada, 2018). ***Note***: Approximately half of the plains bison present in BNP during the population spike from 1920-42 were infected with TB and brucellosis.

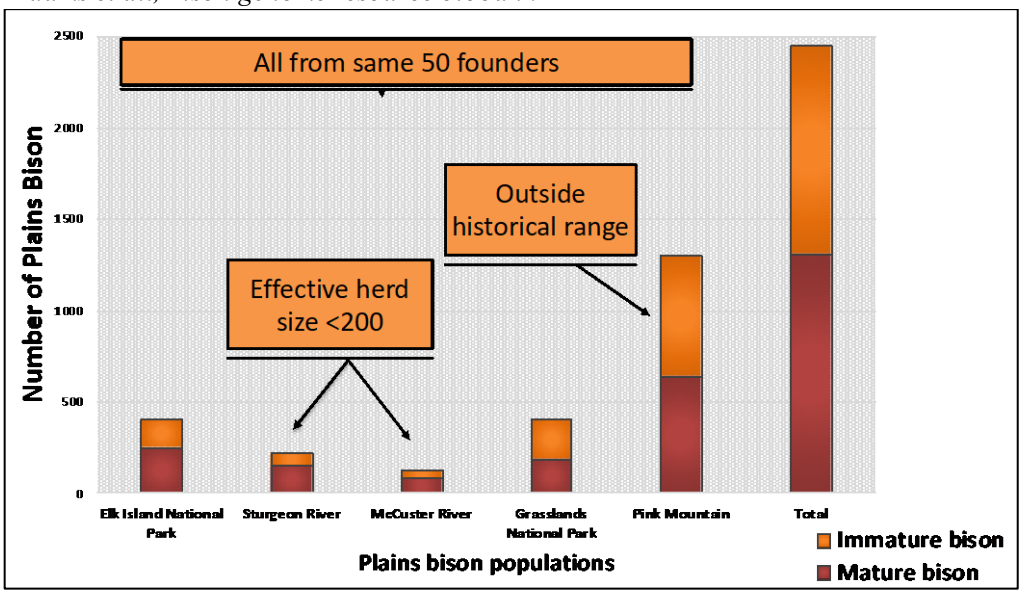

Figure 3.3.2. Plains bison populations in conservation herds in Canada (from COSEWIC 2013).

Wood bison

Historically, wood bison inhabited a region encompassing one of the largest fresh-water deltas in the world – the Peace-Athabasca Delta where the Peace and Athabasca Rivers merge to form the Slave River that ultimately joins the Mackenzie River in the Canadian north. In 1922, the continent's largest national park (17,000 square miles) was established and named Wood Buffalo National Park (WBNP) after its keystone species (Fig. 3.3.3). This park contains the largest remaining wood bison population in the world and the only herd in North America that has never been without wood bison. This can only be compared to Yellowstone National Park (YNP) which has maintained the only continuous herd of plains bison in the world in an area 1/5th the size of WBNP (Moynihan, 1963). All of the world's wild wood bison reside in Canada except for two populations. One was sent from EINP in 3 shipments (n=30 each) to Lenski Stolby Nature Park near Yakutsk, Sahka Republic, Russia in 2006, 2011 and 2013 (Markewicz, 2017). The other was sent from EINP (n=130) and released into the wild in Alaska in 2015 (Government of Canada - Species at Risk Public Registry, 2016; Environment and Climate Change Canada, 2016; MacFarland and Seaton, 2018).

The translocation of plains bison from the Wainwright herd in the 1920s had two very serious consequences for the long-term viability of wood bison as a species: 1) hybridization and 2) introduction of endemic bovine diseases. While the introduction of plains bison into WBNP resulted in a transient increase in the total bison population within the park, the population in general has not thrived and remains at less than 6% of historic numbers (Fig. 3.3.4). Today, 5 main sub-populations of wood bison inhabit geographically distinct areas within Wood Buffalo National Park: Little Buffalo, Needle Lake,

Pine Lake, Peace Athabasca Delta and Garden River (Figs. 3.3.3 and 3.3.5). There are also herds around the park that are considered part of the Greater Wood Buffalo National Park region (Fig. 3.3.3); these herds are known by several names (Fig. 3.3.3) and their disease status is illustrated in Fig. 3.3.5.

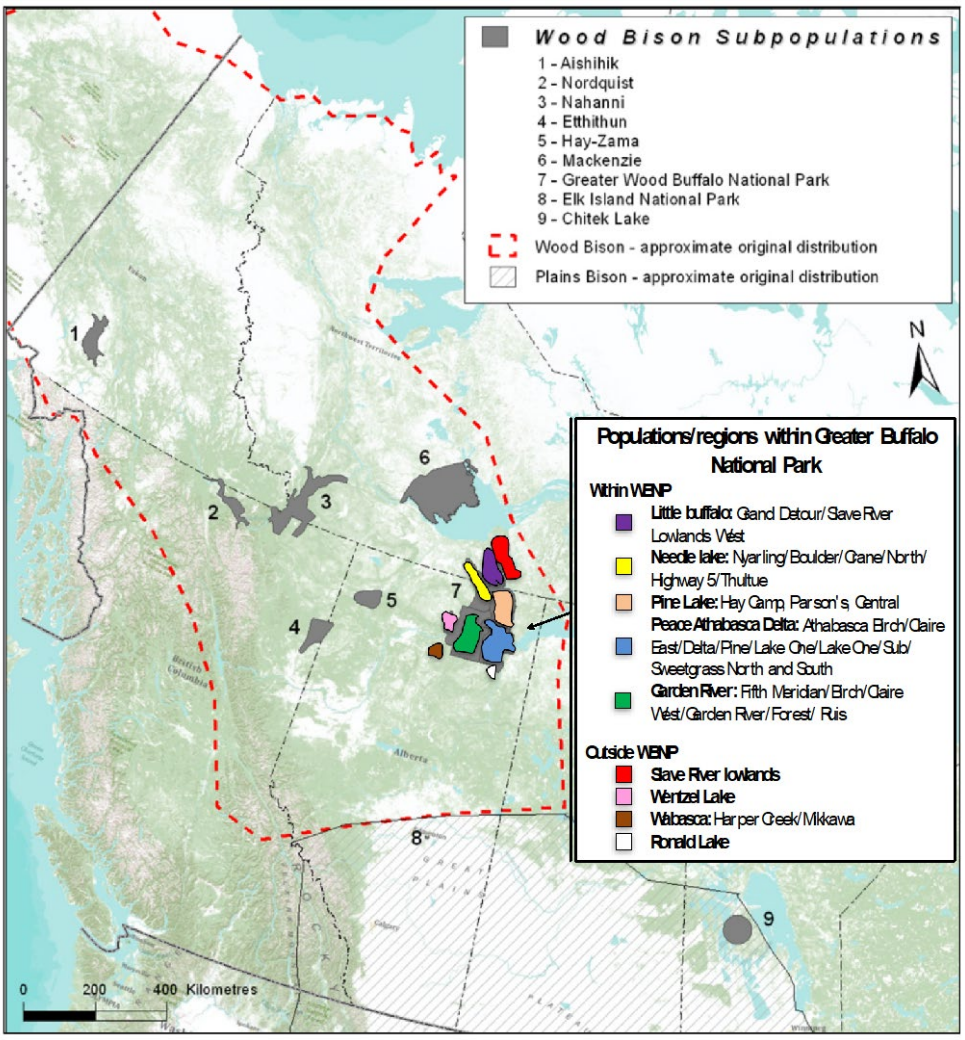

Figure 3.3.3. Wood bison herds (wild and conservation herds) in Canada (adapted from COSEWIC 2013 and 2016 Sharon Irwin, Parks Canada personal communication).

In 1957, an isolated herd of ~200 bison was discovered in the Nyarling River/ Needle Lake area at the northwestern edge of WBNP (Larter et al., 2000; COSEWIC, 2013). The hope at the time was that this group was sufficiently isolated from plains bison introduced into the southeastern corner of WBNP in the 1920s that they escaped hybridization. Genetic testing done more recently has shown that they were unable to avoid hybridization (Wilson and Strobeck, 1999). Seventy seven of the Nyarling River/Needle Lake bison were captured in 1962 to establish a conservation herd (Larter et al., 2000). Following disease testing, 18 of these animals were transferred to the newly established Mackenzie Bison Sanctuary (MBS)

in the Northwest Territories in 1963 (2 died within their first year). After a second roundup in 1965, 23 more Nyarling bison were transferred to EINP in Alberta (Lothian, 1981; Van Camp, 1989; Larter et al., 2000). In 1968, however, bovine tuberculosis was detected in the wood bison herd at EINP and all the adults were killed leaving only a founder population of 11 female and 19 male calves. These wood bison, are the founders for every wild, disease-free wood bison herd in the world outside of the greater Wood Buffalo National Park region, with the exception of MBS which originated from the same Nyarling River/Needle Lake herd (Fig. 3.3.6).

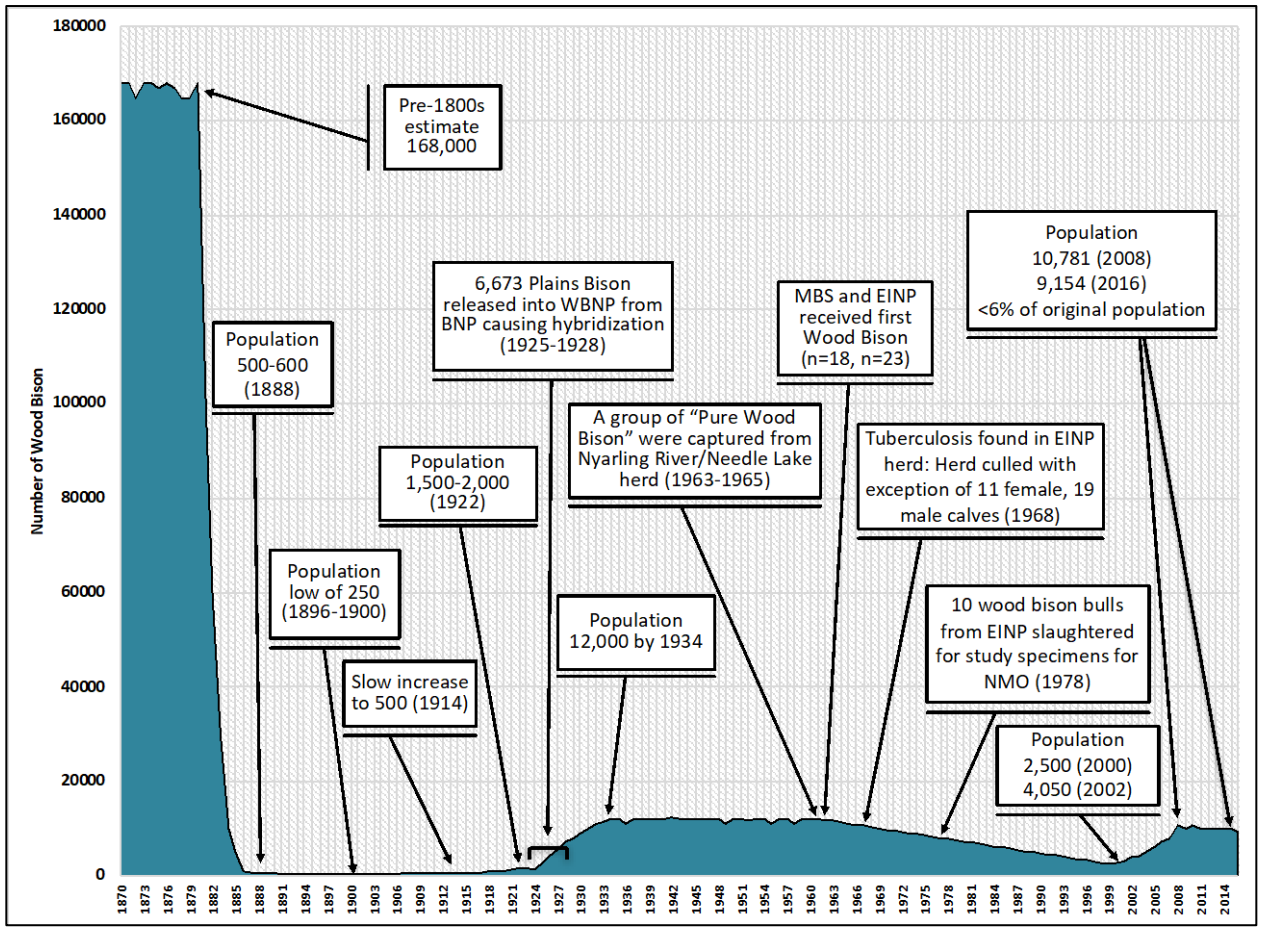

Figure 3.3.4. Population dynamics of wood bison in Canada from 1870 to present (EINP=Elk Island National Park, MBS=Mackenzie Bison Sanctuary, BNP=Buffalo National Park, WBNP=Wood Buffalo National Park, NMO=National Museum collection in Ottawa (adapted from Soper, 1941; Joly and Messier, 2004; Brower, 2008; Gates et al., 2010; COSEWIC, 2013; Environment and Climate Change Canada, 2016; Markewicz, 2017)

In 1978, 10 wood bison bulls were selected from the EINP herd based on the phenotypic traits most characteristic of wood bison, and were slaughtered for use as study specimens for the National Museum collection in Ottawa. This resulted in the loss of 41% of the males in the herd at the time, and removal of those with the highest phenotypic fidelity to the wood bison subspecies. In a recent genetic analysis,

significant bottleneck signatures were evident in all wood bison populations, and even moderate inbreeding in the WBNP population - the world's most diverse population (Ball and Wilson, 2016). The population dynamics of Canada's wood bison wild and conservation herds are show in Figure 3.3.4.

The herds within WBNP are free-ranging, but the extent to which genetic transfer occurs between herds is unclear and has important implications for the genetic diversity within each subpopulation. Cluster analysis of radio-telemetry data suggest that there are five distinct groups of bison in WBNP (Joly, 2001). While it is clear that some individuals move great distances within the park, the exchange rate between groups (calculated as the number of exchanges divided by the number of residents) indicates varying degrees of demographic discontinuity. For instance, no radio-collared bison moved from the Nyarling River group (north-west) to either the Hay Camp (east-central) or Delta (south-east) groups (~ 60 bison-years of monitoring), but the exchange rate between the latter groups averaged about 5% per season (Joly, 2001). The free-ranging Ronald Lake wood bison herd represents an example of apparent isolation despite overlapping territory with herds within WBNP. The Ronald Lake herd is free of bovine brucellosis and tuberculosis and displays a distinctive genetic fingerprint based on microsatellite data, suggesting that mixing has not occurred despite their proximity to diseased herds in WBNP (Ball and Wilson, 2016). If a degree of geospatial isolation exists among the herds of the greater WBNP region, then herds of <1000 individuals are in jeopardy of losing sufficient genetic diversity to support long-term survival. In this regard, only 1 of 7 diseased herds in the greater WBNP and only 1 of 10 disease-free herds outside the park are composed of ≥1000 individuals, and 10 of the 17 herds have fewer than 500 individuals (Fig. 3.3.5). Of the 7 conservation herds founded from EINP, 5 do not exceed 500 individuals including the EINP herd itself, and these herds are clearly geographically and genetically isolated (Fig. 3.3.6). This situation, particularly the latter, is not consistent with stated long-term population and distribution objectives of the Recovery Strategy for the Wood Bison in Canada (2018) *to ensure the existence of at least five disease-free, genetically diverse, connected, self-sustaining, free-ranging populations distributed throughout their original Canadian range, with a minimum of 1,000 animals per herd*.

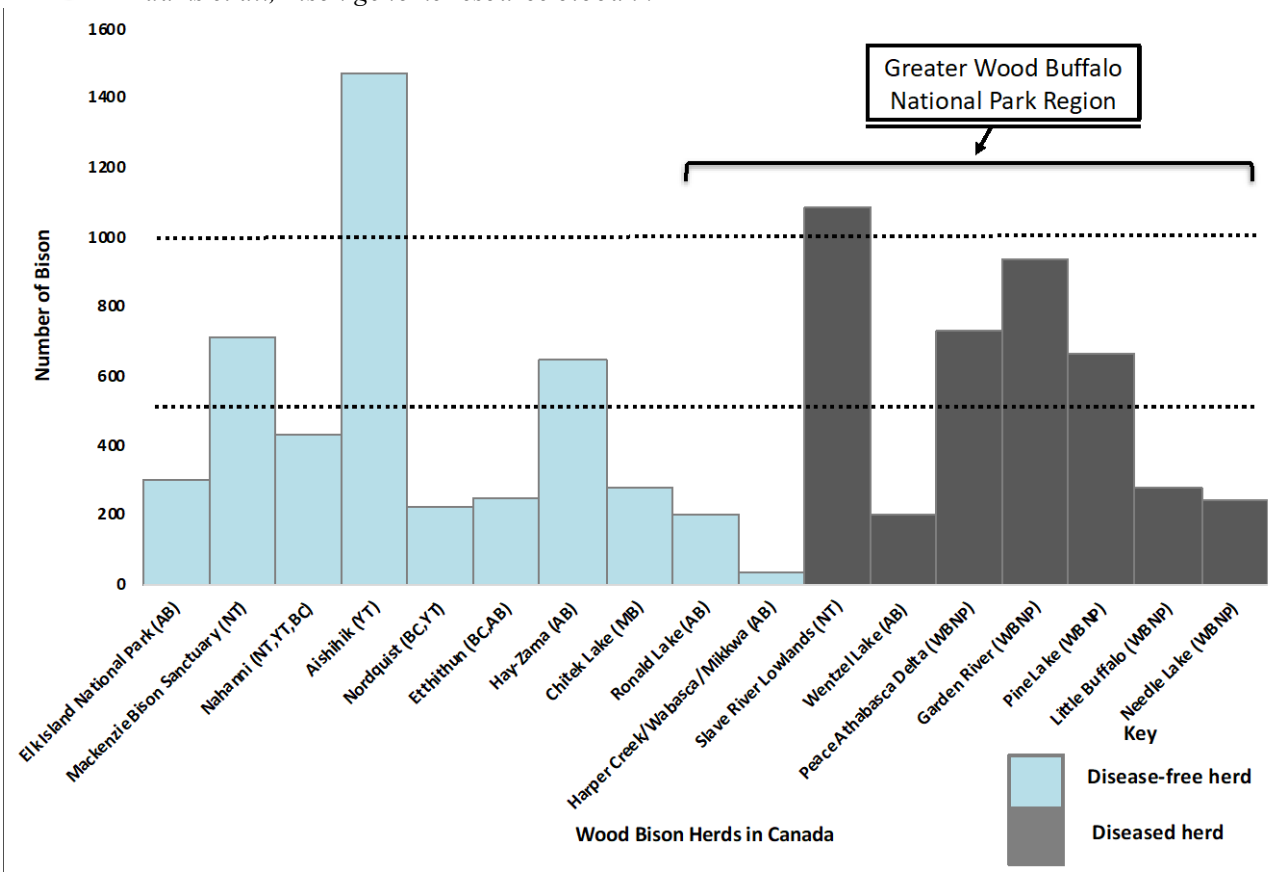

Fig. 3.3.5. Population sizes and disease status for all wood bison herds in Canada as of 2016. AB: Alberta, NT: Northwest Territories, YT: Yukon, BC: British Columbia, MB: Manitoba, WBNP: Wood Buffalo National Park (Adapted from Environment and Climate Change Canada, 2016; Sharon Irwin, Parks Canada personal communication, 2016 estimate).

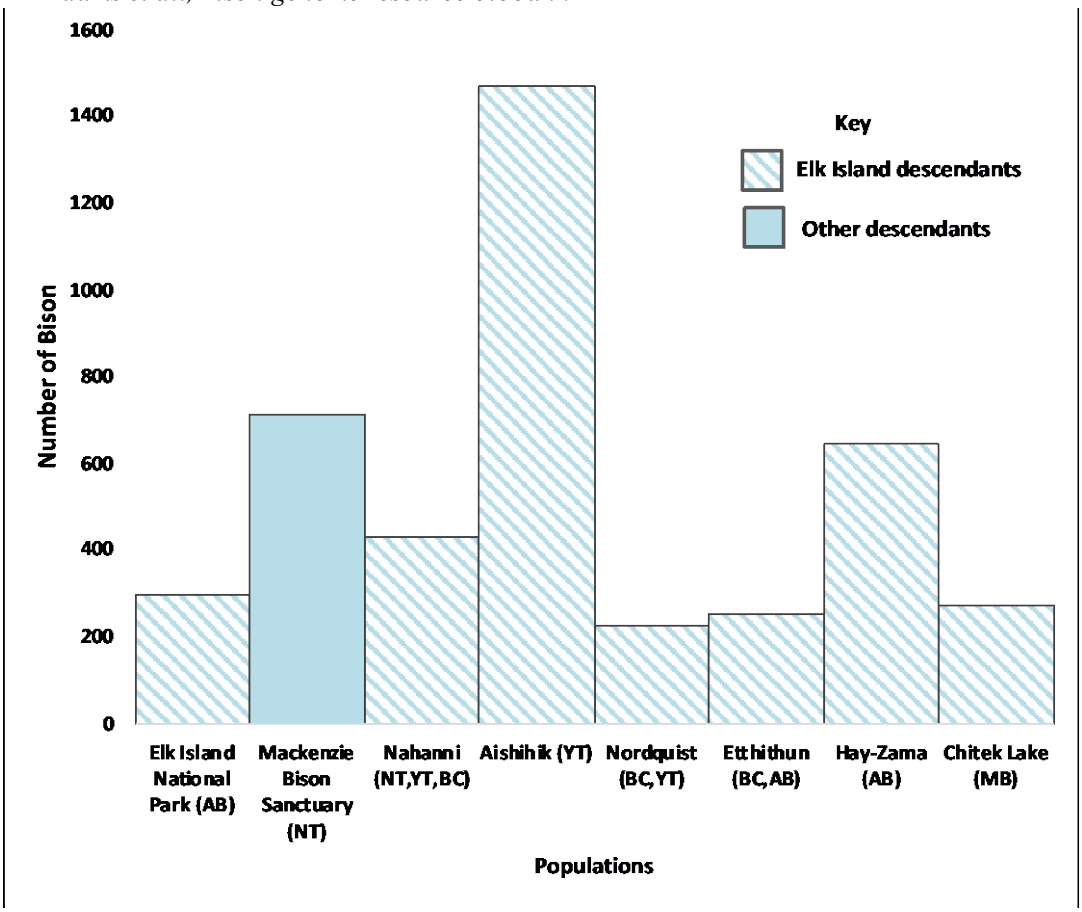

Figure 3.3.6. Disease-free wood bison populations outside of the Greater Wood Buffalo National Park region. (Adapted from COSEWIC, 2013; Ball and Wilson, 2016; Environment and Climate Change Canada, 2016)

4. Phenology of wild bison

4.1 The male

Behaviour, breeding success and herd compositional dynamics of male bison are governed by the existence of a dominance hierarchy. Bison males spend the majority of the year in small bull groups of up to 20 individuals, away from the larger mixed group of cows, calves and juveniles (Fuller, 1960). Approximately half of the bison bulls observed in Wood Buffalo National Park were lone bulls with the remaining in groups of no more than 10 individuals (Fuller, 1960; Shult, 1972). Solitary bulls tend to be

mature in age, likely a reflection of their lack of vulnerability to predation (Lott, 1991). Juvenile bison males will remain with the mixed group until approximately 3 years old (Fuller, 1960; Shult, 1972).

Little information is available on the onset of puberty in male bison. Puberty in bison bulls living under natural conditions is reflected when they begin to exhibit breeding behaviour and achieve hierarchal dominance over other companion bulls. Sperm smears from the caudal epididymis from 20 young males demonstrated that only a small proportion of males reached sexual maturity as yearlings while all three-year-olds were sexually mature (Fuller, 1961). The commercial bison industry successfully uses 2-year-old bulls in their breeding programs indicating that male bison have reached puberty from the standpoint of reproductive capability and semen quality ~26 months of age (Haigh and Grinde, 2007). More recent studies have focused on determining the age of puberty when defined as the point where testicular development has progressed sufficiently to support the production of an ejaculate containing a minimum of 50 x 10^6 sperm cells with at least 10% motility. With this definition, the average age of puberty in a group of plains bison was determined to be 16.5 ± 2.5 months (Helbig et al, 2007a).

Display of sex-specific breeding behaviour was apparent in bulls ≥1 year of age (Rothstein and Griswold, 1991). Olfactory investigation followed by the flehmen response was more common when interacting with females while aggressive displays were more frequent with other males. Younger calves displayed primarily play behaviour. Buller syndrome was also observed in male bison up to 4 years of age with a higher frequency among 2 to 3 year-olds (Lott, 1983). The term *buller syndrome* refers to excessive mounting of a steer by its pen-mates, sometimes resulting in injury or death. It is a syndrome that results in economic loss in beef feedlots, but it appears to be normal behaviour for developing bulls. This syndrome was absent in bison bulls 5 years or older (Lott, 1983). In free-ranging bison, most of the breeding is done by the older, larger males (Ungerer et al 2013, Bowyer et al 2007, Maher and Byers, 1987; McHugh, 1958); however, younger bulls have been observed tending and breeding females particularly in the absence of more mature bulls (Berger and Cunningham, 1994; Wes Olson, personal communication).

Although there is considerable confusion in the literature, bison are seasonal breeders (reviewed below in *Phenology of the female*); the breeding season is generally reported to be between late July and early October with most cows calving in April to May (McHugh 1958; Rutberg, 1984). The *rut* or *rutting season* is broadly defined as the period when males display increased sexual behaviour, compete with other males and seek out females to copulate. Similar to most large ruminants, bison have a polygynous mating system. During the rut, bull groups merge with mixed groups to form large herds with an average cow:bull ratio of between 10:1 to 3:1 (McHugh 1958, Lott, 1981; W. Olson, personal communication). Early indications of the rut in sexually mature bulls include wallowing, urinating in the wallow before pawing and rolling, uprooting of small trees and increased vocalization (Lott 1981; McHugh 1958; Fuller, 1960). The “bellow” is most commonly heard during the rut, particularly between competing bulls (Fuller, 1960; McHugh, 1958; Shult, 1972; Wyman et al., 2012). Bulls within mixed groups have also been observed to display aggressive behaviour towards outside bulls attempting to join (Herrig and

Haugen, 1969). A series of courtship behaviours will occur before copulation (Fuller, 1960; Herrig and Haugen, 1969; Lott, 1991, 1981; Shult, 1972). Bulls will approach females to lick and smell their external genitalia to determine receptivity. In a process that appears to result in increasing receptivity from the female, the bull will "tend" a female of interest by following her around. There may be multiple younger or competing bulls surrounding the female and the tending bull. It is not uncommon for another mature bull to challenge the tending bull and take his place. Various breeding behaviours such as rubbing, wallowing, and bellowing from both the male and female will occur before mounting and mating. After mating, bulls will sequester the female for approximately 35 minutes, after which, 20% of the females will copulate again with another bull (Lott, 1981).

In an early observational study, two thirds of the females were bred by the highest-ranked one third of the males (Lott, 1979). However, in a more recent study of free-range plains bison over a 6-year period, data on breeding behaviour during the rut were correlated with genetic paternity analysis of calves the following season, and authors concluded that while estimates of mating success were positively correlated with reproductive success, copulatory success was a poor predictor of the actual number of offspring sired by individual males (Mooring and Penedo, 2014). They reported that 44% of observed matings did not result in the birth of offspring, and 60% of the copulations that did produce a calf did not accurately predict the sire bull. Microsatellite study of four distinct bison herds demonstrated that a single bull is the predominant breeder for each group. Increasing the number of competing bulls and receptive cows decreases the dominant male's ability to monopolize females. However, in groups of 19 to 54 females and 2 to 5 bulls, at least 73% of the calves were sired by a single bull (Roden et al. 2003). Female preference for high ranking males may play a role (Wolff, 1998).

Little information is available on the reproductive senescence of bison bulls. Authors of one study of the Mackenzie Bison Sanctuary categorized adult bulls as those between 6-12 years of age, and old bulls as those between 10-16 years of age (Komers et al., 1994). In plains bison, the number of copulations and offspring sired was high between 8 and 12 years of age, maximal at 9 years of age, and near nadir by 15 years of age (Mooring and Penedo, 2014). It is unclear if the reduction is due to reproductive senescence or decreased ability to compete for dominance.

4.2 The female

Puberty

The age of puberty in female bison has been inferred by the age at which they first calve. Various reports exist that indicate puberty in female bison occurs somewhere between 24 and 30 months of age such that the first calf is born during the spring of their 3rdrd or 4th year. In one study (Shaw and Carter, 1989),

authors reported that only 12% of 2-year-old bison from the Wichita Mountains Wildlife Refuge in Oklahoma produced a calf, but suggested later in the same report that these females may actually have been 3 years of age when they calved due to the lack of precision associated with tooth eruption patterns used to estimate age. Based on post-mortem examinations of bison harvested in Wood Buffalo National Park during December and January of 1952 to 1956, 36% of 2-year-olds and 52% of 3-year-olds were pregnant and would have calved as 3-year-olds and 4 year olds, respectively (Fuller 1962). In another study on wood bison, done in Elk Island National Park in Canada, the mean age at first calving was 3.7 years (Wilson et al 2002). In that report examining the age structure of reproductive success, successful breeding at 2 years of age was observed once in each year (1996 and 1999), with the majority of 3-year-old bison becoming pregnant over the course of the study. In a more recent study using microsatellite loci to examine plains bison over a 3-year period at the Konza Prairie Biological Station in Kansas (Ungerer et al 2013), female bison typically reached reproductive maturity after 2 years of age and delivered their first calf when they were 3 years old. The onset of puberty in female bison, and factors that affect it, remain unclear. Systematic investigation of factors that influence the onset of puberty in bison will require serial examination to detect ovulation with respect to specific age, body weight and body condition, subspecies (plains vs wood), and latitude within the northern hemisphere. In this regard, an initial study involving serial transrectal ultrasonography was done (McCorkell et al., 2013), and while ovulation may not usually occur until the breeding season in which bison females are 3 years of age, follicle development within the ovary was clearly evident much earlier than that time (see Section 6.1).

Seasonality

Though no reports were found in the literature specifically characterizing the calving distribution in captive or free-roaming bison, bison calves are born in a relatively synchronous pattern during the spring and early summer months suggesting a seasonal pattern to bison fertility. The seasonal pattern is thought to be a physiological response to photoperiod and perhaps climate rather than the effect of predation pressure (Rutberg, 1984). Despite an early report based on the examination of corpora lutea from slaughtered bison suggesting that bison are not polyestrous (Haugen 1974), results of more recent studies indicate that bison are seasonally polyestrous (Kirkpatrick et al., 1991; Rutley and Rajamahendran 1995, Matsuda et al., 1996; Vervaecke and Schwarzenberger, 2006; see Section 6.1). The concept and timing of seasonality in bison, however, have been clouded by use of non-specific terms that relate to sexual behaviour (the rut, anestrus, estrus, anestrus periods and estrous cycle periods), lactational status (i.e., lactational vs spring anestrus), and endocrine status (luteal vs non-luteal phases). The 'rut' (sexual and aggressive behaviour of mature bison bulls) has been variously reported to begin sometime in July or August. While the onset of the rut is a common reference, no reference was found regarding the end of rut. Changes in seasonal and day-to-day sexual behaviour of female bison are much less obvious, and have not been critically characterized using estrus-detection methods currently used for domestic cattle. Interestingly, based on the birth and paternity test of bison calves, 60% of sire matings the previous season were never observed despite *intensive dawn-to-dusk observations conducted on this herd throughout the rut and the nearly ideal conditions for observations* (Mooring and Penedo, 2014).

Until recently (see Section 6.1), studies of bison reproductive patterns have involved the use of fecal or urinary steroid analysis because of the difficulty in frequent handling of the animals (Kirkpatrick et al. 1991; Rutley and Rajamahendran 1995; Matsuda et al., 1996). Before the breeding season, progesterone concentrations remained at nadir and the onset of estrous behaviour was followed by a marked elevation in progesterone concentrations in urine, feces and blood (Matsuda et al., 1996). Despite the variation in geographic locations from which data on bison have been gathered, the beginning of the breeding season appears to be very consistent. In a report of plains bison from the Delta Junction Bison Range in the interior of Alaska (64º North latitude) the mean date of first breeding was August 18 ± 5.1 days (mean ± SD) in 1996 and August 20 ± 6.2 days in 1997 (Bowyer et al., 2007). Earlier reports from Wood Buffalo National Park (64º North latitude) indicated that peak of the rut occurred between August 10 and 20 (Fuller, 1962), and a more recent report from Dawson Creek, British Columbia (56º North latitude) stated that the first elevation in progesterone was recorded around August 8 for the two years of the study (Rutley and Rajamahendran, 1995). A rise in progesterone concentrations beginning in early August was also reported in wood bison studied at the Toronto Zoo (45º North latitude; Matsuda et al., 1996) and from a plains bison located in the Belgian Ardennes (50º North latitude; Veraecke and Schwarzenberger, 2006).

Based on fecal progesterone concentrations, the beginning of the breeding season was characterized by an initial short cycle. This initial cycle was reported to be less than 10 days (Rutley and Rajamahendran 1995) and the duration of progesterone production was 4.1 ± 0.9 days (Vervaecke and Schwarzenberger, 2006). Subsequently, multiple and longer estrous cycles were reported, with a cycle duration ranging from of an a range of 16 to 31 days, and a mean of 21.1 days (Kirkpatrick et al., 1992) or 20.8 ± 0.3 days (Matsuda et al., 1996). Very little information has been published on the duration of the ovulatory season, or the onset of the anovulatory season in bison. In unmated females, regular intervals of high and low fecal progesterone continued well into the winter (Rutley and Rajamahendran, 1995). Luteal activity ceased by the end of April and progesterone concentrations remained at nadir for the spring and summer until the resumption of sponataneous ovulation in August (Rutley and Rajamahendran 1995; Matsuda et al., 1996).

Gestation

Estimates of gestation length in bison have been based on observational studies of the intercalving interval or attempts to correlate copulation during the rut with subsequent births the following season. However estimates of the intercalving interval do not account for a period of natural seasonal anestrus (reviewed above and in Section 6.1), and most copulations resulting in pregnancy and birth of offspring are not detected (reviewed in Section 4.1). Hence, reports of gestation length vary widely from 257 to 293 days (reviewed in Vervaecke and Schwarzenberger, 2006). By correlating dates of detected copulation with fecal progesterone data the mean gestation length for 10 plains bison was 266 days (range: 262–272; Vervaecke, 2006). Similar results were observed for wood bison calves derived by fixed-time AI and by embryo transfer; the mean gestation period was 266 days (range: 264-269, n=6; GP Adams, unpublished).

5. The male: Reproductive physiology and technology

Very little information is available on bison male reproductive physiology; hence, breeding soundness evaluation and other reproductive technologies for bison have been conducted using protocols and procedures developed for dairy and beef bulls.

5.1 Age and seasonal effects on testicular function and semen characteristics

In beef bulls, puberty is defined as the time when the semen ejaculates consistes of >50x10^6 total sperm with >10% motility (Wolf et al., 1965). In an early study of semen quality in 28-30 month-old bison bulls, approximately 65% had attained sexual maturity (Keen et al., 1999). In a longitudinal study of captive plains bison at the University of Saskatchewan, semen was collected from 12 bulls by electroejaculation once a month from 13 to 24 months of age to characterize semen volume, sperm gross and individual motility, morphology, live:dead ratio, and concentration (Helbig et al., 2007a). According to the definition of puberty mentioned above, bison bulls attained puberty at 16 ± 2.5 months of age and 353 ± 53 kg body weight. Around the onset of puberty, sperm motility, concentration and normal morphology were low (i.e. 35 ± 24%, 167 ± 153 million/ml and 21 ± 16%, respectively), but semen quality improved significantly by 24 months of age (49 ± 20% motility, 468 ± 487 million sperm/ml and 68 ± 15% morphologically normal). However, wide variation in each semen characteristic was still observed at 24 months of age. Commercial bison farmers decide on the future usefulness of their breeding bulls between 19 to 21 months of age (Helbig et al., 2007a), but due to the potential for improvement in semen quality, the authors suggested that bison bulls should not be rejected until ≥24 months of age. Scrotal circumference is a reliable indicator of pubertal development and semen production in beef and dairy bulls (Coe, 1999; Arteaga et al., 2001); however, scrotal circumference is difficult to measure in bison due to their aggressive behaviour and safety risks, and thus, has been challenging for bison producers to implement on a routine basis.

The effect of season on bison reproductive physiology is more evident in females than males. Two approaches were used at the University of Saskatchewan to study seasonality in plains and wood bison bulls. First, semen characteristics were evaluated in testicles obtained from bison bulls slaughtered at different times of the year, and second, semen ejaculates were collected from live bison bulls over a period of 1 year (Helbig et al., 2007b). In the slaughterhouse study, both carcass and testes weights were highest during the summer season (July-September; 309 ± 11 kg and 524 ± 67 gm, respectively), and lowest during the winter season (January-March; 278 ± 10 kg and 358 ± 53 gm, respectively). However, the proportion of morphologically normal sperm harvested from the epididymis did not change

significantly between seasons (71 ± 19% vs 57 ± 16% during the summer and winter seasons, respectively). Semen ejaculates collected via electroejaculation from live bison bulls had higher ($P<0.05$) morphologically normal sperm and individual sperm motility (74 ± 9% and 69 ± 14%, respectively) during the pre-breeding season (June) than during the post-breeding (November), winter (January) and spring (April) seasons. Based on seasonal trends in testicular parenchyma and semen quality, results documented that the summer season is most favourable for semen production in bison bulls. No changes were detected in semen quality during the breeding season itself; i.e., sperm motility remained >70% between July-September in bison (Lessard et al., 2009).

5.2 Semen collection and evaluation

Sperm from domestic animals are commonly collected by either artificial vagina or electroejaculator from live animals, or harvested from the epididymis of slaughtered bulls. Since bison bulls are extremely aggressive, semen collection by artificial vagina has not been attempted. Viable sperm have been harvested successfully from epididymides of slaughtered bison bulls as a method of salvaging genetic material from threatened populations (Aurini et al., 2009; Kozdrowski et al., 2011; Krishnakumar et al., 2011; Table 5.2.1). However, slaughtered bison bulls are not as common as beef bulls and information on individual animals (i.e. genetics, age, reproductive history) is generally not available, making research and development difficult. Electroejaculation is the most appropriate and informative approach for bison semen collection.

Standard electroejaculation procedures used in dairy and beef bulls have been very effective in bison bulls restrained in a hydraulic chute-system custom-designed for bison (Lessard et al., 2009; Hussain et al., 2011; 2013). To minimize the stress of chute-restraint and electroejaculation, a study was done to evaluate the use of a long-acting neuroleptic tranquilizer, pipothiazine palmitate (Toosi et al., 2013). Administration of 100 mg and 200 mg of pipothiazine reduced restraint time and duration of semen collection ($P<0.05$) compared to untreated controls. Sperm motility parameters and serum concentrations of testosterone, cortisol and corticosterone were not significantly affected by 100 mg of pipothiazine, but a dose of 200 mg reduced restraint time for semen collection ($P<0.05$), improved total and progressive sperm motility, and reduced serum corticosterone but increased testosterone concentrations compared to untreated controls ($P<0.05$). Results highlighted the value of administering pipothiazine palmitate to manage the stress response and enhance semen collection during electroejaculation in bison. A summary of fresh semen characteristics collected by electroejaculation is provided in Table 5.2.1.

Table 5.2.1: Characteristics of fresh bison sperm (mean ± SEM) collected by epididymal aspiration from slaughtered animals or by electroejaculation in live animals.

Bison	Collection method	# of bison	Volume (ml)	Total motility (%)	Progressive motility (%)	Sperm concentration	Reference
Plains	Epididymal	14	-	-	-	$468x10^6 \pm 207$/bull	Aurini et al. 2009
European	Epididymal	2	-	75	-	-	Kozdrowski et al. 2011
Plains **Wood**	Electroejac	3 3	-	50 70	45 60	-	Lessard et al. 2009
Plains & Wood combined	Electroejac Pipothiazine + Electroejac	6	6.9 ± 0.4 6.8 ± 0.5	65 75	60 70	$694x10^6 \pm 97$/ml $871x10^6 \pm 131$/ml	Toosi et al. 2013

5.3 Chilled and cryopreserved semen

As a member of the bovidae family, it was anticipated that standard cryopreservation procedures available for dairy and beef semen would be effective for bison semen. While the quality of fresh bison semen collected via electroejaculation is similar to that of domestic cattle semen, bison sperm do not survive the cryopreservation procedure well using common extenders and cryoprotectants for dairy and beef bull semen. In early efforts, post-thaw longevity of bison sperm was short and a major hurdle in the application of artificial insemination (Dorn, 1995). Cryopreservation of bison epididymal and electroejaculated sperm has been attempted using commercial extenders, including Triladyl®, BioXcell® and Andromed®, with modest success (Aurini et al., 2009; Lessard et al., 2009; Kozdrowski et al., 2011). In addition, minimizing the risk of transmission of infectious diseases via semen is critical for both bison conservation and international trade. In this regard, Andromed, a commercially available semen extender consisting of plant protein, eliminates exposure to any animal products and related disease; however, survival of either epididymal or electroejaculated bison sperm cryopreserved with Andromed has been poor (Lessard et al., 2009; Krishnakumar et al., 2011). A summary of post-thaw bison semen characteristics is provided in Table 5.3.1.

Cryopreservation involves dilution with semen extenders, equilibration, and freeze-thaw stages. Semen extenders act to extend both the longevity and volume of sperm, and are integral to sperm cryo-survival. An ideal semen extender should have optimum pH, buffering capacity, suitable osmotic pressure and protection against cold shock (Salamon and Maxwell, 2000). The freeze-thaw process is considered the most damaging stage during sperm cryopreservation where approximately 50% of sperm die in most species. However, in bison, sperm motility and velocity were adversely affected during both the dilution and freeze-thaw stages (Hussain et al., 2011).

Table 5.3.1. Characteristics of post-thaw bison sperm (mean ± SEM) with different semen extenders.

Bison	Extender	Collection method	Number of bison	Total motility (%)	Progressive motility (%)	Reference
Wood	Triladyl	Epididymal	-	37 ± 4	26 ± 4	Bogle et al. 2010
Plains	Andromed	Electroejac	3	11	-	Lessard et al. 2009
	Triladyl			34	-	
Wood	Andromed	Electroejac	3	14	-	
	Triladyl			36	-	
European	BioXcell	Epididymal	1	11	5	Kozdrowski et al. 2011
Plains	Andromed	Electroejac	3	45 ± 11	39 ± 8	Pegge et al. 2011
Wood	Andromed	Electroejac	2	48 ± 10	44 ± 9	
Plains	Andromed	Epididymal	6	45 ± 10	35 ± 10	Krishnakumar et al. 2011
	Triladyl			54 ± 7	39 ± 8	
Wood	Andromed	Epididymal	5	16 ± 10	11 ± 7	
	Triladyl			24 ± 12	13 ± 9	

In a comprehensive study to improve the post-thaw quality of bison sperm, cooling, freeze rate, buffer, temperature of glycerol addition, and addition of glutathione in the extender were investigated (Hussain et al., 2011; Hussain et al., 2013). Zwitterion-based extenders (Tes-Tris and HEPES-Tris), temperature of glycerol addition (22 °C vs. 4 °C), and addition of glutathione in extenders did not improve post-thaw quality of bison sperm, but in a comparison of three different freeze-rates, the highest freeze rate (-40°C/min) yielded the best post-thaw sperm quality (Hussain et al., 2011). To minimize damage associated with cooling of bison sperm, an initial experiment was done to investigate the membrane-stabilizing properties of cholesterol (Hussain et al., 2013). Cholesterol is an integral part of the sperm plasma membrane and loss of cholesterol from the sperm plasma membrane during cryopreservation results in premature acrosome reaction and reduced longevity (Bailey et al., 2000). Pretreatment of bison sperm with cholesterol-loaded cyclodextrin (CC) before dilution in an egg yolk-containing extender for cryopreservation resulted in significantly greater post-thaw motility than without pretreatment (48% vs 33%, respectively), and a greater proportion of sperm with an intact plasma membrane and acrosome (46% vs 32%, respectively; Hussain et al., 2013).

Research and development on the incorporation of CC in bison semen extenders have continued because of its efficacy in cryopreservation as well as its potential to minimize the risk of disease transmission from animal biologicals; i.e., replacement of standard egg yolk-based extenders with egg yolk-free CC extenders (animal protein vs animal protein-free). Results to-date are encouraging; post-thaw sperm motility and retention of intact acrosome and plasma membrane were similar in semen frozen in TEYG and CC-TG (Yang et al., 2016, 2018; Table 5.3.2). Furthermore, in tests of sperm fertility using a heterologous *in vitro* fertilization assay, no differences in cleavage and blastocyst rates were observed between bison semen cryopreserved with TEYG vs CC-TG (Table 5.3.2). These findings have led to the

preparation of frozen bison semen, free of animal protein, that can be used for fixed-time artificial insemination of synchronized bison cows (see Section 6.2). Similar findings to those in beef cattle, in which comparable fertility was documented following fixed-time artificial insemination using both egg yolk and CC extenders (Yang et al., 2018), are expected with bison.

Table 5.3.2. Comparison of post-thaw sperm characteristics (mean ± SEM; n=5 replicates, pooled ejaculates from 4 bison), and *in vitro* fertilization rates with bison semen frozen in tris-egg yolk-glycerol extender (TEYG) vs cholesterol-cyclodextrin tris-glycerol extender (CC-TG).

Post-thaw sperm characteristic*	Extender	
	TEYG (%)	CC-TG (%)
Total motility	47 ± 6	44 ± 3
Progressive motility	36 ± 6	36 ± 3
Intact acrosome and plasma membrane	31 ± 5	34 ± 5
Cleavage rate	50 ± 2	40 ± 5
Blastocyst rate	18 ± 2	17 ± 3

*No statistical difference between TEYG and CC-TG extenders for any sperm characteristic

6. The female: Reproductive physiology and technology

6.1 Ovarian and endocrine dynamics during the ovulatory and anovulatory seasons

Although informative, the results of past studies involving behaviour or the use of fecal or urinary steroid analysis provide a limited understanding of the seasonal variation in physiological parameters underlying reproductive function in bison (see Section 4.2). More recent work has demonstrated that wild-caught wood bison may be acclimated to a captive environment and examined daily by transrectal ultrasonography and endocrine profiling to establish a better understanding of the reproductive physiology of bison during the ovulatory (i.e., estrous cycle) and anovulatory seasons, and the transitions between the two (McCorkell et al., 2013).

In the first report of the use of serial ultrasonographic examination of the ovaries in bison, 2½-year-old female wood bison were studied during the months of January and February (McCorkell et al., 2013). Ovarian follicle development was characterized by the regular and synchronous development of a group of follicles in temporal relationship with surges in serum FSH concentration (McCorkell 2013). This

rhythmic wave-like pattern is similar to that described in prepubertal domestic heifers which exhibit an anovulatory follicle wave pattern beginning as young as 2 weeks of age and continuing until the first ovulation at around 13 months of age (Adams et al., 1994; Evans et al., 1994). In bison, a wave of ovarian follicles was detected initially at a diameter of 4 mm, a dominant follicle was selected 3 days later and reached a maximum diameter of 10 mm while subordinate follicles regressed. Successive waves emerged at 7-day intervals; no ovulations were detected (Fig. 6.1.1).

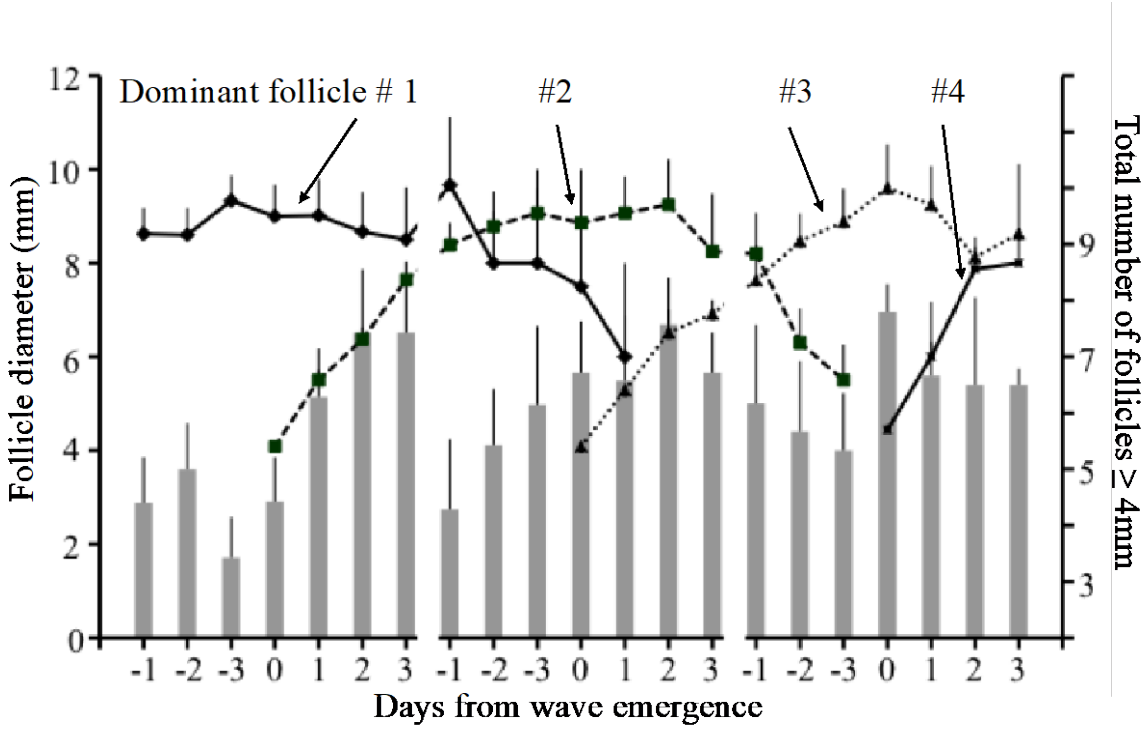

Figure 6.1.1. The ovarian follicular wave pattern in 2 ½-year-old wood bison (n=7) during January and February. Diameter profiles (mean ± SEM) of successive dominant follicles (lines) and the total number of follicles (≥4 mm; bars) were detected during daily ultrasonography of the ovaries. Data were centralized to the day of emergence of the dominant follicle of each wave (wave emergence = Day 0 on the x axis). Day-to-day changes were detected in follicle numbers ($P<0.05$) in association with growth and regression ($P<0.05$) of successive dominant follicles. Ovulation was not detected. (from McCorkell et al., 2013).

Table 6.1.1. Ovarian dynamics (mean ± SEM) during the transition from anovulatory to ovulatory seasons in bison based on serial ultrasonography (n= 7 wood bison; McCorkell et al., 2018).

End point	**1st ovulatory cycle**	**2nd ovulatory cycle**

Date of ovulation	August 24 ± 2.6 days (range, Aug. 11 to Aug. 28)	Sept. 4 ± 2.7 days (range, Aug. 19 to Sept. 13)
Pre-ovulatory follicle diameter	16.1 ± 0.6 mm	14.5 ± 0.7 mm
Maximum CL diameter	17.6 ± 0.6 mm	20.2 ± 0.5 mm
Inter-ovulatory interval	8.4 ± 0.2 days	20.0 ± 0.3 days
Number of follicular waves	1 (100%)	2 (100%)
Emergence of follicular wave* (Day 0 = ovulation)	Day 0	Day 0.1 ± 0.2 (Wave 1) Day 10.5 ± 0.8 (Wave 2)

*Follicular wave emergence was defined as the day on which the dominant follicle was first identified, retrospectively, at a diameter of 4-5 mm.

In a follow-up study designed to correlate ovarian dynamics with endocrine and behavioral changes during the transition from the anovulatory season to the ovulatory season, 3-year-old female wood bison were examined daily from July 15 to October 4 at the University of Saskatchewan (52º02´N, 106°28´W; McCorkell et al., 2018). Ovarian dynamics are summarized in Table 6.1.1. The first ovulation of the season for individual bison occurred between August 11 and August 28, and was not associated with behavioral signs of estrus (Fig. 6.1.2).

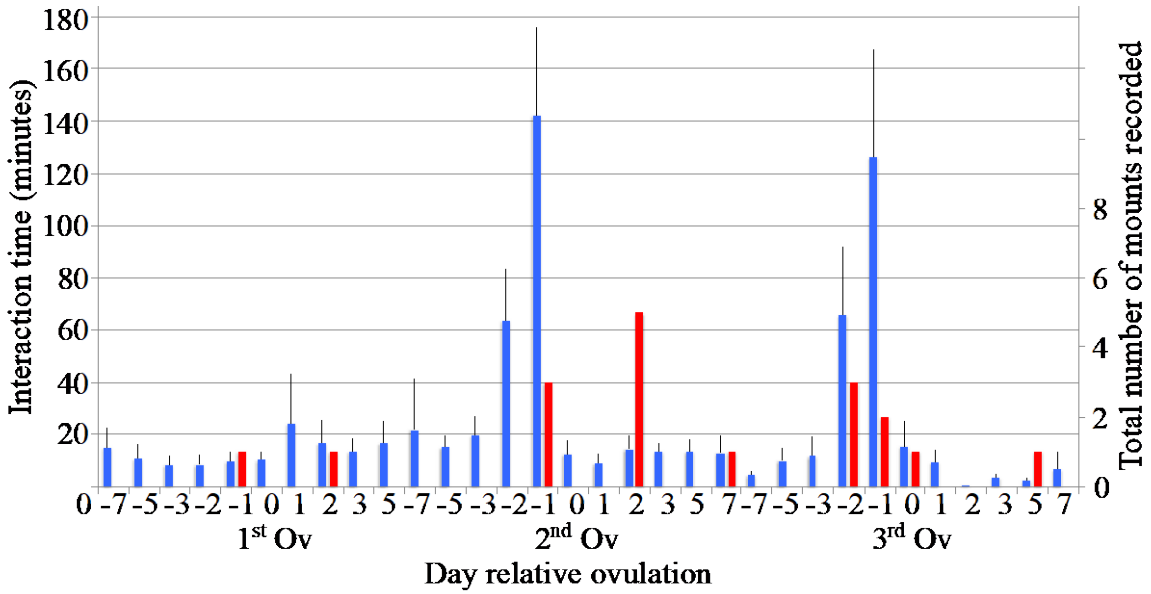

Figure 6.1.2. Display of sexual behaviour in wood bison (mean ± SEM minutes of interaction; n=7 females, n=1 male) during transition from the non-breeding to the breeding season. Interactions were recorded using proximity radio collars (blue), and the number of mounts (red) was recorded by male-induced compression of a tail-head radio transmitter on the female. Data were centralized to the day of ovulation (from McCorkell et al., 2018).

The first interovulatory interval (cycle) of the season was short (8 days) and composed of only a single wave of follicle development, whereas the second cycle was 20 days and composed of 2 waves of follicle development (Table 6.1.1 and Fig. 6.1.3). The diameter profile of the corpus luteum (CL) of the first cycle was smaller than that of the subsequent cycle, and plasma progesterone concentrations were elevated for only 6 vs 16 days in the successive cycles (Table 6.1.1 and Fig. 6.1.3). Unlike the first ovulation of the season, the second and third ovulations were preceded by estrous behaviour (Fig. 6.1.2). Based on these data from 3-year-old bison, the earliest opportunity for conception was the first week of September.

In a recent study involving ultrasonographic examination of the ovaries of a herd of 53 wood bison at roughly 2-week intervals, a corpus luteum (evidence of transition to the ovulatory season) was detected in an increasing proportion of animals from early August to early October. Nearly a third of mature bison cows (3-14 years of age) had a corpus luteum on Aug. 7, but it was more than one month later that 100% of the bison cows were cycling. None of the 2-year-old females had a CL until the end of August and the proportion with a CL did not exceed 44% by the time of the last examination in early October (Fig. 6.1.4; Adams GP, Zwiefelhofer M, 2018, unpublished).

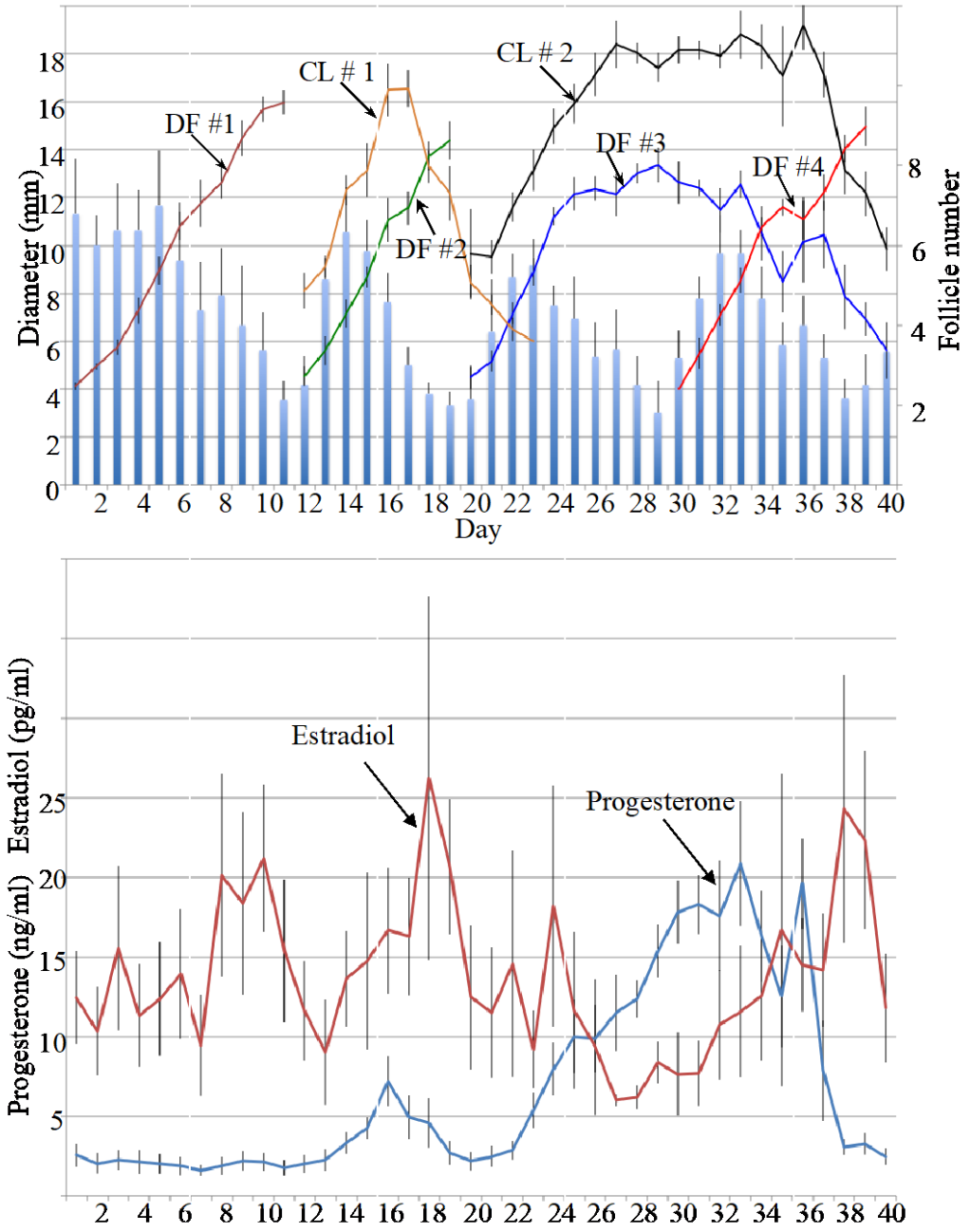

Figure 6.1.3. Ovarian follicular and luteal dynamics (upper panel) and plasma hormone concentrations (lower panel) in wood bison (mean ±SEM; n=7) during the transition from anovulatory to ovulatory seasons in late August to early September. DF#1 is the dominant follicle that was the first to ovulate; DF#2 is the dominant follicle of the single follicular wave that composed the first (short) cycle; DF#3 and #4 are dominant follicles of the waves that composed the second (normal-length) cycle of the ovulatory season (McCorkell et al., 2018).

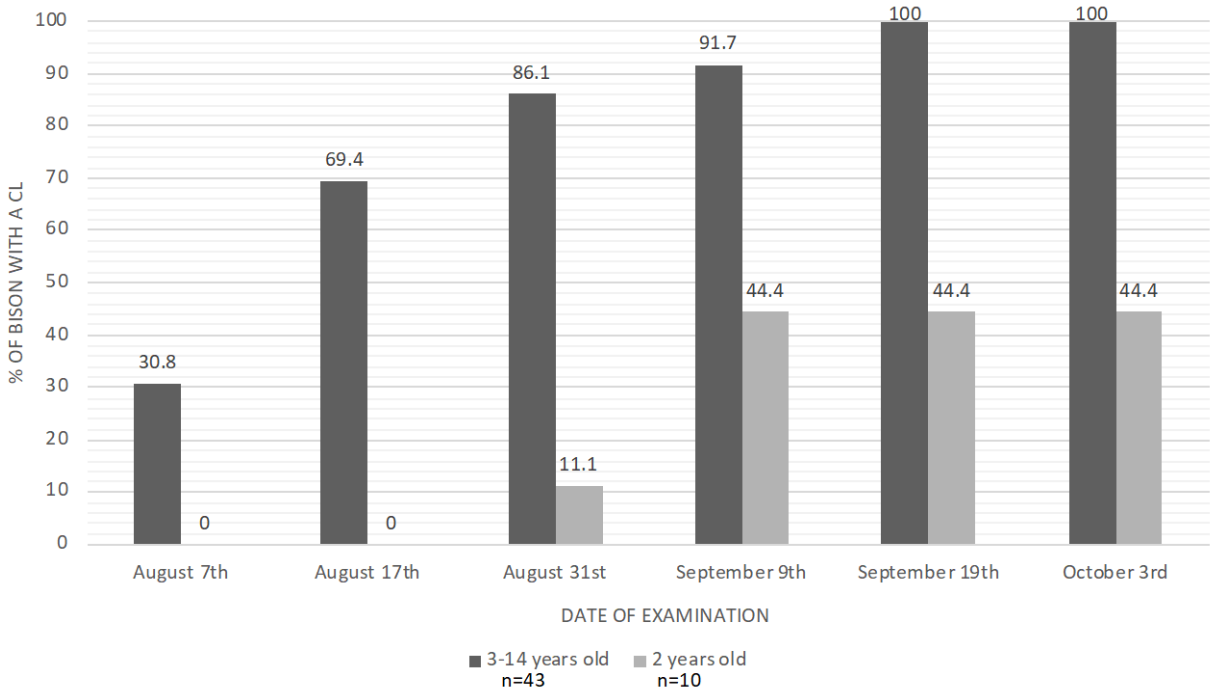

Figure 6.1.4. Onset of the ovulatory season in a herd of wood bison as indicated by the detection of a corpus luteum by transrectal ultrasonography (52º02´N, 106°28´W; Adams GP, Zwiefelhofer M, 2018, unpublished).

6.2. Elective control of ovarian function

Knowledge of the annual reproductive pattern in bison has permitted the development of rational schemes for ovarian synchronization during both the ovulatory and anovulatory seasons. The technology is based on the control of ovarian follicle and luteal dynamics developed for cattle (Adams, 1994; Adams et al., 2012; Bo et al., 1995). The principal implication of being able to control ovarian function is the ability to synchronize ovarian status to optimize the response to a given treatment or breeding management strategy. For instance, the greatest sources of variability in response to ovarian stimulation (designed to induce multiple follicle growth and ovulation) are follicular wave status at the time stimulatory treatment is initiated, and the intrinsic number of follicles present at the time of wave emergence in a given individual (Adams et al., 2012; Singh et al., 2004). Results of studies in cattle have documented that the superstimulatory response is greater when treatment is initiated at the time of wave emergence rather than 1 or 2 days later, after selection of the dominant follicle, and the onset of subordinate follicle regression. Based on bison data illustrated in Fig. 6.1.1, therefore, only approximately 20% (4 or 5 days) of the estrous cycle is available for achieving an optimal superovulatory response. By being able to electively induce the emergence of a new follicular wave, hormonal treatments may be scheduled when the greatest

number of small antral follicles are capable of responding. Other examples of the importance of elective control of ovarian function are group synchronization to optimize oocyte (egg) collection for *in vitro* fertilization and embryo production, synchronization between donors and recipients (surrogates) in embryo transfer programs, ovulation synchronization to enable fixed-time insemination, and contraception strategies to control local populations.

Unlike domestic cattle, bison are seasonal breeders with distinct ovulatory and anovulatory seasons (Sections 4.2 and 6.1). The first attempts to synchronize ovarian function in bison were done during the breeding season using a synthetic progestogen ear implant (Synchro-Mate B, SMB) and estradiol valerate (Matsuda et al., 1996; Othen et al., 1999). Estrus was detected in 25%, 55%, and 20% of bison 2, 3, and 4 days after SMB removal, respectively. However, ovulation was not monitored, and subsequent study in cattle has shown that display of estrus after progestogen withdrawal occurs regardless of whether ovulation ensues (Bo et al., 1995). More, recent synchronization protocols for bison have involved ultrasonographic monitoring of ovarian events and protocol development for both ovulatory and anovulatory seasons.

6.2.1 Anovulatory season – follicular wave synchronization and induction of ovulation

Since ovulation and CL development do not occur during the anovulatory season, synchronization protocols for this time period focus on the control of follicular wave emergence without the need to control luteal function. In the first study of its kind in bison (McCorkell et al., 2010), two methods of synchronizing ovarian follicular development were tested during the anovulatory season. Using a cross-over design, female wood (n=14) and plains bison (n=10) were rotated through each of three treatment groups in which: 1) ovarian follicles ≥5 mm were ablated by ultrasound-guided transvaginal follicle aspiration (Bergfelt et al., 1994), 2) 5 mg estradiol-17ß in canola oil was given im, or 3) no treatment was given (control). In addition, half of the bison in each group were given a single dose of pipothiazine palmitate (150 mg) im to determine the effect of a long-acting tranquilizer on ovarian function. The ovaries were examined daily by transrectal ultrasonography.

The interval to follicle wave emergence was shortest (1 day, $P<0.05$) and least variable ($P<0.05$), in the follicle ablation group, and tended to be less variable in the estradiol group ($P=0.09$) than in the control group (Table 6.2.1). Authors concluded that i) ovarian follicular wave emergence may be effectively synchronized in bison during the anovulatory season, ii) pipothiazine palmitate had no discernable effect on ovarian function and may be useful in reducing the effects of handling stress on untrained animals, and iii) there was no difference between wood and plains bison for any end point.

Table 6.2.1. Day on which follicular wave emergence occurred (mean ± SEM) after synchronization treatment in wood and plains bison with or without a long-acting tranquilizer (pipothiazine palmitate), during the anovulatory season (McCorkell et al., 2010).

	Without pipothiazine		With pipothiazine		Combined
Treatment	Wood bison	Plains bison	Wood bison	Plains bison	(n=24/gp)
Ablation	1.0 ± 0.3	1.0 ± 0.2	1.1 ± 0.3	1.0 ± 0.2	1.0 ± 0.2[a]
Estradiol-17β	3.3 ± 0.5	2.8 ± 0.4	2.6 ± 0.2	4.2 ± 0.8	3.3 ± 0.3[b]
Control	4.3 ± 0.8	4.5 ± 0.3	4.5 ± 1.2	2.8 ± 0.9	4.0 ± 0.4[b]

[ab] Within column, values with no common superscript are different (P<0.05).

However, unintended ovulations occurred after estradiol treatment in 43% of bison which may have confounded the synchronizing effect of this treatment. A follow-up study was done to determine if a lower dose of estradiol-17β (2 mg) alone or in combination with 100 mg progesterone would eliminate unwanted ovulation and better synchronize follicular wave emergence (Palomino et al., 2014a). Again, the interval to follicle wave emergence was shortest (1.2 days, P<0.05) and least variable (P<0.05) in the follicle ablation group (Fig. 6.2.1). Unwanted ovulation occurred in only 1 bison (in the estradiol-only group); hence, wave emergence in the hormone-treated group was also sooner (3.3 days; P<0.05) and less variable (P<0.05) than in controls (Fig. 6.2.1). Both methods of follicular synchronization require individual animal handling. The advantage of the follicle ablation technique is that it consistently induced the earliest and most synchronous response. The advantage of estradiol + progesterone treatment is that it is quick and does not require ultrasound equipment and expertise.

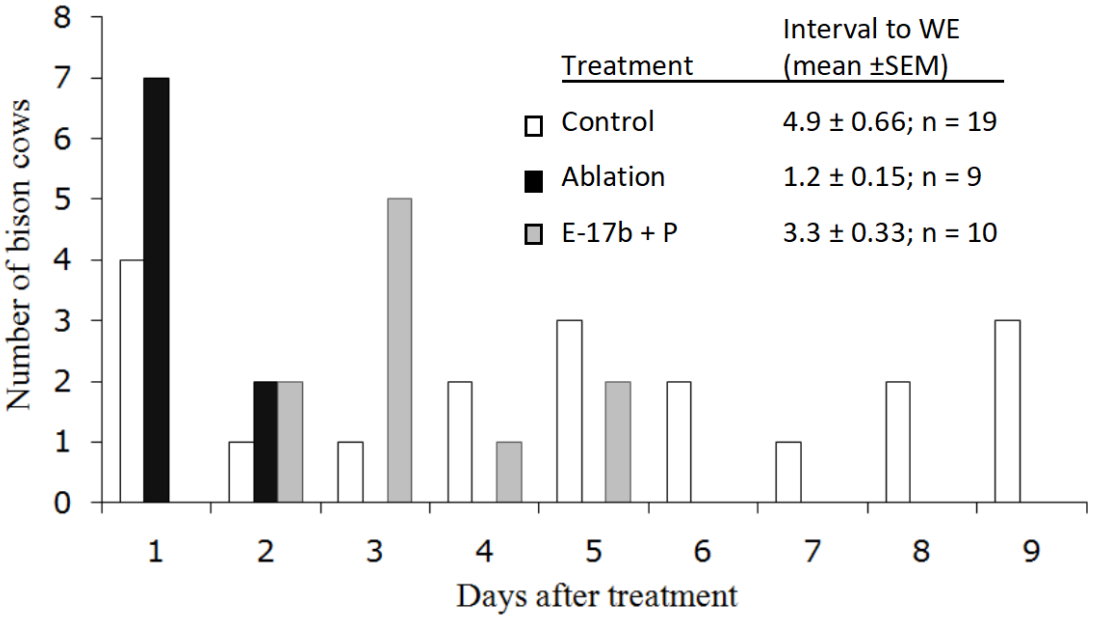

Figure 6.2.1. Distribution of new follicular wave emergence (WE) in wood bison during the anovulatory season in the control phase (no treatment) and after transvaginal ultrasound-guided follicular ablation or estradiol + progesterone treatment (adapted from Palomino et al., 2014a).

In a following study designed to determine the effects of ovulation induction agent and follicle maturity during the anovulatory season in wood bison, the ovulatory response was more consistent when the pre-ovulatory follicle diameter was ≥10 mm (compared to 8-9 mm) and with the use of hCG rather than pLH or GnRH (Palomino et al., 2015a). Similar results were reported in bison undergoing ovarian superstimulation; hCG induced a greater ovulatory response than pLH in both the ovulatory and anovulatory seasons (Palomino et al., 2016). Results clearly demonstrate that ovulation can be induced effectively during both the anovulatory and ovulatory seasons.

6.2.2 Ovulatory season – ovulation synchronization and fixed-time artificial insemination

Ovarian synchronization protocols for the ovulatory season must be modified from that of the anovulatory season to incorporate the control of CL function and untimely spontaneous ovulation. Based on previous results from the anovulatory season (cited above), a series of studies were done in bison to test ovulation synchronization schemes during the ovulatory season, specifically for fixed-time insemination.

Table 6.2.2. Effect (mean ± SEM) of an estradiol+progesterone protocol for ovarian synchronization in bison (n=10 per group) during the ovulatory season (Adams et al., 2010).

	Control group (n=10)		E + P group (n=10)	
	Mean	Synchrony	Mean	Synchrony
Interval from start to follicle wave emergence (days)	4.1 ± 2.5 [a]	1.9 ± 0.5*	4.1 ± 0.9 [a]	0.7± 0.2*
Interval from pLH treatment to ovulation (days)	5.4 ± 1.9 [a]	5.3 ± 0.8*	2.7 ± 0.6 [b]	1.2 ± 0.4*
Diameter of ovulatory follicle (mm)	15.2 ± 1.1		15.2 ± 1.0	
Diagnosed pregnant at 30 days	2/10		3/10	

[ab] Within rows, values with different superscripts are different (P<0.05)

* Within rows, variation (mean ± sem of residuals) differed (P<0.05)

In the first study (Adams et al., 2010), done in the early ovulatory season (September), wood bison (n=14) and plains bison (n=9) were blocked by subspecies and assigned randomly to a control group (no treatment) or a treatment group given estradiol 17β (2.5 mg) + progesterone (50 mg) in canola oil im with a progesterone-releasing intravaginal device on Day 0 to mitigate untimely ovulation (n=10 per group). On Day 8, the progesterone-releasing device was removed and PGF (500 mg Estrumate) was given to induce luteolysis. On Day 10, bison in both groups were given 5 mg of pLH (Lutropin V) and artificially inseminated 12 hours later with semen collected and frozen previously from wood bison of the same herd. The ovaries were examined daily by transrectal ultrasonography beginning 5 days before treatment, and thereafter, until the first post-treatment ovulation. Ultrasonographic pregnancy diagnosis was done 30 days post-insemination. Two bison in the treatment group and one in the control group were excluded because of handling difficulty. No differences were detected between wood and plains bison for any end point, and data were combined (Table 6.2.2). The interval to new wave emergence was less variable (P<0.05), and the interval from pLH treatment to ovulation was shorter (P<0.05) and less variable (P<0.05; Table 6.2.2) in bison treated with estradiol + progesterone than in untreated controls. While treatment with estradiol and progesterone improved ovulation synchrony, pregnancy after a single fixed-time artificial insemination was diagnosed in only 3 bison in the treatment group and 2 in the control group; all gave birth to live calves the following spring (Fig. 6.2.3).

Based on these findings, a second synchronization/fixed-time AI study was done during the month of November using a 2 x 2 design to compare the efficacy of two ovulation synchronization techniques and two semen cryopreservation protocols (Adams et al., 2016). Wood bison were assigned randomly to two groups (n=24 per group) in which ovarian synchronization was induced by ultrasound-guided follicle ablation or intramuscular treatment with 2.5 mg estradiol-17B + 50 mg progesterone. A progesterone-releasing intravaginal device was placed at the time of treatment and maintained for 5 days and 8 days in the follicle ablation and E+P treatment groups, respectively. A luteolytic dose of prostaglandin was given at the time of vaginal device removal, and 2500 IU hCG was given im 3 days later. Bison were inseminated 24 and 36 h after hCG treatment using frozen-thawed semen. The semen was collected by electroejaculation from 4 wood bison bulls, pooled and divided into aliquots diluted in either egg-yolk extender (EY) or cholesterol-cyclodextrin complex (CC; see Section 5.3). Half the bison in each synchronization group were inseminated with either EY- or CC-extended semen. The ovaries were examined by ultrasonography every 12 hours beginning on the day of hCG treatment until ovulation was detected. Pregnancy diagnosis was done by ultrasonography 34 to 36 days after insemination. Two bison were excluded during the experiment because of handling difficulty; results are summarized in Table 6.2.3. The ovulation rate was not different between synchronization groups (combined mean, 37/46 [80%]), nor was the degree of synchrony, as assessed by the residuals (variation from the mean) in the respective groups. However, the diameter (mean ± SEM) of the dominant follicle at the time of hCG treatment was smaller ($P \leq 0.04$), in the follicle ablation group than in the E+P group and the interval from hCG treatment to ovulation tended to be longer ($P \leq 0.10$).

Table 6.2.3. Efficacy of transvaginal ultrasound-guided follicle ablation vs estradiol+progesterone in ovulation synchronization protocols in bison (mean ± SEM; n= number of bison; from Adams et al., 2016).

End point	Follicle ablation (n= 24)	E+P (n=22)	P-value
Follicle diameter at time of hCG (mm)	10.5 ± 0.6	13.9 ± 0.6	≤0.04
Number of bison that ovulated after hCG	17/24 (71%)	20/22 (91%)	NS
Interval, hCG to ovulation (hours)	35.3 ± 1.6	31.8 ± 1.3	≤0.1

Pregnancy rate was not affected by synchronization procedure, but pregnancy was detected only in the EY-inseminated group (9/23 vs 0/23; $P<0.01$). Despite post-thaw sperm motility being similar for EY and CC semen (41.7 ± 2.9% and 44.6 ± 3.3%, respectively), CC-treated semen failed to produce pregnancy in bison *in vivo*. Of the 23 bison inseminated with EY-extended semen, 21 ovulated (91%), and of those that ovulated, 9 became pregnant (43%). Both synchronization schemes were effective, but we concluded that the ablation protocol may be improved by an additional day of follicle growth between ablation and hCG treatment, and by modification of the CC semen extender.

A third synchronization/fixed-time AI study was done late in the ovulatory season (late November and early December) using a 2x2 design to determine the effects of two ovulation synchronization techniques

and two semen cryopreservation protocols on ovulation and pregnancy (Huanca et al., 2016). Wood bison were assigned randomly to a follicle ablation group (n=15) or estradiol + progesterone group (n=14), as described in the previous study except that the progesterone-releasing intravaginal device was left in place for 6 days (rather than 5) in the ablation group (Fig. 6.2.2). Prostaglandin and hCG treatments were given as before, but bison were inseminated at 12 and 24 h (rather than 24 and 36 hr) after hCG treatment. Semen was collected and handled as before, but the concentration of cholesterol-cyclodextrin used in the CC-extended semen was half that used previously (see Section 5.3). Bison were examined by ultrasonography as in the previous study.

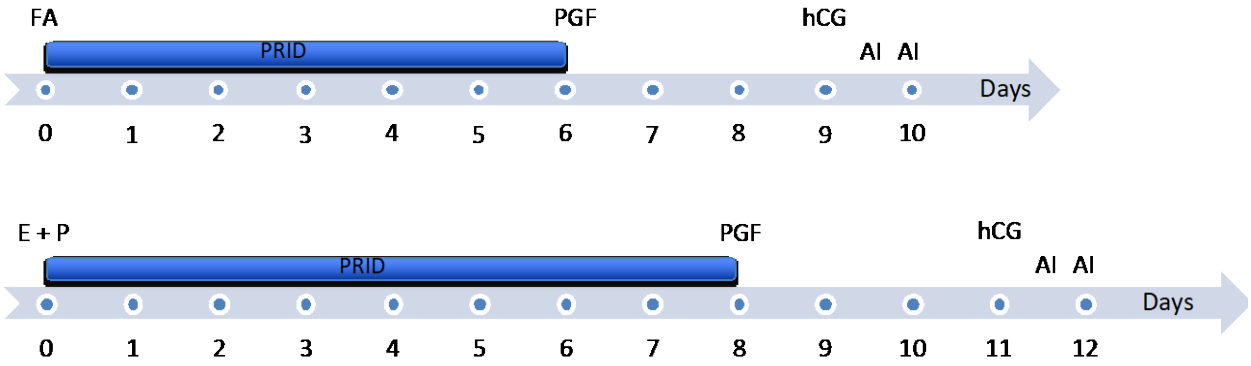

Figure 6.2.2. Protocols for synchronizing ovulation in bison for fixed-time artificial insemination. FA: transvaginal ultrasound-guided follicle ablation. E+P: 2.5 mg estradiol 17B in + 50 mg progesterone in canola oil, im. PRID: progesterone-releasing intravaginal device (1.55 g progesterone/device). PGF: 500 ug cloprostenol, im. hCG: 2500 IU human chorionic gonadotropin, im. AI: artificial insemination 12 and 24 h after hCG.

No differences between synchronization groups were detected for any end point (Table 6.2.4). The combined ovulation rate was high, and all bison ovulated ≤36 hours after hCG treatment. The overall pregnancy rate was 43% and did not differ between the EY and CC semen extender groups (6/11 vs. 4/12, respectively). Both synchronization methods and both semen cryopreservation protocols were effective for producing pregnancy in bison after fixed-time insemination. Of the 23 bison inseminated with EY- and CC-extended semen, 22 ovulated (96%), and of those that ovulated, 10 became pregnant (45%).

Table 6.2.4. Effect of two synchronization protocols for fixed-time artificial insemination in bison (mean ± SEM; n= number of bison; Huanca et al., 2016).

End point*	Follicle ablation (n= 12)	E+P (n=11)	Combined
Follicle diameter at time of hCG (mm)	12.3 ± 0.5	12.6 ± 0.8	12.4 ± 0.4
Number of bison that ovulated after hCG	12/12 (100%)	10/11 (91%)	22/23 (95.6%)
Interval, hCG to ovulation (hours)	28.8 ± 2.6	27.6 ± 4.4	28.3±2.3
Number pregnant	6/12 (50%)	4/11 (36%)	10/23 (43.5%)

*No significant differences between groups for any end point.

These results are exciting and document for the first time the ability to produce bison calves by fixed-time artificial insemination. To test the feasibility of synchronization and fixed-time artificial insemination in a field setting, two small trials were done on a privately owned bison herd south of Saskatoon during late August and early September of successive years; the bison had little or no previous experience in a handling chute. In the first trial, 8 bison were selected from a group of 16 based on detection of a corpus luteum during an initial ultrasonographic examination; i.e., evidence that they had entered the ovulatory season. The bison were synchronized using the estradiol + progesterone combination described above and inseminated at 12 and 24 h after hCG treatment; 4/8 (50%) became pregnant and delivered a calf the following spring. A similar trial was done the following year but included bison in which a CL was detected (cycling, n=12) as well as those in which a CL was not detected (non-cycling, n=7), and insemination was done once only at 12 h after hCG. The pregnancy rate was 4/12 (33%) in the cycling group, and 1/7 (14%) in the non-cycling group (Fig. 6.2.3). Based on these results and studies on the transition from anovulatory to ovulatory seasons (see Section 6.1), synchronization for fixed-time AI will be more effective when initiated after the first week of September in mature bison cows, and later in 2-year-olds after confirming the presence of a CL.

Figure 6.2.3. Live bison calves born following artificial insemination of synchronized bison cows with frozen semen at Native Hoofstock Center, University of Saskatchewan (wood bison, upper panel) and at commercial farm near Saskatoon, Canada (plains bison, lower panel).

7. Embryo technology

7.1 Superovulation for embryo collection and transfer

Early attempts at embryo collection and transfer in bison involved the use of domestic cattle methods (Dorn et al., 1990; Robison et al., 1998). In the first report, 14 bison cows were superovulated using a multiple-dose regime of FSH developed for cattle, but only 5 morphologically normal embryos were collected (Dorn et al., 1990). The embryos were transferred immediately to synchronized cattle recipients, but no pregnancies were reported. It was suggested that poor results may have been associated with the stress of handling (Dorn, 1995). In an effort to minimize handling, equine chorionic gonadotropin (eCG) was used in a single dose to induce superovulation in bison, but resulted in a mean of only 2 CL and 0.6 transferable embryos per bison (Robison et al., 1998).

Ovarian superstimulation enables more efficient embryo production by either *in vivo* or *in vitro* fertilization. During the development of a natural ovarian follicular wave in bison, 8 to 12 antral follicles begin to grow simultaneously but by 2 days after wave emergence, one follicle is selected as dominant and continues to grow while the other (subordinate) follicles cease to grow and ultimately regress (*see Section 6.1*). Co-dominance (more than one dominant follicle) is rare in bison; hence the single ovulation that occurs at the end of the estrous cycle results in the potential for collecting only a single embryo. In cattle, the use of gonadotropin treatment has

been used to 'rescue' approximately 70% of the follicles within a wave that would otherwise regress, thus allowing them to reach ovulatory size (multiple co-dominance; Singh et al., 2004). Gonadotropin-induced multiple follicle growth, or ovarian superstimulation, is used to increase the number of large follicles available for oocyte collection for the purposes of *in vitro* fertilization or to induce multiple ovulation for *in vivo* fertilization and embryo collection (Mapletoft et al., 2007, 2015; Chaubal et al. 2007; Garcia-Guerra et al., 2015). In cattle, a traditional superstimulation protocol consists of twice daily treatments of follicle-stimulating hormone (FSH) over a period of 4 or 5 days (Mapletoft et al., 2015). Unfortunately, this scheme of treatment scheme may adversely affect the results in wild species (i.e., bison) because of the suppressive effects of handling stress (Dorn, 1995).

In a recent series of studies, protocols of ovarian superovulation with a reduced number of treatment/handling events have been developed for bison to mitigate the effects of stress (summarized in Table 7.1.1; Toosi et al., 2013; Palomino et al., 2013; 2014b; 2016; 2017a; 2017b). In all instances, superstimulatory treatment was initiated on the day of follicle wave emergence synchronized among bison by transvaginal ultrasound-guided follicle ablation or by treatment with estradiol + progesterone (*see Section 6.2*). Approaches involved superstimulation of follicle growth by administration of a single large dose of gonadotropin (FSH) or 2 smaller doses given 48 hours apart, followed by ovulation induction treatment with GnRH, LH or hCG (Fig. 7.1.1). All reduced-treatment/handling protocols induced multiple ovulation during both the ovulatory and anovulatory seasons (Table 7.1.1), and live calves have been produced after transfer of fresh (Toosi et al., 2013) and frozen embryos (National Post, 2016; U of News, 2016).

Table 7.1.1. Summary of studies on ovarian superovulation for embryo collection in bison (in vivo-derived embryo production; mean ± SEM per bison). In all instances, superstimulatory treatment was initiated on the day of follicle wave emergence induced by transvaginal follicle ablation or by treatment with estradiol + progesterone (*see Section 6.2*).

Season	Protocol	Follicles ≥9 mm*	Ovula-tions	Transfer rable embryos	Conclusion (Reference)
Ovulatory (Sept.-Nov.)	**Expt. 1:** 1 x 400 mg FSH sc SRF** 2 x 200 mg FSH sc (48h) **Expt. 2:** 2 x 200 mg FSH sc (48h) 4 x 100 mg FSH sc (48h) ± PRID**		 3.3 ± 0.5[a] 2.9 ± 0.8[a] 8.9 ± 1.5[a] 7.1 ± 1.6[a]	 7 from 20 donors 6 from 5 donors	1st study in wood bison. Reduced FSH treatment frequency effective. Use of PRID had no effect. 5 embryos transferred fresh (1/recipient) and 2 live calves born (Toosi et al., 2013).
Anovulatory (May-June)	1 x 400 mg FSH im no PRID** with PRID** Ovulation induced with: hCG LH	 8.5 ± 1.7[a] 7.7 ± 1.6[a]	 6.0 ± 1.5[a] 2.7 ± 1.0[b] 6.6 ± 1.8[a] 2.8 ± 0.8[b]	 0.5 ± 0.2[a] 0.6 ± 0.4[a] 0.6 ± 0.3[a] 0.5 ± 0.3[a]	Greater ovulatory response with hCG than pLH. PRID did not improve embryo quality. (Palomino et al., 2016).
Ovulatory (Sept.-Oct.)	1 x 400 mg FSH im 2 x (300 & 100 mg) FSH im (48h) Ovulation induced with: hCG LH	5.9 ± 1.1[a] 10.5 ± 1.4[b]	3.0 ± 0.8[a] 7.1 ± 0.9[b] 6.3 ± 0.8[a] 3.8 ±1 .2[b]	0.8 ± 0.4[a] 0.6 ± 0.3[a] 0.8 ± 0.4[a] 0.4 ± 0.3[a]	Better superstimulation with split-dose FSH than single dose FSH and greater ovulatory response with hCG than

					pLH (Palomino et al., 2016).
Anovulatory (May-June)	300 & 100 mg FSH im (48h) with: PRID eCG eCG and PRID**	 14.8 ± 2.4[a] 14.3 ± 4.6[a] 16.0 ± 1.4[a]	 10.6 ± 1.7[a] 9.7 ± 2.8[a] 7.2 ± 1.0[a]	 1.6 ± 0.9[a] 1.0 ± 0.5[a] 1.2 ± 0.3[a]	Ovarian response and embryo production not increased by addition of eCG or PRID (Palomino et al., 2017a).
Ovulatory (Sept.-Oct.)	2 x (300 & 100 mg) FSH im (48h) with: no eCG eCG	 9.8 ± 1.5[a] 9.1 ± 1.3[a]	 6.9 ± 1.1[a] 5.4 ± 0.9[a]	 2.6 ± 0.7[a] 2.0 ± 0.4[a]	Ovarian response and embryo production not increased by addition of eCG (Palomino et al., 2017a).
Anovulatory (June)	2 x 200 mg FSH im (48h) 3 x 133 mg FSH im (48h)	10.4 ± 1.7[a] 10.1 ± 1.1[a]	4.4 ± 0.7[a] 5.5 ± 0.9[a]	0.4 ± 0.3[a] 0.2 ± 0.2[a]	Low embryo production during the anovulatory season (Palomino et al., 2017b).
Ovulatory (Sept.)	2 x 200 mg FSH im (48h) 3 x 133 mg FSH im (48h)	7.4 ± 1.2[a] 9.1 ± 1.4[a]	4.3 ± 0.7[a] 5.8 ± 1.0[a]	1.2 ± 0.4[a] 2.5 ± 0.6[b]	Extended FSH treatment increase embryo production (Palomino et al., 2017b).

[ab] Within experiment and end point, values with different superscript are different (P<0.05)
*Number of large follicles (≤9 mm) at the time of ovulation induction with hCG or LH
**Sustained-release formulation (SRF). Progesterone-releasing intravaginal device (PRID) for 5 d.

A single or split dose of FSH was as effective or more effective than 3 or 4 daily doses, and there was no evidence to support the use of a sustained-release form (SRF; 1% hyaluronan) of FSH nor subcutaneous vs intramuscular administration (Table 7.1.1). In a direct comparison in one study, the superovulatory response to a single 400 mg dose of FSH was lesser than that to a split dose (2 x 200 mg) given 48 hrs apart (Palomino et al., 2016). Although not compared directly, there appears to be no difference in the response to a decreasing split dose of FSH (300 mg and 100 mg) vs a static dose (2 x 200 mg; Table 7.1.1). The ovarian response and embryo production were not increased by addition of eCG or a progesterone-releasing intravaginal device (PRID) to the superovulatory protocol during the ovulatory or anovulatory season (Palomino et al., 2017b).

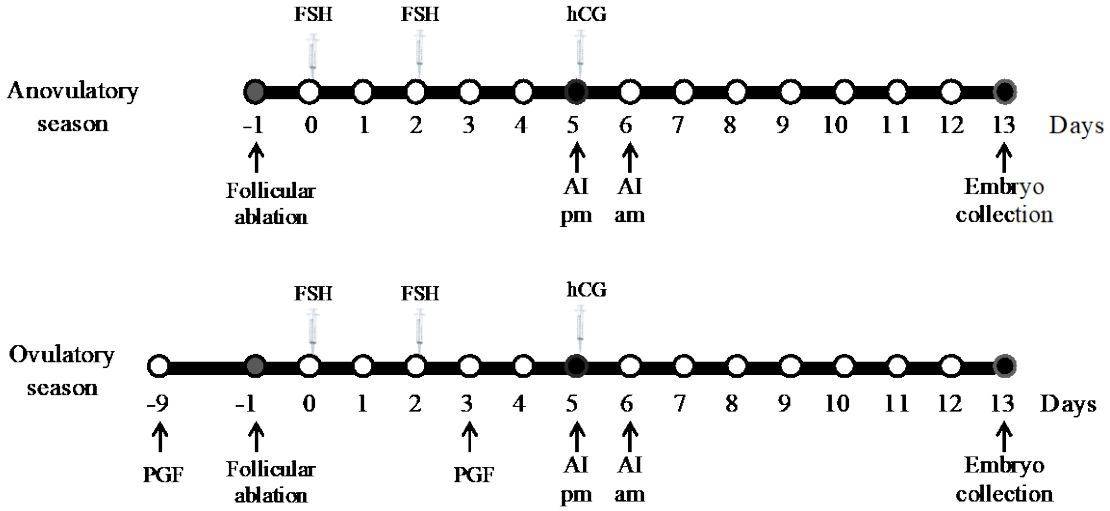

Figure 7.1.1 Protocols for superovulation and embryo collection in bison during ovulatory and anovulatory seasons. Split doses of FSH of 200 mg each or 300 and 100 mg provide similar superstimulatory responses, and doses of 2000 to 3000 IU of hCG are effective for inducing ovulation.

Table 7.1.2. Seasonal comparison of protocols that resulted in the best ovarian response and embryo collection in bison.

Season	Protocol	Follicles ≥9 mm	Ovulations	Total ova/ embryos	Transfer-rable embryos	Reference
Anovulatory	1 x 400 mg FSH im	8.5 ± 1.7	10.3±1.9	2.5 ± 1. 9	0.8 ± 0.5	Palomino et al., 2016
Ovulatory	2 x (300 & 100 mg) FSH im (48h)	10.5 ± 1.4	8.0 ± 0.6	2.2 ± 1.0	1.2 ± 0.7	
Anovulatory	2 x (300 & 100 mg) FSH im (48h)	14.3 ± 4.6	9.7 ± 2.8	4.9 ± 2.3	1.0 ± 0.5	Palomino et al., 2017a
Ovulatory	2 x (300 & 100 mg) FSH im (48h)	9.1 ± 1.3	5.4 ± 0.9	3.3 ± 0.5	2.0 ± 0.4	
Anovulatory	3 x 133 mg FSH im (48h)	10.1 ± 1.1	5.5 ± 0.9	2.8 ± 0.7	0.2 ± 0.2[a]	Palomino et al. 2017b
Ovulatory	3 x 133 mg FSH im (48h)	9.1 ± 1.4	5.8 ± 1.0	4.3 ± 0.8	2.5 ± 0.6[b]	

[ab] Within experiment and end point, values with different superscript are different (P<0.05)

Numerically, the best response during the *anovulatory season* was with a split-dose of FSH (300 mg + 100 mg) no PRID and ovulation induction with hCG (10.3 ± 1.9 ovulations, 2.5 ± 1.9 ova/embryos, and 0.8 ± 0.5 transferrable embryos; Table 7.1.2; Palomino et al., 2016). The best response during the *ovulatory season* was with a split-dose of FSH (300 mg + 100 mg) and ovulation induction with hCG (8.0 ± 0.6 ovulations, 2.2 ± 1.0 ova/embryos, and 1.2 ± 0.7 transferrable embryos; Table 7.1.2, Fig. 7.1.1; Palomino et al., 2016). The antral follicle 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 (15.8 ± 1.1 vs 12.1 ± 0.8; P<0.01) was associated with a greater number of CL at the time of embryo collection. Despite this, however, the total number of ova/embryos collected was similar between seasons, but only 10 to 50% of the number of freezable embryos were collected during the anovulatory season (Palomino et al., 2017b).

Table 7.2.1. Summary of studies on ovarian superstimulation for transvaginal ultrasound-guided collection of cumulus-oocyte complexes (COC) in bison (mean ± SEM per bison).

Season	Protocol	Follicles ≥5 mm[1]	COC collected	Collection efficiency[2]	Conclusion (Reference)
Anovulatory (July)	1x 2500 IU eCG im 2 x 200 mg FSH sc (48h)	8.1 ± 0.6[a] 14.6 ± 1.4[b]	3.8 ± 0.7[a] 6.7 ± 1.0[b]	56%	FSH more effective than eCG; LH induced *in vivo* COC maturation (Palomino et al, 2013).
Ovulatory	**Expt. 1:**				**Expt. 1:**

(Sept.-Dec.)	2 x 200 mg FSH sc (48h) 2 x 200 mg FSH SRF** im (48h) **Expt. 2:** 1 x 2500 IU eCG im 2 x 200 mg FSH SRF** sc (48h)	12.4 ± 1.5[a] 13.8 ± 1.2[a] 5.8 ± 0.5[a] 12.2 ± 1.7[b]	6.5 ± 1.1[a] 6.3 ± 1.0[a] 3.4 ± 0.6[a] 7.2 ± 1.4[b]	59%[a] 52%[a] 67%[a] 61%[a]	Both FSH preparations effective **Expt. 2:** FSH more effective than eCG (Palomino et al, 2014b).
Anovulatory (Apr.-May) **Ovulatory** (Sept.-Nov.)	2 x (300 & 100 mg) FSH im (48h) 2 x (300 & 100 mg) FSH im (48h)	17.5 ± 1.3[a] 10.8 ± 1.1[b]	10.5 ± 1.3[a] 5.6 ± 0.8[b]	71%[a] 56%[b]	*In vivo* maturation maximal by 30-34 h after hCG treatment (Cervantes et al, 2017a)
Anovulatory (March-May)	2 x (300 & 100 mg) FSH im (48h)	12.8 ± 0.6	6.0 ± 0.8	58%	COC morphology reflects competence (Cervantes et al, 2017b).

[ab]Within experiment and end point, values with different superscript are different (P<0.05).
[1]Number of follicles ≥5 mm on the day of COC collection.
[2]Number of COC collected/number of follicles aspirated (expressed as %).
**Slow-release formula (SRF).

7.2 Ovarian superstimulation for oocyte collection

The technique of transvaginal ultrasound-guided oocyte collection was first described in bison in 2013 as a method that would permit repeated collection from live bison for the purpose of *in vitro* embryo production (Palomino et al., 2013; see Section 7.3). Briefly, bison were restrained in a squeeze chute, caudal epidural anesthesia was induced and the vulva was washed before placing the ultrasound probe into the vagina. The cumulus-oocyte complexes (COC) were aspirated and collected through a disposable needle connected via silicone tubing to a filter using a regulated vacuum pump. The aspirate was diluted and washed by passing collection medium through the filter, and the COC were located and morphologically classified by stereomicroscopy. The procedure was typically completed in about 15 minutes and the bison was released with no untoward effects or recovery period required.

For reasons similar to those given above for embryo collection, ovarian superstimulation increases the number of follicles available for aspiration and COC collection. A series of studies were done in wood bison to determine factors affecting the efficiency of oocyte collection, including superstimulatory protocol and season, and results are summarized in Table 7.2.1. In all instances, superstimulatory treatment was initiated on the day of follicle wave emergence synchronized among bison by transvaginal ultrasound-guided follicle ablation or by treatment with estradiol + progesterone (Fig. 7.2.1).

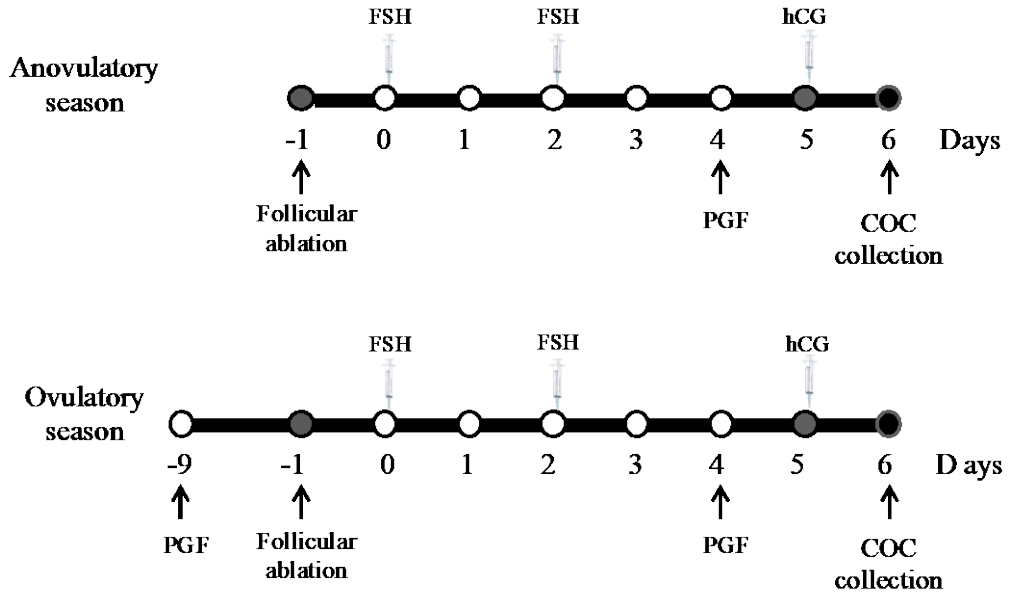

Figure 7.2.1 Protocols for superstimulation and COC collection in bison during ovulatory and anovulatory seasons. Split doses of FSH of 200 mg each or 300 and 100 mg were effective, and doses of 2000 IU hCG were effective for inducing *in vivo* oocyte maturation.

In summary, i) the superstimulatory response and COC collection rate were better with FSH than eCG, ii) FSH treatments as a static or decreasing dose were equally effective, iii) hCG effectively induced in vivo maturation, iv) more follicles were available for aspiration, and COC collection efficiency was higher during the anovulatory vs ovulatory season, and v) the number of COC collected was greater during the anovulatory season.

7.3. In vitro embryo production in bison

In vitro production of embryos (IVP) is a more recent term commonly used interchangeably with *in vitro* fertilization (IVF) as a process of generating embryos in the laboratory or outside the body (Hasler and Barfield, 2014). The term IVP is more inclusive and refers to a process that includes three main steps: oocyte maturation, oocyte fertilization, and zygote/embryo culture.

An important prerequisite for IVP is a viable oocyte. Oocytes may be obtained from the ovaries of immediately deceased animals (e.g., slaughterhouse-derived or hunter-killed) or living animals (by transvaginal follicular aspiration). In the first IVP study reported in bison, oocytes from slaughterhouse-derived wood bison ovaries were matured *in vitro* and fertilized with either frozen-thawed or chilled epididymal spermatozoa, resulting in 7.5% and 10.0% blastocyst rates, respectively, with only six blastocysts being produced in total (Thundathil et al., 2007). Comparable results were reported in plains bison using similar sources of oocytes and sperm (6.3% and 8% blastocyst rates; Aurini et al., 2009). An

improvement in the development of *in vitro* matured oocytes fertilized with frozen-thawed epididymal sperm was reported in plains bison after zygotes were cultured in medium supplemented with 5% fetal calf serum (16% blastocyst rate; Barfield and Seidel, 2011). In the same study, the percentage of blastocysts increased to 20% when embryos were cultured in medium supplemented with 5% calf serum after having reached the 8-cell stage.

In an effort to determine whether species-specific differences impact the development of bison embryos produced using domestic cattle IVP protocols, bison-cattle hybrid embryos were produced by *in vitro* fertilization of *in vitro* matured cattle oocytes with wood and plains bison spermatozoa (Seaby et al., 2012). Wood bison and plains bison hybrid blastocysts (x cattle) had significantly fewer cells than those of non-hybrid cattle blastocysts, with wood bison hybrid blastocysts having a greater incidence of apoptosis than non-hybrid cattle blastocysts. The authors suggested that decreased developmental competence in the wood bison hybrid embryos was due to inadequate culture conditions (Seaby et al., 2012). Because of limited availability of bison oocytes, the technique of producing bison x cattle hybrid embryos *in vitro* has been effective to test the fertility of fresh and frozen-thawed bison semen (Yang et al., 2016; 2018). Following *in vitro* fertilization of cattle oocytes, bison semen and commercially produced dairy semen (internal control) yielded cleavage rates of 50% and 52%, respectively, and blastocyst rates of 18% and 22%, respectively.

In cattle, there is evidence that oocytes collected by transvaginal ultrasound-guided follicle aspiration have greater developmental competence *in vitro* than slaughterhouse-derived oocytes (Neglia et al., 2003). In a series of recent studies done in wood bison, transvaginal ultrasound-guided follicle aspiration was used to collect oocytes from live superstimulated bison for the purposes of *in vitro* production of blastocysts. In the first study, temporal changes in oocyte morphology and nuclear status were investigated to determine the capability of bison oocytes to undergo maturation *in vitro* as well as *in vivo*; an initial step to develop IVP procedures in this species (Cervantes et al., 2016). Results indicated that *in vivo* maturation (oocyte aspiration post-hCG) in wood bison was associated with more extensive cumulus cell expansion and a slower onset of the resumption of oocyte meiosis than *in vitro* maturation. Whereas nuclear maturation (metaphase II) was maximal after 24 h of *in vitro* culture in oocytes that had been aspirated immature, the majority (≥50%) of oocytes matured *in vivo* did not reach metaphase II until 30 h after hCG treatment.

In a second study (Cervantes et al., 2017a), *in vivo* matured oocytes collected 34 hours after hCG treatment of superstimulated wood bison were more competent to develop into blastocysts following *in vitro* fertilization and culture than those collected 30 hours after hCG treatment (54.1 vs. 37.2%). Findings also demonstrated that 4 hours of additional *in vitro* maturation of oocytes collected 30 hours after hCG treatment contributed to higher blastocysts rates (26-37% vs. 10%), but not to the same extent as an additional 4 hours of *in vivo* maturation (54%).

In a third study (Cervantes et al., 2017b), immature oocytes (no hCG treatment) collected by follicle aspiration from superstimulated bison and matured *in vitro* for 24 h also supported embryo development to the blastocyst stage (28%). Morphologic characteristics of wood bison COC at the time of collection (no hCG treatment) were predictive of the potential of oocytes to develop to the blastocyst stage after *in vitro* maturation, fertilization and culture. Compact COC with ≥3 layers of cumulus cells resulted in the highest blastocyst rate (54%).

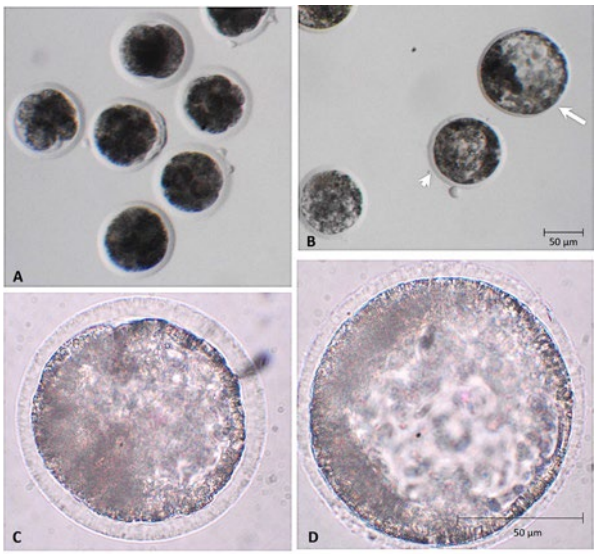

Fig. 7.3.1. *In vitro*-produced wood bison embryos from *in vivo* matured oocytes at A) 56 h after *in vitro* fertilization, and B) the blastocyst (arrowhead) and expanded blastocyst (arrow) stages on Day 8 (evaluated by stereomicroscope, magnification x 80), C) blastocyst and D) expanded blastocyst on Day 8 (evaluated by light microscope, magnification x 400) of *in vitro* culture in Charles Rosenkran's aminoacid (CR1aa) medium with 5% calf serum containing amino acids, L-Glutamic acid, BSA and gentamicin (Day 0=day of *in vitro* fertilization).

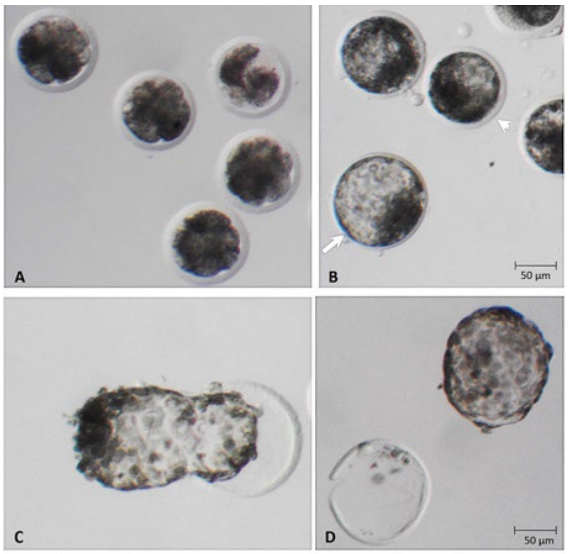

Fig. 7.3.2. *In vitro*-produced wood bison embryos from *in vitro* matured oocytes at A) 56 h after *in vitro* fertilization, and B) the blastocyst (arrowhead) and expanded blastocyst (arrow) stages on Day 8, C) the hatching and D) hatched blastocysts between Days 9 and 11 of *in vitro* culture in Charles Rosenkran's aminoacid (CR1aa) medium with 5% calf serum containing amino acids, L-Glutamic acid, BSA and gentamicin (Day 0=day of *in vitro* fertilization).

The series of wood bison studies listed above were done during both the anovulatory and ovulatory seasons, providing an opportunity to assess the effect of season on IVP. There was no seasonal effect on the *in vitro* or *in vivo* maturational capacity of wood bison oocytes (Cervantes et al., 2016). Although blastocyst-stage wood bison embryos were produced *in vitro* during both the ovulatory and anovulatory seasons (Cervantes et al., 2017a; 2017b), the overall efficiency of IVP was better during the anovulatory season because of i) a greater number of follicles ≥5 mm available for transvaginal ultrasound-guided aspiration, and ii) greater COC collection efficiency (see Table 7.2.1). While there was no difference between seasons in the developmental competence of oocytes, twice as many oocytes were collected per bison during the anovulatory season resulting in nearly a two-fold increase in the number of embryos produced compared to the ovulatory season (Table 7.3.1; Cervantes et al., 2017a).

Interestingly, these findings are in contrast to those of another study in which the rate of blastocyst development was greater from oocytes collected from slaughterhouse-derived bison ovaries during July to Sept. than during Jan. to Mar. (25% vs 7%; breeding season vs non-breeding season, respectively; Krishnakumar et al., 2015). However, the proportion of slaughtered bison that had a corpus luteum (indicative of the ovulatory season) was not reported and data may be confounded by a mixture of animals in the ovulatory vs anovulatory season (see Section 6.1). The difference may also be attributed to the effect of superstimulatory treatment used to increase the number of ovarian follicles available for transvaginal aspiration in the live-animal studies.

Table 7.3.1. *In vitro*-produced wood bison embryos from oocytes collected transvaginally from live wood bison superstimulated during the anovulatory and ovulatory seasons. Oocytes were matured for 30 h *in vivo* (before collection) and 4 h *in vitro* (after collection; adapted from Cervantes et al., 2017a).

End point*	Anovulatory season	Ovulatory season
Oocytes submitted to *in vitro* fertilization (n)	86	38
Cleaved oocytes	49/86 (57.0%)	23/38 (60.5%)
Morulas on Day 7	23/86 (26.7%)	12/38 (31.6%)
Blastocysts on Day 7	9/86 (10.5%)	9/38 (23.7%)
Blastocysts on Day 8	32/86 (37.2%)	17/38 (45.9%)
Blastocysts on Day 8/cleaved embryos	32/49 (65.3%)	17/23 (73.9%)
Blastocysts on Day 8 (n)	32	17
Grade 1	22/32 (68.8%)	12/17 (70.6%)
Grade 2	7/32 (21.9%)	3/17 (17.6%)
Grade 3	3/32 (9.4%)	2/17 (11.8%)

Grade 1: embryos with symmetrical and spherical cell mass, uniform blastomeres

Grade 2: embryos with moderate irregularities in size, shape, color and density of cell mass

Grade 3: embryos with major irregularities in size, shape, color and density of cell mass

*No significant differences between seasons for any proportional end point.

Using non-surgical oocyte collection and non-surgical embryo transfer, researchers at the University of Saskatchewan produced the world's first bison calves by *in vitro* embryo production (Cervantes, 2016). Ten IVP wood bison embryos were transferred fresh into 10 wood bison recipients (1 embryo per recipient) on October 24, 2015, and after a gestation of 265-266 days (8-day-old embryos + days in utero), 3 healthy calves ("Hope", "Moon" and "*Storm*"; Fig. 7.3.3) were born on July 7 and 8, 2016 (National Post, 2016; University of Saskatchewan News, 2016). The following year saw the birth of the world's first bison calves from transfer of frozen IVP embryos; 1 plains bison (Benham et al., 2018) and 1 wood bison (Mastromonaco et al., 2018). In both instances, the IVP embryos were produced during the nonbreeding season and were vitrified and stored in liquid nitrogen until the following breeding season when they were transferred to bison recipients. In the plains bison, oocytes and sperm cells were collected within 24h of slaughter from Yellowstone National Park animals. In wood bison, oocytes were aspirated trasvaginally from live superstimulated bison after inducing *in vivo* maturation, and sperm was collected by electroejaculation of live bulls. The frozen wood bison IVP embryos were transported by air from the IVP laboratory at the University of Saskatchewan to the Toronto Zoo, located 2,880 km away for embryo transfer.

Despite the dramatic progress that has been achieved in the IVP of bison embryos in recent years, more studies are needed to improve the production of transferable embryos (Grades 1 and 2; IETS Manual, 2010), particularly from oocytes matured *in vitro* in both standard and portable incubators. In other species, the overall quality of the transferred embryo has been related to pregnancy success (Hasler et al., 1995; Scenna et al., 2008). In cattle, for example, Grade 1 *in vitro*-produced embryos at the blastocyst stage were shown to yield the highest pregnancy rates when transferred either fresh or frozen-thawed (Hasler et al., 1987; Hasler et al., 1995). The composition of culture media is also considered critical for embryo development (Gordon, 2004), as well as embryo cryotolerance in cattle (Rizos et al, 2003; Nedambale et al., 2004). Important aspects that remain to be investigated in bison are the effects of culture media on embryo development and cryotolerance, and establishment of cryopreservation protocols for IVP embryos with confirmation of post-thaw viability and pregnancy outcome.

Figure 7.3.3. World's first bison calves born after *in vitro* fertilization ("Hope", "Moon" and "*Storm*") on July 7 and 8, 2016 (National Post, 2016).

7.4. Somatic cell nuclear transfer

Conventional reproductive technologies, including AI and IVF and embryo transfer, are effective for producing live offspring from desired genetic material. However, these techniques rely on the acquisition of viable sperm and oocytes from donor animals. There are circumstances where acquisition of gametes is not possible, including animals that are infertile, reproductively senescent, prematurely deceased, or neutered (Mastromonaco and King, 2007). Similarly, there are many species in which successful cryopreservation of sperm and oocytes hasn't been achieved. In these instances, the use of somatic cell nuclear transfer (SCNT), also known as reproductive cloning, may provide a valuable alternative strategy for *in vitro* production of embryos. Furthermore, SCNT preserves the entire genome from an important individual, avoiding the dilution of alleles that occurs with fertilization (Mastromonaco and King, 2007). Although SCNT success rates are significantly lower than conventional reproductive technologies (i.e.,

domestic cattle calving rates are 5-13% by SCNT, 24-35% by multiple ovulation and embryo transfer, and 20-22% by in vitro embryo production (Watanabe and Nagai, 2011)), the contribution of otherwise lost genetic material in a conservation breeding population may warrant the effort and expense of this technique.

Compared to the collection and cryopreservation of gametes and embryos, establishment and cryopreservation of fibroblast cell cultures is fairly routine for most species and does not require specialized protocols or equipment (Mastromonaco et al., 2014). Studies in domestic and wild cattle species have involved primarily the use of adult fibroblasts to produce SCNT embryos, a sample that is easily acquired from a skin biopsy of a living or recently deceased animal by biopsy dart, hand-held biopsy punch, or forceps and scissors (Wong et al., 2012). Viable SCNT embryos produced from tissue obtained from carcasses stored at 4^{o}C up to 48h post-mortem highlights the technique's potential for rescuing genetics from unexpectedly deceased animals (Mastromonaco, unpublished data; Adams et al., 2004). Furthermore, transport of biopsy tissues from the field to the laboratory is easily done since they may be stored without significant loss of viability for up to 7 days in phosphate buffered saline with antibiotics at 4^{o}C (Mastromonaco, unpublished data) or up to 3 weeks in tissue culture medium plus antibiotics at 4^{o}C (Wong et al., 2012).

Attempts to produce SCNT embryos in wild cattle species have involved the use of domestic cattle enucleated oocytes as recipients of the wild cattle donor cell (interspecies somatic cell nuclear transfer; iSCNT) (reviewed in Mastromonaco et al., 2014). This is due primarily to insufficient numbers or lack of access to oocytes from wild cattle donors to effectively carry out the technique, and because of the ability of domestic cattle oocytes to reprogram somatic cells from closely and distantly related species (Dominko et al., 1999). The application of iSCNT is most beneficial in cases where female gametes are not easily retrievable or are too valuable to be used in a technique that currently results in high loss rates at each stage of development. Examples of this are two threatened wild cattle species, gaur (*Bos gaurus*) and banteng (*Bos javanicus*), that are managed in zoo-based conservation breeding programs where oocyte aspiration from super-stimulated females was not possible. Several attempts carried out in gaur resulted in iSCNT blastocyst rates ranging from 12.0-37.5% (Sririttana et al., 2012; Mastromonaco et al., 2007; Lanza et al., 2000). However, detailed evaluation of gaur iSCNT embryos showed evidence of disrupted cellular processes, including delayed development and decreased cell numbers, compared to domestic cattle SCNT controls indicative of alterations in mitochondrial function in the interspecies reconstructed embryos (Mastromonaco et al., 2007). Of the 10 confirmed pregnancies at day 60 from the transfer of 150 embryos, only two live calves resulted but none survived past the first week (Sririttana et al., 2012; Lanza et al., 2000). This may not have been solely due to the effects of iSCNT as the embryos were transferred into domestic cattle recipients (i.e., interspecies embryo transfer) (Sririttana et al., 2012; Lanza et al., 2000), which has been shown to impact pre- and post-natal survival (Hammer et al., 2001). Similar

outcomes were observed in the banteng. Blastocyst development rates ranged from 20.0-28.0%, but interspecies transfers of 38 iSCNT embryos into domestic cattle recipients resulted in only 2 pregnancies confirmed on day 30, both of which were subsequently lost before day 90 of gestation (Sansinena et al., 2005). An earlier attempt by another group of researchers resulted in the birth of 2 male banteng calves following iSCNT and interspecies embryo transfer, one of which survived past the first week (Janssen et al., 2003). Interestingly, the calves were found to be cryptorchid (surviving calf, bilateral; deceased calf, unilateral) (Janssen et al., 2003).

In bison, iSCNT attempts using domestic cattle oocytes have resulted in the production of blastocysts from fibroblasts derived from adult wood bison, plains bison and European bison (*Bison bonasus;* wisent) (Seaby et al., 2013). No significant differences were observed between bison iSCNT embryos and domestic cattle SCNT controls at any developmental stage (cleavage, 8-16 cells, morulae, blastocysts), with the exception of lower blastocyst development in the plains bison iSCNT group (13.3% vs 27.6-33.9%). However, the authors suggest this reduced blastocyst development many not have been due to species-specific differences, but to variability in the donor cell cultures (Seaby et al., 2013). Unlike the gaur study, temporal developmental patterns were similar between the bison iSCNT and domestic cattle SCNT embryos (2-4 cells at 28 hours post-activation (hpa), 8-16 cells at 80 hpa, morulae at 120 hpa, blastocysts at 196 hpa) (Seaby et al., 2013). In an attempt to mitigate potential adverse effects of heterospecific ooplasm on iSCNT embryo development up to the 8-16 cell stage (time of maternal-embryonic transition), plains bison ooplasm was transferred with a plains bison somatic cell nucleus into a domestic cattle oocyte during iSCNT, but no significant benefits were observed (Gonzalez-Grajales et al., 2016). The results suggest that bison iSCNT embryos may not be impacted by the domestic cattle ooplasm and therefore, oocytes from domestic cattle may be valuable resource for the production of bison offspring. However, long-term effects of domestic cattle ooplasmic organelles (e.g. mitochondria) can be evaluated only following the birth and post-natal development of bison clones. Further studies on the pre- and post-implantation developmental potential of bison iSCNT and SCNT embryos (using bison enucleated oocytes) transferred into bison recipients are necessary to better understand the potential of SCNT as a strategy for genetic preservation of bison.

8. Factors affecting restoration of genetic diversity in North American bison

On-going threats to genetic diversity in bison include geographic isolation of herds with small effective population sizes, interspecies introgression, subspecies hybridization, disease, and importantly, a lack of solidarity among stakeholders regarding solutions to the problem. With a total world population today of

about 500,000, the North American bison appears to be making a comeback, but appearances are deceiving (Fig. 2.2.1). Recent expansion of commercial bison farming accounts for 95% of the bison population in North America today, but because of mixed ancestry (cattle and plains x wood hybrids), they are not useful for conservation of the species as it existed before the near-extinction event a century ago. Only 5% currently exists in conservation herds across North America - far less than 1% of the historic population size. Canada has not had new plains bison genes added to its herds since the introduction of the Pablo-Allard herd over 110 years ago (Lothian, 1981), and the largest and most diverse population of wood bison is "locked" inside WBNP because of endemic zoonotic diseases (McFarlane et al., 2006; Hedrick, 2009).

8.1 Geographic isolation and small effective population size

Currently, bison occupy less than 1% of their original range in North America. Population growth and the addition of new subpopulations is impeded by fragmented or unsuitable habitat that is often managed to exclude bison. Random events, like disease outbreaks and extreme weather, may be tolerated in large herds, but are catastrophic to small populations, resulting in an unsustainable rate of allelic fixation and the loss of vital genetic diversity.

The total *effective population size* of Canada's plains bison is less than 1500, but the population is distributed among 5 conservation herds (Fig. 3.3.2.), all of which were derived from a single founder population of 50 bison that originated at Elk Island National Park. Further, the plains bison conservation herds have remained isolated from each other and from new genetic input since their inception, and the effective population size is less than 200 in all but one of the herds (Fig. 3.3.2).

The total *effective population size* of wood bison is approximately 9,000, but again, the population is distributed among geographically distinct herds. Seven of the wood bison conservation herds were derived from a single founder population of 23 bison that originated at Elk Island National Park, and 5 of these herds do not exceed 500 individuals. Like the plains bison conservation herds, these disease-free wood bison herds are geographically and genetically isolated (Fig. 3.3.3). It is unclear to what extent the herds within WBNP intermingle, but recent evidence that the Ronald Lake herd has remained isolated from neighboring WBNP herds suggests that geospatial boundaries exist that prevent genetic mixing. Only 1 of

7 diseased herds within the greater WBNP region, and only 1 of 10 disease-free herds outside the park are composed of ≥1000 individuals (Fig. 3.3.5.).

8.2 Inter- and intra-species hybridization

Domestic cattle introgression

A devastating side-effect of the initial conservation effort was the intentional hybridization of bison with cattle. There is no evidence that natural crosses between domestic cattle and bison would occur (Hedrick, 2009), but hybridization was actively pursued in herds from which the Pablo-Allard herd was derived. Although hybrids were difficult to produce, hybridization experiments were continued in the plains bison herd at Wainwright from 1916 to 1935. Of 42 pregnancies that were achieved, only 6 F1 hybrid calves were born alive (Hedrick, 2009). The cross-breeding effort was abandoned, but the effect on the genetic make-up of present day plains bison has been profound. In a genetic study of the 11 federal conservation herds in the US, only 4 did not have detectable cattle introgression, but authors suggested that they may be reasonably confident of no introgression in only 2 herds – Yellowstone National Park in Wyoming and Wind Cave National Park in South Dakota (Halbert and Derr, 2007). More recent genetic testing with the use of single-nucleotide polymorphism (SNP) chips, however, revealed that approximately 70% of plains bison derived from Wind Cave National Park were positive for bovine introgression (American Prairie Reserve, personal communication). Only one private herd, owned by Turner Enterprises in New Mexico, has been shown to be free of bovine introgression (Freese et al., 2007).

The physiologic effects of cattle x bison hybridization have not been critically examined, but bison with cattle mitochondrial DNA have the phenotypic effect of being consistently smaller than bison with bison mitochondrial DNA (Derr et al., 2012). Based on the supposition that mating between bison and domestic cattle represents a threat to the survival of the wild species, *bison from populations with evidence of cattle ancestry should not be introduced into populations with no evidence of cattle ancestry* (Hedrick, 2009). To-date, domestic bovine introgression has not been detected in Canada's conservation herds of plains bison, but a small and apparently insignifican amount of introgression has been detected in wood bison (Todd Shury, personal communication).

Plains x wood bison hybridization

After the introduction of plains bison into WBNP in the 1920s, there was a fear that the true wood bison (non-hybrids) were disappearing (Lothian, 1981). Based on comparison of microsatellite genotypes (Wilson and Strobeck 1999; Cronin et al. 2013; Ball and Wilson, 2016) and mitochondrial DNA (Forgacs et al., 2016), it has been suggested that "genetically pure" wood bison may no longer exist, and that all herds fall into a spectrum of genetic admixture. However, both phenotypic and genotypic data document distinct clustering of populations as plains and wood bison. The sharpest phenotypic and genotypic distinctions were evident in comparisons between known plains bison (i.e., EINP and US conservation herds) and wood bison herds (i.e., greater WBNP area; van Zyll de Jong et al., 1995; Ball and Wilson, 2016). Phenotypic characteristics of plains vs wood bison have been identified based on differences in pelage, hump style, and body conformation (van Zyll de Jong et al., 1995; Olson, 2013), and quantitative differences in phenotype have been used to document retention of taxonomic traits despite prolonged displacement from their historic range (e.g., 16 generations of wood bison in central Alberta, 36 generations of plains bison in Alaska; Olson, 2013).

To-date, no genetic markers of purity have been identified, but genomic tools are changing rapidly and hold promise for distinguishing between subspecies and rapid identification of cattle introgression. One recommendation of a scientific workshop on reproductive strategies for addressing genetic diversity in Canadian cattle and bison was the need to link phenotypic characteristics with genotype data (MacPhee and Adams, 2016). Recent quantitative scoring of phenotypic traits of bison in the Ronald Lake herd indicated a strong wood bison type with less variability than that of other conservation herds (Olson, 2018), consistent with a distinctive genotype based on microsatellite analysis (Ball and Wilson, 2016). Genetic clustering of populations within WBNP was not apparent using the microsatellite approach, but clustering may become apparent by direct correlation with phenotypic traits.

8.3 Disease

Disease has played an important role in the rise and fall of both plains and wood bison populations. During the early 1800s, Texas tick fever was a scourge of domestic cattle that spread in epidemic proportions to both cattle and bison as a result of cattle drives from Texas northward (Koucky, 1983). Specific diseases such as anthrax, bovine brucellosis and bovine tuberculosis have had substantial effects on wood bison populations, and remain a threat today. Further, wild ungulates like bison and elk remain a reservoir of bovine brucellosis and tuberculosis and represent a threat to the disease-free status of livestock in both Canada and the US (Tessaro et al. 1990; Lees 2004). Detection of bovine tuberculosis in

an Alberta cow in 2016 illustrates the gravity of this threat. Two years after the case was identified, the Canadian Food Inspection Agency finally issued a release from quarantine of 79 trace-out herds and 71 trace-in herds involving roughly 30,000 animals. Approximately 11,500 animals were destroyed as a result of the disease investigation, with $55 million in compensation from federal and provincial governments (https://www.realagriculture.com/2018/02/last-quarantine-lifted-in-alberta-bovine-tb-investigation/).

Bovine tuberculosis and brucellosis

The plains bison population derived from the original Pablo-Allard herd had been co-mingled with domestic cattle before leaving Montana as well as after arriving at Wainwright, resulting in the transfer of bovine diseases (brucellosis and tuberculosis) to bison. The introduction of infected plains bison into the wild in the 1920s resulted in endemic infection of the wood bison in WBNP that persists to this day. Herds within WBNP, the largest reserve of wood bison in the world, have an intransigent prevalence of infection of 30-60% for both brucellosis and tuberculosis, depending on age class but irrespective of population density (McCormack, 1992; Tessaro et al., 1993; Mitchell and Gates, 2002; Joly and Messier, 2001; 2004). Authors of one study estimated that the 1971–1999 population decline would have occurred in the absence of disease (Bradley and Wilmshurst, 2005), but based on dramatic pregnancy loss in bison newly infected with *Brucella* (Davis et al., 1990; 1991), others concluded that brucellosis and tuberculosis in bison herds in and around WBNP limited growth of the metapopulation as a result of increased mortality, reduced fecundity, and increased vulnerability to predation in affected animals (Joly and Messier, 2005).

The issue is further complicated by the risk of disease transmission to neighboring healthy free-ranging bison herds and cattle ranches (Mitchell and Gates; 2002; Shury et al., 2015). In 1987, the Northwest Territories instituted a Bison Control Area to prevent the spread of brucellosis and tuberculosis to disease-free herds (Gates et al., 2001). The provinces of Alberta and British Columbia have similar Agricultural Area Surveillance Zones to monitor and restrict movement of free-roaming bison. By killing bison that enter into these "buffer zones", the programs effectively prevent establishment of free-ranging herds in over 40% of the original wood bison range (Hartop et al., 2009). Of the 9,000 free-ranging wood bison today, approximately half reside in populations affected by bovine tuberculosis and brucellosis, and 2/3rd of the remaining (disease-free) wood bison are descendants of 11 female calves in Elk Island National Park (Environment and Climate Change Canada, 2018; Parks Canada, 2018).

Table 8.3.1. Regional wood bison anthrax deaths by year (adapted from Dragon and Elkin, 2001; New, 2014).

Year	Slave River Lowlands	Wood Buffalo National Park	Mackenzie Bison Sanctuary	All regions
1962	281	0		562
1963	257	47	Started Sanctuary	608
1964	303	60	0	726
1967	0	120	0	240
1968	0	1	0	2
1971	33	0	0	66
1978	39	40	0	158
1991	0	32	0	64
1993	0	0	172	172
2000	0	100	0	200
2001	12	92	0	208
2006	26	0	0	52
2007	0	64	0	128
2010	45	6	10	112
2012	0	0	451	451

Anthrax

The first confirmed incident of anthrax *(Bacillus anthracis)* in Canadian bison was documented in July 1962 in a herd just outside the border of Wood Buffalo National Park (Dragon and Elkin, 2001). Deaths were recorded 60 miles north of Fort Smith in an area with an estimated population of about 10,000 bison (Moynihan, 1963). A total of 281 bison were found dead by air surveillance between July and August in a region called Hook Lake (Dragon and Elkin, 2001) located between the Slave River in the west and the Talston River in the east. Efforts were made to quarantine the area and to properly dispose of the bodies using techniques of the time. In the summer of 1963, 15 more carcasses were found in the Hook Lake region, and the anthrax outbreak spread to neighboring herds with 242 carcasses in the Grand Detour region and 47 in the Park Central region. In 1964, a total of 363 carcasses were found in the Slave River Lowlands and WBNP. Anthrax-infected carcasses have been found in the Slave River Lowlands and WBNP regions 13 times since 1962 (Table 8.3.1). Early anthrax outbreaks provided the impetus to capture the Nyarling/Needle Lake herd in 1963 and 1965 with subsequent translocations to Makenzie Bison Sanctuary and Elk Island National Park, in an effort to salvage this isolated herd before it was too

late (Ashley, 1966). Since then, the Makenzie Bison Sanctuary, located across the Great Slave Lake, has been affected by anthrax on a large scale (Dragon and Elkin, 2001). In 1993, there were 172 deaths in this region out of a population of ~ 2,000 (New, 2014). No new incidents were documented until 10 carcasses were found in 2010 and 451 in 2012 (New, 2014). Half the Makenzie Bison Sanctuary population, the largest free-ranging population of wood bison in the world outside of WBNP, was lost to anthrax during 2012 and 2013 (COSEWIC, 2013; Government of Canada, 2018 https://faune-especes.canada.ca/registre-especes-peril/species/speciesDetails_e.cfm?sid=143# ot18l).

8.4. Inadvertent selection

Inadvertent selection is an insidious process that may undermine efforts to increase genetic diversity and population fitness. This is particularly true in the management of captive herds where individuals that adapt poorly to the management style are inevitably culled, whether in a commercial bison farm or in a conservation herd. An example of potential inadvertent selection is that of the Elk Island National Park herd where care is taken during annual population-reduction round-ups to ensure unbiased selection. An estimate or actual count of calves is conducted before the handling operation and a decision is made regarding the number to be removed. For example, if 100 calves were born and 50 are to be removed, the first 25 male and 25 female calves to pass through the facility are culled. Referred to as a "chute run", this process is intended to randomly select bison from the population with no bias for any specific trait or weight. However, in a retrospective analysis of data from annual chute runs from 1997 to 2003, the plains bison calves that were shipped weighed more than the calves that were retained in the park. A similar process was used for herd reduction in the wood bison herd, and in a comparison of the mean weight of all wood bison calves and yearlings handled in 2018 vs those during the period from 1965 to 2005, the mean weight is lower now vs then (from Olson, 2018a). As well, visual examination of data on the calving rate of wood bison cows from 1967 to the present suggest a downward trend. These observations are preliminary and will require statistical verification, but point out the value of interrogation of longitudinal data to test for unintended selection or previously unnoticed trends in overall fitness.

Studies of diseased wildlife populations have focused on the ecology of host/pathogen interactions, but the effects of disease on host population genetic structure may be equally important (reviewed in McKnight et al., 2017). Studies have shown that disease itself exerts a selection pressure on wildlife populations that influences overall fitness through decreased population size, and by extension, genetic diversity. The use of culling as a disease management technique may exacerbate this loss of genetic variation within a population, and contribute to the perils of small, fragmented populations that are at increased risk of inbreeding and lack of gene flow (McKnight et al., 2017).

8.5 Solidarity – social, geo-political, commercial

The physical factors that currently threaten the bison species have been clearly documented and are largely uncontroversial. North American bison today are threatened by geographically unconnected herds with small effective population sizes, genetic introgression from domestic cattle and sub-species hybridization, endemic and epidemic diseases that affect not only bison but domestic livestock and human health as well, and by intentional or unintentional selection. These factors place extant herds of wood and plains bison in danger of potential catastrophic consequences of stochastic events (severe winters, floods, disease outbreaks).

Short-term conservation objectives have necessarily focused on protecting existing bison and minimizing the spread of disease, but do not address the root causes of the threats. Hence, the threats remain and the uncomfortable status quo of the last 50 years persists. The single most important impediment to addressing the long-term survival of bison has been the lack of solidarity among interested parties about how to address the threats directly. A laudable example of solidarity is the Hook Lake Wood Bison Recovery Project. The conservation project was initiated in 1996 and co-managed by the Government of the Northwest Territories and the First Nations communities of Fort Resolution. The objective was to derive disease-free bison from a diseased herd that could then be introduced into existing disease-free wood bison herds to augment their genetic diversity and establish new disease-free wood bison herds in their historic range in the Canadian northwest. From 1996 to 1998, bison calves (n=60) were captured when they were a few days old from the Hook Lake herd, a free-roaming wood bison herd located northeast of WBNP known to be infected with bovine tuberculosis and brucellosis. The calves were taken to a facility in Fort Resolution where they were managed as a breeding herd and tested repeatedly for both diseases over a period of 8 years. In a memorandum of understanding dated April 2005, 50 bison from the Hook Lake recovery herd were to be shipped to the University of Saskatchewan's new Specialized Livestock Facility to continue the recovery project. However, in March 2005, tuberculosis was detected in one of the bison as part of a routine cull of surplus animals. Subsequent epidemiologic investigation and herd depopulation revealed 13 additional cases of TB in the herd (Himsworth et al. 2010; Shury et al., 2015). Despite neonatal capture, separate rearing under strict isolation, and repeated testing for more than 8 years, the approach was unsuccessful in establishing a disease-free herd from an infected population. Notably, however, no infection with *Brucella* was detected in the recovery herd.

The Hook Lake Wood Bison Recovery Project was an important trial that illustrated the difficulty, cost, and risk of transporting live animals for the purposes of establishing a genetic resource from a diseased

population. With recent progress on the development of reproductive technologies, we now have an opportunity to create and deploy a bison genome biobank using sperm and embryos rather than live animals to derive disease-free bison calves from diseased herds (see Section 9). Sperm and embryos may be collected, washed free of disease organisms, stored frozen, and transported more safely and economically than live animals. Furthermore, the use of artificial insemination and embryo transfer will enable long-term preservation of unique alleles and more effective dissemination of valuable genetics. These tools may be employed to create surrogate herds as an ongoing source of seed-stock, or to directly and safely populate existing wild herds. The approach ensures restoration of genetic diversity by enabling critical re-connection among herds that have been genetically isolated for more than a century. The risk of disease transmission is minimized by transport of semen and embryos rather than live animals, and the use of an intermediate or "surrogate" herd to propagate valuable genetics provides the opportunity for an additional layer of disease surveillance before offspring are translocated to the wild or to other conservation herds. The genetic resources within the biobank provide insurance against future stochastic events. The approach also presents an opportunity for local communities, private industry, and commercial bison producers to participate in the restoration process by providing surrogate herds, thereby creating an interconnected North American bison network that will permit movement of bison back into their native territory without posing a disease threat to livestock producers and human health. The approach, if managed appropriately, will provide a measure of confidence that respective bison lineages will survive and thrive for generations to come.

9. Managing and deploying a bison genome resource biobank

9.1 Feasibility and long-term goals

Numerous reviews of the economic and ecologic benefits and potential of reproductive technologies for species conservation have been published (Lasley et al., 1994; Loskutoff et al., 1995; Bainbridge et al., 1998; Holt et al., 1999; Solti et al., 2000; Thundathil et al., 2007), but to-date, there are few examples of implementation. One example of successful application of a genome resource biobank is the black-footed ferret (*Mustela nigripes*), bred in captivity since the 1980's from a founding population of seven animals (three males, four females), it has been successfully reintroduced to the wild due to the co-operative breeding efforts of six zoological and government facilities (Marinari, 2014). Artificial insemination with fresh and frozen-thawed sperm played a key role in the production of 139 ferret kits from 49 of 82 females (59.8%) inseminated between 1996–2008 (Howard and Wildt, 2009). Recently, live ferret kits produced using semen banked for 20 years from a male of the founding population will significantly enhance the current population's genetic diversity and reduce inbreeding levels with their incorporation into the

breeding pool (Howard et al., 2016). The benefits of reproductive technologies are also well-documented in the breeding management of the whooping crane (*Grus americana*) and Wyoming toad (*Bufo baxteri*), the offspring of which have been produced by artificial insemination and reintroduced to the wild (reviewed by Wildt et al., 2010). In what may be the first example of genetic management using a complete program of reproductive biotechnologies for wildlife conservation, oocytes from captive-bred European mouflons *(Ovis orientalis musimon)* were collected surgically after hormonal priming (ovarian synchronization and superstimulation). Following surgical transfer of 20 IVP mouflon embryos to domestic ewes, 4 live offspring were born (Ptak et al., 2002). These examples emphasize the power of reproductive technologies to enhance breeding outcomes and build genetically and demographically healthy populations *ex situ* and, ultimately, *in situ*.

A common theme among the above-cited reviews is the need for understanding the basic natural reproductive pattern of the species of interest before effective reproductive techniques can be developed and applied. Beginning with studies of the fundamental reproductive physiology of bison, tremendous progress has been achieved in the last decade on development of effective reproductive technologies for the restoration of genetic diversity in threatened bison:

- Recovery and cryopreservation of epididymal sperm as a model for salvaging the genetics of wood bison (Aurini et al., 2009)
- Successful cryopreservation of sperm from ejaculates of live bison (Hussain et al., 2013)
- First serial ultrasonographic examination of bison to characterize ovarian and endocrine events of the ovulatory and anovulatory seasons (McCorkell et al., 2010; 2013; Section 6.1)
- First bison calves born from fixed-time artificial insemination using frozen semen (Adams et al., 2010; Yang et al., 2016; Section 6.2)
- First transcontinental shipment of chilled semen (from the University of Saskatchewan to the Toronto Zoo) and artificial insemination results in birth of a wood bison calf at the Toronto Zoo (Mastromonaco et al., 2019 – in preparation)
- First wood bison calves born from embryo transfer (Toosi et al., 2013; Section 7.1)
- First bison calves born after transfer of embryos derived from *in vitro* fertilization (Cervantes et al., 2016; National Post, 2016; Section 7.3)
- First bison calf born after transfer of a frozen embryo (National Post, 2016; University of Saskatchewan News, 2016)
- First demonstration that bison semen and embryos can be effectively washed free of *Brucella abortus* (Palomino et al., 2015; Section 8.2)
- First bison calf born after transfer of a frozen embryo derived by *in vitro* fertilization. A vitrified wood bison IVF embryo produced at the University of Saskatchewan was shipped to the Toronto Zoo and transferred to a wood bison recipient in Nov. 2016. Live calf born in July, 2017 (Canadian Geographic, 2018)

The recent birth of bison calves after artificial insemination using chilled or frozen semen, and after transfer of fresh or frozen-thawed embryos derived by *in vivo* or *in vitro* fertilization are important milestones for future conservation programs in bison. In particular, these events document the feasibility of collecting and preserving germ plasm during both the ovulatory and anovulatory seasons, and demonstrate the ability to successfully transport bison genetic material between distant places. While some conservation herds are managed in a captive or semi-captive environment with handling facilities, and even IVP laboratories on-site or nearby, many important bison populations are in free-roaming, wild settings without access to this important infrastructure, which adds a layer of complexity to the retrieval of genetic material from these animals. Protocols for shipping oocytes currently used for domestic species, such as cattle (e.g., Boviteq Canada), are being investigated and adapted for use in bison. In a recent study (Cervantes et al., 2018), results demonstrated that wood bison oocytes collected from non-superstimulated bison and matured *in vitro* using portable incubators were competent to develop into embryos (morula plus blastocyst rates on Day 7: 45%) following *in vitro* fertilization and culture. These latest results are important for future plans that require transporting oocytes from remote collection sites to an IVP laboratory.

Opportunistic collection of gametes may occur either during routine handling of bison in captive herds, or in the field during surveillance procedures or following a cull or hunt. In live animals, retrieval of oocytes can be accomplished by transvaginal aspiration and retrieval of sperm by electroejaculation in animals restrained in a chute or anesthetized animals during remote capture procedures. The gametes will be placed in holding/transport solutions and transferred to a laboratory for further processing and cryopreservation. Gamete retrieval from animals post-mortem is feasible if the ovaries or testes are removed within a few hours of death, placed on cold packs, and transported to a laboratory for further processing and cryopreservation. Experiments done to-date have attempted to mimic field conditions and have provide exciting results, but attempts must be made under actual field conditions to provide a more realistic assessment of the feasibility of germplasm collection in less controlled settings. However, results to-date provide confidence of consistent and reproducible protocols for gamete collection from female and male bison in either captive or wild settings.

The long-term goal for effective deployment of a bison genome resource bank is to re-establish connectivity between populations; that is, to provide a method for genetic exchange so populations do not become so isolated that they succumb to negative consequences of a loss of genetic diversity (Ball and Wilson, 2016; Hedrick and Kalinowski, 2000). Storage of cryopreserved genetic resources, including sperm, oocytes, embryos and somatic cells, in a properly catalogued biobank is an important process that can support the maintenance of the original genetic diversity of a population indefinitely (Ballou, 1992). Representation of remaining animals within the biobank provides insurance against any sudden loss of genetic diversity due to disease, natural disaster, and other unforeseeable events (Wildt, 1992). More

importantly, introduction of genetic material from the biobank into the remaining population via reproductive technologies over time will help offset any loss of alleles due to genetic drift (Wildt, 1992), a significant problem when managing small populations which lose genetic variation more rapidly than larger populations (Lacy, 1987).

A repository of bison genetic material will be instrumental for establishing a national management strategy for bison populations. Use of biobanked samples for reproductive technologies will not only readily permit gene flow between *ex situ* bison herds, but also between *in situ* and *ex situ* bison herds. This will not only ensure the contribution of all animals into the national gene pool, but also prevent inbreeding and unintentional selection that can occur in small populations. Maintaining an active genome resource bank with samples being continuously "deposited" and "withdrawn" has other benefits: elimination of live animal translocations between populations, decreased space requirements (i.e. reduced numbers of live animals) for breeding herds, and increased reproductive efficiency, to name a few (Wildt, 2000).

9.2 Biosecurity

Sanitary measures to minimize the risk of disease transmission through embryos and semen is pivotal for successful deployment of a genome resource bank. This is particularly relevant to valuable conservation herds of plains bison in the US and wood bison in Canada, which harbor endemic disease. Bison in and around WBNP have overall apparent prevalence rates of 49% and 31% for tuberculosis and brucellosis, respectively (Jolly et al, 2004), representing the most important limiting factor to the reestablishment of other healthy free-roaming herds in the region (Gates et al. 2001, Hartop et al., 2009).

In this regard, the objective of a recent study (Cervantes et al., 2019, in preparation) was to determine the effectiveness of washing procedures for removing a field strain of Brucella bacteria from wood bison embryos, produced either by *in vivo* or *in vitro* fertilization, that were experimentally exposed to the pathogen. *In vivo*-derived and *in vitro*-produced embryos with an intact zona pellucida (ZP) were placed in Petri dishes containing holding medium and incubated with *Brucella abortus* biovar 1 (originally isolated from a wild wood bison from WBNP; Forbes and Tessaro, 1996) for 2 h at 37ºC in 8% CO_2. After incubation, embryos were subjected to a 10-step washing procedure using medium without antibiotics or with antibiotics, according to the guidelines of the International Embryo Technology Society (Stringfellow and Givens, 2010). A sample of medium was cultured at wash steps 1, 3, 6, and 9. After the 10th wash, embryos were cultured individually on sheep blood agar and specific identification of *Brucella* organisms was tested by PCR. *Brucella abortus* was not detected in media after the 3rd wash in either wash group

(with or without antibiotics). *Brucella abortus* was detected by PCR in two *in vivo*-derived embryos from the group washed without antibiotics (2/27), but in none of the *in vivo*-derived embryos in the group washed with antibiotics (0/27). All of the in vitro-produced embryos in both wash groups were culture-negative (0/84). In summary, *Brucella abortus* was removed from 92% of in vivo-derived and 100% of in vitro-derived bison embryos washed with medium without antibiotics, and from 100% of all embryos washed with medium containing antibiotics.

For the purposes of developing a procedure to harvest pathogen-free sperm from potentially diseased bison, a sperm separation and purification product, BoviPure, was tested to determine its effect on bison sperm viability before and after cryopreservation (Bogle et al., 2010). BoviPure (NidaCon International AB, Mölndal, Sweden) is a density centrifugation gradient system that contains trypsin, and is designed to separate motile from non-motile sperm and remove infectious pathogens (De la Rey et al., 2005; Fourie et al., 2012). BoviPure gradient with or without trypsin did not adversely affect total motility or progressive motility of bison sperm either before or after cryopreservation, and has potential as a method of harvesting pathogen-free sperm from wild bison of unknown disease status (Bogle et al., 2010). In an initial study to determine the effectiveness of the BoviPure washing procedure, semen was collected by electroejaculation from non-diseased wood bison and was incubated fresh with *Brucella abortus* biovar 1 for 1 hour (Adams et al., unpublished). Infected semen samples were then divided into three groups (n = 18 per group) and diluted in extenders containing antibiotics + trypsin, only antibiotics, or neither, and then submitted to the Bovipure washing procedure. *Brucella* culture-positive samples were detected in 3/18, 1/18, and 0/18 samples in the group extended without antibiotics, with antibiotics, and with antibiotics + trypsin, respectively. The density gradient technique (Bovipure) was an effective method of removing *Brucella* bacteria from bison semen contaminated *in vitro*, particularly when using a semen extender containing both antibiotics and trypsin.

These findings are encouraging and provide impetus to begin gamete collection and embryo production from diseased animals to confirm the efficacy of removing infectious agents from material derived from naturally infected bison.

9.3 Genetic fidelity and diversity

Quantification of genetic variation is integral to managing the genetic fitness of a herd and measures such as expected heterozygosity, probability of identity, allelic richness, number of private alleles, and allelic proportion will be needed to monitor the success of implementing a

strategy to restore genetic diversity in bison (McFarlane, 2006). In a recent DNA microsatellite study that compared the genetic variation among parent-descendant herds, 3 transplanted wild bison populations had lesser heterozygosity and allelic richness than their respective parent populations, but based on results in cattle, loss of genetic variation from founder effects or inbreeding can be restored with crossbreeding and gene flow (Cronin et al., 2016; Weller et al., 2017). Recent efforts to identify individuals or subgroups as 1st or 2nd generation migrant or of non-migrant ancestry (Ball and Wilson, 2016) will be important for identifying candidate sources of germ plasm that are unrelated.

In a strategy involving the collection of germ plasm in the field from live or recently killed bison, criteria used for selection of candidate donors will include historical knowledge of herd provenance, assumed geospatial isolation, phenotype and genotype. Rapid development of tools for genomic selection, including the use of high-density SNP chips and full genome sequencing provides the exciting possibility of resolving the phylogenetic distinction between subspecies and establishment of markers of interspecies and intraspecies introgression.

It is interesting that the presence of endemic bovine tuberculosis and brucellosis has been used as a proxy for genetic introgression in wood bison herds and vice versa (Ball and Wilson, 2016; Shury et al, 2015). While there is no direct relationship between infectious disease and genetic introgression, evidence of bovine diseases implies that the herd was in contact, directly or indirectly, with the infected plains bison introduced in the 1920s. Hence, efforts to identify pure wood bison have been aimed at locating herds most likely to be geographically isolated and disease-free. Paradoxically, the remote Nyarling River herd from the northern border of WBNP, used to establish the EINP herd in the 1960s, turned out to harbor bovine TB while the not-so-remote Ronald Lake herd at the southern border of WBNP is apparently disease-free.

The importance of maintaining the genetic ancestry of the WBNP population, despite its disease-positive status, was highlighted in a recent study because the population has the highest mean number of alleles of all the herds examined, and may harbor ancestral diversity not captured in any of the translocated herds (Ball and Wilson, 2016). Regarding the Ronald Lake Herd, three attributes make it potentially very valuable: i) it appears to be free of bovine brucellosis and tuberculosis, ii) it has a genetically distinctive fingerprint (Ball and Wilson, 2016), and iii) and it displays phenotypic characteristic distinctive of wood bison (Olson, 2018). The Ronald Lake Herd represents an interesting example where identification of donor animals may involve *a priori* assessment of biobank candidates.

9.4 Strategic use of a genome resource biobank

The development and use of a genome biobank, as outlined in this review, is offered as a first step in a new phase of bison recovery, and is expected to evolve over a period of 5 to 10 years to make a

meaningful and long-term contribution to bison as a species, the environment, and to the health of humans and our livestock. While the approach offers the possibility of widespread redistribution of bison genetics and the promise of sustainable diversity among isolated bison populations, it can also have the opposite effect. The domestic Holstein cow is a good example of a critical narrowing of the gene pool brought about by selection pressure for a singular characteristic (milk production) that was intensified through the use of genomics and reproductive technologies (Stachowicz et al, 2011; MacPhee and Adams, 2016). This underlines the importance of genetic monitoring in the deployment of a bison genome biobank to minimize over-representation of a few individuals and the effect of intentional or unintentional selection for "preferred" characteristics. Effective tools for genomic selection in wild bison are those that will help distinguish between subspecies (i.e., plains vs wood), detect bovine introgression, and permit research into the genetic characteristics of populations of different geographic locations and ecotypes.

On-going threats to the long-term sustainability of genetic diversity of bison in Canada warrants the need to integrate *in situ* (i.e. free-ranging herds) and *ex situ* (i.e. captive herds, and cryo-stored germ plasm) conservation efforts. Establishment and operation of an effective bison genome resource biobank requires the participation of diverse stakeholders to ensure the operational and genetic sustainability of the biobank, which should therefore include federal and provincial governments, zoological institutions, academic institutions, local communities and private owners.

A successful species conservation strategy that involves implementation of a genome resource biobank for the long-term sustainability of bison in Canada will require the following (FAO, 2012; IUCN/SSC, 2014):

1. Biobank operation:

   a) Establishing cooperation among partners
   b) Identifying funding sources
   c) Providing infrastructure (sample processing laboratory, cryostorage facility, security, information management system)
   d) Creating biobanking procedures (sample acquisition, cryopreservation, disease testing and other protocols)
   e) Determining legal issues (ownership and other property rights)
   f) Establishing accessibility (deposit/withdrawal of samples)
   g) Finalizing business plan (cost recuperation, not-for-profit, etc.)

2. Genetic resource management:

a) Determining genetic and demographic structure in herds of interest (free-ranging and captive)
b) Establishing a genetic goal (e.g., preserving 90% of the source population's heterozygosity for 200 years; Soulé et al., 1986)
c) Identifying collection goals (number of animals, number of samples per animal, types of samples)
d) Identifying collection sites to ensure capture of rare/specific alleles
e) Establishing a studbook for long-term management of captive animals and biobank material

Preservation of Canada's natural resources, specifically genetic resources, should be supported by the Canadian government (i.e., a basic expectation of financial support over time). Supplemental funds to enhance sampling and support research may be obtained from private and commercial donors, cost-recovery programs (sample disbursement fees), and grants, to name a few. There are already several examples of Canadian biobanks supported by the federal government, such as the Canadian Tumour Repository Network and the Canadian Animal Genetic Resources program. Recently, several countries have enhanced their commitments to their natural genetic resources and established or provided funds to wildlife biobanks (CryoArks Biobank, United Kingdom; Ian Potter Australian Wildlife Biobank, Australia).

Wildlife genome resource banks that have been successfully in operation for more than 20-30 years have been either initiated by zoological institutions (San Diego Zoo's Frozen Zoo®; Smithsonian Centre for Species Survival's Genome Resource Bank; Cincinnati Zoo and Botanical Garden's CryoBioBank®) or established as a joint venture between zoological and academic institutions (Monash University and Zoological Parks Board of New South Wales' Animal Gene Storage and Resource Centre of Australia). Conservation programs integrating *in situ* and *ex situ* strategies would benefit from inter-institutional partnerships in the genome resource bank since each partner can bring specific skills and resources to the operation of the biobank.

9.5 Roles of conservation herds, zoological and heritage parks, and commercial producers

One of the key factors influencing the success of conservation breeding programs is the space required to maintain significant numbers of animals for long-term genetic sustainability of the species. To reduce genetic drift and unintentional selection, larger populations should be maintained in minimally managed environments, and preferably divided into several subpopulations (Lacy, 1987). Conservation of

threatened bison populations would, therefore benefit from the help of multiple partners in an effort to arrest and reverse the loss of genetic diversity. A new model for the implementation of collaborative breeding partnerships for endangered species preservation is the Conservation Centers for Species Survival (C2S2) established in 2005 (http://conservationcenters.org/about-c2s2/). Today, C2S2 includes the founding five zoological institutions along with other zoos, conservation centres, private landowners, and government agencies. The aims are to provide large areas, natural group sizes, minimal public disturbance, and encourage scientific research in its species conservation programs. This approach can be strengthened by the implementation of genome resource banks and reproductive technologies; i.e., large populations divided among key stakeholders all following a national genetic management plan implemented via reproductive technologies.

Division of populations among collaborating facilities is also beneficial for several other reasons. The costs of species recovery and long-term sustainability can be shared among the partners. Likewise, recruitment of funding to carry out conservation activities has a broader base of support. Education and public outreach on the conservation status of the species can extend to a much larger audience across the country. But most importantly, distribution of animals across different geographical areas provides protection against significant loss of animals from disease epidemics or natural events in one area. For bison, there is an excellent opportunity to invest the help of commercial bison producers who have as a part of their vision statement *to bring together stakeholders to restore bison to North America in a way that sustains the cultural health of First Nations and contributes to regenerative health of ecosystems* (National Bison Association, 2017). The challenge will be to establish a system that rewards maintenance of genetic fidelity (i.e., a functional registry) within commercial herds that have been 'certified' contributors to bison conservation. Working together, a bison consortium can meet the necessary goals to maintain optimal numbers of healthy, genetically valuable animals in the wild for the next 100 years.

Our work in bison reproduction has highlighted the importance of strong partnerships, particularly between academic institutions and conservation organizations. Academic institutions focus on hypothesis-driven research and higher-level learning, and can provide access to theoretical knowledge, technical expertise, and specialized equipment. Zoos focus on understanding the species in their care and developing tools to support breeding goals, small population management, and the necessity for maintaining demographically and genetically healthy *ex situ* populations. Governmental agencies prioritize habitat management along with species restoration, and are instrumental for evaluating and promoting long-term species sustainability *in situ*. Collaboration between facilities with diverse priorities and functions is mutually beneficial and will typically result in enhanced outcomes. Along with the added knowledge, technical expertise and equipment, partnerships increase access to genetically valuable animals, funding from different sources, and exposure to a larger community to disseminate conservation messaging and garner project support. Furthermore, inter-institutional partnerships are especially valuable

for projects spanning decades (and generations) as they strengthen the ability to maintain long-term goals despite changes in government (i.e. regulations, policies, priorities), financial resources, personnel, and other unforeseen challenges.

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Appendix 1: Cost of implementing a bison biobank

Costs will vary according to specific objectives, location, facilities, and partnerships. For the purposes of this report, a budget will be described for a 5-year Research & Development Project which represents the final stage before implementation. This final stage offers the advantages of training highly qualified personnel and establishing partnerships that will be necessary for long-term management of the genetic rescue project. Initial objectives include:

1. *Increasing proficiency in producing bison calves by artificial insemination and embryo transfer:*
    a. Collect >15 artificial insemination doses of semen per collection per bison bull, with a post-thaw sperm motility of ≥50%
    b. Produce >1.5 transferrable embryos per collection (oocyte or embryo collection)
    c. Achieve a pregnancy rate of >30% after artificial insemination or embryo transfer
2. *Documentation of effectiveness of gamete and embryo disinfection:*
    a. *In vitro* exposure, *in vitro* documentation (Cervantes et al., 2019, in preparation)
    b. *In vitro* exposure, *in vivo* documentation; i.e., use *in vitro*-exposed and washed embryos and semen in disease-free bison females
    c. *In vivo* (potential) exposure, *in vivo* documentation; i.e., use washed embryos and semen collected from endemically infected population in disease-free bison females
3. *Rescuing genetics from wild herds by producing disease-free bison calves using semen and oocytes/embryos derived from isolated and genetically important conservation herds.*

Assuming the production rates listed in Table 10.1 (based on results reported in Sections 6 and 7), it is feasible to produce 300 live bison calves over a 5-year period.

Table 10.1. Live calf production based on semen collection from 100 bison bulls, *in vivo*-derived embryos from 100 bison cows and *in vitro* embryo production from 100 bison cows.

Production by AI				
	# bulls	Doses/collection	# collections	Total semen doses
semen	100	15	1	1500
	# cows	Pregnancy rate	Live-born rate	**Calves born**
	1000	30%	90%	270

Production by *in vivo* ET (in subsequent years from offspring raised in captivity)

	# cows	Embryos/collection	# collections	# transferable embryos
embryos	100	1.5	1	150
	post-thaw viability	Pregnancy rate	Live-born rate	**Calves born**
	50%	30%	90%	20.3

Production by *in vitro* ET

	# cows	emb/collection	# collections	# transferable embryos
embryos	100	1	1	100
	post-thaw viability	preg rate	Live-born rate	**Calves born**
	50%	30%	90%	13.5
Total calves born				**303.8**

5-year Research & Development Project focused on herds in greater WBNP

Project Budget:					
	Year One	Year Two	Year Three	Year Four	Year Five
Salaries & Benefits					
Principal Investigator (50%)	$100,000.00	$100,000.00	$100,000.00	$100,000.00	$100,000.00
Graduate Students (2)	$ 52,000.00	$ 52,000.00	$ 52,000.00	$ 52,000.00	$ 52,000.00
Professional Research Associate (1)	$ 80,000.00	$ 80,000.00	$ 80,000.00	$ 80,000.00	$ 80,000.00
Technical Assistant (50%)	$ 25,000.00	$ 25,000.00	$ 25,000.00	$ 25,000.00	$ 25,000.00
Summer veterinary student assistant	$ 9,500.00	$ 9,500.00	$ 9,500.00	$ 9,500.00	$ 9,500.00
Equipment &					
Purchase ultrasound	$ 8,596.70	$ 8,596.70	$ 8,596.70	$ 8,596.70	$ 8,596.70
Stereo microscope	$ 800.00	$ 800.00	$ 800.00	$ 800.00	$ 800.00
Light microscope	$ 800.00	$ 800.00	$ 800.00	$ 800.00	$ 800.00
Electroejaculator	$ 400.00	$ 400.00	$ 400.00	$ 400.00	$ 400.00
Portable COC collection system	$ 2,900.00	$ 2,900.00	$ 2,900.00	$ 2,900.00	$ 2,900.00
Controlled temp shipper	$ 800.00	$ 800.00	$ 800.00	$ 800.00	$ 800.00
Rental (portable lab)	$ 10,000.00	$ 10,000.00	$ 10,000.00	$ 10,000.00	$ 10,000.00
Brucella testing, InterVac/VIDO	$15,185.00	$15,185.00	$15,185.00	$15,185.00	$15,185.00
Bison facilities and bison	$63,875.00	$63,875.00	$63,875.00	$63,875.00	$63,875.00
Materials & Supplies					
Semen handling supplies	$ 10,000.00	$ 10,000.00	$ 10,000.00	$ 10,000.00	$ 10,000.00
Bovipure/ProInsert	$ 6,000.00	$ 6,000.00	$ 6,000.00	$ 6,000.00	$ 6,000.00
Liquid nitrogen & tanks	$ 5,400.00	$ 5,400.00	$ 5,400.00	$ 5,400.00	$ 5,400.00
In vitro embryo production	$ 5,000.00	$ 5,000.00	$ 5,000.00	$ 5,000.00	$ 5,000.00
In vivo embryo production	$ 2,300.00	$ 2,300.00	$ 2,300.00	$ 2,300.00	$ 2,300.00
Supertim & synchr tx	$ 22,200.00	$ 22,200.00	$ 22,200.00	$ 22,200.00	$ 22,200.00
Miscellaneous	$ 1,500.00	$ 1,500.00	$ 1,500.00	$ 1,500.00	$ 1,500.00
Travel					
Professional Conferences	$ 4,000.00	$ 4,000.00	$ 4,000.00	$ 4,000.00	$ 4,000.00
Native Hoofstock Centre	$ 3,429.65	$ 3,429.65	$ 3,429.65	$ 3,429.65	$ 3,429.65
Field Trips	$ 143,720.00	$ 143,720.00	$ 143,720.00	$ 143,720.00	$ 143,720.00
Technology Transfer					
Publications	$ 1,500.00	$ 1,500.00	$ 1,500.00	$ 1,500.00	$ 1,500.00
Stakeholder Meeting & Workshops	$ 9,414.00	$ 9,414.00	$ 9,414.00	$ 9,414.00	$ 9,414.00
TOTAL EXPENDITURES	$584,320.35	$584,320.35	$584,320.35	$584,320.35	$584,320.35

Explanation

Salaries & Benefits:

- Includes salary for a principal investigator, 2 full-time graduate students, a research associate, laboratory technician, and summer student assistants

*Equipment & Facilities (*costs amortized over 5 years in above budget):*

- Ultrasound equipment including 2 probes, batteries, charger and hard case $42,983*
- Stereo-microscope for examining oocytes and embryos $ 4,000*
- Light microscope with phase contrast for examining sperm $ 4,000*
- Electro-ejaculator for semen collection $ 2,000*
- Oocyte collection pump & portable incubator $ 14,500*
- MicroQ shipping device (cooler/incubator) for field transport of embryos $ 4,000*
- Rental of a portable lab unit (truck or trailer) for lab work in the field $ 10,000
- Brucella testing, InterVac/VIDO.
  - Buffered plate agglutination test: 100 samples x $9 $ 900
  - Brucella culturing (semen, embryos): 100 samples x $40 $ 4,000
  - Submission set-up fee 10 x $28.50 $ 285
  - InterVac/VIDO bio-containment level 3 lab facility rental $10,000
- Bison facilities and animal maintenance at the Native Hoofstock Centre
  - 50 bison x $3.50 x 365 days $63,875

Materials & Supplies (average per year for 5 years):

- Semen handling supplies: tubes, pipettors & tips, semen extender, antibiotics, microscope slides, hemocytometer, AI equipment, straws $10,000
- Semen disinfection supplies with antibiotics and trypsin
  - BoviPure: $10/ejaculate x 200 ejaculates /yr $10,000

ProInsert: $20/ejaculate x 200 ejaculates /yr $20,000

- Liquid nitrogen & tanks for cryopreserving embryos and semen $ 4,000
- Cryogenics portable dry shipper/short-term storage ($2,400 over 5 yrs) $ 480
  Large (47 litre) storage tank ($3,600 over 5 yrs) $ 720

  Goblets, canes, cryo tubes, straws and straw handling $ 200

- *In vitro* maturation, fertilization and culture (IVM/IVF/IVC)
  Media, glass- and plastic-ware, CO_2 and N gas cylinders $ 5,000

- *In vivo* embryo production
  Embryo flush/holding media $ 1,500

  Transfer catheters, filters, tubing, stylets $ 800

- Superstimulation treatment - FSH = $140/bottle; hCG = $ 45/ bottle $16,200
- Synchronization treatments
  Intravaginal devices, estrogen, progesterone, prostaglain, hCG $ 6,000

- Miscellaneous
  Sleeves, gloves, lubricant, syringes, needles, scrub, electrical, etc $ 1,500

Travel

- Professional Conferences. Two national or international conferences per year to report on our research and development*:* $2000 x 2 $ 4,000
- Native Hoofstock Centre (local): 240 trips x 34 km x $0.42/km $ 3,430
- Field Trips in and around Wood Buffalo National Park
  Flight to Ft. Mac or Ft. Smith: $1400 x 3 persons x 2 trips $ 8,400

  Accommodations: 10 nights x $120/night x 3 persons x 2 trips $ 7,200

  Food: 10 days x $52/day x 3 persons x 2 trips $ 3,120

  Helicopter fees: 25 bison x $2,500/bison x 2 trips $125,000

Technology Transfer

- Publications: 1 scientific manuscript/yr $ 1,500
- Stakeholder meetings & workshops (Saskatoon, Calgary, Edmonton, Ft. MacMurray)
  Flight: $350 x 3 persons x 3 meetings $ 3,150

  Accommodations: $180/night x 3 nights x 3 persons x 3 meetings $ 4,860

  Food: $52/day x 3 days x 3 persons x 3 meetings $ 1,404

Note: Costs of genomic testing for bovine introgression and parentage have not been included. Genomic tools are evolving rapidly and accurate estimation of cost and feasibility will benefit from direct interview with scientists currently involved in the field (e.g., Drs. James Derr and David Forgacs of Texas A&M University)

Future Bison Biobank Business Plan (preliminary)

Operating:

Infrastructure (secure facility with utilities)	committed by hosting institution
Equipment (veterinary/laboratory)	purchased during final R&D phase
Cryogenic tanks	purchased during final R&D phase
Liquid nitrogen	$1,500/year
Inventory system (LIMS)	$10,000
Part-time assistant	$25,000/year

Genome Banking:

Sperm collection and banking	$500/animal + travel expenses
Embryo collection/production and banking	$1500/animal + travel expenses
Breeding (AI/ET)	$500/animal + travel expenses

Inter-institutional shipments	$400 each
Disease testing	$100/sample
Genomic testing	???

Cost Recovery:

Semen	$100/straw
Embryo	$1000 each

Potential Partners

NGO AND NOT-FOR-PROFIT

- Fort Peck Indian Reservation, Montana http://www.fortpecktribes.org
- Wanuskewin Heritage Park https://wanuskewin.com
- Toronto Zoo http://www.torontozoo.com
- World Wildlife Fund http://www.wwf.ca
- Wildlife Preservation Canada https://wildlifepreservation.ca/
- Turner Foundation http://www.turnerfoundation.org
- Genome Canada https://www.genomecanada.ca/en/programs
- Mitacs https://www.mitacs.ca/en

INSTITUTIONAL AND GOVERNMENT

- Provinces of Saskatchewan, Alberta, Northwest Territories, Yukon
- University of Saskatchewan (and others)
- Parks Canada
- Environment and Climate Change Canada
- Agriculture and Agri-Food Canada

COMMERCIAL BISON

- Canadian Bison Association https://www.canadianbison.ca
- National Bison Association https://bisoncentral.com
- Individual bison producers

OIL, ENERGY & MINING

- Teck Resources https://www.teck.com/responsibility/sustainability-topics/biodiversity/
- Canadian Natural Resources https://www.cnrl.com/corporate-responsibility/environment
- Census Energy https://www.cenovus.com/responsibility/environment/wildlife-biodiversity-land.html
- Suncor https://sustainability.suncor.com/2017/en/environment/land-biodiversity.aspx?_ga=2.5583924.669787848.1521143817-329783032.1521143817
- Shell Canada https://www.shell.ca/en_ca/sustainability/environment.html
- Enbridge https://www.enbridge.com/About-Us/Community-Investment/Everyones-Environment.aspx
- TransCanada Corp https://www.transcanada.com/en/commitment/environment/land-and-wildlife/
- Trans Alta https://www.transalta.com/sustainability/
- Husky Energy http://www.huskyenergy.ca/responsibility/environment/land-habitat.asp

UTILITIES

- BC Hydro https://www.bchydro.com/about/accountability_reports/2011_gri/f2011_environmental/f2011_environmental_EN13.html
- Manitoba Hydro https://www.hydro.mb.ca/environment/wildlife_stewardship.shtml
- Yukon Energy https://yukonenergy.ca/sustainability/conservation/stewardship-biodiversity

FORESTRY

- Weyerhaeuser https://www.weyerhaeuser.com/timberlands/forestry/canada/
- Canfor http://www.canfor.com/sustainability-report/environment/protecting-habitat
- West Fraser https://www.westfraser.com/responsibility/environment
- Western Forest Products http://www.westernforest.com/sustainability/environmental-stewardship/planning-and-practices/biodiversity-strategy/
- Millar Western https://millarwestern.com/environment/