Mammal Research (2020) 65:869–874
https://doi.org/10.1007/s13364-020-00530-x

SHORT COMMUNICATION

# Use of a portable ultrasound machine to determine pregnancy and uterine litter size for wild Gunnison's prairie dogs (*Cynomys gunnisoni*)

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Received: 3 January 2020 / Accepted: 18 August 2020 / Published online: 25 August 2020


**Abstract**
Accurate estimates of population dynamics play a major role in formulating conservation strategies for Gunnison's prairie dogs (*Cynomys gunnisoni*) and other keystone species. Over 5 years (2014–2018) at Valles Caldera National Preserve in NM, USA, we used a portable ultrasound machine (PUM) to investigate the presence or absence of pregnancy, and to determine uterine litter size for 238 pregnant female Gunnison's prairie dogs from two colonies. We compared litter size at weaning for 168 of these 238 females and we found no significant difference between uterine litter size and litter size at weaning. For 76 of these 168 females (45%), estimates of uterine litter size and litter size at weaning were identical. We detected no significant variation among years for uterine litter size versus litter size at weaning. To our knowledge, our research is the first to compare uterine litter size to litter size at weaning for the same group of females living under natural conditions.

**Keywords** *Cynomys gunnisoni* · Gunnison's prairie dog · Litter size at weaning · Portable ultrasound machine (PUM) · Uterine litter size

## Introduction

Accurate estimates of pregnancy rate and litter size can be important for a better understanding of population dynamics (Festa-Bianchet and King 1991; Griffin et al. 2003). Obtaining these estimates from animals living under natural conditions, however, is challenging. The presence of hormones indicative of pregnancy occurs in the blood serum, but the process of extracting blood can cause significant stress to the sampled females (Derocher et al. 1992; Stephenson et al. 1995; Sweitzer and Holcombe 1993). A less invasive method to detect pregnancy quantifies fecal hormones in captive and wild mammalian females (Beehner et al. 2006; Garnier 2001; Mahmoud 2017).

Hormone concentrations allow researchers to detect pregnancy, but they provide no estimate for uterine litter size. The number of fetuses within pregnant mammalian females has been estimated from postmortem examinations (e.g., Fuller et al. 2003; Rausch 1967), but techniques such as visual inspection, body mass, palpation, and ultrasonography are obviously preferable (Greer and Hawkins Jr 1967; Milner et al. 2013). By using a portable ultrasound machine (PUM), we attempted to verify presence or absence of pregnancy, and also to determine uterine litter size, for female Gunnison's prairie dogs (*Cynomys gunnisoni*, hereafter simply "GPDs") living under natural conditions at Valles Caldera National Preserve in New Mexico USA.

GPDs are medium-sized, hibernating, colonial rodents of the squirrel family (Sciuridae) and inhabit parts of Arizona, Colorado, New Mexico, and Utah (Hoogland 1996). They live in harem-polygynous family groups called clans that typically contain 1 adult (≥ 1 year old) sexually mature male, 3–4 adult sexually mature females, and 1–2 sexually immature yearling

Communicated by: Shuiqiao Yuan

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s13364-020-00530-x) contains supplementary material, which is available to authorized users.

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males (Fitzgerald and Lechleitner 1974; Hoogland 1998; Rayor 1985, 1988). Like black-tailed prairie dogs (*Cynomys ludovicianus*; Kotliar et al. 2006), GPDs are keystone species of their grassland habitats (Davidson et al. 2012, 2014). GPDs hibernate for approximately 4 months of every year, from November through February, and they typically arouse from hibernation in March and April. Female GPDs usually copulate for the first time as 1-year-olds in the spring of their first year, but males typically do not copulate for the first time until they are 2 years old. Like females of other ground-dwelling squirrels (Holekamp and Sherman 1989; Hoogland 2013a), female GPDs usually remain in the natal territory for their entire lives. This philopatry of GPD females enabled us to observe the same females across consecutive years (Hoogland 2013a).

An ultrasound machine allows a researcher to determine uterine litter size within commercial facilities such as zoos, aquariums, research labs, and veterinary offices (Flores et al. 2014; Radcliffe et al. 1997). Field biologists have used a PUM to detect pregnancy in a variety of mammalian species of large size (> about 20 kg) such as gray wolves (*Canis lupis*), northern fur seals (*Callorhinus ursinus*), and moose (*Alces alces*) (Adams et al. 2007; McNay et al. 2006; Stephenson et al. 1995). However, no previous study has compared estimates of uterine litter size with litter size at weaning for the same females over time under natural conditions.

With no information about copulations, previous research with ultrasound machines has not been able to control for stage of pregnancy (Drew et al. 2001; Inzani et al. 2016; Smith and Lindzey 1982). To address this shortcoming, we compared uterine litter sizes with litter sizes at weaning for GPDs for which we knew the exact date of copulation. Knowing when females copulated allowed us to perform our ultrasounds when females were at approximately (within 4 days) the same stage of late pregnancy.

## Materials and methods

The GPD mating season usually lasted for approximately 3 weeks each year in late March through mid-April, but each female was sexually receptive for several hours of a single day (Hoogland 1998, 2013b). Parturition usually occurred after a gestation of 29 or 30 days following estrus and copulation (Hoogland 1999). The nearly weaned young emerged from their natal burrows for the first time approximately 5.5 weeks after parturition. We determined maternity and sibships by documenting the burrow in which a lactating female consistently spent the night with no other adults and from which a litter eventually appeared aboveground. Rarely, at a frequency of < 5% per year, GPD mothers reared offspring in the same burrow, and burrows sometimes had more than one entrance—so that assignment of weaned juveniles to their mothers in those rare cases was difficult or impossible (Hoogland 1997, 2001).

### Study sites

We performed our research in study-sections of two colonies of GPDs within Valles Caldera National Preserve, Sandoval County, NM, USA: the Redondo Meadow Colony (RMC, 2014–2016) and the Visitor Center Colony (VCC, 2017–2018). Both colonies contained approximately 500 adult prairie dogs, were approximately 2500 m above sea level, and were separated by approximately 15 km.

### Livetrapping, handling, and marking

To capture adult GPDs, we followed the example of previous investigators (Fitzgerald and Lechleitner 1974; Hoogland 1995) and used 15 × 15 × 60-cm double-door livetraps (Tomahawk Livetrap Company, WI, USA). For bait, we used a mixture of oats and sunflower seeds. To capture juveniles, we used unbaited 13 × 13 × 40-cm single-door Tomahawk livetraps.

To handle adult GPDs, we used a conical canvas bag that could be unzipped from either end (Hoogland 1995). We handled juveniles directly without a bag. For permanent identification, we inserted one uniquely numbered fingerling eartag (National Band and Tag Company, Newport, KY, USA) per ear.

For visual identification from a distance, we used Nyanzol fur dye (Greenville Colorants, Clifton, NJ, USA) to paint a unique number or symbol on each flank of every GPD (Hoogland 1995). To identify burrows where mothers reared their offspring (nursery burrows), we used jumbo Ritchey cattle eartags (Brighton, CO, USA) mounted on 30-cm sections of clothesline wire.

### Observations of copulations

The mating season (from the day of the first copulation until the day of the last copulation) for the GPDs at our two study colonies started in late March each year and lasted until mid-April. Each female copulated with 1–5 different males on her single day of estrus and sexual receptivity (Hoogland 1998). In an attempt to record all copulations for every female, we observed marked GPDs with binoculars from elevated deer blinds (Sportsman's Condo, West Point, MS, USA) every day from dawn until dusk.

Some copulations occurred aboveground, and therefore were easy to document. Most copulations, however, occurred underground. We inferred a copulation when ≥ 3 of the following 5 criteria were satisfied (Hoogland 1998, 2013b): (1) a sexually mature male sniffed or licked a sexually mature female's vulva, and then (2) followed her into a burrow for ≥ 5 min, and usually for ≥ 30 min; (3) with the estrous female nearby (≤ 2 m away), the male gave a unique vocalization within 2 min before or after the underground consortship; (4) the male or the female licked its genitals within 5 min after emerging from the underground consortship; and (5) the female remained aboveground much later than usual, typically 60–90 min after nonestrous females living in her territory had submerged into burrows for the night. Females that copulated aboveground consistently satisfied ≥ 3 of these same criteria (except criterion 2) (Hoogland 1998, 2013b). Determining the date of copulation for every female was crucial so that we could identify day 25 after copulation, and then capture each pregnant female when she was in late pregnancy. Consequently, our ultrasounds were all from females at approximately the same stage (within 4 days) of pregnancy.

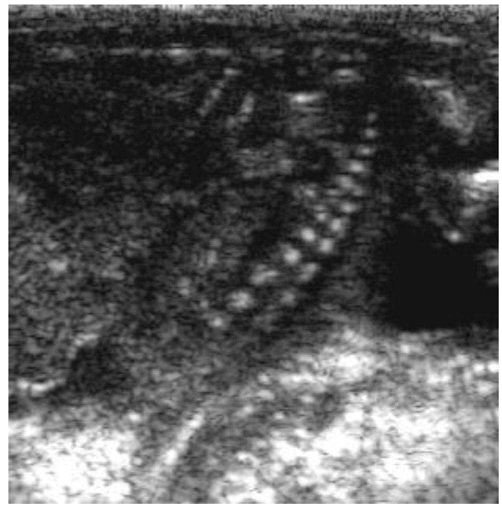

**Fig. 1** Ultrasound from pregnant Gunnison's prairie dog (*Cynomys gunnisoni*) approximately 25 days after copulation. Note the distinctive vertebral column. A skull and heartbeat for each fetus were usually visible during the ultrasound as well

## Ultrasound examinations

To obtain uterine counts of fetuses for pregnant GPD females, we used a Sonosite M-Turbo PUM (Bothell, WA, USA). We used a HFL38X transducer (probe) and used the following M-Turbo setttings: Gen, SmP, and depth = 2.2. We examined a total of 238 pregnant GPD females, of which 168 (70.6%) eventually weaned a litter. For our analyses, we excluded all duplicate ultrasounds and all cases for which uterine litter size was 0. Usually, we obtained an ultrasound from a female 25 days after her single day of estrus and copulation(s). Because certain females were difficult to livetrap, however, we sometimes were unable to perform an ultrasound until 1–4 days later. We never attempted an ultrasound beyond day 29 after a female's estrus.

To perform an ultrasound, John Hoogland used the canvas bag described above to hold the pregnant female's rear legs. Hoogland applied Aquasonic 100 Ultrasound Transmission Gel (Clinton Township, MI, USA) to the female GPDs abdomen, and then moved the Sonosite probe up and down both horns of the uterus. One research assistant used the same canvas bag to hold the pregnant female's front legs, and (s)he and another research assistant watched the screen of the PUM for definitive signs of a fetus (spine, skull, or beating heart) (Fig. 1). Accurate counts of fetuses required several attempts, and both observers needed to agree before we recorded a final count.

Using a PUM to determine uterine litter size required about 15 min per pregnant female. The process of getting an ultrasound was similar to the process of marking that we used for hundreds of GPDs each year, and we detected no evidence that getting an ultrasound caused any harm or stress to the examined female. We returned each female to her home territory soon after obtaining an ultrasound, and we observed all 238 examined females foraging aboveground later in the same day of their ultrasounds, or early the following day. The frequency of examined pregnant GPD females that did not eventually wean a litter (70/238 = 29%) is similar to the frequency of unexamined pregnant females that did not eventually wean a litter in previous long-term research with GPDs living under natural conditions at Petrified Forest National Park in Arizona from 1989 through 1994 (Hoogland 1999, 2001). As documented below, notice that uterine litter sizes estimated with the PUM were similar to litter sizes at weaning for the same females.

While obtaining our ultrasounds, GPD fetuses usually moved, and often we could see the heartbeat. We did not detect any fetuses that were obviously dead, nor did we detect any evidence that females sometimes had aborted certain fetuses and then attempted to resorb them.

## Statistical analyses

We used the Wilcoxon matched-pairs ranked sign test to compare uterine litter size and litter size at weaning for the same females, and the Kruskal-Wallis analysis of variance to investigate differences among years for uterine litter size versus litter size at weaning. We used the Mann-Whitney *U* test to compare uterine litter size and litter size at weaning between our two study colonies. *P* values are from two-tailed statistical tests. As in previous long-term research (Hoogland 1999, 2001), most pregnant GPD females were yearlings, but a few were 2- or 3-year-olds; we considered data from the same females in different years ($N = 22$) to be independent. For two pregnant GPD females, we obtained ultrasounds for three consecutive years.

## Results

From 2014 through 2018, we used our PUM to estimate uterine litter size for 238 GPD females at our two study colonies (Fig. 2). Over the same 5-year period, we also determined litter size at weaning for 168 females that weaned litters (Fig. 3). For these 168 females, we had information for both uterine litter size at approximately 25 days after copulation and litter size at weaning. Seventy of the 238 pregnant females (29%) from which we obtained an ultrasound lost their litters at some point before weaning. Uterine litter sizes ranged from 2 to 8 (Fig. 2), and litter sizes at weaning ranged from 1 to 8 (Fig. 3). The most common uterine litter size was 4 (86/238 = 36%; Fig. 2). The most common litter size at weaning was also 4 (60/168 = 36%; Fig. 3).

For the 168 GPD females for which we had both estimates of litter size, we found no significant difference between uterine litter size and litter size at weaning ($P = 0.267$, Wilcoxon matched-pairs signed rank test; Fig. 4). For 76 of these 168 females (45%), estimates of uterine litter size and litter size at weaning were identical (Fig. 4).

We also compared uterine litter size to litter size at weaning across years (Fig. 5). These two estimates were most similar in 2015 (identical for 31/32 = 97% of females), but we detected no significant variation among years for uterine litter size versus litter size at weaning ($P = 0.059$, Kruskal-Wallis analysis of variance; Fig. 5).

Litter size at weaning was significantly higher for RMC than for VCC (mean ± SD = 4.31 ± 1.22, $N = 110$ versus mean ± SD = 3.67 ± 1.10, $N = 58$, $P = 0.003$, Mann-Whitney $U$ test). Estimates of uterine litter size, however, did not significantly differ between our two study colonies (mean ± SD = 4.31 ± 1.17 at RMC, $N = 110$ versus mean ± SD = 3.98 ± 0.99 at VCC, $N = 58$, $P = 0.054$, Mann-Whitney $U$ test). For our other statistical analyses, we combined data from RMC and VCC.


**Fig. 2** Frequency of uterine litter sizes for Gunnison's prairie dog (*Cynomys gunnisoni*) determined by using a portable ultrasound machine from 2014 through 2018 at Valles Caldera National Preserve, NM, USA

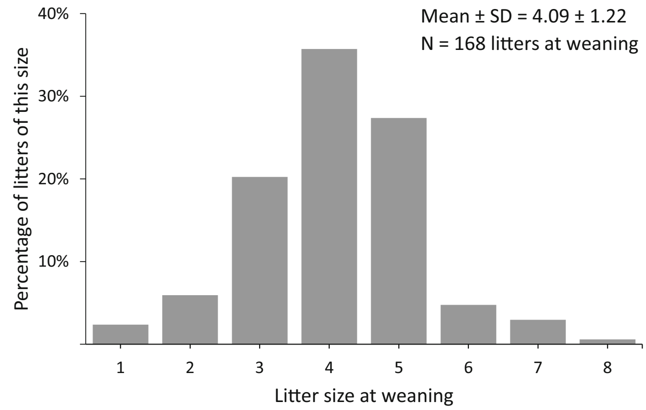


**Fig. 3** Frequency of litter size at weaning for Gunnison's prairie dogs (*Cynomys gunnisoni*) at Valles Caldera National Preserve, NM, USA, from 2014 through 2018. Data from this figure are only from those females that weaned at least one offspring and for which we had obtained an earlier ultrasound in late pregnancy. We scored weaning for each litter on the date when ≥ 1 juvenile first appeared aboveground from the natal nursery burrow

## Discussion

Our research shows that using a PUM is an easy, accurate method to verify the presence or absence of pregnancy for GPDs living under natural conditions. The PUM also can provide an estimate of uterine litter size in late pregnancy. Previous studies have documented the efficacy of using a PUM to document pregnancy (Drew et al. 2001; Smith and Lindzey 1982; Stephenson et al. 1995), but only a few studies have used ultrasonography to estimate uterine litter size (Griffin et al. 2003; Inzani et al. 2016; Smith and Lindzey 1982).

Of the 238 GPD females for which we recorded uterine litter size via ultrasounds, 168 (71%) weaned ≥ 1 offspring. For the percentage of litters per year for which uterine litter size was − 1, equal to, or + 1 from the litter size at weaning, only 1 year (2018) fell below 90%. All previous years (2014–2017) showed concordance of ≥ 90%, with the highest

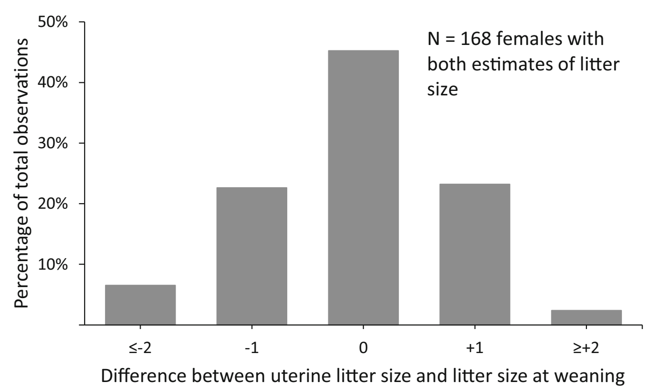


**Fig. 4** Difference between uterine litter size versus litter size at weaning for the same 168 female Gunnison's prairie dogs (*Cynomys gunnisoni*) from 2014 through 2018 at the Valles Caldera National Preserve, NM, USA

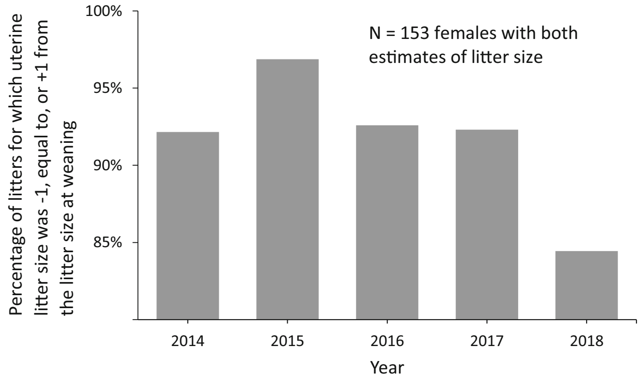


**Fig. 5** Percentage of Gunnison's prairie dog (*Cynomys gunnisoni*) litters per year for which uterine litter size was − 1, equal to, or + 1 from the litter size at weaning for 2014 through 2018 at the Valles Caldera National Preserve, NM, USA

concordance being 97% in 2015. For GPD females that weaned a litter, our results show that uterine liter size determined by a PUM in late pregnancy can be a good estimate of eventual litter size at weaning.

Litter size at weaning was higher at RMC than at VCC. One possible explanation for this intercolonial difference is that food was more plentiful or of higher nutrition at RMC. We could not test this hypothesis because we did not collect any information regarding the availability or nutritive quality of food for our two study colonies. From earlier research (Hoogland 2013b), we know that GPD litter size at weaning is affected by maternal age and maternal body mass. Perhaps, intercolonial differences in these latter factors, in combination with intercolonial differences in availability or nutritive quality of food, contributed to intercolonial differences in litter size at weaning.

For 70 cases for which we estimated uterine litter size in late pregnancy, the pregnant females did not wean a litter. For 49 other cases, litter size at weaning was smaller than our estimate for uterine litter size (Fig. 4). What happened to all those GPD juveniles that we detected in the uterus, but we did not see and capture at weaning? If the litter size at weaning for a marked female was smaller than our estimate of uterine litter size for the same female, the implication is that ≥ 1 of the uterine offspring died before or during parturition, or died for some unknown reason between parturition and weaning. Perhaps, certain GPD females selectively aborted, and then possibly resorbed, certain fetuses after the ultrasound but before parturition—as has been reported for black-tailed prairie dogs and Belding's ground squirrels (*Urocitellus beldingi*) (Anthony and Foreman 1951; Knowles 1987; McKeever 1964). We have no information before or after our ultrasounds regarding abortion and possible resorption of fetuses for the female GPDs at our study colonies. Sources of mortality of juveniles after parturition but before weaning and first appearance aboveground probably included disease, predation, and infanticide (Hoogland 1995). We have no information of diseases that might have eliminated GPD fetuses or young juveniles before weaning, but we did document many cases of infanticide of unweaned GPD juveniles by male and female GPD adults. We also documented predations of unweaned GPD juveniles by bull snakes (*Pituophis catenifer*) and possibly by long-tailed weasels (*Mustela frenata*) as well.

What about the 43 cases in Fig. 3 for which litter size at weaning for a marked female was larger than our estimate of uterine litter size? Possible sources of error that might have caused this inconsistency include an inaccurate count of uterine fetuses, and the occasional underground mixing up of juveniles from litters of different mothers before the juveniles first appeared aboveground at weaning (see above and Hoogland 1995)—so that estimates of litter size at weaning for each affected female were elusive and possibly imprecise.

To our knowledge, no previous study has compared uterine litter size to litter size at weaning for mammals living under natural conditions. Our research shows that a PUM and careful research with marked individuals can provide estimates of the presence or absence of pregnancy, uterine litter size, and litter size at weaning for GPDs living under natural conditions. This information will be important for conservation and management of a rare keystone species such as the GPD (Davidson et al. 2012; Knowles 2002; Seglund et al. 2006; Seglund and Schnurr 2010).

**Acknowledgments** For help with recording ultrasounds, we thank T. Cornwell, M. Dart, R. Farid, J. Gedert, I. Hirschler, B. Jones, C. Poje, K. Rave, M. Rivera-Rodriguez, and T. Townsend. We are grateful to D. Boesch, E. Davidson, Robert Gardner, Peter Goodwin, and the University of Maryland Center for Environmental Science for facilitating JLH's long-term research with prairie dogs. For assistance with our research, we thank the following personnel at Valles Caldera National Preserve: D. Davis, D. Mccray, R. Parmenter, and Jorge Silva-Bañuelos.

**Funding information** We received financial assistance from the Denver Zoological Foundation, the National Science Foundation, and the University of Maryland Center for Environmental Science.

## Compliance with ethical standards

Our research was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Maryland Center for Environmental Science (Protocol number F-AL-15-13), and complied with current guidelines of the American Society of Mammalogists (Sikes and The Animal Care and Use Committee of the American Society of Mammalogists 2016). The number of our Scientific Research Permit at Valles Caldera National Preserve, US Department of the Interior, was VALL-2017-SCI-0004.

## References

Adams GP, Testa JW, Goertz CEC, Ream RR, Sterling JT (2007) Ultrasonographic characterization of reproductive anatomy and early embryonic detection in the northern fur seal (Callorhinus ursinus) in the field. Mar Mamm Sci 23(2):445–452

Anthony A, Foreman D (1951) Observations on the reproductive cycle of the black-tailed prairie dog (Cynomys ludovicianus). Physiol Biochem Zool 24:242–248

Beehner JC, Nguyen N, Wango EO, Alberts SC, Altmann J (2006) The endocrinology of pregnancy and fetal loss in wild baboons. Horm Behav 49:688–699

Davidson AD, Detling JK, Brown JH (2012) Ecological roles conservation challenges of social, burrowing, herbivorous mammals in the world's grasslands. Front Ecol Environ 10:477–486

Davidson AD, Friggens MT, Shoemaker KT, Hayes CL, Erz J, Duran R (2014) Population dynamics of reintroduced Gunnison's prairie dogs in the southern portion of their range. J Wildl Manag 78: 429–439

Derocher AE, Stirling I, Andriashek D (1992) Pregnancy rates and serum progesterone levels of polar bears in western Hudson Bay. Can J Zool 70:561–566

Drew ML, Bleich VC, Torres SG, Sasser RG (2001) Early pregnancy detection in mountain sheep using a pregnancy-specific protein b assay. Wildl Soc Bull 29:1182–1185

Festa-Bianchet M, King WJ (1991) Effects of litter size and population dynamics on juvenile and maternal survival in Columbian ground squirrels. J Anim Ecol 60:1077–1090

Fitzgerald JP, Lechleitner RR (1974) Observations on the biology of Gunnison's prairie dog in central Colorado. Am Midl Nat 92:146–163

Flores LE, Hildebrandt TB, Kuhl AA, Drews B (2014) Early detection and staging of spontaneous embryo resorption by ultrasound biomicroscopy in murine pregnancy. Reprod Biol Endocrinol 12:38

Fuller TK, Mech LD, Cochrane JF (2003) Wolf population dynamics. In: Mech LD, Boitani L (eds) Wolves: behavior, ecology and conservation. University of Chicago Press, Chicago, pp 161–191

Garnier JN (2001) Non-invasive reproductive monitoring of black rhinoceros females in the wild: patterns of fertility and the influence of environmental factors. Doctoral dissertation, University of London

Greer KR, Hawkins WW Jr (1967) Determining pregnancy in elk by rectal palpation. J Wildl Manag 31:145–149

Griffin PC, Bienen L, Gillin CM, Mills LS (2003) Estimating pregnancy rates and litter size in snowshoe hares using ultrasound. Wildl Soc Bull 31:1–7

Holekamp KE, Sherman PW (1989) Why male ground squirrels disperse. Am Sci 77:232–239

Hoogland JL (1995) The black-tailed prairie dog: social life of a burrowing mammal. University of Chicago Press, Chicago

Hoogland JL (1996) Why do Gunnison's prairie dogs give antipredator calls? Anim Behav 51:871–880

Hoogland JL (1997) Duration of gestation and lactation for Gunnison's prairie dogs. J Mammal 78:173–180

Hoogland JL (1998) Estrus and copulation of Gunnison's prairie dogs. J Mammol 79:887–897

Hoogland JL (1999) Philopatry, dispersal, and social organization of Gunnison's prairie dogs. J Mammal 80:243–251

Hoogland JL (2001) Black-tailed, Gunnison's, and Utah prairie dogs reproduce slowly. J Mammal 82:917–927

Hoogland JL (2013a) Prairie dogs disperse when all close kin have disappeared. Science 339:1205–1207

Hoogland JL (2013b) Why do female prairie dogs copulate with more than one male? Insights from long-term research. J Mammal 94: 731–744

Inzani EL, Marshall HH, Sanderson JL, Nichols HJ, Thompson FJ, Kalema-Zikusoka G, Hodge SJ, Cant MA, Vitikainen EI (2016) Female reproductive competition explains variation in prenatal investment in wild banded mongooses. Sci Rep 6:20013

Knowles CJ (1987) Reproductive ecology of black-tailed prairie dogs in Montana. Great Basin Naturalist 47:202–206

Knowles CJ (2002) Status of white-tailed and Gunnison's prairie dogs. National Wildlife Federation, Missoula, MT and Environmental Defense, Washington, DC, pp 30

Kotliar NB, Miller BJ, Reading RP, Clark TW (2006) The prairie dog as a keystone species. In: Hoogland JL (ed) Conservation of the Black-Tailed Prairie Dog. Island Press, Washington, pp 53–64

Mahmoud MA (2017) A review on non-invasive pregnancy diagnosis in wild cats. J Dairy Vet Sci 1:1–3

McKeever S (1964) Seasonal changes in body weight, reproductive organs, pituitary, adrenal glands, thyroid glands, and spleen of the Belding ground squirrel (*Citellus beldingi*). Am J Anat 113:153–173

McNay ME, Stephenson TR, Dale BW (2006) Diagnosing pregnancy, in utero litter size, and fetal growth with ultrasound in wild, free-ranging wolves. J Mammol 87(1):85–92

Milner JM, van Beest FM, Solberg EJ, Storaas T (2013) Reproductive success and failure: the role of winter body mass in reproductive allocation in Norwegian moose. Oecologia 172:995–100

Radcliffe RW, Czekala NM, Osofsky SA (1997) Combined serial ultrasonography and fecal progestin analysis for reproductive evaluation of the female white rhinoceros (*Ceratotherium simum simum*): Preliminary Results. Zoo Biol 16:445–456

Rayor LS (1985) Dynamics of a plague outbreak in Gunnison's prairie dog. J Mammal 66:194–196

Rayor LS (1988) Social organization and space-use in Gunnison's prairie dog. Behav Ecol Sociobiol 22:69–78

Rausch RA (1967) Some aspects of the population ecology of Wolves, Alaska. American Zoologist 7(2):253–265

Seglund AE, Schnurr PM (2010) Colorado Gunnison's and white-tailed prairie dog conservation strategy. Colorado Division of Wildlife, Denver

Seglund AE, Ernst AE, O'Neil DM (2006) Gunnison's prairie dog conservation assessment. Western Association Fish and Wildlife Agencies. Laramie, Wyoming. vii + 84 page

Sikes RS, The Animal Care and Use Committee of the American Society of Mammalogists (2016) Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J Mammal 97:663–688

Smith RB, Lindzey FG (1982) Use of ultrasound for detecting pregnancy in mule deer. J Wildl Manag 46:1089–1109

Stephenson TR, Testa JW, Adams GP, Sasser RG, Schwartz CC, Hundertmark KJ (1995) Diagnosis of pregnancy and twinning in moose by ultrasonography and serum assay. Alces 31:167–172

Sweitzer RA, Holcombe DW (1993) Serum-progesterone levels and pregnancy rates in great basin porcupines (*Erethizon dorsatum*). J Mammal 74:769–776