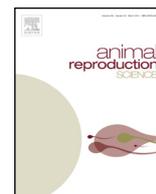




ELSEVIER

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Nutritional effects on reproductive performance of captive adult female coyotes (*Canis latrans*)

Eric M. Gese^{a,*}, Beth M. Roberts^{b,1}, Frederick F. Knowlton^a^a United States Department of Agriculture, Wildlife Services, National Wildlife Research Center, Department of Wildland Resources, Utah State University, Logan, UT 84322, USA^b Department of Wildland Resources, Utah State University, Logan, UT 84322, USA

ARTICLE INFO

Article history:

Received 27 October 2015

Received in revised form

16 December 2015

Accepted 22 December 2015

Available online 29 December 2015

Keywords:

Canis latrans

Coyote

Litter

Nutrition

Reproduction

Pup survival

ABSTRACT

Interactions between animals and their environment are fundamental to ecological research. Field studies of coyote (*Canis latrans*) reproductive performance suggest mean litter size changes in response to prey abundance. However, this relationship has been assessed primarily by using carcasses collected from trappers. The objective of this study was to assess whether nutritional manipulation prior to mating affected reproduction in adult female coyotes. We examined the effects of caloric restriction during the 7 months prior to estrus on the reproductive rates of 11 captive female coyotes and the subsequent initial survival of pups through two reproductive cycles. This was a 2-year study with a cross-over design so each female was monitored for reproductive performance on each of the two diet treatments. We assessed the number of implantation scars, number of pups born, sex ratios of pups, average pup weight at birth and 2- and 6-weeks of age, and the survival rates between implantation and 2-weeks of age for two diet treatments. We found the mean number of implantation sites and pups whelped during a reproductive cycle was influenced by food-intake prior to conception. Additionally, we found evidence suggesting the effects of nutritional stress may persist for additional breeding cycles. We also provided evidence suggesting well-fed females tended to have more male pups. Understanding how environmental factors influence reproductive output may improve model predictions of coyote population dynamics.

Published by Elsevier B.V.

1. Introduction

Understanding the interaction between animals and their environment is a central tenet of ecological study. How environments influence an animal's reproductive performance and subsequent fitness is of interest to ecologists. Population demographics and reproductive parameters of

coyotes (*Canis latrans*) have been studied throughout the United States and Canada using carcasses collected from hunters (e.g., Gier, 1968; Knowlton, 1972; Todd et al., 1981; Todd and Keith, 1983; Windberg, 1995; Bartel and Knowlton, 2005). Reproductive parameters from these studies suggested the proportion of females ovulating and becoming pregnant was positively correlated with prey abundance and negatively correlated with coyote density for both multiparous and primiparous females (Gier, 1968; Knowlton, 1972; Todd and Keith, 1983; Windberg, 1995; Sacks, 2005). Reproduction among primiparous females was especially influenced by prey abundance and coyote density, with the percentage of such females

* Corresponding author. Fax: +1 435 797 3796.

E-mail address: eric.gese@usu.edu (E.M. Gese).¹ Present address: Memphis Zoo, 2000 Prentiss Place, Memphis, TN 38112, USA.

ovulating and becoming pregnant ranging from <10% to >80% among years differing in prey abundance and coyote density; mean litter size was influenced as well. These studies indicated changes in mean litter size of primiparous females and adults in some locations (Alberta and Kansas) was significantly correlated to prey abundance (Gier, 1968; Todd and Keith, 1983), while litter size of adult females in other locations, such as a high coyote density area in Texas, was not correlated to prey abundance (Windberg, 1995).

Litter size is a complex process dependent on interactions among many factors. Determining direct correlations and mechanisms of reproductive change is difficult using one-time carcass sampling; hence we employed captive studies to try to better understand different variables on reproductive output in coyotes. Results from individual-based population modeling suggested litter size and juvenile survival were the predominant parameters influencing population flux in coyote populations (Pitt et al., 2003). Understanding how environmental and behavioral factors influence reproductive output of coyotes may provide more complete information to improve future model predictions of coyote population dynamics.

Coyotes are territorial, monogamous (Bekoff and Wells, 1986; Gese, 2001), and seasonally monestrous (Hamlett, 1938; Gier, 1968; Kennelly and Johns, 1976) and generally successful breeding attempts are limited to the dominant mate pairs within a territory (Camenzind, 1978; Kleiman and Brady, 1978; Bekoff and Wells, 1986; Hodges, 1990). One captive study found subordinate females were reproductively limited by physical or psychological suppression from sexual activity, with subordinate behavior leading to failure to express overt estrus behavior or even ovulate in the presence of a dominant female (Hodges, 1990). These findings along with the aforementioned correlations between ovulation rates and social and nutritional stress suggested the “decision” to ovulate, especially for primiparous animals, may depend on both opportunity and a threshold energy balance. “Deciding” to ovulate (i.e., reproduce) as well as “determining” the number of ova to shed may be under the control of different mechanisms or thresholds. Litter size may be affected by many factors including genetics, age (Green et al., 2002; Sacks, 2005), stress (Chatterjee and Chatterjee, 2009; Sayles, 1984), parental food environment (Mech et al., 1991; Plaistow et al., 2006), and the current food environment (I'Anson et al., 1991; Lucy, 2003). The influence of nutrition on reproduction has long been recognized, but the mechanisms are not fully understood even in highly studied domestic animals. Although there are numerous studies relating prey abundance to reproductive fitness of wild coyotes, and captive research has explored the effects of poor nutrition during pregnancy and lactation on litter size and maternal physiology, there has been a paucity of research investigating the effects of nutritional plane prior to mating on reproductive output.

The objective of our study was to assess whether nutritional manipulation prior to mating affected reproduction in adult female coyotes. Ovulation rate, oocyte maturation, embryo survival, and implantation rates in livestock (Cox, 1997; Lucy, 2003; Kosior-Korzecka and Bobowiec, 2006; Scaramuzzi et al., 2006) were affected by nutritional

manipulation during the follicular phase prior to ovulation. But unlike these domestic animals, the coyote is seasonally monestrous having only one estrous cycle per year consisting of extended follicular and luteal phases followed by several months of anestrus. Follicular dynamics of the dog are poorly understood, but results suggest the follicles grow and change during late anestrus (Okkens and Kooistra, 2006) and both numbers and size of follicles continue to increase throughout proestrus (Concannon, 2011). Because the estrous cycle is likewise extended in the coyote and follicular dynamics likely influence reproductive output during the proximal breeding season, we chose to apply the experimental diets from July through mid-February in order to include late anestrus through estrus into the time period of nutritional manipulation. We examined the effects of a calorie restricted diet during the period 7 months prior to estrus on the reproductive rates of 11 captive female coyotes through two reproductive cycles, incorporating a crossover design so each female was subjected to each of the two diet treatments. Several parameters were assessed including the number of implantation scars, number of pups born, sex ratios of pups, average pup weight at birth, 2- and 6-weeks of age, and the survival rates between implantation and 2-weeks of age for the two diet treatments.

2. Materials and methods

2.1. Animal subjects

The role of caloric food intake on reproductive outcome was assessed for 11 captive-born female coyotes. Subjects were provided by the USDA National Wildlife Research Center (NWRC) facility where they were born and hand-reared. Each female was permanently identified with uniquely numbered ear tags. All females were from the same cohort, 3–4 years of age during the study, and were multiparous. Initially, we intended to use 12 coyote pairs from a cohort of animals and proven breeders, but one pair failed to produce pups or implantation scars for two breeding seasons prior to the start of this study and hence censored from the study. A suitable replacement was not available. Each female was paired with a hand-reared, non-sibling male from the same cohort.

2.2. Facilities and animal care

The coyotes were housed alternately in pens or kennels at the NWRC facilities near Millville, Utah. The coyotes were hand-reared in outdoor pens. Six months prior to the normal breeding period, the coyotes were transferred and housed individually in kennels (1.2 × 1.8 × 3.0 m). Each kennel included a den box and a “lix-it” fixture for continuous water availability. In January, prior to the breeding period, coyotes were placed in 0.1 ha outdoor pens, as non-sibling male/female pairs allowing them to mate. The outdoor pens contained shade shelters, den boxes, and had water available ad libitum. In mid-May, after the whelping season was over and pups were more than 6-weeks of age, the pairs were separated and returned to the kennels. Females were paired with the same male each year

of the study. Animal care and use guidelines of the American Society of Mammalogists were followed (Sikes and Gannon, 2011), and research protocols were approved by the Institutional Animal Care and Use Committees at Utah State University and the National Wildlife Research Center.

2.3. Diet treatments

Two diet levels were used to assess the effects of reduced food intake on reproductive fitness. In mid-May, 6-weeks prior to the start of the experimental treatments, the 11 females and their mates were moved to the kennels to allow for acclimation to housing, handling, and dry food pellets; the males were housed in kennels adjacent to their female mates. The females were converted to the dry dog food (Black Gold Premium Dog Food, Vienna, Missouri) over a 2-week period. The females were started on the differential diet treatments after 4 additional weeks of acclimation. In the first week of July, each of the 11 females was assigned to one of two dietary treatments. A female received either the “high diet,” 440 g of dry dog food (110% of a maintenance diet), or the “low diet,” 260 g of dry dog food (65% of maintenance); 400 g of dry dog food was considered to be the maintenance diet based upon the energetic requirements of a female coyote and the caloric information provided by the manufacturer. Females were handled routinely to perform body condition assessments and collect serum.

Females were paired with their respective mates in 0.1 ha pens in mid-January for the breeding season. The differential diet treatments were continued until all pairs ceased mating, and then pairs were converted to normal feeding regimes for pregnant and lactating animals in accordance with standard animal care practices at the facility. A second year of the study was repeated with a cross-over design. Group 1 ($n=6$) was fed the low diet the first year and the high diet the second year, while Group 2 ($n=5$) was fed the high diet the first year and the low diet the second year.

2.4. Mating behavior

Observations of mating activity (i.e., copulatory ties; Bekoff and Diamond, 1976) were recorded from mid-January through March, with routine observations of the 11 coyote pairs conducted each day for 2 h near dawn and dusk (total of 4 h/day). Copulatory ties observed for each pair were recorded. Once mating activity had been recorded for a pair, the female was considered to have ended estrus if one week passed without any further mating activity observed. The expected whelping date was calculated as 62 days after the last day that ties were recorded (Hamlett, 1938; Kennelly, 1978).

2.5. Offspring assessment

Observations for parturition behavior were increased during the expected whelping period of each pair. Three days after whelping was documented, pups were counted, sexed, and weighed. At 2 weeks of age, litters were reassessed: counted, sexed, weighed, and number of pups

with eyes open recorded. At this time, litters were standardized for both treatment groups to 4 pups: 2 males and 2 females. This standardization was done to minimize litter-size effects on growth rates between 2 weeks and 6 weeks. At 6 weeks of age, the pups were again counted, sexed, and weighed. The following June or July of each year, implantation scars on the uterus were counted via laparotomy (Green et al., 1979; Kennelly et al., 1977).

2.6. Statistical analysis

We made statistical comparisons of the reproductive performance, including number of implantation sites, number of pups whelped, 2-week survival rates and growth rates of pups whelped from females fed the high diet versus the low diet prior to conception. All but one female produced live pups both years. Abdominal examination of the female that failed to produce live pups confirmed the female to be pregnant one week prior to her exhibiting denning and whelping behavior. We presumed the pups were born, but not found versus being reabsorbed as fetuses, therefore we chose to use the scar counts of this female for statistical analysis for both pup and scar counts. Obviously this litter was not sexed or weighed, so the diet group sample sizes for the 2 years were 10 and 11 females for the high and low diets, respectively, for the gender and weight analyses.

All ANOVA models were fitted using either mixed or GLIMMIX procedure in SAS software (SAS Version 9.2 for Windows). The effects of diet sequence and year on litter size, number of male pups, number of female pups, and number of implantation scars were assessed using a generalized linear mixed model, with a two-way factorial (diet sequence and year) in a split-plot design for each response. The whole plot unit was a female coyote; the whole plot factor was diet sequence. The subplot unit was an annual period for a female coyote; the subplot factor was year. Several pertinent covariance structures for repeated measures through time were assessed based on the small-sample-size corrected version of Akaike Information Criterion (AICc); for all the counts, standard variance component structure provided the best fit. All counts were square root-transformed prior to analysis to better meet assumptions of normality and homogeneity of variance. The interaction between diet sequence and year was also assessed and this interaction represented the diet effect on the dependent variables. The models were fitted using the GLIMMIX procedure.

The effects of diet sequence, year, and diet sequence \times year interactions on average 3-day old pup weight, average 2-week old pup weight, and 6-week old pup weights were assessed using a generalized linear mixed model, with a two-way factorial (diet sequence and year) in a split-plot design, for each response. We analyzed pup weights at 3 days, 2 weeks, and 6 weeks in separate split-plot ANOVA models, instead of running repeated measures for weight within the split-plot ANOVA because loss of litters would cause the ANOVA to drop whole subjects from the analysis, and the numbers of pups were standardized at 2-weeks. Standard variance component structure provided the best covariance structure fit. All

Table 1

Total and mean (\pm SE) number of scars, pups, and male and female pups produced by female coyotes on high and low diets. Different letter within column denotes significance ($P < 0.05$).

Total	# Scars	# Pups	# Males	# Females
Low diet	56	53	24	29
High diet	70	66	33	26
Total	126	119	57	55
Mean	Scars	Pups	Male pups	Female pups
Low diet	5.1 \pm 0.31 ^a	4.8 \pm 0.26 ^a	1.2 \pm 0.22 ^a	2.6 \pm 0.34
High diet	6.4 \pm 0.34 ^b	6.0 \pm 0.50 ^b	3.3 \pm 0.49 ^b	2.6 \pm 0.50

pup weight measurements were log-transformed prior to analysis to better meet assumptions of normality and homogeneity of variance. The models were fitted using the GLMIX procedure. Counts are reported as mean \pm SE.

3. Results

3.1. Diet effect on reproductive timing and behavior

All females were observed in at least one copulatory tie and exhibited denning and whelping behavior each trial year. Dietary plane did not appear to affect the timing of estrus or parturition. All pairs mated between January 20 and February 20 during both years with an average peak tie date of February 3 the first experimental year and January 30 the second year. The average whelping date was April 4 the first year, and March 29 the second year. During the first year, parturition dates of the females were in a 2-week time period (March 30–April 15), while during the second year, parturition dates covered a 1-month period (March 12–April 20).

3.2. Reproductive output

All study subjects became pregnant, as determined by the presence of pups at the den or laparoscopic visualization of recent implantation scars on the uterus. Reduced food intake affected the implantation and whelping rate. Females fed the high diet had more fetuses implanted than the low diet (Table 1) with the mean number of fetuses implanted being significantly more for females on the high diet than females of the low diet (Tables 1 and 2; Seq \times Year [Diet]: $F_{1,9} = 22.98, P = 0.001$). This higher implantation rate carried over to litter size with females on the high diet whelping 66 pups and females on the low diet whelping fewer pups (Table 1). Mean litter size was significantly reduced by food restriction with smaller litter sizes for females on the low diet and larger litters for females on the high diet (Tables 1 and 2; Seq \times YR [Diet]: $F_{1,9} = 6.39, P = 0.03$).

In the pregnancies in which gender was determined, more male pups were whelped when the subjects were fed the high diet (Tables 1 and 2; $F_{1,17} = 4.48, P = 0.05$). Diet did not affect the number of female pups whelped per litter (Tables 1 and 2; $F_{1,84} = 0.05, P = 0.83$). Overall, the high diet females produced 27% more male pups, and the litter sex ratio was skewed towards males (0.55) when females were fed the high diet versus the low diet (0.45).

Table 3

Average pup weight (g) at 3-days, 2-weeks, and 6-weeks of age for observation year 1, year 2, and both years combined. Different letters represent values that were significantly different ($P < 0.05$) using a split-plot ANOVA model.

Year	Seq group	3 days old	2-weeks old	6-weeks old
Low yr 1	Low:high	314 \pm 10	748 \pm 40	2,362 \pm 110
High yr 1	High:low	288 \pm 30	609 \pm 70	2,309 \pm 50
Low yr 2	High:low	308 \pm 20	732 \pm 90	2,370 \pm 110
High yr 2	Low:high	308 \pm 30	675 \pm 50	2,331 \pm 100
Low both yrs		311 \pm 10	741 \pm 50 ^a	2,366 \pm 80
High both yrs		298 \pm 20	642 \pm 40 ^b	2,320 \pm 60

Table 4

Fixed effects for offspring weights at 3-days, 2-weeks, and 6-weeks of age produced by female coyotes on two diet regimes.

Effect	3-days		2-weeks		6-weeks	
	F-value _(df)	P	F-value _(df)	P	F-value _(df)	P
Seq	0.28 _(1,9,21)	0.609	0.76 _(1,9,17)	0.405	0.00 _(1,9,03)	0.996
Year	0.17 _(1,8,59)	0.692	0.19 _(1,7,94)	0.677	0.02 _(1,8,99)	0.887
Seq \times yr	1.22 _(1,8,59)	0.299	4.73 _(1,7,94)	0.062	0.16 _(1,8,99)	0.695

3.3. Offspring survival

Pup loss from implantation to 2-weeks of age was not different between diet treatments ($P > 0.05$). Over the two trial years, the total number of pups whelped accounted for 94% of the implantation scars (119 pups from 126 scars, Table 1). The females successfully reared 104 pups from whelping to 2-weeks of age (87% survival).

Offspring weights

Mean pup weight at 3 days of age was not different between diet treatments (3-day old mean \pm SE, weight: 311 \pm 10 and 298 \pm 26 g, low and high diets, respectively; $F_{(1,8,59)} = 1.22, P = 0.30$; Tables 3 and 4). Mean pup weight at 2-weeks post-whelping tended to be higher for pups from the females fed the low diet versus the high diet females (2-week weight: 741 \pm 45 and 642 \pm 41 g, low and high diets, respectively; Tables 3 and 4; $F_{1,7,94} = 4.73, P = 0.06$). This difference in weight was likely due to the low diet females having smaller litters. To reduce the confounding effect of litter size on growth, litter sizes were standardized to 4 pups at 2-weeks of age. At 6-weeks of age, average pup weights were not different between treatment groups (Tables 3 and 4; $F_{1,8,99} = 0.16, P = 0.67$).

4. Discussion

The study's objective was to isolate nutritional stress prior to ovulation to determine if reduced caloric intake would affect estrus timing, reproductive behavior, and reproductive output of adult female coyotes. The results showed that even with access to male mates, denning space, and nutrition to maintain pregnancy, the reproductive outcome of a female coyotes were affected by a reduced calorie diet prior to ovulation. Mating, denning, and maternal care did not appear to be overtly affected by the reduced diet; however, the females had 20% fewer implantation scars and live pups when fed the reduced diet prior to

mating. Interestingly, when fed the high diet the females produced 27% more male pups, skewing the sex ratio (0.55 versus 0.45, males versus females). Fetal reabsorption and low pup survival has been documented in coyotes nutritionally stressed during pregnancy and lactation (Sayles, 1984). In the current study, females were fed a pregnancy and lactation maintenance diet. We found that the females had low fetal reabsorption, with 94% of the implantation scars counted being represented by live pups, suggesting that the reduced litter size when fed the low diet was due to either a reduced ovulation or fertilization rates. Likewise pup survival rate to 2 weeks was high and similar for both groups.

Several field studies investigating the reproductive biology of coyotes report that the proportion of females ovulating and becoming pregnant was significantly affected by coyote density and prey abundance. In areas of low coyote density or during high prey abundance, 80–100% of adult females ovulated; whereas <50% ovulated in regions with high coyote density or during years with low prey abundance (Knowlton, 1972; Todd and Keith, 1983; Windberg, 1995). Field studies have also found evidence that prey abundance and coyote density influence litter size. Knowlton (1972) found females from low density populations had more fetal implantations per female than those from high density populations (6.6 versus 5.3 implantation scars per female, respectively). Likewise, Todd and Keith (1983) found that the mean number of ovulations per coyote female increased to 8.3 from 4.3 during years of high snowshoe hare abundance in Alberta versus when prey was scarce. Age and social status confound the effects of nutritional stress. A 10-year study of a high density coyote population in Webb County, Texas, found that although litter size was correlated to prey abundance for primiparous females, for adult females there was no clear correlation between litter size and changes in food abundance or coyote density (Windberg, 1995). Mean litter size appeared to be affected by changes in prey and coyote abundance but field studies were unable to isolate variables making it difficult to determine how influential nutrition alone was on litter size. High coyote density can affect both social stress and nutritional intake by increased competition and resource partitioning among pack members (Gese et al., 1996a,b).

The current study focused on the influence of caloric-intake on reproductive outcome in a captive environment. Mates and territorial space was provided to reduce the confounding influence of density and social stress. We found that the restricted diet prior to estrus did not affect the proportion of females mating and ovulating, but reduced mean litter size. These findings were comparable to the

reduction in corpora lutea and implantations observed in wild coyote during years of low prey abundance (Gier, 1968; Todd and Keith, 1983; Windberg, 1995). Our results indicated the reduced diet did not affect the “decision to ovulate” but effectively changed “how many” pups were produced. These two “decisions” may be influenced at different caloric thresholds and the low diet in this study was not restrictive enough to mimic the nutritional deficiency experienced by wild coyotes associated with a reduction in the proportion of females ovulating. Alternatively, procuring a mate and interacting in courtship behavior may play a permissive role for proestrus and estrus, and ultimately ovulation. A mate influence on coming into estrus and ovulating may be especially influential for peripubertal and nutritionally stressed females. An experimental group isolated from mates during nutritional manipulations would be needed to test this hypothesis.

After ovulation, maternal nutritional status can also affect embryo and fetal survival and influence live litter size (Almeida et al., 2001; Robinson, 1990; Sayles, 1984; Van Lunen and Aherne, 1987). The number of implantation scars represent the maximum number of pups expected from a female in that cycle; therefore a discrepancy between implantation scars and the number of pups whelped would be indicative of the reabsorption of embryos or fetuses, still births, or cannibalized litters (Green et al., 1979). A captive study (Sayles, 1984) investigating the effect of maternal diet during pregnancy on fetal survival found that female coyotes fed 1200 g, 800 g, or 500 g of ground meat during pregnancy after implantation had 75%, 65%, and 30% of the implantations accounted for at whelping, respectively (maintenance diet = 700 g). In the current study, all females were fed a pregnancy maintenance diet after estrus ended. We found that there was not a significant change from implantation to whelping in either diet group. This suggested the observed reduction in litter size was due to reduced ovulation rates or embryo survival prior to implantation rather than fetal reabsorption after implantation. The current captive study and previous field studies (Gier, 1968; Todd and Keith, 1983; Windberg, 1995) strongly suggested that coyotes have physiological mechanisms to incrementally change litter size before and after implantation in accordance to current food resources.

Reduced maternal nutrition prior to pregnancy may affect fetal and early postnatal growth and survival; therefore, pup growth was monitored through 6-weeks post-parturition. We found that females produced larger litters after the high diet regimen than the low diet but average pup weight at birth was the same. The reduction in litter size after restricted maternal food-intake did not appear to be compensated with increased fetal growth and

Table 2

Fixed effects and covariance parameter estimates for the numbers of pups, implantation scars, male and female pup counts, for the reproductive output of captive females on two diet regimes.

Effect	Scars		Pups		Male pups		Female pups	
	F-value _(df)	P	F-value _(df)	P	F-value _(df)	P	F-value _(df)	P
Seq	2.62 _(1,9)	0.14	4.11 _(1,9)	0.07	4.77 _(1,17)	0.04	0.17 _(1,9.13)	0.69
Year	4.94 _(1,9)	0.05	1.65 _(1,9)	0.23	1.76 _(1,17)	0.20	0.00 _(1,8.84)	0.96
Seq × yr	22.98 _(1,9)	0.001	6.39 _(1,9)	0.03	4.48 _(1,17)	0.05	0.05 _(1,8.84)	0.83

higher birth weights but may have allowed for the pups produced to reach an ideal birth weight and increase survival probability. Pups from smaller litters gained weight faster during the first 2-weeks, but after the litter sizes were standardized to 4 pups per female at 2-weeks, average pup weight at 6-weeks was not different between treatment groups indicating reduced food competition of smaller litters allowed for increased growth rate. Overall, pup survival was not affected by maternal nutrition prior to estrus. The negative correlation of litter size to weight gain often does not persist after weaning (Epstein, 1978; Huber et al., 2011) so the pup monitoring was concluded as the pups began to switch to solid food.

Interestingly, the pup counts collected over two trial years suggested the effect of diet restriction on reproductive output may extend into subsequent breeding season(s). The sequence in which the diets were administered was found to be significant for implantation rates (Table 2). Females had similar implantation, live pup, and male pup counts when each group was treated with the low diet but the same was not found for the high diet. Females treated with a high diet the first year and low diet the second year (Sequence High:Low, Group 1) had a marked decrease in the implantation rate between treatments with mean scar count decreasing from 7.2 ± 0.5 to 5.2 ± 0.4 ; whereas the females fed the low diet the first year and the high diet the second year (Sequence Low:High, Group 2) only increased from 4.9 ± 0.4 to 5.6 ± 0.4 scars. Both groups of females had more implantations and pups when fed the high diet versus the low diet, but the smaller variation in scar and pup counts between diets for females in group 2 suggested that the initial low diet may have had a carry-over effect on litter size for these females.

The sequence in which the diet was administered also appeared to affect gender counts among the litters. Females in Group 1 (High:Low, $n=5$) produced a mean of 4.2 ± 0.57 males per litter the first year and 2.4 ± 0.43 males per litter the second year; whereas, females in Group 2 (Low:High, $n=6$) produced similar numbers of males per litter both years, 2.0 ± 0.36 and 2.4 ± 0.44 . The number of female pups was similar for both groups for both years. This time sequence suggested the coyote reproductive system exhibited an acute down regulation in response to proximate food stress, while displaying evidence of slower recovery after the restoration of diet. Additionally, the sequential results also suggested coyote females nutritionally stable enough to produce large litters may augment their litters with more males. Trivers and Willard (1973) hypothesized that mammalian parents would gain fitness if they were able to skew offspring sex ratio as environmental factors changed. They predicted the production of more males during times of high maternal social dominance or good body condition (Trivers and Willard, 1973). Although we observed an increase in the production of male pups in one year of the experiment, further investigation of sex-allocation in the coyote would be needed before it could be concluded that female coyotes exhibit sex-allocations as predicted by Trivers and Willard (1973).

In conclusion, we found that the mean number of fetuses implanted during a reproductive cycle was sensitive to reduced food-intake prior to conception. Additionally, we

found evidence that the effects of nutritional stress may persist for additional breeding cycles. We also found evidence that well-fed females tended to have more male pups. More definitive results may have been gleaned if several years of high nutrition were observed after the low diet was administered to determine if the effect did indeed persist for both groups and for how long the effect might persist. Also, because females typically increase litter size until the third breeding season (Green et al., 2002), waiting until the fourth reproductive year to start treatments would have given us one untreated year (i.e., baseline) with which to compare subsequent nutritional manipulations. Understanding how environmental factors influence reproductive output of the coyote should provide more complete understanding and allow improved model predictions of coyote population dynamics.

Conflict of interest

None of the authors have a financial or personal relationship with other people or organizations that could bias their work.

Acknowledgements

Funding and logistical support provided by the U.S. Department of Agriculture, Wildlife Services, National Wildlife Research Center, Logan Field Station, Utah. We thank K. Grant for her care and diligence in hand-rearing all coyotes used in this study, and the staff and students at the Millville Predator Research Facility, especially S. Brummer, T. Christensen, P. Darrow, L. Gilbert-Norton, J. Schultz, and E. Stevenson for assistance with animal care and observations.

References

- Almeida, F.R.C.L., Mao, J., Novak, S., Cosgrove, J.R., Foxcroft, G.R., 2001. Effects of different patterns of feed restriction and insulin treatment during the luteal phase on reproductive, metabolic, and endocrine parameters in cyclic gilts. *J. Anim. Sci.* 79, 200–212.
- Bartel, R.A., Knowlton, F.F., 2005. Functional feeding responses of coyotes, *Canis latrans*, to fluctuating prey abundance in the Curlew Valley, Utah, 1977–1993. *Can. J. Zool.* 83, 569–578.
- Bekoff, M., Diamond, J., 1976. Precopulatory and copulatory behavior in coyotes. *J. Mammal.* 57, 372–375.
- Bekoff, M., Wells, M.C., 1986. Social ecology and behavior of coyotes. *Adv. Stud. Behav.* 16, 251–338.
- Camenzind, F., 1978. Behavioral ecology of coyotes on the National Elk Refuge, Jackson, Wyoming. In: Bekoff, M. (Ed.), *Coyotes: Biology, Behavior, and Management*. Academic Press, New York, pp. 267–294.
- Chatterjee, A., Chatterjee, R., 2009. How stress affects female reproduction: an overview. *Biomed. Res.* 20, 79–83.
- Concannon, P.W., 2011. Reproductive cycles of the domestic bitch. *Anim. Repro. Sci.* 124, 200–210.
- Cox, N.M., 1997. Control of follicular development and ovulation rate in pigs. *J. Repro. Fert. Suppl.*, 52.
- Epstein, H.T., 1978. The effect of litter size on weight gain in mice. *J. Nutr.* 108, 120–123.
- Gese, E.M., 2001. Territorial defense by coyotes (*Canis latrans*) in Yellowstone National Park, Wyoming: who, how, where, when, and why. *Can. J. Zool.* 79, 980–987.
- Gese, E.M., Ruff, R.L., Crabtree, R.L., 1996a. Foraging ecology of coyotes (*Canis latrans*): the influence of extrinsic factors and a dominance hierarchy. *Can. J. Zool.* 74, 769–783.
- Gese, E.M., Ruff, R.L., Crabtree, R.L., 1996b. Social and nutritional factors influencing the dispersal of resident coyotes. *Anim. Behav.* 52, 1025–1043.

- Gier, H.T., 1968. Coyotes in Kansas. In: *Agri. Exper. Stat. Kan. State Univ., Agri. App. Sci.*, Manhattan, KS, USA.
- Green, J.S., Barnum, D.A., Gates, N.L., Flinders, J.T., 1979. Coyote laparotomy: in vivo determination of reproductive success. *Theriogenology* 11, 291–296.
- Green, J.S., Knowlton, F.F., Pitt, W.C., 2002. Reproduction in captive wild-caught coyotes (*Canis latrans*). *J. Mamm.* 83, 501–506.
- Hamlett, G.W.D., 1938. The reproductive cycle of the coyote. *USDA Tech. Bull.* 616, 1–11.
- Hodges, C.M., 1990. The Reproductive Biology of the Coyote (*Canis latrans*). PhD Dissertation. Texas A&M University, College Station, TX, USA.
- Huber, S., Hoffmann, I.E., Millesi, E., Dittami, J., Arnold, W., 2011. Explaining the seasonal decline in litter size in European ground squirrels. *Ecography* 24, 205–211.
- l'Anson, H., Foster, D.L., Foxcroft, G.R., Booth, P.J., 1991. Nutrition and reproduction. *Oxf. Rev. Repro. Bio.* 13, 239–311.
- Kennelly, J.J., Johns, B.E., 1976. The estrous cycle of the coyote. *J. Wildl. Manage.* 40, 272–277.
- Kennelly, J.J., 1978. Coyote reproduction. In: Bekoff, M. (Ed.), *Coyotes: Biology, Behavior, and Management*. Academic Press, New York, pp. 73–93.
- Kennelly, J.J., Johns, B.E., Breidenstein, C.P., Roberts, J.D., 1977. Predicting female coyote breeding dates from fetal measurements. *J. Wildl. Manage.* 41, 746–750.
- Kleiman, D.G., Brady, C.A., 1978. Coyote behavior in the context of recent canid research: problems and perspectives. In: Bekoff, M. (Ed.), *Coyotes: Biology, Behavior, and Management*. Academic Press, New York, pp. 163–186.
- Knowlton, F.F., 1972. Preliminary interpretations of coyote population mechanics with some management implications. *J. Wildl. Manage.* 36, 369–382.
- Kosior-Korzecka, U.R., Bobowiec, C.L., 2006. Fasting-induced changes in ovulation rate, plasma leptin, gonadotropins, GH, IGF-1 and insulin concentrations during oestrus in ewes. *J. Vet. Med.* 53, 5–11.
- Lucy, M.C., 2003. Mechanisms linking nutrition and reproduction in postpartum cows. *Repro. Suppl.* 61, 415–427.
- Mech, L.D., Nelson, M.E., McRoberts, R.E., 1991. Effects of maternal and grandmaternal nutrition on deer mass and vulnerability to wolf predation. *J. Mamm.* 72, 146–151.
- Okkens, A.C., Kooistra, H.S., 2006. Anoestrus in the dog: a fascinating story. *Repro. Domestic Anim.* 41, 291–296.
- Pitt, W.C., Box, P.W., Knowlton, F.F., 2003. An individual-based model of canid populations: modelling territoriality and social structure. *Ecol. Modell.* 166, 109–121.
- Plaiستow, S.J., Lapsley, C.T., Benton, T.G., 2006. Context-dependent intergenerational effects: the interaction between past and present environments and its effect on population dynamics. *Am. Nat.* 167, 206–215.
- Robinson, J.J., 1990. Nutrition in the reproduction of farm animals. *Nut. Res. Rev.* 3, 253–276.
- Sacks, B.N., 2005. Reproduction and body condition of California coyotes (*Canis latrans*). *J. Mamm.* 86, 1036–1041.
- Sayles, N.D., 1984. The Effect of Nutrition on Maternal Behavior Among Captive Coyotes. MS Thesis. Utah State University, Logan, UT, USA.
- Scaramuzzi, R.J., Campbell, B.K., Downing, J.A., Kendall, N.R., Khalid, M., Munoz-Gutierrez, M., Somchit, A., 2006. A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. *Repro. Nut. Dev.* 46, 339–354.
- Sikes, R.S., Gannon, W.L., 2011. Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J. Mamm.* 92, 235–253.
- Todd, A.W., Keith, L.B., 1983. Coyote demography during a snowshoe hare decline in Alberta. *J. Wildl. Manage.* 47, 394–404.
- Todd, A.W., Keith, L.B., Fischer, C.A., 1981. Population ecology of coyotes during a fluctuation of snowshoe hares. *J. Wildl. Manage.* 45, 629–640.
- Trivers, R.L., Willard, D.E., 1973. Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179, 90–92.
- Van Lunen, T.A., Aherne, F.X., 1987. Effect of long-term feed restriction on age at puberty of gilts. *Can. J. Anim. Sci.* 67, 797–801.
- Windberg, L.M., 1995. Demography of high-density coyote populations. *Can. J. Zool.* 73, 942–954.