THE REPRODUCTIVE PERFORMANCE OF BISON AT THE NATIONAL BISON RANGE

by

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APPROVAL

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency and is ready for submission to the Division of Graduate Education.

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ABSTRACT

Bison (*Bison bison*) calf production at the National Bison Range (NBR) near Moiese, MT has dropped from the historic average of 87 to 32 calves per 100 breeding-age cows in 2008. The purpose of this project was to determine specific stages of the reproductive cycle of female NBR bison that may be responsible for low reproductive rate and calf production. The reproductive cycle was divided into 3 stages: conception to early embryogenesis; gestation; and perinatal calf survival. From 2008 to 2010, we used transrectal ultrasonography to determine pregnancy rate in cows ages 3 to 12 yr in October of each year. Sixty-eight percent (28/41), 63% (56/89) and 71% (50/70) were pregnant in 2008, 2009 and 2010, respectively. In 2008, pregnant cows were painted with a unique number using hair bleach. Fecal samples collected in Oct. 2008, and Jan. and Mar., 2009, were analyzed for progesterone (P4) concentrations. Because bleach marks did not persist through calving, in 2009 radio collars were secured to 27 pregnant and 10 non-pregnant cows. Fecal samples collected in Oct. 2009, and Jan., March, Apr., and May, 2010, were analyzed for P4 concentrations. Based on fecal P4 analyses, 26% of cows pregnant in October 2008 lost their pregnancy during the winter 2009. In 2009, 26 pregnant cows retained their collars. Of these, we confirmed 23 produced calves, suggesting a 12% (3/26) loss during gestation. Furthermore, 2 (20%) cows classified non-pregnant by ultrasonography in Oct. produced a calf demonstrating bison females may breed later than early September. Serum samples of breeding-age cows collected during the 2000 to 2007 roundups were also analyzed for serum P4 concentrations to assess herd-wide corpora lutea (CL) activity. Serum P4 during roundup was highly correlated ($R^2 = 0.89$; $P < 0.05$) with calf production the following year, indicating fall estrus cycling with CL production as the likely major determinant of calf production. Body weights (BW) of 4- to 7-year old cows during roundup from 1999 to 2008 were highly correlated ($R^2 = 0.98$; $P < 0.05$) to calf production the following year, indicated that the changes in calf production and fall estrus cycling were likely related to cow body weights. Calf production recovered to 56% and 67% in 2009 and 2010, respectively. However, historic records from 1956 to 2010 revealed calf production began declining around 1980. In conclusion, the decrease in calf production at the NBR appears to be due primarily to the number of breeding-age females that exhibit breeding and ovulatory activity during the breeding season, presumably due to cow body condition. The specific causes for these effects are not known but may be related to range resources.

INTRODUCTION

The 7,500 hectare National Bison Range (NBR) near Moiese, MT was established by Congress in 1908. In 1909-1910, 40 bison (*Bison bison*) were brought to the NBR, and by the early 1920's the herd size was approximately 300 animals. Current management objective is for a herd size of 300-350 bison after the annual roundup in October of each year (United States Department of Interior, 1988). The United States Department of Interior National Bison Range Fenced Animal Management Plan (1988) states that between 1956 and 1987 average calf production was 87%. The lowest calf production recorded during that same period of time was 72% in 1970. In 1980, calf production fell to 74% and at least part of the problem was attributed to a presumptive leptospirosis outbreak in 1979. The refuge vaccinated bison with a *Leptospira* bacterin for an unknown length of time, but by 1981 calf production returned to 85%.

For the 10 years preceding the start of this study, calf production had gradually declined. Average calf production from 2005 to 2007 was 54%, with an all time low of 32% in 2008 (U.S. Fish and Wildlife Service, unpublished data).

Calf production, in a coarse sense, is the culmination of a series of temporally synchronized processes that start with successful mating and conception and end with a live calf at the time calf counts are made. Failure anywhere along that biological path results in a decrease in calf production. With no data aside from the observed decrease in production and the multitude of possible etiologies that can cause decreased production, it is essential to narrow down where in the reproductive cycle losses are occurring to narrow the list of possible etiologies warranting investigation.

The objectives of this study were to determine at which stage of the reproductive cycle bison at the NBR are experiencing reproductive failures, and characterize the temporal trends in calf production across the last half century. Our hypotheses are that reproductive losses during 3 time intervals (conception to early embryonic development, embryo to calving, and perinatal to roundup in October) did not differ and that the decrease in calf production started in 1999.

This review of literature will encompass: 1) an overview of the characteristics of reproductive processes in bison; 2) a review of some of the causes for decreases in calf production; and 3) a description of the National Bison Range.

LITERATURE REVIEW

Characteristics of Bison Reproduction

In sexually mature bison successful reproduction involves reproductive behavior, copulation, gestation, parturition and maternal energy investment. This section will review the reproductive tract anatomy, reproductive behavior, and factors that are thought to limit reproductive rate in bison.

Reproductive Tract Anatomy of Female Bison

Bison have been shown to be anatomically and physiologically similar to domestic cattle, with a few differences. Female bison have a bicornuate uterus that is the uterus has two distinct horns separated by a uterine septum. Right and left uterine horns are fused caudally into a common uterine body (for review, see Senger, 2005). The uterine submucosa is differentiated into “button-like” structures known as caruncles; sites of placentation. The uterine endometrium covers the uterine caruncular and inter-caruncular surface of the uterus. Uterine glands, both superficial and deep, are found in the inter-caruncular areas of the uterus (for review, see Senger, 2005). According to Dorn (1995), adult female bison have ovaries and reproductive tracts comparable in size to those of beef heifers (ovaries are approximately 3 cm in length). Matsuda et al. (1996) reported that the planar size of adult bison ovaries and corpora lutea (CL) averaged 26 mm x 18 mm and 22 mm x 19 mm, respectively. Planar size of large antral follicles ranged from 7 mm x 7 mm to 10 mm x 14 mm, within the range of mid- to large-sized follicles observed in domestic cattle (Salisbury et al., 1978). Similar to the domestic cow, bison females exhibit spontaneous estrus and are generally monovulators. Ovulation tends to occur slightly more often from the right ovary than left (54 and 46%, respectively; Matsuda et al., 1996).

<u>Puberty</u>

Puberty in female bison has been characterized by the age at which a heifer has her first successful pregnancy. First successful calving usually occurs at around 3 years of age (McHugh, 1958; Haugen, 1974); although there are a few documented cases of heifers producing calves at 2 years of age. Shaw and Carter (1989) reported that calving rate for 2-year-old heifers was 12% at Wichita Mountains National Wildlife Refuge (WMNWR), OK and McHugh (1958) reported rare cases of calving at 2 years of age at the NBR. Haugen (1974) supported these observations when he examined the ovaries from 17 yearling heifers collected in October, and found that only one had a CL; however, there was no evidence that she had conceived or developed an embryo. Examination of 39, 2-year-old heifers indicated that 34 (87%) were pregnant and the other five non-pregnant heifers had CL. The other five lacked evidence of embryonic or fetal tissue.

Age at puberty in bulls has been determined more subjectively by observing either copulations or the tending (guarding) of a cow. Both McHugh (1958) and Berger and Cunningham (1994) reported that bulls entering their third breeding season could successfully sire calves. Lott (2002) expanded on this observation by adding that while bison bulls at the NBR can successfully breed cows at 3 years of age, “they usually don’t until around 5 years of age due to competition with older bulls.” Helbig et al. (2007)

addressed the physiological capabilities of bison bulls by examining various characteristics of semen quality including volume, density, gross motility, individual motility, morphology and live/dead ratio. They defined puberty as the point in testicular development where an ejaculate contains a minimum of 50×10^6 sperm cells and contained at least 10% progressively motile sperm. Using these criteria, they reported that bison bulls reach puberty at 16.5 ± 2.5 mo ($\pm$ SD).

Collectively, the data suggest that female bison attain puberty between 2 to 3 years of age and generally produce their first calf at 3 years of age while male bison attain puberty between 1 and 2 years of age though their chances of mating at this young age are reduced by competition with more mature males.

Reproductive Behavior

Bison exhibit a male dominance polygyny breeding strategy. During the breeding season or “rut”, bulls seek cows that are approaching estrus by sniffing the anogenital region and performing flehmen. When a cow “interests” a bull, he tends or guards her from other males by staying close alongside her until she displays overt signs of estrus (Mooring et al., 2006). During tending, bulls display various courtship behaviors that include: bellowing, scent-urination, pawing, rubbing, and wallowing. Rival bulls frequently surround and challenge tending bulls and head-to-head fights are common. A tending bull will guard a cow until being displaced by a more dominant bull or mating (Wolff, 1998).

Copulation lasts less than 10 seconds from mount to dismount, and most cows breed only once in a breeding season (Mooring et al., 2006). Immediately following a copulation, the cow arches her back, expels a small volume of clear or milky secretions from the vulva (presumably vaginal fluids and semen) and erects her tail (Mooring et al., 2006; Komers et al., 1992; Wolff, 1998). This ‘tail-up’ response is distinct from tail elevation performed by bison in other contexts (e.g., defecation, urination, or agonistic interactions) in that the angle of the raised tail may be 135° or higher (180° is straight up vertical), and the duration of the tail-up behavior lasts from 1 to 2 days. So reliable is this behavioral indicator of copulation that it can be used to infer copulation in the absence of direct observation (Berger, 1989; Berger and Cunningham, 1991; Wolff, 1998).

Breeding Season

Unlike cattle, bison are seasonally polyestrus generally between July and September (McHugh, 1958). However, like cattle the length of the estrous cycle is approximately 21 days (Kirkpatrick et al., 1993). Vervaecke and Schwartzenberger (2006) characterized the transition into the breeding season for cows using patterns of fecal progesterone (P4) concentrations. As is the case in domestic sheep and cattle, bison females first exhibit a so-called “short” cycle followed by a normal length cycle. This “short” cycle is not fertile, as Vervaecke and Schwartzenberger (2006) reported that 9 of 10 (90%) conceived during their first normal-length cycle and one cow conceived during her third estrous cycle. Progesterone from the first CL formed after the first ovulation and after seasonal anestrous “primes” the brain so that its sensitivity to estrogen is optimized (Senger, 2005). When estrogen from the second group of follicles after anestrus appears, the cow will display behavioral estrus (Senger, 2005).

Gestation

Data on the gestation length for bison vary widely, possibly because most estimates are determined by observing or inferring breeding and calving dates. Vervaecke and Schwartzenberger (2006) summarized the literature on the gestation length of bison (Table 1). Berger and Cunningham (1994) reported that gestation length of cows at Badlands National Park varied between 276.6 ± 2.7 days and 292.5 ± 4.1 days (± SE) based on behavioral observations and calving. Rutley (1995) estimated gestation at 257 days, based on observed matings and calving dates. Vervaecke and Schwartzenberger (2006) verified that behavioral observations were reasonable indicators

Table 1. Summary of gestation lengths in bison[1]

Species	Gestation period	Method	Source
Bison bonasus	264.3 ± 4.2 days (range: 254 to 272)	Observed copulations and calving, n = 132	Krasinski & Raczynski, 1967
Bison bison	285 days	Estimated from embryo sizes (n = 121) with cattle embryo growth curves	Haugen, 1974
Bison bison	262 to 272 days	Observed copulations or inferred copulations (tail-up) and observed calving with possible 1 day error, n =28	Rutberg, 1986
Bison bison	276.6 ± 2.7 days 277.8 ± 3.3 days 286.2 ± 4.0 days 292.5 ± 4.1 days for each year n =17	Observed copulations or inferred copulations (tail-up) and observed calving with possible 3 day error, n =17 x 4	Berger & Cunningham, 1994
Bison bison	257 days	Observed copulation and calving, fecal progestins, n = 1	Rutley, 1995

[1] Adapted from Vervaecke and Schwartzenberger (2006).

of conception by associating behavioral observations to fecal progesterone concentrations and estimated the gestation length to be 266 $\pm$ 1 days ($\pm$ SD). Berger (1992) reported that cows in good condition, bred within 14 days after the mean conception date, would shorten their gestation by up to 6 days. The mechanism for such an adjustment in gestation is not clear. Presumably, reducing gestation by 6 days lowers the individual risk to predation by increasing birth synchrony. However, cows in good condition bred before or more than 14 days after the mean conception date, and cows in poor condition bred anytime during the breeding season did not shorten their gestation.

Haugen (1974) estimated the conception date of fetuses in bison cows by extrapolating growth curves of embryos from those of cattle. Conception dates using this method were compared to field observations of tending behavior of these cows (Shult, 1972) in the same area in the same year. Haugen (1974) surmised that because growth differed little in the first 3 months of gestation that gestation length of bison was probably similar to cattle at 285 days. According to his estimates, this meant that 60% of calving should have occurred between May 7 and May 21 and Shult (1972) reported 33% of calves were born by May 7 and that 66% of calves were born by May 13. These findings suggest that gestation of bison is about 7 to 10 days shorter than gestation in domestic cattle, averaging 275 to 278 days.

Collectively, the data suggest that the gestation of female bison is between 265 and 275 days, although cows may be able to shorten gestation by up to 6 days.

Reproductive Synchrony

Reproductive synchrony is birth of offspring in a short period of time (Berger and Cain, 1999). Two nonexclusive theories have been put forth to explain reproductive synchrony: predator satiation and plant phenology (Green and Rothstein, 1993; Rutberg, 1984, 1987). Because predators are resource limited at other times of year, the predator population is insufficient to consume all the offspring, ensuring that at least some of the offspring survive (Estes, 1976). While surplus killing does occur it is argued that predator satiation prevents active hunting and killing limiting the number of prey killed (Zuhn, 1972). Plant phenology implies that the timing of parturition corresponds with optimum forage conditions (Bowyer, 1991).

In bison, reproductive synchrony has been measured in confined and free-ranging herds (Berger and Cain, 1999; Green and Rothstein, 1993; Rutberg, 1984; 1987). Studying the effect of predation on bison reproductive synchrony has been difficult because few herds experience the diversity of predators that once preyed on bison calves in the past. Data from studies related to reproductive synchrony indicate that bison still experience synchrony. However, the authors of these studies concluded that plant phenology likely played a larger role than predation in this phenomenon (Green and Rothstein, 1993; Rutberg, 1984; 1987).

Using methods outlined by Rutberg (1984) wherein reproductive synchrony was defined as the length of time that the peak 80% of offspring are born, Green and Rothstein (1993) found that reproductive synchrony varied widely by year at Wind Cave National Park. During 1982, 1983 and 1984 they found that reproductive synchrony was

8, 10, and 5 weeks, respectively. The mean calving season for all 3 years was 53.7 ± 10.2 days (± SE). Although this estimate is quite different from that reported by Rutberg (1984) for one season at the NBR (23 days) the mean calving seasons were not statistically different. As mentioned previously, Berger (1992) reported that cows in good condition bred within 14 days after the mean conception dates were able to shorten their gestation by up to 6 days, presumably to calve closer to the peak or average of the herd.

Another factor that may play a role in reproductive synchrony is disease, although this has not been thoroughly researched. Berger and Cain (1999) attempted to synthesize work on synchrony from the Wind Cave National Park, the NBR, and Badlands National Park bison herds and compared calving windows to the *Brucella*- infected herd in the southern Greater Yellowstone Ecosystem (sGYE). They found a difference in synchrony between the sGYE and Badlands National Park herds. The 95% confidence interval of the calving season for the sGYE herd was 61 days while non-*Brucella* infected herds were 40 days at Wind Cave National Park, 42 days at NBR, and 89 days (significantly longer) at Badlands National Park. While the results from this study are inconclusive on the effect of *Brucella* on birth synchrony, the importance of disease cannot be overlooked and needs further research. Diseases such as trichomoniasis are likely to increase calving windows (Rice, 1981; Sprott and Field, 1998).

Collectively, it seems as though reproductive synchrony varies by year and is greatly affected by plant phenology, although the influence of disease and predation is not fully understood.

Maternal Energy Investment

In 1973, Trivers and Willard proposed that females with small litter sizes, like bison, in better body condition would be more likely to produce larger-bodied male offspring than females in poor condition due to maternal energy investment. Rutberg (1986) examined the reproductive tracts of bison cows slaughtered in December 1964 to 1967 at the NBR and found that the overall fetal sex ratio was 51 male and 31 female (1.65:1), but calf sex ratios at roundups in 1965 to 1968 were 0.88:1. This suggests that the informal culling policy in place at that time at the NBR to minimize the orphaning of calves (Rutberg, 1986) artificially selected for cows with male fetuses. This leads to an interesting question as to whether cows that produce male calves are less likely to bear a calf in the next calving season due to the additional energy required to raise a male offspring. Wolff (1998) reported that bison cows at the NBR nursed male calves for significantly longer periods of time during their first three months and that cows bearing a male calf were less likely to become pregnant or bear an offspring in the following year compared to cows bearing female calves. On the other hand, Green and Rothstein (1991) evaluated both maternal body condition and parental energy investment of bison at Wind Cave National Park over an 8-year period and found that there was little support that either of these factors had any influence on sex ratios of calves. While there isn't complete consensus there does seem to be an additional energetic cost to producing males over females.

Diagnosis of Pregnancy in Bison

To study changes in calf production rate of females it is important to obtain estimates of embryonic and fetal losses. To get these estimates requires diagnosis of the presence of an embryo or fetus in individual females. These estimates provide a basis for determining pregnancy rate at different times in the reproductive cycle. Pregnancy rate is the number of breeding-age females that have an embryo or fetus out of the total number of breeding-age females available to become pregnant. This estimate is different than calving rate, which is the number of cows giving birth out of the total number of breeding-age females available to produce a calf. The tractability of animals is an important consideration when determining the appropriate method for pregnancy estimation during different times of pregnancy.

Diagnostic Methods

Pregnancy in bison cows can be determined in a restrained animal by rectal palpation (Wolfe et al., 1999) and/or ultrasonography of the uterus (Dorn, 1995), by assaying concentrations of serum P4 (Dorn, 1995), and pregnancy specific protein b (PSPb; Haigh et al., 1991). Pregnancy can be determined in unrestrained bison by assaying feces and urine for P4 metabolites (Kirkpatrick et al., 1992).

Physically Restrained Animals. Depending on the tractability of the animal, accurate real-time pregnancy diagnosis can be accomplished by a trained technician through rectal palpation at 30 to 45 days of gestation and any time thereafter (Dorn, 1995). Ultrasonography can detect 100% of pregnancies between 25 and 35 days of gestation and any time thereafter and is considered the preferred method for pregnancy determination when animals can be physically restrained (Dorn, 1995).

Pregnancy can also be determined by PSPb. Using radioimmunoassay (RIA), PSPb was identified in 25 of 27 pregnant wood bison with only a minor crossreaction in 1 of 9 non-pregnant cows (Haigh et al. 1991). For intractable animals the use of fecal and urinary steroid analyses has proven to be effective at detecting pregnancy after 90 days of gestation (Kirkpatrick et al., 1992).

Remote Pregnancy Determination. Handling individual bison throughout gestation can be costly, time consuming, and dangerous to both the animals and the researchers, making a remote sample collection procedure appealing. Progesterone is essential for maintenance of pregnancy (Rabiee et al., 2001). It is a gonadal hormone that is primarily produced by the CL and the placenta during pregnancy in most ruminant species. However P4 is also synthesized by the maternal adrenal gland as a precursor for adreno-steroids although it is not normally secreted in appreciable quantities unless an animal is under distress (for review, see, Hadley and Levine, 2006). In the bovine, the CL is maintained throughout most of gestation, although its contribution to systemic concentrations of P4 decreases gradually between 190 and 275 days of gestation, primarily as a result of a reduction in the number of viable luteal cells, their secretory activity, and responsiveness to luteotropic factors (Shemesh, 1990). As luteal-derived P4 wanes placental-derived P4 becomes more important for maintaining pregnancy until parturition (Shemesh, 1990).

The concentration of a hormone is directly related to the secretory rate minus the degradation rate (in the liver, kidney, gut). Once at equilibrium steroids easily pass through the mucosa of the intestine and become "trapped/bound" in fecal material. This provides a non-catabolic mechanism for removal of the steroid from circulation and at the same time allows us an opportunity to measure the trapped/bound steroid at the time when equilibrium occurred.

Circulating P4 is primarily catabolized by the liver. Progesterone is quite hydrophobic and catabolic mechanisms not only render it inactive, but also make the molecule more hydrophilic through conjugation with glucuronide, (Norman and Litwack, 1987). The water soluble conjugated form increases transfer of P4 back into the blood for renal excretion (for review, see Senger, 2005). This metabolite is also eliminated in feces through excretion by the biliary system into the intestine. Once in the alimentary canal it is believed that bacterial action further modifies or catabolizes the structure (for review, see Senger, 2005; Figure 1). Fecal concentrations of P4 and/or its metabolites allow researchers to investigate reproductive endocrinology without the need to restrain the animal.

Fecal P4 metabolite assays have been validated in many species including cattle (Desauliners et al., 1989), moose (Schwartz et al., 1995), bighorn sheep (Shoenecker et al., 2004), elk (White et al., 1995; Garrott et al., 1998; Creel et al., 2007), baboon (Wasser et al., 1994), giraffe (Dumonceaux et al., 2006), avian species (Wasser et al., 2000) and carnivores (Berger et al., 1999) including felids (Brown et al., 1994).

Kirkpatrick et al. (1992) evaluated the use of urine and feces to determine if levels of pregnanediol-3-glucuronide (PdG), urinary estrone conjugates (EC), and fecal total

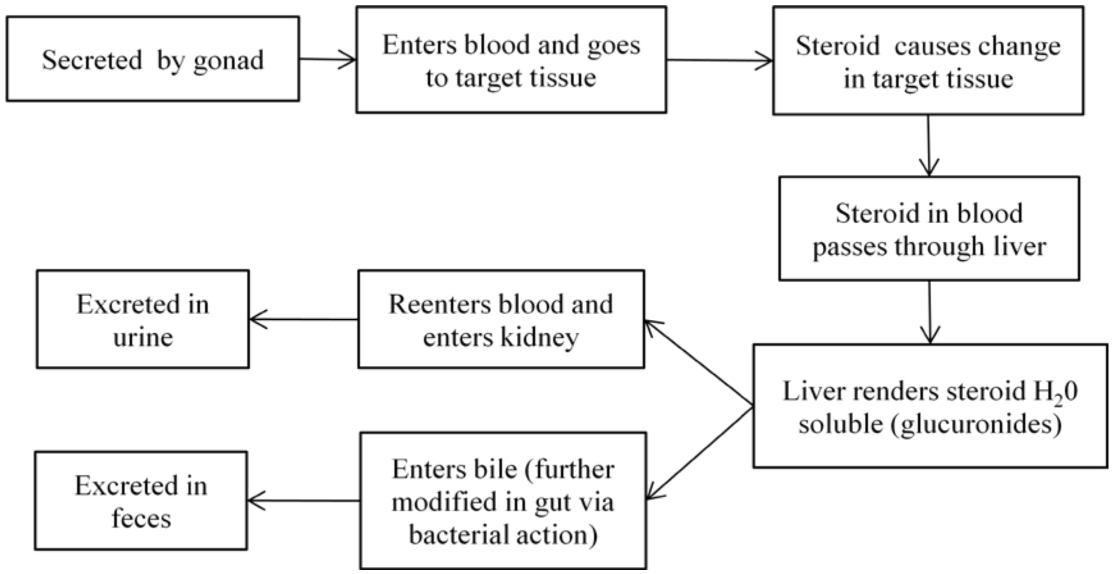

Figure 1. Flowchart of the pathway of progesterone catabolism (Adapted from Senger, 2005).

estrogens (TE) could accurately predict pregnancy in female bison by 3 months gestation. They found that PdG was 100% accurate, EC was 89% accurate and TE was 100% accurate in predicting pregnancy in bison.

As Vervaecke and Schwartzenberger (2006) point out, most attempts to establish an approximation of gestation in bison have been done by observing matings or "tail up" behavior to establish the date of conception and duration from mating to calving. They validated the accuracy of inferred conception from observed copulation by comparing observed copulations to fecal progesterone metabolite concentration data. These authors determined that fecal progesterone metabolite data was closely related to their observations of breeding behavior and could adequately be used to determine conception. Conceptions were documented during the second full-length cycle and possibly a third using fecal progesterone metabolites.

White et al. (1995) attempted to validate a one sample enzymeimmunoassay

(EIA) for determining pregnancy status in elk. Fecal samples were collected from each cow elk during 3 periods of gestation, 3 to 4 months, 5 months and 6 to 7 months. Concentration of progesterone and PdG were elevated in pregnant animals in all 3 periods ($P < 0.003$) but differences were greatest ($P < 0.001$) in March and April. Fecal progesterone provided the most accurate determination along with PdG but discrimination from a single sample was only 74 to 84% accurate. The authors suggest that multiple sampling would be necessary to ensure accurate results using this method. Garrott et al. (1998) expanded on the single sample method and use of native P4 by employing a more sensitive RIA. They found that during late gestation (April) concentrations of "free progestagin" were 0.43 ± 0.26 μg/g dry fecal weight and 2.96 ± 1.49 μg/g dry fecal weight for non-pregnant and pregnant cow elk, respectively. They suggested that fecal P4 using RIA would be a valid method for pregnancy rates in free-ranging elk. However, Garrott et al. (1998) point out that even though both studies used antibodies specific to the same class of progesterone metabolites (20-oxo-pregnanes) that their antiserum seemed to provide better discrimination for pregnancy detection in elk, stressing that laboratory validation is necessary before accurate estimates of pregnancy rates can be made.

Thermoregulation: An Important Consideration When Using Fecal Progesterone

Thermoregulation is the ability of an organism to keep its body temperature within certain boundaries, even when the surrounding ambient temperature is very different (for review, Hadley and Levine, 2006). Processes for thermoregulation in bison differ from those in domestic cattle. During winter months motor activity levels and metabolic rates of bison decrease, while both of these variables increase in cattle. Christopherson et al. (1978, 1979) exposed both bison and Hereford cattle, ages 6 to 18 months of age, to -30, 0 or 10^{o} C throughout each season of the year in Alberta, Canada and measured their metabolic rate, heart rate and respiration rate. They found that in most seasons Hereford cattle showed a marked increase in metabolism when exposed to -30^{o} C compared to metabolism at 10^{o} C. Winter and spring metabolic rates showed the most dramatic increase from 658.5 ± 68.2 to 829.5 ± 47.8 KJ per kg$^{.75}$ per day and 760.1 ± 27.0 to 938.3 ± 41.8 KJ per kg$^{.75}$ per day (SE), respectively. In summer and fall Hereford metabolism remained relatively stable when exposed to -30^{o} C compared to metabolism at 10^{o} C. Conversely, bison metabolism showed no change or a decrease in metabolic rate when exposed to -30^{o} C in the winter and spring. Interestingly, bison metabolism increased markedly when exposed to 10^{o} C in the winter from 718.3 ± 72.6 at -30^{o}C to 934.4 ± 184.6 KJ per kg$^{.75}$ per day (± SE), suggesting that in the winter, bison have an upper critical temperature around 10^{o}C where they then increase metabolism to maintain appropriate body temperature. Christopherson et al. (1979) concluded that bison at 6 mo of age are as cold tolerant as 13-mo to 17-mo-old Herefords. Changes in temperature-induced metabolic rate can significantly alter catabolic processes and in turn alter catabolism of steroids. One could speculate that in the winter bison significantly decrease their metabolic rate and might decrease catabolic processes. In doing so, steroid catabolism may be diminished during the colder winter months and decrease the difference between fecal P4 concentrations in pregnant and non-pregnant female bison.

Causes for Decreases in Calf Production

The primary factors that cause decreases in reproductive rate of any mammalian species include disease and malnutrition, both of which compromise energy utilization. Other factors include inbreeding, lethal genes, environmental stresses and predation (Krieg, 1988). The following section is a review of calving rate and primary factors that are related to decreased reproductive rate in bison.

Calving Rate

Reports on bison pregnancy rate vary from 35% on Santa Catalina Island, UT (Lott and Galland, 1987) to 88% at NBR (Rutberg, 1986); Table 2 summarizes pregnancy rates for some of the free- or semi-free ranging bison herds of North America. The low

Table 2. Pregnancy rates of some free-ranging North American bison herds[1]

Location	Pregnancy Rate (%)	Source
National Bison Range	88	Rutberg (1986)
Fort Niobrara National Wildlife Refuge	78	Haugen (1974)
Wichita Mountains Wildlife Refuge	72	Shaw and Carter (1989)
Badlands National Park	64	Berger and Cunningham (1994)
Henry Mountains	63	Van Vuren and Bray (1986)
Yellowstone National Park	52	Meagher (1973)
Antelope Island	46	Wolfe et al. (1999)
Santa Catalina Island	35	Lott and Galland (1987)

[1]Adapted from Wolfe et al. (1999)

pregnancy rates at Santa Catalina Island and Antelope Island were thought to be due to poor nutrition (Lott and Galland, 1987; Wolfe et al., 1999). The Utah Department of

Parks and Recreation started culling animals and feeding calves but calving rate did not improve (Wolfe et al., 1999) suggesting that nutrition alone was not the primary factor responsible for the productivity. Since the report of Haugen (1974), calving rate at Fort Niobrara National Wildlife Refuge has increased to approximately 82% (K. McPeak, U.S. Fish and Wildlife Service, personal communication). Dorn (1995) reported that average calf production varies between 50 and 80%.

Mortality in juvenile (less than 2 years of age) bison is relatively low. Berger and Cunningham (1994) reported juvenile survival ranging from 95.8 to 97.5%, while at the NBR first year survival varied from 94 to 100% and averaged 98% for the 2004 to 2008 calf cohorts (U.S. Fish and Wildlife Service, unpublished data). This suggests that perinatal mortality is not a significant source of bison mortality.

Disease

Diseases affecting bison calf production can be categorized as either venereal or non-venereal. Venereal diseases include vibriosis and trichomoniasis. Vibriosis (*Campylobacter fetus*) causes early embryonic death and infertility. Repeat breeding is generally observed along with irregular estrous cycles. Other clinical signs include retained placentae and varying degrees of vaginitis. Diagnosis is confirmed by culture of the causative organism from cervical mucus or an aborted fetus. Trichomoniasis (*Trichomonas fetus*) also causes abortions, infertility, inflammation and uterine discharge. This protozoan is harbored in the prepuce of bulls and is transmitted to cows during mating (Rice, 1981; Sprott and Field, 1998).

Other infectious, non-venereal diseases that can affect reproduction include:

brucellosis, leptospirosis, infectious bovine rhinotracheitis (IBR), and bovine viral diarrhea (BVD). Brucellosis is caused by the bacterium *Brucella abortus* and spreads through vaginal discharge, or contact with an aborted fetus, contaminated environment or fomites. Brucellosis can cause abortions, retained placentas, weak calves and infertility (Rice, 1981; Sprott and Field, 1998). In the U.S., leptospirosis is usually caused by one of 3 serovars, *Leptospira pomona*, *L. hardjo, L. grippotyphosa,* although other serovars have been isolated, *L. canicola*, *L. bratislava* , *L. autumnalis* and *L. icterohaemorrhagiae*. Clinical signs of leptospirosis include anemia, icterus, hemoglobinuria, milk may become thick, yellow and blood-tinged. Leptospirosis can cause abortions 2 to 5 weeks after infection but most occur about the seventh month of gestation (Rice, 1981; Sprott and Field, 1998). Transmission of leptospirosis in cattle is usually through the urine of an infected animal. The organisms gain entry through the membranes of the eyes, nose, mouth and even the skin (Rice, 1981; Sprott and Field, 1998).

Although IBR normally causes respiratory disease in cattle, this herpesvirus can also cause vulvovaginitis and abortion. Abortions usually occur about 20 to 45 days after infection. Respiratory disease and abortion are the most economically significant forms of IBR infection. IBR virus also causes conjunctivitis and mild genital infections and is usually transmitted through respiratory secretions. Temporary infertility can occur after an infection with IBR (Rice, 1981; Sprott and Field, 1998). Lastly, BVD can cause abortions, weak calves at birth, cerebellar hypoplasia in calves and other fetal anomalies. Once a herd becomes infected with BVD it can be difficult to eradicate. If a pregnant cow is infected between 60 and 120 days of gestation, before the immune system of the fetus is fully developed, the infected fetus can become persistently infected, intermittently shedding virus throughout the rest of its life and periodically re-infecting the rest of the herd (Stokka et al., 2000).

Nutrition

Most of the information available on bison nutrition is based on beef cattle requirements, bison forage selectivity, knowledge of the bison digestive system, growth, and seasonal adaptations (Feist, 2008). From December to April bison lower their metabolism and lose from 10 to 15% of pre-winter body weight (Christopherson et al., 1978). Activity, growth and basic energy stores take priority over reproduction (Grimard et al., 1997; Guedon et al., 1999). An energy balance that does not meet these requirements reduces the availability of energy metabolites, particularly glucose, for resumption of ovarian cyclicity and maintenance of pregnancy (Yavas and Walton, 2000). In managed, disease-free bison herds, females not bearing a calf in one year will be in better than average condition the next year (Rutberg, 1986; Kirkpatrick et al., 1993). Halloran (1968) concluded that calving rates at the WMNWR were influenced by a tendency of cows to produce calves in alternate years, a pattern that he attributed to superior body condition of non-lactating females. Green and Rothstein (1991) examined the cost and benefit of reproduction in bison females and found that fecundity or reproductive rate was not generally lower in cows that calved in consecutive years than in cows that had calves in alternate years, despite weight loss of cows calving in consecutive years. They concluded that a high reproductive rate in female bison does not impose significant individual fitness costs. On the other hand, they also found that offspring weight was lower when dams calved in consecutive years compared to calves from dams that calved in alternate years suggesting that there is a trade-off between the number of calves and quality of offspring. During periods of poor forage quality female bison that were already in poor body condition during any one pregnancy may not be of sufficient body condition or have sufficient body reserves and may not be able to support successive pregnancies. Certainly this would reduce calving rate in any fixed population of bison.

Poor nutrition has been implicated for low pregnancy rates in several bison herds (Lott and Galland, 1987; VanVuran and Bray, 1986; Wolfe et al., 1999), although management changes were only implemented at Antelope Island, UT (Wolfe et al., 1999). The Utah Department of Parks and Recreation initiated limited hunting, annual culling, and supplemental feeding of calves in an attempt to improve calf production, but after 6 years, pregnancy rates were still similar to the 6 years prior to management changes. Wolfe et al. (1999) concluded that only large-scale range improvements could sufficiently increase cow body condition to improve calf production.

Description of the National Bison Range

The 7,500 hectare NBR was established in 1908 "for a permanent national bison range for the herd of bison" (Dratch and Gogan, 2010). In 1909, the bison herd was started with 37 animals; 36 bison purchased from or donated by the American Bison Society from the Conrad herd near Kalispell, MT, and an additional animal from the Goodnight herd in Texas. Three more animals were introduced from the Corbin herd in

1910. Additional bison introductions include, “2 bison in 1939 (7-Up Ranch, origin unknown), 4 in 1952 (Fort Niobrara National Wildlife Refuge), 2 in 1953 (Yellowstone National Park), and 4 in 1984 (Maxwell State Game Refuge),” (United States Department of Interior, 1988).

The NBR supports many other ungulate species. Twenty-one elk were introduced from Jackson Hole, WY between 1910 and 1913. An additional introduction of 26 elk from Yellowstone National Park occurred in 1916. Current management objectives call for a total herd size of 130 elk. Bighorn sheep were first brought onto the NBR in 1922 when 12 were brought from Banff National Park in Canada. Current management calls for a peak population of 75 bighorn sheep. Pronghorn antelope were initially introduced between 1910 and 1916 with several further additions throughout the years. White-tailed and mule deer have also been introduced to NBR with population objectives of 175 and 100, respectively. Culling is performed on these species when population estimates are higher than the species objectives (United States Department of Interior, 1988).

Bison management includes a pasture rotation grazing program. Animals are moved by horseback between pastures on a schedule reflecting each pasture’s available forage as perceived by refuge staff. The bison herd is “rounded up” each October. Animals are handled as part of the comprehensive herd health monitoring program that has been in effect since 2000, and to cull animals to maintain the herd within the NBR’s carrying capacity (United States Department of Interior, 1988). Bison are individually marked as calves with radio frequency identification (RFID) chips and a metal ear tag. Thus management decisions can be made at the individual level.

The NBR bison have historically had little problem with calf production. The United States Department of Interior, National Bison Range Fenced Animal Management Plan (1988) states that over 32 years (1956 to 1987) the average calf production was 87%. The lowest calf production recorded during that same period was 72% in 1970. In 1980, calf production fell to 74% and at least part of the problem was attributed to a presumptive leptospirosis outbreak in 1979. The refuge vaccinated bison with a *Leptospira* bacterin for an unknown length of time thereafter, but by 1981 calf production had returned to 85%. (United States Department of Interior, 1988). More recently, the production of calves has decreased from the historic average of 87% to 32% in 2008 (US Fish and Wildlife Service, unpublished data). The reason for and exact timing of this decrease in production is unknown and the primary focus of this thesis.

STATEMENT OF PROBLEM

Between 1956 and 1987 production of calves by female bison at the NBR averaged 87%. The lowest calf production recorded was 72% in 1970. For the 10 years preceding this study, calf production had gradually declined. The average calf production from 2005-2007 was 54%, with an all time low of 32% in 2008 (U.S. Fish and Wildlife Service, unpublished data; Figure 2).

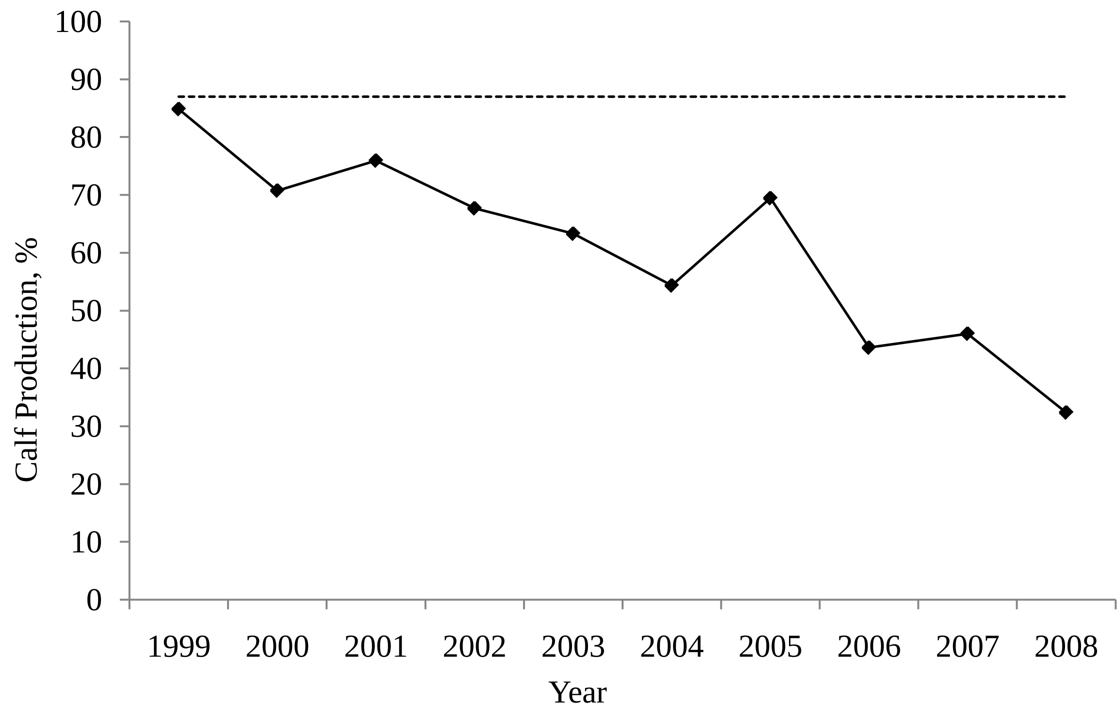

Figure 2. Calf production, the numbers of calves per 100 breeding-age cows, at the yearly roundup in early October, for the National Bison Range bison herd over the10-yr period from 1999 to 2008. The horizontal dashed line within the graph represents the average calf production for 1956 to 1987 (87%).

Calf production, in a coarse sense, is the culmination of successful conception, maintenance of gestation and perinatal calf survival. Failure in any one of these biological processes results in a decrease in calf production. With no data aside from the observed decrease in production and the multitude of possible etiologies that can cause decreased production, it is essential to narrow down where in the reproductive cycle losses are occurring to narrow the list of possible etiologies warranting investigation.

Additionally, personnel at the NBR maintain records of calf production dating back over 50 years, cow weights since the late 1990’s and climatological records from at least 40 years. Historic records and archives of biological samples could provide valuable insight into which or combination of several of these biological processes is/are responsible for the continuing reduction in calf production at the NBR.

The goals of this study were: 1) to determine which of these biological processes or combination of biological processes is/are involved with the reduction in calf production at the NBR, and 2) using historical records and archived biological samples to identify when the reduction in calf production actually began. Meeting these goals would give insight into a solution to the problems associated with reproductive rate of bison at the National Bison Range.

The specific objectives of this study were to: 1) determine pregnancy rates and luteal activity of breeding-age bison cows at roundup in October of 2008, 2009 and 2010; 2) characterize temporal pattern of fecal P4 concentrations, determine if failure of cows to produce calves is related to the temporal pattern of fecal P4 concentrations, and establish criteria for accurately assessing pregnancy by fecal P4; 3) determine if the reduction in calf production at roundup during October is related to perinatal survival; 4) determine if pregnancy rates determined by ultrasonography and/or fecal P4 concentrations predict calf production the following year; and, 5) evaluate relationships among luteal activity, cow body weights and calf production.

The specific hypotheses were that: 1) pregnancy rates assessed by ultrasonography, fecal P4 concentration, and observations of cows tending calves do not differ among years; 2) temporal patterns of fecal P4 concentrations do not differ between pregnant and non-pregnant cows, and the accuracy of fecal P4 concentration predict when embryonic or fetal losses occur during pregnancy; 3) long-term trends in calf production, cow body weight and luteal activity at roundup do not differ among years between 1999 and 2010; and, 4) there are no relationships between long-term calf production, cow body weight or proportion of cows that exhibited luteal activity at roundup.

MATERIALS AND METHODS

For the purpose of this study, the biological processes involved in the reproductive cycle of female bison were divided into 3 consecutive, temporal stages: Stage 1, conception to early embryogenesis; Stage 2, gestation; and, Stage 3, perinatal calf survival. Cows were handled during the yearly roundup in October according to the United States Department of Interior, National Bison Range Complex, Animal Care and Use Committee standards.

Study Site

The NBR is 7,500 hectares located at 47^{o} 19' N 114^{o} 13' W and ranges in elevation from approximately 830 m to 1500 m. According to the Western Regional Climate Center the area around the NBR receives an annual precipitation of 40 cm, and average high and low temperatures are 14^{o} C and 0.6^{o} C, respectively. The habitat consists of native Palouse prairie, forests, wetlands, and streams providing a wide range of habitats for wildlife.

Animals

In 2008, the bison herd had 124 breeding-age females (3 years and older), of which 41 were sent through the handling facilities to be pregnancy tested by ultrasonography at roundup in October. Each pregnant cow received a number on their right flank using commercial hair bleach (Silvy et al., 2005). This allowed identification of individuals through most of gestation. Cows were then released back into the herd for the remainder of the study (October, 2008 to October, 2009).

In 2009, the herd had 114 breeding-age females, of which 89 breeding-age female bison were sent through the handling facilities to be pregnancy tested by ultrasonography at roundup in October. A unique VHF radio frequency collar was applied to 27 pregnant and 10 non-pregnant breeding-age female bison to allow individual identification throughout gestation and calving (Silvy et al., 2005). Cows were then released back into the herd for the remainder of the study (October, 2009 to October, 2010).

In 2010, the last year of the study, the herd had 127 breeding-age (3 years and older), females of which 70 breeding-age female bison were sent through the handling facilities to be pregnancy tested by ultrasonography at roundup in October.

<u>Stage 1: Handling, Blood and Fecal Sampling and Ultrasonography at Roundup</u>

During each roundup study females were restrained in a hydraulic squeeze-chute. A blood sample was collected from each female by jugular venepuncture in 2008 and 2009. Blood samples were allowed to clot at ambient temperature for a minimum of 4 hours, and then centrifuged at 1600 x *g* for 10 minutes. Serum was decanted, labeled and stored frozen at -20^{o} C until assayed for P4.

During roundups in 2008 and 2009, fecal samples were collected from each female and 3 bulls for assay of P4 by using a gloved hand to pull fecal material directly from the rectum while the animal was restrained in the chute. Fecal samples were placed into a 50 mL polypropylene, conical, centrifuge tube, labeled and frozen at -20^{o} C until assayed for P4.

In 2008, 2009, and 2010 pregnancy diagnoses were performed by visualization of the uterine contents of each cow by ultrasonography using a Titan Ultrasound Imaging System (Sonosite, Wallawalla, WA) equipped with a selectable 5 to 10 MHz transducer. Images of embryos or fetuses were saved to estimate conception date. Measurements of embryos or fetuses were obtained using Sigma Scan Pro 5.0 (Sigma Stat, Inc., Point Richmond, CA). Embryo and fetal development in bison differs little from cattle early in pregnancy (Haugen, 1974; Gogan et al., 2005). Estimates of embryonic and fetal ages were based on embryonic and fetal measurements described in cattle by Lamb (2001). There was the opportunity during this study to ultrasound the same cows in multiple years. Some cows were observed in 2008, 2009 and 2010. However, some cows that were examined in 2008 and 2009 were not evaluated in 2010, and some cows that were evaluated in 2009 and 2010 were not evaluated in 2008.

Stage 2: Fecal Sampling of Free-ranging Bison

In 2008, fecal samples were collected from cows with bleached numbers in January and March; whereas, in 2009 fecal samples were collected from collared cows in January, March, April and May.

Field collections of fecal samples consisted of personnel monitoring marked or collared cows and waiting for them to defecate. We observed cows at ranges of 50-500 m by means of a spotting scope or binoculars until they voided and the site of defecation was noted. After the bison had moved a safe distance away, personnel were directed to the site by means of hand-held radios. Samples were collected from fecal material on the ground by gloved hand and placed in a 50 mL polypropylene, conical, centrifuge tube and frozen at -20^{o} C until assayed for P4.

Stage 3: Perinatal Calf Survival

In 2009, perinatal calf survival was assessed using two different methods. The first method involved counting a minimum of 200 bison excluding calves and bull-only groups. Then calves were counted for those 200 bison. The ratio of calves to the minimum 200 bison was extrapolated to the entire herd to estimate the total calf production. This was performed monthly beginning in late March until mid-September. The second method involved monitoring collared cows monthly to determine if they were tending calves. Once observed with a calf, failure to find a calf with those cows on subsequent visits was our second index of perinatal calf mortality which was subtracted from 100% to yield an estimate of perinatal calf survival.

Assays for Fecal and Serum Progesterone

Progesterone Extraction from Feces

Extraction of P4 from fecal samples was performed by modifying the method described in Brown et al. (2005). Samples were homogenized by thoroughly mixing the entire sample with an icing spatula, in a sterile 250 mL glass beaker. Approximately 15 grams of wet fecal material was split into 2 mortars and dried for 24 hours in an incubator (Thelco; Thermo Fisher Scientific Inc.) at 37^{o} C. Once dry, samples were pulverized with a pestal and 0.5 grams (+/- 0.03 grams) was weighed in quadruplicate and each placed into 4 identically labeled 16 mm x 125 mm borosilicate tubes. Samples were refrigerated at 4^{o} C until extracted.

The extraction procedure consisted of adding 4.5 mL of 100% ethanol (EtOH) and 0.5 mL of ultrapure water to each tube. Samples were vortexed and the solution volume height was marked on the side of each tube. Samples were boiled for 20 minutes at 95^{o} C and 100% EtOH was added as necessary to avoid samples from boiling dry. Samples were brought back to the pre-boiled volume on the tube with 100% EtOH and refrigerated overnight at 4^{o} C. Samples were centrifuged at 1600 x *g* for 20 minutes. The supernatant of each sample was poured into an identically labeled 16 mm x 125 mm tube. The pellet of each sample was reconstituted with 5 mL of 100% EtOH vortexed and centrifuged for 20 minutes. This supernatant was added to the first supernatant and dried in a Dubnoff Metabolic Shaking incubator (Thermo Fisher Scientific, Inc.) in 95^{o} C water under nitrogen. Dried samples were reconstituted with 3 mL 100% EtOH, vortexed and dried in 95^{o} C water under nitrogen. Once dry, samples were reconstituted with 1 mL of 100% methanol. Samples were briefly vortexed and placed in an ultrasonicator (Cole Parmer, Vernon Hills, IL) for 15 minutes before being dried down in 95^{o} C water under nitrogen. Finally, samples were reconstituted with 1 mL of 0.01 M phosphate-buffered saline (PBS; pH = 7.1) with 0.1% bovine serum albumin (BSA). Samples were vortexed and again placed in the ultrasonicator for 15 minutes. Each sample was then poured into an identically labeled 12 mm x 75 mm borosilicate tube and centrifuged for 15 minutes. This step was necessary to remove large sedimentary particles from the supernatant for pipetting purposes.

Progesterone Assay of Serum and Fecal Extracts

Serum. Serum samples for P4 were assayed in duplicates using solid-phase radioimmunoassay (RIA) kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). The assay was validated for bison serum in our laboratory. Details for these assays were described by Custer et al (1990) in cattle. Recovery of known concentration of P4 was 95%. Intra- and inter-assay CV for pool of serum that contained 2 ng/mL were 4.7 and 11.2%, respectively.

Fecal Extracts. One-hundred uL of the PBS-BSA extract of 3 of the 4 quadruplicates was pipetted into12 mm x 75 mm anti-body coated assay tubes. Progesterone concentrations were assayed using solid-phase RIA kits described for serum. For these assays standard curves were prepared with the PBS-BSA used for rehydrating dried extracts. Displacement curves of serial dilutions of fecal extracts were parallel (b_1 = -2.1 logits/log, ng/mL of P4) to standard curve prepared with PBS-BSA (b_1 = -2.2 logits/log, ng/mL of P4). Extraction efficiency for each sample was determined in one quadruplicate by adding 1 mL of radiolabeled P4-I^{125}. At the same time, 1 mL of P4-I^{125} was placed into a plastic 12 mm x75 mm tube. The supernatant for each of the extraction efficiency quadruplicate was poured into a non-antibody coated 12 mm x 75 mm plastic tube. Counts per minute of the extraction efficiency quadruplicate were compared to those of the 1 mL of P4-I^{125} set aside at the beginning of the assay to determine the procedural loss of P4-I^{125}. Extraction efficiencies of P4- I^{125} from fecal samples were 43%. Intra- and inter-assay CV for fecal P4 concentration from samples of anovular cows were 10 and 20%, respectively; and, for fecal samples from cows that had a CL were 11 and 25%, respectively.

Concentrations (ng/g dry feces) of P4 in fecal samples were calculated by dividing the ng/mL of P4 in supernatant by the sample extraction efficiency then dividing this number by the dry weight of the sample before extraction.

Stage 1: Assessment of Luteal Activity and Pregnancy Rate

Serum Progesterone

Serum samples collected at roundup were used to evaluate the proportion of breeding-age cows that exhibited luteal activity. The criterion for establishing that a cow had an active CL was a P4 concentration of greater than or equal to 1 ng/mL (Custer et al., 1990; Garrott et al. 1998). Cows with P4 concentrations less than 1 ng/mL were considered to be anovular or lacked an active CL at roundup.

Ultrasound of the Uterus

In 2008, 2009 and 2010 uterine contents of cows were examined using ultrasonography for the presence of an embryo or fetus. Cows that had an embryo or fetus were considered pregnant; whereas, cows that did not were considered non-pregnant.

Stage 2: Assessment of Maintenance of Pregnancy

In 2009, temporal patterns of fecal P4 concentrations were used to determine embryonic or fetal loss during pregnancy. First we monitored collared cows that were determined to be pregnant by ultrasonography until they were observed tending a calf. Collared cows determined to be not pregnant by ultrasonography were monitored until September to verify that they weren't tending a calf. Second, fecal samples were assayed from known pregnancy status standards for P4 to determine what P4 concentration corresponded to a successful pregnancy. Third, the assay was applied to collared cows that were determined to be pregnant at roundup by ultrasonography but were not observed tending a calf to assign a temporal pregnancy status.

Long-term Trends in Calf Production, Cow Body Weight and Archive Serum Samples

Calf Production

Calf production records were compiled from 1956 to 2010. These data were obtained from 3 sources: 1) 1956 to 1987, from the United States Department of Interior National Bison Range Fenced Animal management Plan (1988); 2) NBR yearly narratives (1987 to 1998); and, 3) the U.S. Fish and Wildlife Service (USFWS) Wildlife Health database (1999 to 2010). These records were used to evaluate calf production over the last 55 years.

Cow Body Weight

Body weights (BW) are collected from bison before they are released back to the range during each year's roundup. Body weights of both 'prime' breeding-age cows (4- to 7-years-old; 1999 to 2010) were compared to calf production the following year to evaluate whether changes in cow BW were related to changes in calf production.

Luteal Activity

The U.S. Fish and Wildlife Service Wildlife Health office maintains an archive of serum samples collected at yearly roundups, from 2000 to 2007 for bison at the NBR. Serum from these samples were assayed for P4 concentration.

Statistical Analyses

Pregnancy Rates at Roundup

Data for pregnancy rates, among years (2008, 2009 and 2010), determined by ultrasonography, were analyzed using PROC FREQ of SAS (SAS, Inst., Inc., Carey, NC). Pregnancy rates for individual cows that were examined in 2008, 2009 and 2010, either in 2008 and 2009 or 2009 and 2010 were analyzed using PROC FREQ of SAS.

Temporal Pattern of Fecal Progesterone

Data for fecal P4 samples collected in 2009 for pregnant cows that were observed tending a calf and cows that were not pregnant and were not observed tending a calf exhibited heterogeneity of variance using Bartlett's test for homogeneity of variance using SAS. Square root transformations were made on fecal P4 data and analyzed by the PROC MIXED procedure for repeated measures of SAS. The model included pregnancy status, month and the interaction between pregnancy status and month. Animal was the experimental subject and month was the repeated measure. Means were separated by Bonferronni's multiple comparison tests.

To evaluate when during gestation loss of pregnancy occurred in 2008 and 2009 a minimum fecal P4 concentration was established by calculating the overall mean of fecal samples collected in October, January, March, April and May. A cow was considered to be pregnant if her fecal P4 concentration was greater than 1 SD below the mean for known pregnant cows. A cow was considered not pregnant if her fecal P4 concentration was less than 1 SD greater than the mean for known non-pregnant cows. If the fecal P4 concentration of a cow was between 1 SD below the mean of pregnant cows and more than 1 SD above the mean of non-pregnant cows then the fecal P4 concentration from the next month's sample was used to evaluate whether she was pregnant. Individual t-tests were used to evaluate differences in fecal P4 concentrations between pregnant and non-pregnant cows for each month using the two-sample procedure of SAS.

Calf Production

Calf production defined as the number of calves recorded at the yearly roundup per 100 breeding-age females for 2008, 2009 and 2010 were analyzed using PROC FREQ of SAS. Data for pregnancy rate and calf production for 2008 and 2009 at Stages 1, 2 and 3 were analyzed using separate PROC FREQ analyses of SAS. Actual calf production at roundup was compared to pregnancy rates determined by ultrasonography and fecal P4 concentration using PROC FREQ of SAS.

Long-term Trends in Calf Production, Cow Body Weight and Luteal Activity

Long-term calf production expressed as the number of calves per 100 breeding-age cows recorded at the October roundups were plotted by year from 1956 to 2010. There was an obvious visual downward trend in the data beginning in approximately 1980 and a dramatic decline between 1999 and 2008. The data were divided into 3 temporal components, from 1956 to 1978, from 1979 to 1998 and 1999 to 2010. Then linear regressions were calculated for each of these temporal intervals using the trendline-fitting function in Excel. Furthermore, it was apparent that there were cyclic trends during each interval. The minimum and maximum calf productions were modeled by fitting a polynomial curve using the trendline-fitting function in Excel.

Mean BW of 4- to 7-year-old cows between 1999 and 2009 were regressed on year using PROC REGRESS of SAS. Calf production from 2000 to 2010 was regressed on year using PROC REGRESS of SAS. The correlation of calf production (year + 1) with mean BW of cows between 1999 and 2009 was obtained using PROC CORR of SAS. Calf production was regressed on mean cow BW using PROC REGRESS of SAS.

Proportions of cows that exhibited luteal activity at roundup in October in each year from 2000 to 2009 were analyzed by chi-square analyses using PROC FREQ of SAS.

Proportions of cows that exhibited luteal activity at roundup in October between 2000 and 2009 were regressed on year using PROC REGRESS of SAS. Calf production, defined as the number of calves per 100 breeding-age cows, from 2001 to 2010 was regressed on year using PROC REGRESS of SAS. The correlation of calf production (year + 1) with proportion of cows that exhibited luteal activity at roundup between 2000 and 2009 was obtained using PROC CORR of SAS. Calf production was regressed on proportion of cows that exhibited luteal activity using PROC REGRESS of SAS.

RESULTS

Stage 1: Conception to Early Embryogenesis

Pregnancy rates of breeding-age cows at roundup in October did not differ ($P > 0.10$) among years, (Table 3). The proportion of breeding age cows that exhibited a serum P4 concentration ≥ 1 ng/mL at roundup in 2008 (36 of 41 cows; 88%) did not differ ($P > 0.10$) from that in 2009 (36 of 45 cows; 80%). Thirty-four of the 41 from 2008 were reexamined in 2009 and 2010. Of these 34 cows, 14 (42%) were pregnant in all 3 years, 11 (32%) were pregnant in 2 of these years, 8 (24%) were only pregnant 1 year and only 1 (3%) animal was not pregnant in any of the 3 years (Table 4).

Table 3. Pregnancy rates of breeding-age bison cows at the National Bison Range at October roundups in 2008, 2009 and 2010[1]

Variable	Year 2008	Year 2009	Year 2010	Total
n	41	89	70	200
Pregnancy rate	68%[a]	63%[a]	71%[a]	67%

[1] Pregnancy rates determined by ultrasonography.
[a] Percentages with different superscripts differ ($P < 0.05$).

Table 4. Proportion of breeding-age cows that were pregnant in 2008, 2009 and 2010, pregnant in 2 of these years, pregnant in 1 of these years or not pregnant in any of these years at roundup in October at the National Bison Range[1,2]

Pregnant all 3 years	Pregnant in 2 years	Pregnant in 1 year	Not pregnant in any year
14 (42%)[a]	11 (32%)[a]	8 (24%)[a]	1 (3%)[b]

[1] n = 34 for 2008, 2009 and 2010.
[2]Pregnancy determined by ultrasonography.
[a] Percentages with different superscripts differ ($P < 0.05$).

The proportion of cows that were pregnant in only 1 year, 2 years or 3 years did not differ ($P > 0.10$). However, the proportion of cows that were not pregnant in any year was lower ($P < 0.05$) than that for cows that were pregnant in 3 years, 2 years or 1 year (Table 4).

There were 3 cows examined in 2008 not reexamined in 2009 and 2010, and 14 cows examined in 2009 not reexamined in 2010, were not included in analyses for multiple year examinations. Four cows ultrasounded in 2008 were reexamined in 2009 and 36 of the 37 collared cows examined in 2009 were reexamined in 2010. Of these 40, 23 (58%) were pregnant in both years, 12 (30%) were pregnant in 1 year, and 5 (13%) were not pregnant in either year (Table 5). The proportion of cows that were pregnant in both years was greater ($P < 0.05$) than the proportion of cows that pregnant in 1 year or not pregnant in both years (Table 5).

Table 5. Proportion of breeding-age cows that were pregnant in 2008 and 2009 or 2009 and 2010 at roundup in October at the National Bison Range[1,2]

Pregnant in 2 years	Pregnant in 1 year	Not pregnant in any year
23 (58%)[a]	12 (30%)[b]	5 (13%)[b]

[1]n = 4 in 2008 and 2009; n = 36 for 2009 and 2010.
[2]Pregnancy determined by ultrasonography.
[a] Percentages with different superscripts differ ($P < 0.05$).

Stage 2: Maintenance of Pregnancy

Fecal Progesterone

Using repeated measures analysis of the square roots of fecal P4 concentrations, there was no interaction ($P > 0.10$) between month and pregnancy status for fecal P4 concentrations in 2009, (Figure 3). However, mean fecal P4 concentration during

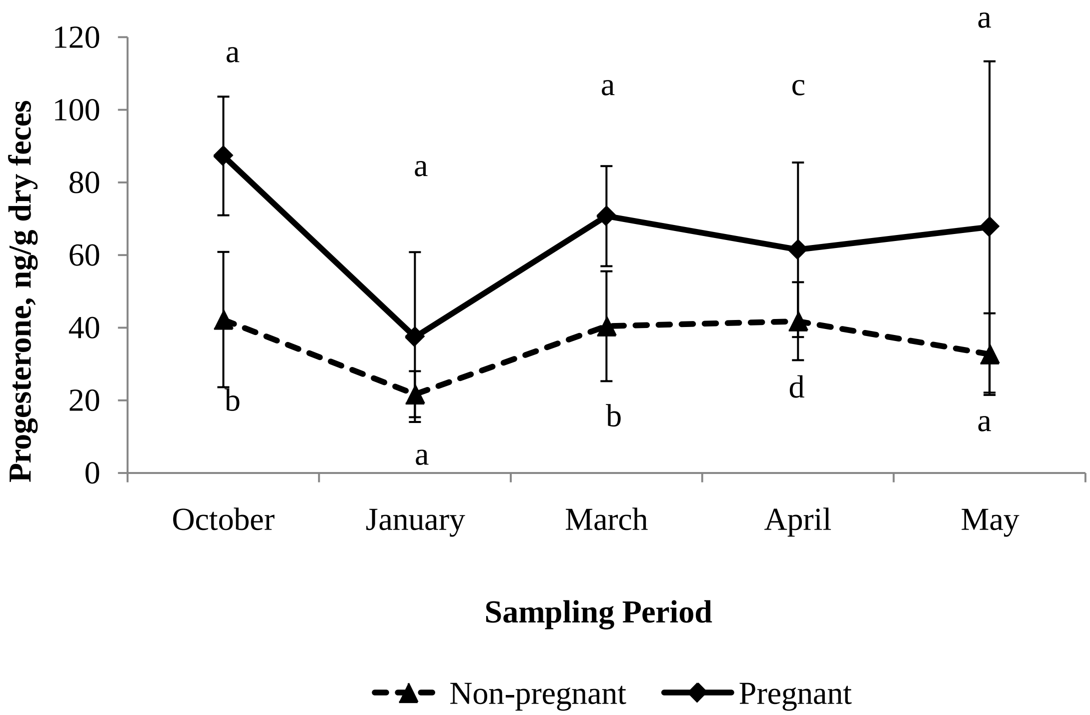

Figure 3. Fecal progesterone concentrations (ng/g dry feces) of pregnant (n=23) and non-pregnant (n=6) cows by month in 2009. Statistical analyses of these raw data were performed on square root-transformed data. Different letters (a,b) associated with concentrations of fecal progesterone for pregnant and non-pregnant cows within each month indicate a difference in the square root data ($P < 0.05$). Different letters (c,d) associated with concentrations of fecal progesterone for pregnant and non-pregnant cows within each month in the square root data tend to differ ($P = 0.08$).

January (27.6 ± 0.12; mean ± SE) was lower ($P < 0.05$) than mean fecal P4 concentrations during October (64.4 ± 0.21), March (53.4 ± 0.12) and April (49.7 ± 0.12) and tended ($P = 0.08$) to be lower than that during May (45.0 ± 0.16). Mean fecal P4 concentration in pregnant cows (60.8 ± 0.02 ng/g dry feces) was greater ($P < 0.05$) than that in non-pregnant cows (34.3 ± 0.08 ng/g dry feces). Even though the pattern of temporal fecal P4 concentrations were parallel between pregnant and non-pregnant cows, fecal P4 concentrations in pregnant cows during October and March were greater ($P <$ 0.05) than fecal P4 concentration in non-pregnant cows. Fecal P4 concentrations tended

(P = 0.08) to be greater in pregnant cows than in non-pregnant cows during April but did not differ (P > 0.10) between pregnant and non-pregnant cows during January or May (Figure 3).

Applying our criteria outlined in statistical treatment for assessing pregnancy by fecal P4 concentration to samples collected from bison ultrasounded in October yielded an accuracy of 77% (18 of 21 pregnant cows, 2 of 3 non-pregnant cows and 2 cows were equivocal). Applying the criteria for samples collected during March was slightly better at 80% (20 of 23 pregnant cows, 4 of 6 non-pregnant cows and 1 cow was equivocal). Finally, when each cow's fecal P4 concentrations were averaged across all months, pregnancy was correctly identified 83% of the time (19 of 23 pregnant cows, 6 of 6 non-pregnant cows and 1 cow was equivocal).

In 2008, maintenance of pregnancy was estimated using fecal P4 concentrations of samples collected in March, because it provided the greatest sample size. In 2008, pregnancy was maintained in 74% of cows (17 of 23).

In 2009, 27 collared cows were determined to be pregnant by ultrasonography in October. Of these 27 cows, 26 retained their collars through calving and 23 (88%) of these were observed tending a calf. Additionally, 2 of 10 (20%) collared cows that were not pregnant at roundup were observed to be tending a calf late in the summer.

Of the 3 collared cows that were pregnant at roundup but failed to be observed tending a calf, 2 had fecal P4 concentrations during October and March similar to the mean for non-pregnant cows; whereas, the third cow had a fecal P4 concentration very similar to the mean fecal P4 concentration for pregnant cows.

Stage 3: Perinatal Calf Survival

Calf production, the number of calves recorded at the yearly roundup during October per 100 breeding-age cows, in 2009 was greater ($P < 0.05$) than calf production in 2008 (Table 6). However, calf production in 2010 did not differ ($P > 0.05$) from calf production in 2009 (Table 6). Pregnancy rates determined by ultrasonography at roundup did not differ from actual calf production the following year in 2008 or 2009 ($P > 0.10$; Table 7).

Table 6. Calf production at roundup during October 2008, 2009 and 2010

	Year		
Item	2008	2009	2010
Number of breeding-age females	105	124	114
Number of calves recorded at roundup	34	70	76
Calf production, %	32%[a]	56%[b]	67%[b]

[a,b] Percentages within row with different superscripts differ ($P < 0.05$).

Table 7. Pregnancy rates (PR) of breeding-age bison cows determined by ultrasonography at roundup in October, during March by fecal progesterone (P4) concentrations and the following roundup by actual calf production at the National Bison Range in 2008 and 2009

	Year		
Item	2008	2009	Pooled over year
PR determined by ultrasonography	68%[a]	63%[a]	65%
Actual calf production[1]	56%[a]	67%[a]	61%

[1]Actual calf production in year + 1.
[a] Percentages within column and rows with different superscripts differ ($P < 0.05$).

Observations of cows tending calves between late March and late September are shown in Figure 4. Seventy-nine percent of the calves were born between early May and mid-June. The remaining 21% were from early August to the first of October (Figure 4).

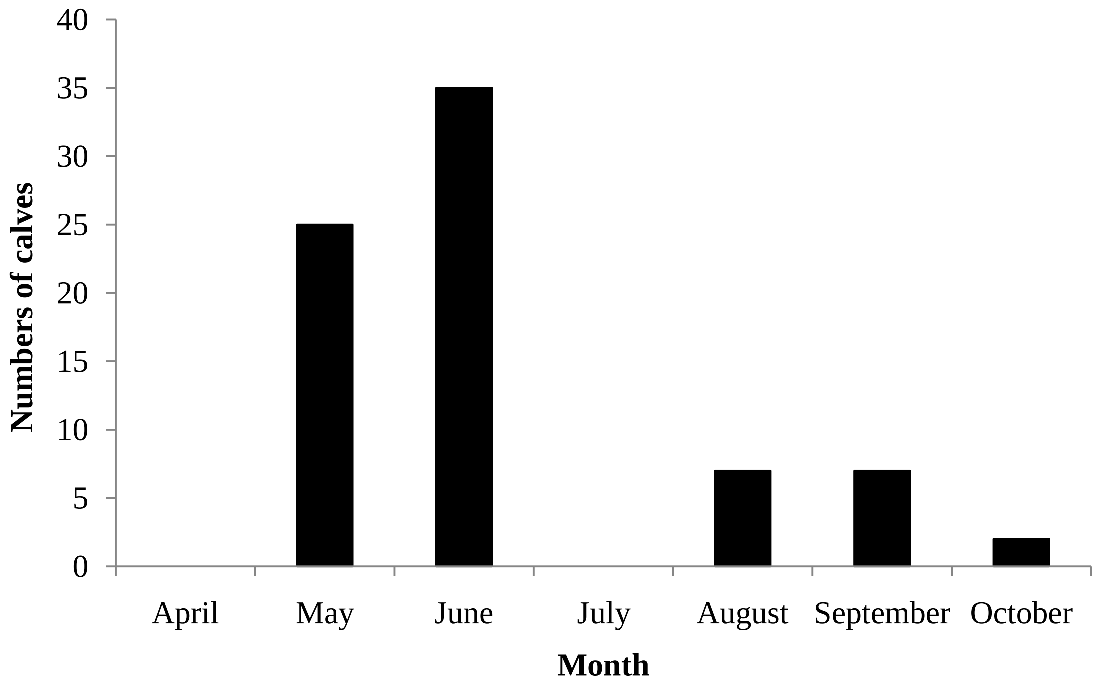

Figure 4. Estimates of calf production by month in 2010. Estimates were based the ratio of calves to a minimum of 200 bison excluding bull-only groups to the entire herd.

Long-term Trends in Calf Production, Cow Body Weight and Luteal Activity

Calf Production

Calf production by year from 1956 to 2010 is shown in Figure 5. Initial regression analysis indicated that the change in calf production followed a decreasing quadratic trend from 1956 to 2010 where calf production $y = 0.87 + 0.0047x - 0.0002x^2$, where x = the slope (b_1) of calf production per year. By visual inspection there seemed to be 3 distinct phases as characterized by differing linear trends (Figure 6). Linear regression analyses were performed on each of these phases. Linear regression of

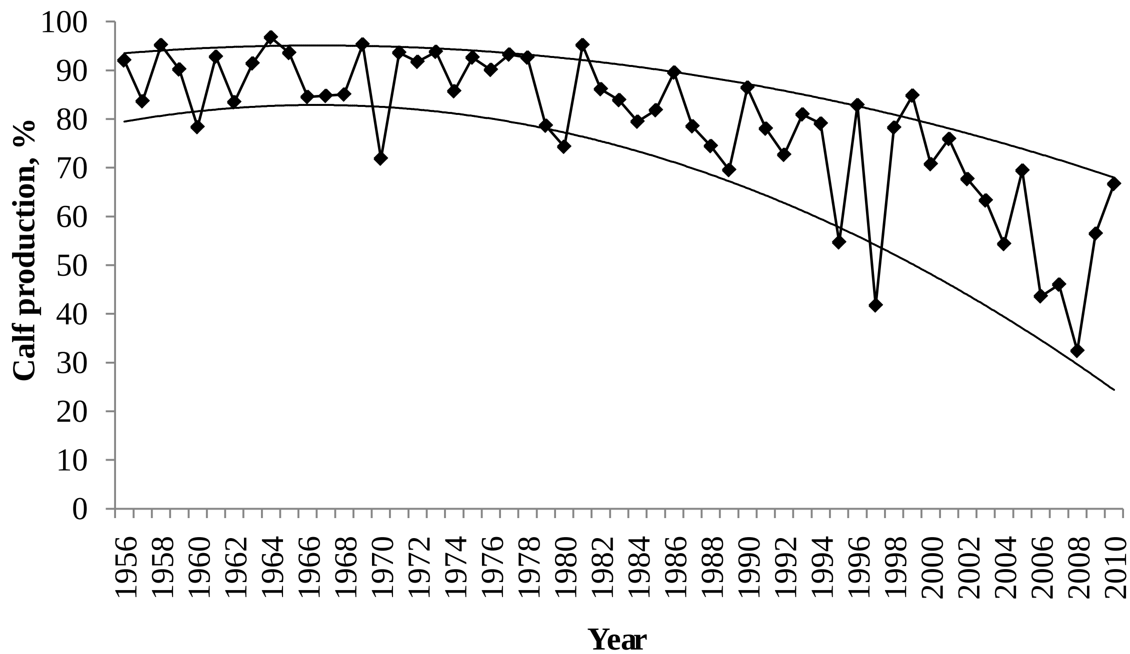

Figure 5. Calf production from 1956 to 2010. Quadratic trendline ($y = -0.0143x^2 + 0.3307x + 93.196$; $R^2 = 0.92$) for peaks was caluclated using calf production in years 1958, 1961, 1964, 1969, 1972, 1977, 1981, 1990, 1993, 1996, 1999 and 2010. Quadratic trendline ($y = -0.0309x^2 + 0.7099x + 78.808$; $R^2 = 0.86$) for troughs was calculated using calf production in years 1957, 1960, 1962, 1967, 1970, 1974, 1980, 1989, 1992, 1995, 1997 and 2010.

calf production by year between 1956 and 1978, indicated that calf production over these years did not differ ($b_1 < 0.01$ calf production/year; $P > 0.10$; Figure 6). Linear regression of calf production during the second phase between 1979 and 1998 indicated that calf production began to decrease ($b_1 = -0.001$ calf production/year; $P < 0.05$; Figure 6). Linear regression of calf production during the third phase between 1999 and 2010 indicated calf production decreased more rapidly ($b_1 = -0.028$ calf production/year; $P < 0.05$; Figure 6). If calf production from 2009 and 2010 are excluded from the analyses then calf production per year shows a more dramatic decrease from 1999 to 2007. The slope for the linear regression of calf production on year from 1999 to 2007 was -0.06

calf production/year with an R^2 of 0.80.

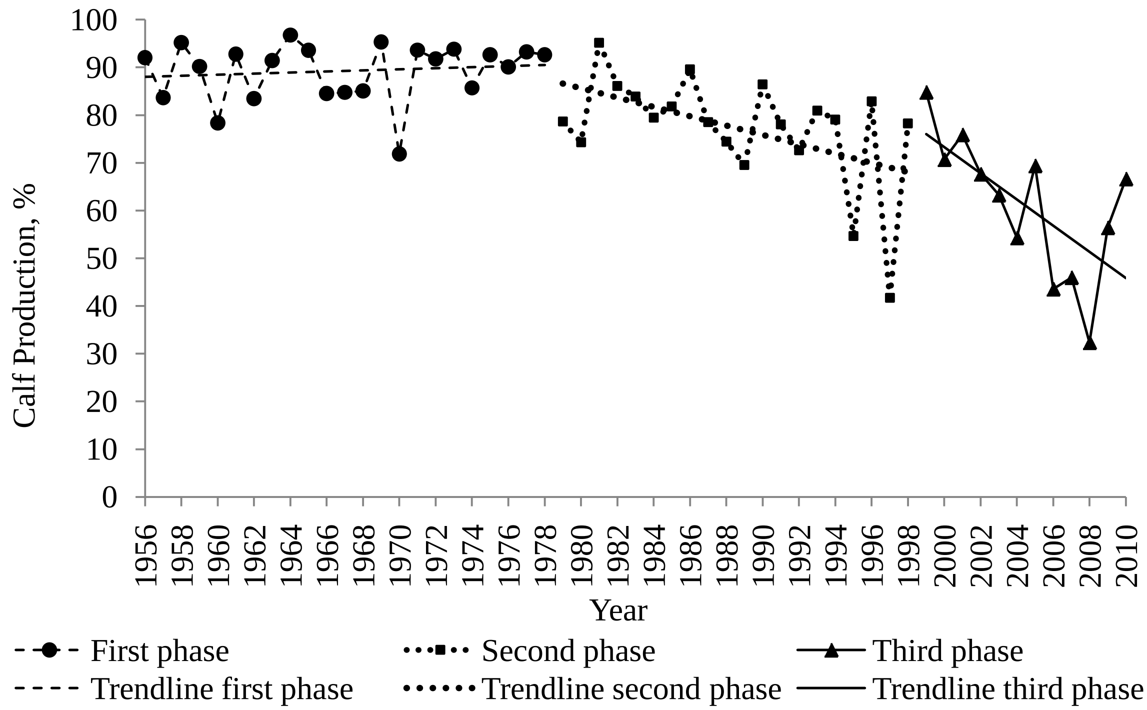

Figure 6. Calf production by year from 1956 to 2010 at roundup in October at the National Bison Range. The quadratic relationship of calf production on year was divided into 3 temporal phases. Linear regression within each phase are shown by trendlines for each phase. The first phase was from 1956 to 1978 (b_1 = 0.001 calf production/year; $P >$ 0.10). The second phases was from 1979-1998 (b_1 = -0.01 calf production/year; $P <$ 0.05). The last phase was from 1999-2010 (b_1 = -0.028 calf production/year; $P < 0.05$).

<u>Cow Body Weight</u>

Body weights of cows (4- to 7-year-old) decreased ($P < 0.05$) linearly (b_1 = -2.9 kg/year; $P < 0.05$; $R^2 = 0.56$) from 1999 to 2009 (Figure 7). If BW of cows from 2008 and 2009 are excluded from the analysis the R^2 was 0.75 (b_1 = -4.3 kg/year; $P < 0.05$). Linear regression of calf production in year + 1 on mean BW of 4- to 7-year-old cows between 1999 and 2009 had a slope of 0.88 and R^2 of 0.69 (Figure 8).

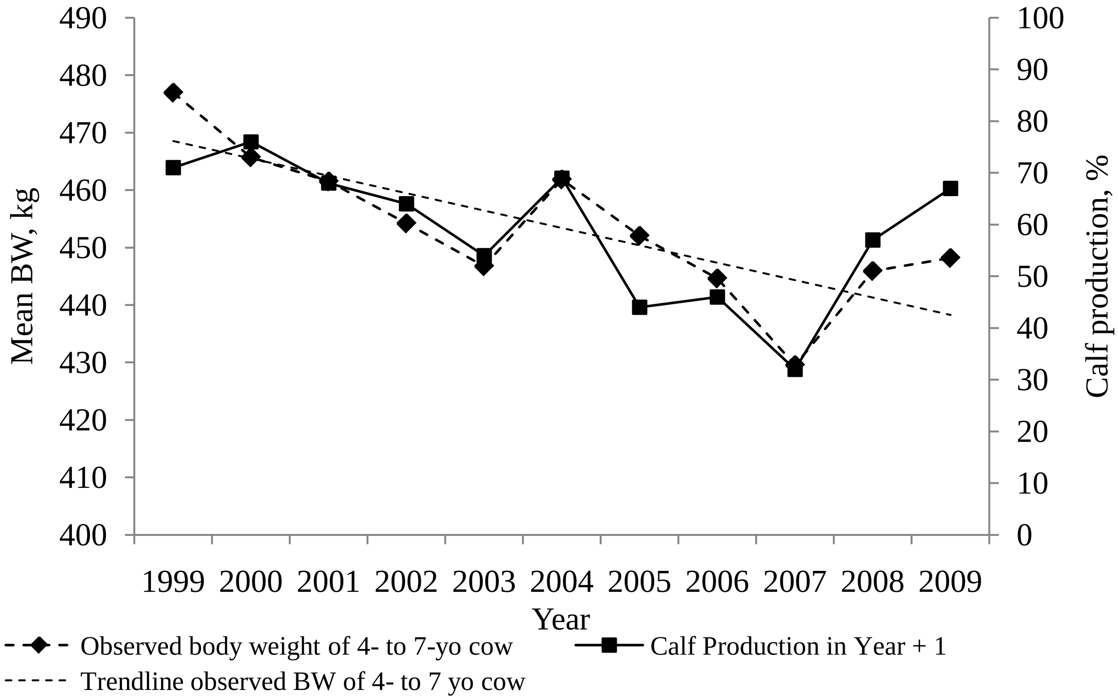

Figure 7. Linear regression for the mean body weight (BW) of 4- to7-year-old cows between 1999 and 2009 (b_1 = -2.7 kg/year; $P < 0.05$; $R^2 = 0.07$) and calf production (year + 1), number of calves produced per 100 cows, on year at the National Bison Range from 2000 to 2010.

Luteal Activity

Three hundred forty-one serum samples from cows collected at roundup in October from 2000 to 2009 were assayed for P4 concentrations to evaluate the proportion of cows that exhibited luteal activity. Proportion of cows that exhibited luteal activity differed ($P < 0.05$) among years (Table 8). The proportion of cows that exhibited luteal activity did not differ ($P > 0.10$) between 2000 and 2004 and the proportion of cows that exhibited luteal activity did differ ($P > 0.10$) between 2008 and 2009. The proportion of

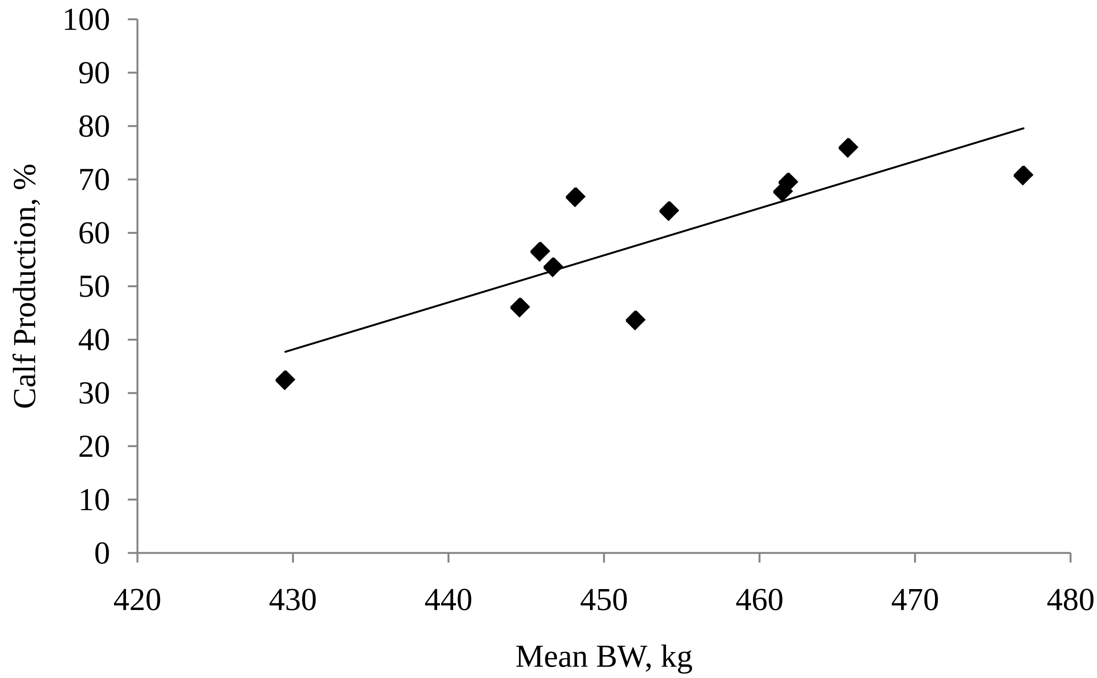

Figure 8. Linear regression of calf production, number of calves produced per 100 cows, for 2000 to 2010 on mean body weight (BW) of 4- to7-year-old cows between 1999 and 2009, (b_1 = 0.88 calf production/kg BW; $P < 0.05$; $R^2 = 0.69$).

cows that exhibited luteal activity among years 2005 and 2007 did not differ ($P > 0.10$), but were less than ($P < 0.05$) the proportion of cows that exhibited luteal activity in years between 2000 and 2004 and 2008 and 2009.

Table 8. Percentages of breeding-age female bison that exhibited a serum progesterone concentration ≥ 1 ng/mL at roundup in October between 2000 and 2009 at the National Bison Range

	Year									
Item	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
n	39	33	16	20	19	42	49	37	41	45
%[1]	87[a]	74[a,c]	81[a]	70[a,c]	90[a]	62[b,c]	63[b,c]	46[b]	88[a]	80[a]

[1] Percentage of cows that exhibited a concentration ≥ 1 ng/mL.

[a,b,c] Percentages with different superscripts differ ($P < 0.05$).

Figure 9 shows the linear regression for the percentage of cows that had a serum P4 concentration ≥ 1 ng/mL on year from 2000 to 2009. The slope (b_1 = -0.01 percentage of cows ≥ 1 ng/mL/year; R^2 = 0.06; Figure 9) of the regression coefficient indicated that the percentage of cows that exhibited luteal activity did not decrease (P > 0.10) from 2000 to 2009. If cows from 2008 and 2009 are excluded from the analysis, the regression coefficient (b_1 = -0.05 percentage of cows ≥ 1 ng/mL/year; R^2 = 0.56) indicated that the percentage of cows that exhibited luteal activity decreased ($P < 0.05$) from 2000 to 2007. Linear regression of calf production in year + 1 on percentage of cows that exhibited luteal activity between 2000 and 2009 had a slope of 0.82 and R^2 of 0.69 (Figure 10) indicating that calf production in year + 1 increased by 0.82% for an increase in 1% of cows that exhibited luteal activity at roundup between 2000 and 2009. If data from 2009 and 2010 for calf production are excluded the slope (b_1 = 0.98 calf production/percentage of cows ≥ 1 ng/mL; R^2 = 0.90) indicated that as the proportion of cows that exhibited luteal activity at roundup increases by 1% , calf production increased by 0.98%.

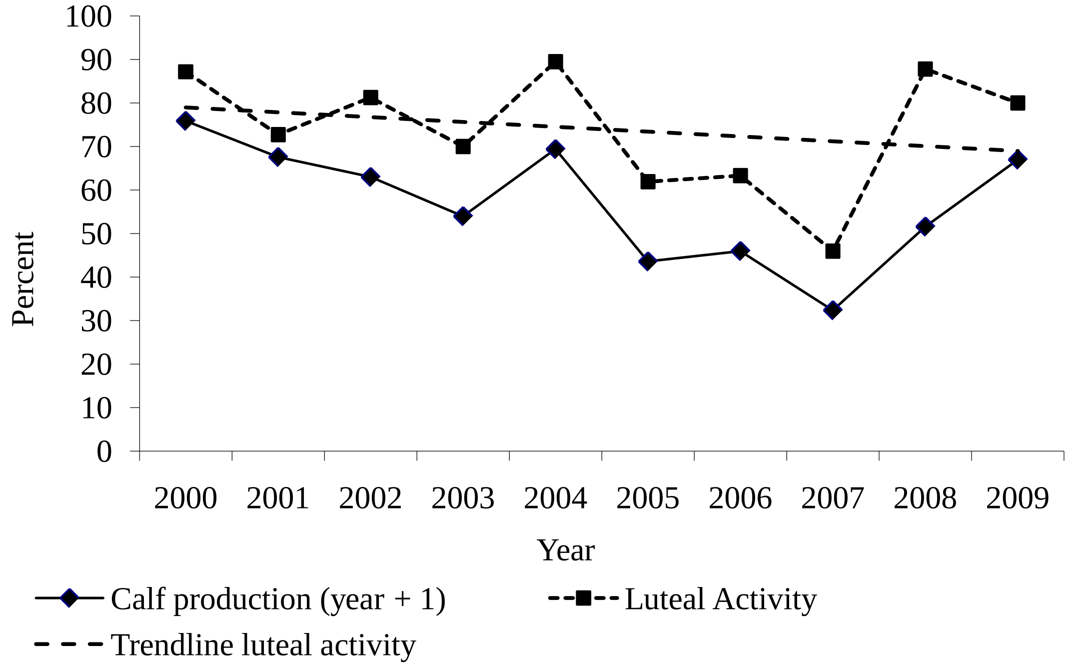

Figure 9. Linear regression for percentages of breeding-age cows showing luteal activity at roundup in October between 2000 and 2009, (b_1 = -4.5 percentage of cows ≥ 1 ng/mL/year; R^2 = 0.56; $P < 0.05$), and calf production, number of calves per 100 breeding-age cows at roundup in October between 2001 and 2010 on year at the National Bison Range.

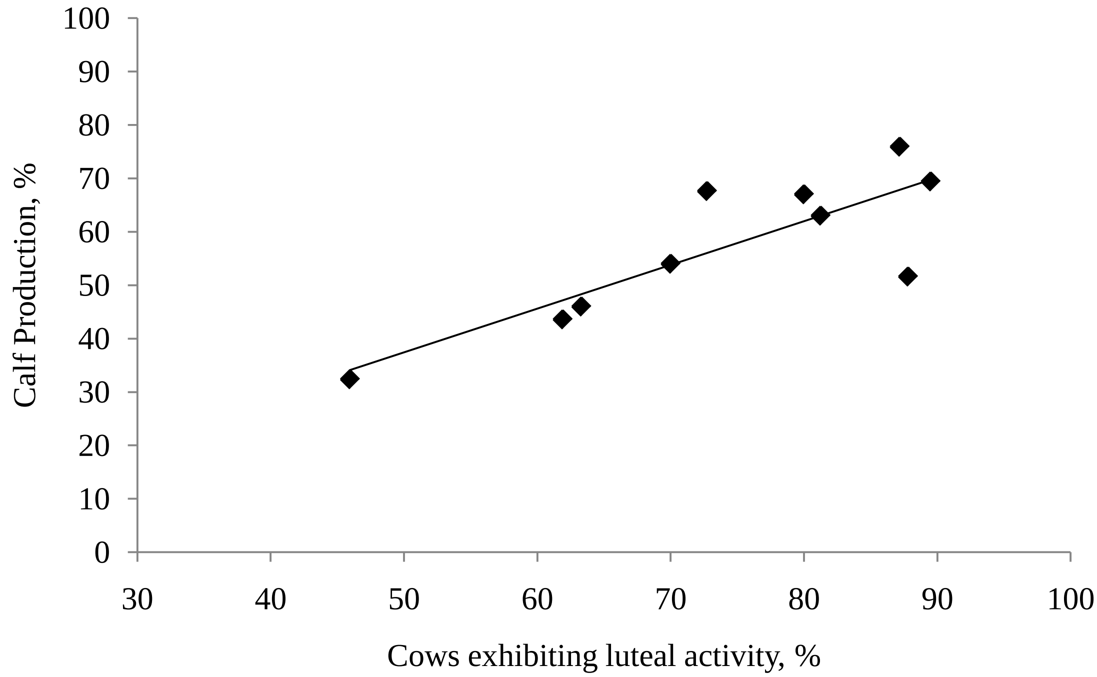

Figure 10. Linear regression for calf production, number of calves per 100 breeding-age cows at roundup in October between 2001 and 2010 on percentage of breeding-age cows that exhibited luteal activity at roundup in October between 2000 and 2009, (b_1 = 0.82 percentage calf production/percentage of cows that exhibited luteal activity; $P < 0.05$; R^2 = 0.69) at the National Bison Range.

DISCUSSION

Calf production at the National Bison Range has shown a dramatic decrease from 85% in 1999 to 46% in 2007. This represents a significant drop in reproduction rate that could eventually cause the loss of a valuable conservation resource. The present study represents a 3-year evaluation of the biological processes that determine calf production in female bison. These biological processes were conception and early embryonic development, maintenance of pregnancy and perinatal calf survival.

Pregnancy rates determined by ultrasonography did not differ among 2008, 2009 and 2010. Additionally, percentages of cows that exhibited luteal activity at roundup in 2008 and 2009 were 88 and 80%, respectively. Thus, it does not appear that the decrease in calf production in female bison at the NBR during these 3 years is related to an anestrous/anovular condition or a failure of cows to become pregnant and maintain early embryonic development. There are no data reported in the literature of estimates of pregnancy rates determined by ultrasonography in semi-free ranging bison at roundup in October.

Some authors have suggested that low calf production in bison may be related to alternate year breeding (Kirkpatrick et al., 1993; Shaw and Carter, 1989; Wolfe et al., 1999). In one study, Kirkpatrick et al. (1993) evaluated pregnancy rates of bison in Yellowstone National Park and concluded that calving rates of 35 to 55% indicate an every other or every third year calving. Furthermore, Wolfe et al. (1999) reported results for the Antelope Island, UT bison herd that indicated that approximately 13% of cows gave birth to calves in a 5- to 8-year interval. In our study, there were a sufficient number of cows that underwent ultrasonographic evaluation in all 3 years and 2 of the 3 years to investigate alternate year breeding. Pregnancy rates for these cows indicated that only 42 to 58% were pregnant in every year, which suggests that cows at the NBR may show some variant of less-than every consecutive year pregnancy pattern. Therefore, changes in alternate year breeding may not be a primary cause for the decrease in calf production at the NBR.

Patterns of fecal P4 concentrations have been used to estimate pregnancy rates in many species described in the literature review, including bison in Yellowstone National Park (Kirkpatrick et al. 1992) and recently by Vervaecke and Schwartzenberger (2006) to evaluate the initiation of luteal activity during the transition from non-breeding to breeding season in bison. In our study it was necessary to establish a lower threshold concentration of progesterone in feces that would allow for accurate assessment of whether an individual cow maintained pregnancy. Using the criteria established in the results there was no interaction between month and pregnancy status for P4 patterns, indicating that changes in P4 during gestation are similar between pregnant and non-pregnant bison. However, mean fecal P4 concentration was higher in pregnant cows than in non-pregnant cows. Further, examination of these patterns in October and March show a clear difference between pregnant and non-pregnant cows. This finding allowed us to evaluate whether losses of pregnancy were related to luteal activity in cows that were determined to be pregnant by ultrasonography at roundup.

Fecal P4 concentrations from cows in 2008 were used to estimate maintenance of pregnancy using the criterion established for cows of 2009. In doing so pregnancy rate in

March based on fecal P4 concentrations was 74% (17 of 23 cows). By taking a loss rate during gestation of 26% and applying that to the pregnancy rate determined at roundup by ultrasonography indicated a net loss of 18%. In 2009, 3 collared cows were pregnant at roundup and failed to produce a calf. The fecal P4 concentration patterns for 2 of these cows were similar in that in October they were lower than the mean fecal P4 concentration of pregnant cows and similar to the mean fecal P4 concentration of non-pregnant cows. Fecal P4 concentrations of these cows for the remaining sampling periods mimic those of non-pregnant cows. Although this occurred in only 2 cows this result may indicate these cows were undergoing some physiological changes that undergoing luteal insufficiency and consequent loss of pregnancy. On the other hand, 1 cow in which fecal P4 concentrations were similar to the mean fecal P4 concentration of pregnant cows in October, was low in January, but increased to a concentration that was similar to the mean fecal P4 concentration of pregnant cows in March. This individual cow might have lost her pregnancy from causes other than luteal insufficiency. Nevertheless, these 3 cows represent an 8% loss during pregnancy between October 2009 and March 2010. There are no data in the literature for pregnancy failures during the second trimester of pregnancy of bison. In cattle, there is a 15% loss that occurs during late embryo development and perhaps 10% fetal loss throughout the rest of pregnancy (Hansen, 2007). Based on our data, it would appear that bison at NBR experience similar gestational losses as cattle. This is the first report on mid-gestational loss of pregnancy in American bison.

An unexpected finding was a decrease in fecal P4 from October to January and subsequent rebound from January to March in all bison. A physiological reason(s) for this decrease in fecal P4 concentration is elusive because there is no evidence for the occurrence of this phenomenon in any ruminant species. However, at least in elk, fecal P4 concentrations increase during mid-gestation (White et al., 1995). This begs the question as to why this large decrease in fecal P4 concentration in pregnant cows between October and January does not result in a loss of pregnancy? Likewise, it is difficult to understand why the fecal P4 concentrations in non-pregnant, anestrous females increase during the winter. This decrease in fecal P4 concentration in pregnant females is apparently not directly related to fetal loss that could result in decreased calf production at the NBR. Bison are known to decrease their metabolism during winter (Christopherson et al. 1978; 1979), and it may be that P4 synthesis and secretion decreases and catabolism increases. Also, this period of time may correspond to a transition from maternal to placental production of P4 and serve as a “reset button” during pregnancy. If a fetus is not developed sufficiently to a point where placental production is delayed then this drop in P4 may result in termination of the pregnancy. Metabolically, this would be advantageous for cows that were bred late in the breeding season and had poor body condition. Removal of the energy requirement for pregnancy allows for the cow to utilize the winter resource for fat deposition and may provide an avenue for more synchronous breeding the next year. Nevertheless, further research is needed to investigate the physiological mechanism(s) involved with this observation.

If losses in other ungulates such as cattle (Hansen, 2007) and moose (Testa and Adams, 1998) are similar to that in bison, the difference between pregnancy rates determined by ultrasonography and actual calf production at the following roundup in 2009 and 2010 fetal losses are probably within the normal limits.

The distribution of calf births for 2009 cows between March 2010 and roundup indicated that most of the calves were born between early May and mid-June, the remainder of which were born between August and October. This distribution is in line with reports of calf distribution from Antelope Island State Park, UT (Wolfe and Kimball, 1989; Wolfe and Shipka, 1999), Fort Niobrara National Wildlife Refuge, NE, Wind Cave State Park, SD, Custer State Park, SD (Haugen, 1974), the NBR (Rutberg, 1984), and Wichita Mountains National Wildlife Refuge, OK (Shaw and Carter, 1989). It does not appear cows bred late in the breeding season would contribute to a decrease in calf production although the data from this study does not represent a large sample size. Evaluation of the calf production records from 1956 to 2010 in the present study indicated that long-term calf production has been decreasing since about 1980. Closer examination of the data for long-term calf production suggests a cyclic pattern in calf production. Production peaks appear to occur approximately once every 4 years, with intervening troughs. More importantly, the fitted curves of maximums and minimums are decreasing but for the function representing the minimums appear to be decreasing more rapidly than the maximums of calf production over years. During our study calf production hit its lowest point in 2008 and has increased in 2009 and 2010. Whether this represents a continued upward trend to reach levels achieved between 1956 and 1978 is unknown because of the cyclic changes within this long-term pattern. It could very well be that the calf production in 2010 is a peak within a cycle and the calf production of

2011 would again decrease. However, the pregnancy rate as determined by ultrasonography at roundup in 2010 was 71%. Based on the predictive value of ultrasonography, calf production in 2011 should be equivalent to or greater than calf production in 2010. One could speculate that there might be a much longer cycle (perhaps 50 to 60 years) for increases and decreases in calf production superimposed on the 1 in every 4 year cycle observed. As far as we know the possible long-term cycle in calf production of bison has never been described in the literature.

We investigated the relationship of calf production from 1999 to 2008 to body weights (as a surrogate for condition) of 4- to 7-year-old cows and found a strong correlation and significant regression. Linear regression of calf production from 2000 to 2008, which represents calves from cows with BW represented the year before, indicated that calf production decreased in a parallel manner to that of the regression for cow BW, so that as cow BW decreases so does calf production the following year. One interpretation of these results is that 4- to 7-year old cow BW could be a good indicator of calf production the following year. It is well known in domestic animal species and in wildlife animal species that reduction in BW are related directly to body condition of females for breeding (Grimard et al., 1997; Guedon et al., 1999). Furthermore, poor body condition of females results in lower offspring production in mammals (for review, see Senger, 2005). Our data indicate that the decreases in cow BW appear to be directly related to decreases in calf production. These results support previous results that have clearly shown that poor nutrition is related to low calving rate in several bison herds (Lott and Galland, 1987; VanVuran and Bray, 1986). Further evidence that is the case at the

NBR is provided by this study in that cow BW increased in 2008 and 2009 and calf production increased in 2009 and 2010. Taken together the dramatic decrease in calf production is closely associated with the body condition of prime breeding-age cows. The cause for the drop in cow BW over this time is not obvious and further research into the range resource or management conditions of the herd are necessary.

How does change in BW affect reproductive processes that might be involved with a decrease in calf production? To answer that question, archived serum samples from cows collected at roundups from 2000 to 2007 were used to determine the percentage of cows that exhibited luteal activity to identify whether there was a relationship between numbers of cows exhibiting luteal activity in one year and calf production in the following year at roundup. Numbers of cows that exhibit luteal activity at roundup seems to provide a reasonable estimate of calf production rate in the following year. This relationship indicates that anestrus or anovular conditions or early conceptus loss during the breeding season may be an important consideration for the reduction in calf production between 1999 and 2008. To support this idea is the observation that in 2008 and 2009, the proportion of cows that exhibited a serum P4 concentration ≥ 1 ng/mL at roundup doubled from that of cows in 2007, with a corresponding doubling of calf production in 2009. The physiological interpretation of these data is that there is an increasing trend to have a high proportion of anestrus/anovular cows in the herd during the breeding season. If cows are anestrus they would not be able to become pregnant because they would not be able to display estrus behavior. The cause for this trend is probably related to nutritionally induced anestrus. This indicates that the decreasing trend in calf production related to the proportion of cows that exhibit luteal activity may be changing, at least in the short-term. There is a possibility that this upward trend could be due to an improvement in the range resources.

A consequence of inadequate nutrition might lead to non-consecutive year calving (Kirkpatrick et al., 1993; Wolfe et al., 1999). This could be the reason for the more dramatic decrease in minimums of long-term calf production. Kirkpatrick et al. (1993) evaluation of pregnancy rates of the bison in Yellowstone National Park led them to suggest that calving rates of 35 to 55% indicate an every other or every third year of calving. In support of this notion were results reported by Wolfe et al. (1999) for the Antelope Island, UT herd that indicated that approximately 13% gave birth to calves in a 5- to 8-year interval. In our study, there were a sufficient number of cows that underwent ultrasonographic evaluation in all 3 years and 2 of the 3 years. The pregnancy rates for these cows indicated that only 42 to 58% were pregnant in every year, which suggests that cows at the NBR may show some variant of less-than every consecutive year pregnancy pattern.

A few conclusions can be made from the results our study. First, pregnancy rate in bison as determined by fall ultrasonography is a good predictor of calf production at the following roundup. Next, calf production has increased from a low in 2008 through 2009 and 2010. Furthermore, reproductive loss during gestation occurs in these bison but not at a higher rate than would be expected in cattle. Fetal losses and maintenance of pregnancy do not appear to be a primary cause for the decrease in calf production at the NBR. The results over the 3-year study support the fact that the trend in decreasing calf production at the NBR from 1999 to 2010 has diminished. If anything, calf production appears to be increasing over these years.

Furthermore, examination of the relationship between cow BW, cows that exhibited luteal activity and the dramatic decrease in calf production from 2000 to 2007 one can infer that the number of cows that were anestrus increased, resulting in a decrease in the proportion of cows bred during breeding season and a decrease in the proportion of cows pregnant at roundup. Additionally, normal losses during gestation exacerbated the decrease in calf production. From the historical trends, it would appear that the cause of anestrus during the breeding season is the result of body condition of cows at the NBR, most likely due to range resources or other environmental factors. Finally, the temporal patterns of fecal P4 concentrations during pregnancy in bison show a distinct decrease between October and January, and an increase between January and March. The reason for this is not known and it would appear that pregnant cows that undergo this change should lose their pregnancy but do not. This is a paradox that certainly deserves further consideration and understanding.

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