

# Fetal development in wolves, *Canis lupus*, of the Keewatin District, Northwest Territories, Canada

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**Abstract:** Of 205 female wolves (*Canis lupus*) shot by Inuit hunters between 1987 and 1989 in the Keewatin District, Northwest Territories, Canada, 97 were parous, and 16 gravid females carried 73 identifiable fetuses. Fetuses grew at a mean rate of 5.17 g/day between day 32 post coitus and parturition. During the same period, fetuses increased in length at a mean rate of 0.204 cm/day. No significant sexual dimorphism in body mass or other morphological features was found at this stage of development. Cranio-caudal length ranged from 3 mm shortly after implantation to approximately 185 mm at parturition. All correlations of morphological parameters with cranio-caudal length were significant, and with the exception of humerus length and contour length, all parameters increased faster than cranio-caudal length. The results are discussed in relation to reproductive and developmental strategies in canids.

**Résumé :** Des 205 femelles de loup (*Canis lupus*) tuées par des chasseurs Inuit entre 1987 et 1989 dans le district de Keewatin, Territoires du Nord-Ouest, Canada, 97 avaient porté des petits et 16 femelles enceintes portaient 73 fœtus identifiables. Le taux moyen de croissance des fœtus était de 5,17 g/jour entre le jour 32 post coitus et la mise bas. Durant cette période, la croissance en longueur des fœtus se faisait à raison de 0,204 cm/jour en moyenne. À ce stade du développement, il n'y avait pas de dimorphisme sexuel entre mâles et femelles quant à la masse corporelle ou aux autres structures morphologiques des fœtus. La longueur cranio-caudale, évaluée à 3 mm peu après l'implantation, était de 185 mm au moment de la mise bas. Toutes les corrélations entre les variables morphologiques et la longueur cranio-caudale étaient significatives et, à l'exception de la longueur de l'humérus et du périmètre de la silhouette, toutes les variables avaient une croissance plus rapide que celle de la longueur cranio-caudale. Les résultats sont examinés en fonction des stratégies de reproduction et de développement chez les canidés.

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## Introduction

Fetal growth is an important component of the annual energy budget of female mammals, and litter mass and litter growth rate are valuable indicators of the magnitude of nutrient transfer from mother to young. Estimation of energy and nitrogen requirements in gravid canids depends upon a knowledge of the development of the uterus and fetuses (Ofstedal and Gittleman 1989). Numerous studies have been conducted on uterine and fetal development during gestation in the domestic dog, *Canis familiaris* (Concannon et al. 1975, 1977; Miller 1979; Noden and LaHunta 1986; Dore 1989); however, little research has been conducted on uterine and fetal development in wild canids (Kennelly et al. 1977; Wayne 1986).

To date, research on wolf developmental biology has focused on growth patterns post partum (Kuyt 1962; Van Ballenberge and Mech 1975; Mech 1980) and little work has been conducted on uterine and fetal development during gestation. The primary objective of this study was to describe uterine and fetal growth patterns in wolves from the central Canadian Arctic in relation to the reproductive strategies of canids.

## Materials and methods

Ninety-seven parous females were identified among 205 female wolves (*Canis lupus*) shot by Inuit hunters between 1987 and 1989 in the Keewatin District, Northwest Territories, Canada, between latitudes 61°07'N and 66°32'N. Parity was established by the presence of fetuses, corpora lutea/albicantia, and (or) the presence of placental scars. Sixteen of the reproductively active females carried identifiable fetuses. The reproductive system was severed at the cervix and the entire uterus and paired ovaries were removed and fixed in neutral buffered formalin. Ovaries were subsequently separated from Fallopian tubes, cleaned of adipose tissue, and weighed to the nearest 0.005 g on an Oertling top-loading balance. In addition, the lower left premolar was removed from each individual and sent to Matson's Laboratory (Milltown, Montana) for ageing using the cementum annuli technique (Johnson et al. 1987).

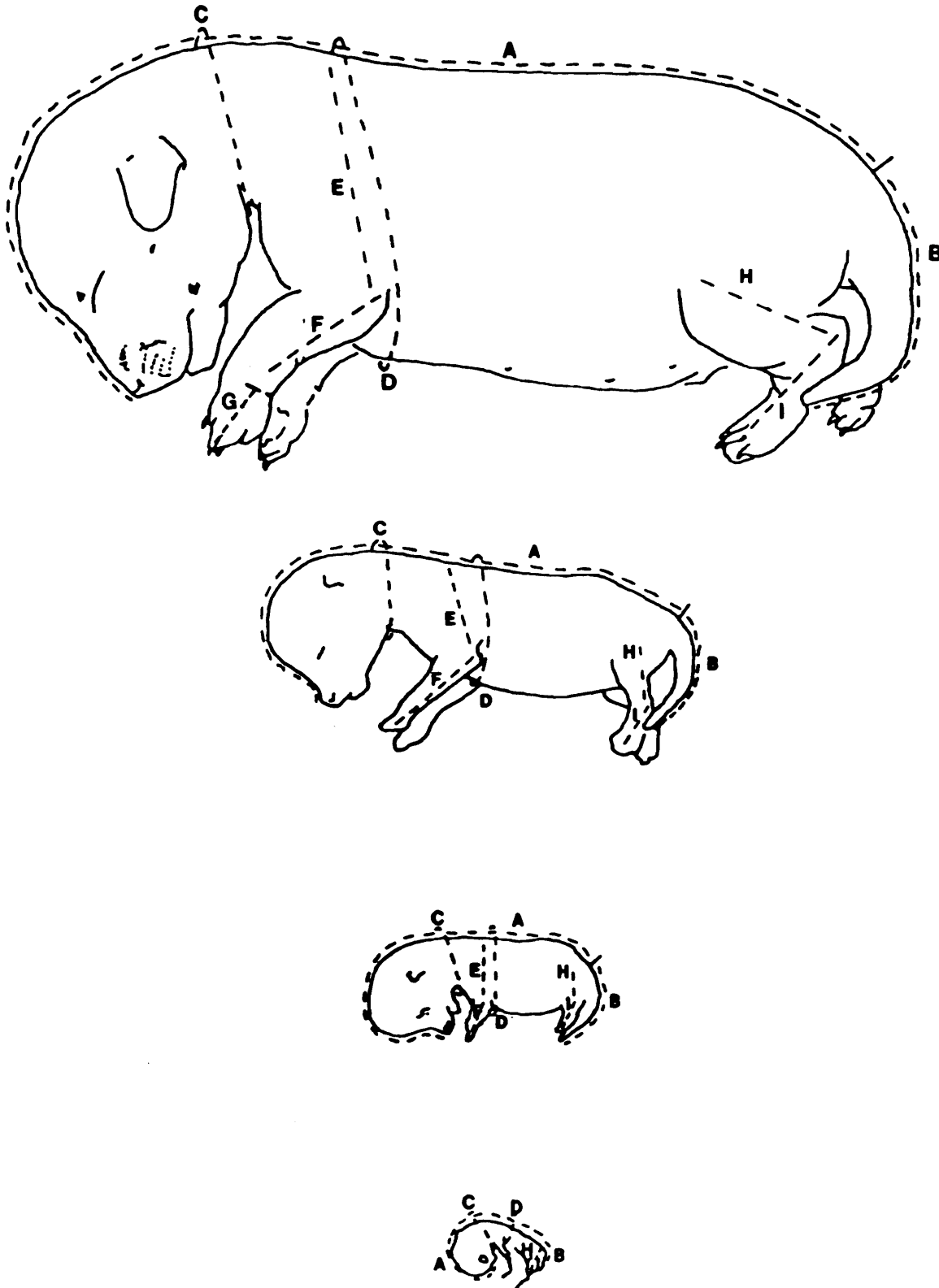
Fetuses were counted, cranio-caudal length was measured to the nearest 0.5 mm using a standard ruler, and individual fetuses were subsequently dissected. Fetal orientation (anterior or posterior) and position within the uterus (left or right uterine horn) were recorded for each female. Fetal measurements were made (to the nearest 0.5 mm) using vernier calipers and a metric ruler and included contour length, tail length, and girth and neck circumferences (Fig. 1). Total body mass (to the nearest 0.05 g) was also recorded using a Sartorius 1219 MP top-loading balance and Sartorius 2474 analytical balance. Where possible, sex, color, right foreleg length, and right hind leg length were determined. Student's *t* tests were conducted to establish whether sexual dimorphism existed in wolf fetuses at the same stage of development. Instances of interuterine mortality and fetal reabsorption were noted and recorded during dissection. Reabsorbed embryos were identified as uterine swellings that contained partial fetuses or undifferentiated tissue.

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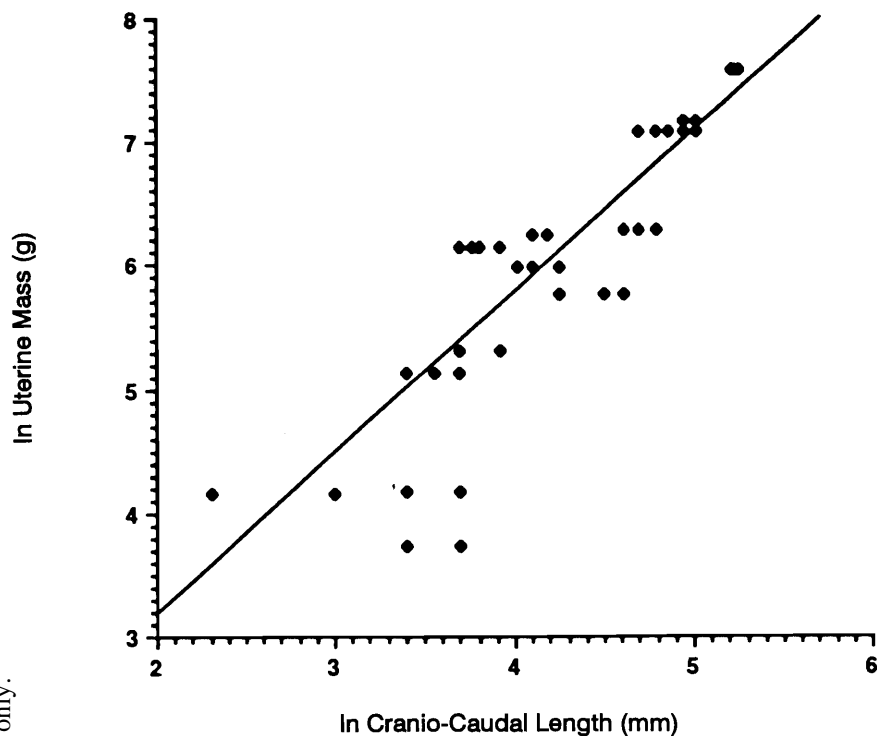
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**Fig. 1.** Standard morphometric measurements taken from fetuses. A, contour length (tip of the lip to the base of the tail); B, tail (base to the tip of the tail); C, neck (circumference at approximately the second cervical vertebra); D, girth (circumference at the sternum); E, humerus (shoulder to elbow); F, ulna/radius (elbow to wrist); G, front paw (tip of the longest phalanx to the metacarpal); H, femur (thigh to knee); I, hind leg (tip of the longest phalanx to the proximal end of the tibia/fibula).



**Fig. 2.** Natural logarithm of uterine mass regressed against the natural logarithm of cranio-caudal length. Line of best fit:  $y = 0.571 + 1.3023x$ ;  $R = 0.906$ ,  $p < 0.001$ .



Gestation length in wolves and the domestic dog has been shown to be approximately  $63.0 \pm 5$  days (Mech 1980; Seal et al. 1987). In addition, implantation in the domestic dog has been observed to occur between days 17 and 18 post coitus (Holst and Plemister 1971), and the correlation between crown-rump length and uterine mass was highly significant (Miller 1979; Robbins and Robbins 1979; Noden and LaHunta 1986). To establish whether a similar relationship existed in wolves from the Arctic, the cranio-caudal lengths of fetuses were plotted against the uterine masses. Owing to the curvilinear nature of the data, both parameters were transformed into natural logarithms. As new corpora lutea were first observed on February 24 (day 0), and other researchers have indicated that gestation length in wolves and domestic dogs is approximately  $63.0 \pm 5$  days (Rausch 1967; Mech 1980), the gestation period was estimated to last from February 24 to April 27 (day 63).

In an attempt to understand differential growth patterns during fetal development and to identify allometric changes in growth characteristics of wolves from this region, morphological parameters were transformed into natural logarithms and correlated with cranio-caudal length. The data were transformed in order to minimize curvilinearity and reduce heteroscedasticity (Sokal and Rohlf 1981).

To determine implantation success (relationship between the number of ova shed at ovulation and the number of fetuses implanted), the number of ova shed, represented by the number of corpora lutea/albicantia, was correlated with the number of fetuses or placental scars present. Placental scars were identified by hematomas formed at the implantation sites post partum (Barrau et al. 1975).

## Results

Sixteen of the 56 uteri of reproductively active females contained 73 identifiable fetuses. Another 41 parous reproductive systems contained corpora lutea and, in some cases, swollen uterine horns without identifiable fetuses. Placentation was zonal. Fetuses from this wolf population grew at a

mean rate of 5.17 g/day between day 32 post coitus (approximately) and parturition. During the same period, fetuses increased in length at a rate of 0.204 cm/day. No significant differences were noted for either contour length ( $F = 1.517$ ,  $p = 0.209$ ) or body mass ( $F = 0.541$ ,  $p = 0.706$ ) in relation to position within the uterine horn (closest to or farthest from the cervix). Changes in orientation (anterior versus posterior) related to stage of gestation were as follows: from days 33 to 39, 44.8% of the fetuses had the head positioned posteriorly, from days 40 to 49, 36.3% had the head positioned posteriorly, from days 50 to 60, 46.6% had the head positioned posteriorly, and from day 60 to parturition, 100% were oriented posteriorly. Among identifiable fetuses, the numbers of males and females were equal. Sexual dimorphism in body mass and the other morphological parameters was not observed.

The natural logarithm of uterine mass was highly correlated with the natural logarithm of cranio-caudal length ( $r = 0.954$ ,  $p < 0.001$ ; Fig. 2), and cranio-caudal length ranged from 3 mm shortly after implantation to a maximum of 185 mm near parturition. Allometric growth patterns in fetuses were also analyzed by correlating body parameters with cranio-caudal length. All correlations with cranio-caudal length were significant, and with the exception of contour length and humerus length, growth rates of all extremities (tail, femur, etc.) and increases in volume measurements (neck, girth, etc.) exceeded that of cranio-caudal length (Table 1). Growth rates in fetuses were significantly different at all developmental stages.

Reabsorbed embryos were observed in reproductively active females at all stages of gestation. The analysis indicated that 42.8% of the gravid yearling females (1–2 years)

**Fig. 3.** Stages in the development of the wolf fetus. (A) About 32 days. (B) About 37 days. (C) About 38 days. (D) About 45 days. (E) About 51 days. (F) About 54 days. (G) About 63 days. Scale bars = 1 cm.



Fig. 3 (concluded).



and 100% of the gravid adult females (>2 years) had at least one reabsorbed embryo. None of the gravid juvenile females (0–1 years;  $n = 1$ ) contained reabsorptions. The relationship between the percentage of fetuses reabsorbed and age-class indicated that 0% ( $n = 1$ ) of the fetuses produced by juvenile females were reabsorbed, while 8.1% ( $n = 7$ ) of those produced by gravid yearling females and 23.8% ( $n = 8$ ) of those produced by adult females were reabsorbed.

Litter sizes varied between age-classes in a similar manner to the frequencies of reabsorbed embryos. The gravid juvenile females had a mean litter size of 4 ( $n = 1$ ), while yearling females had a mean litter size of 5.28

(range 5–6;  $n = 7$ ) and adult females had a mean litter size of 5.25 (range 4–8;  $n = 8$ ).

The average number of ova shed per female was correlated with the average number of fetuses per female to determine the reproductive efficiency of breeding females. Results from the 3 years of the study indicated that these two parameters were significantly correlated ( $r = 0.766$ ,  $p < 0.001$ ) and that reproductive efficiency was 76.6%, indicating that 23.4% of the ova shed during ovulation were lost prior to or during implantation.

Based on the fetuses removed from gravid females, developmental stages can be described as follows (Fig. 3):

**Table 1.** Relationship of the natural logarithm of fetal body parameters against the natural logarithm of cranio-caudal length (cm).

Variable	<i>n</i>	Equation	<i>r</i>	<i>p</i>
Body mass	73	$y = -10.41 + 3.674x$	0.839	<0.001*
Contour length	73	$y = 3.5936 \times 10^{-2} + 0.972x$	0.943	<0.001*
Tail	68	$y = -3.1376 + 1.299x$	0.919	<0.001*
Humerus	59	$y = -11.858 + 0.291x$	0.933	<0.001*
Ulna/radius	59	$y = -4.0199 + 1.419x$	0.863	<0.001*
Paw (front)	52	$y = -4.5949 + 1.426x$	0.848	<0.001*
Femur	52	$y = -5.5213 + 1.757x$	0.931	<0.001*
Tibia/fibula	52	$y = -5.3462 + 1.675x$	0.910	<0.001*
Paw (back)	52	$y = -6.8222 + 1.927x$	0.905	<0.001*
Girth	68	$y = -2.0517 + 1.309x$	0.933	<0.001*
Neck	68	$y = -2.2960 + 1.290x$	0.894	<0.001*

**Note:** Parameters were assessed using correlation analysis; a probability of less than 0.001 indicates significance. Since no sexual dimorphism was observed in fetuses, the data from males and females are pooled. Body mass is given in grams and all linear measurements in millimetres.

\*Significant at the 0.001 confidence limit.

(i) Approximately 32–37 days post coitus (Figs. 3A–3C): Limb buds apparent at the plate stage, pronounced flexion of the head and neural cord evident, vertebral elements chondrify, optic pit and eye formed, heart visible through epidermis, no external evidence of sexual differentiation.

(ii) Approximately 38 days post coitus (Fig. 3D): Epidermis opaque and eyespot pigmented, limb buds paddle-like, but individual digits evident, some interdigital webbing evident, spine starting to straighten from flexed position, genital tubercle present adjacent to anus.

(iii) Approximately 45 days post coitus (Fig. 3E): Rotation and adduction of limbs, oral cavity present, eyelids developed, eye closed, pinna covers ear opening, ossification of temporal, pterygoid, and lacrimal bones, sex of fetus can be determined externally (male genital tubercle more anterior).

(iv) Approximately 51 days post coitus (Fig. 3F): Vibrissa follicles appear, keratinized nails present, tongue apparent, body more darkly pigmented.

(v) Approximately 53 days post coitus (not shown): Body darkly pigmented, body hair apparent, scrotal swellings large in males, labia prominent.

(vi) Approximately 63 days post coitus (Fig. 3G): Development of pigmentation, growth of hair, closure and fusion of eyelids, eye fully developed and pigmented (blue), growth of external ear, elongation of the trunk, differentiation of the sexes, sense organs complete, forelimbs and hind limbs well developed (toes, pads, and keratinized nails).

## Discussion

Reproductive success in carnivores has been shown to be highly correlated with female condition and primary prey density (Kuyt 1962; Mech 1980; Harrington et al. 1983; Hall 1989; Oftedal and Gittleman 1989; Hillis 1990). In the arctic fox (*Alopex lagopus*), litter size varied between 0 and 13 and correlated significantly with small-mammal density and winter subcutaneous fat deposits (Hall 1989). Processes in which the female system can intervene and vary reproductive investment and strategy include ovulation, implantation, fetal reabsorption, parturition, postpartum care (infanticide, abandonment), lactation, and weaning. Generally, these parameters are vari-

able in species inhabiting unstable environments and will influence energy conservation, optimal litter size, and ultimately reproductive success of the individual. In this regard, little information has been published on ovulation rates, implantation, fetal growth and development, fetal reabsorption, or prenatal litter size in wild canids (Oftedal and Gittleman 1989). In addition, little information is available on age at first reproduction and delayed parity in the Canidae (Oftedal and Gittleman 1989).

Neonatal growth may be determined by energy or nutrient availability to the mother. Changes in litter size and growth of wolves have been documented post partum and have been linked to prey availability (Kuyt 1962; Mech 1980; Harrington et al. 1983; Hillis 1990). In this study, data are too limited to allow examination of the effects of varying maternal condition on implantation and fetal growth rates in wolves, but results from Hillis (1990) suggest that maternal condition plays a key role in fetal growth in wolves in the Keewatin District.

Most canids exhibit high rates of litter growth, which are influenced by the maternal diet; carnivores grow more rapidly than herbivores and frugivores (Oftedal and Gittleman 1989). The wolf fetuses in this study grew at an average rate of 5.17 g/day from approximately day 32 to birth. Over the same time period, fetuses increased in length by 0.204 cm/day. No differences were noted in body size (mass and contour length) of the fetuses, regardless of position in the uterine horn. Changes in the orientation of the fetus during development was found to occur on an approximately 10-day cycle. Orientation was calculated from day 33 to birth and occurred as follows: from days 33 to 39, 44.8% of the fetuses were head ventral, from days 40 to 49, 36.3% were head ventral, from days 50 to 60, 46.6% were head ventral, and from day 60 to birth, 100% were head ventral.

The sex ratio of the fetuses was found to be 1:1 (males: females). The population of wolves in this region was increasing and stabilizing during this study (Hillis 1990), and the sex ratio in utero reflects this.

The growth rates of all morphometric parameters in utero were significantly correlated with cranio-caudal length and, with the exception of humerus length, exceeded that of cranio-

caudal length. The slower development of the humerus suggests that postpartum growth occurs soon after birth. Variations in wolf development post partum have been noted by Van Ballenberghe and Mech (1975); members of the same litter quite often show a high degree of variance in body morphology, specifically body and leg lengths and body mass. Results from this study suggest that little or no prepartum variation occurs in fetuses.

During growth, the wolf fetus exhibits a curvilinear relationship between age (body size) and cranio-caudal length. Carnivore species have brief periods of postpartum development to minimize the maternal energy cost of feeding the young and reduce vulnerability to fluctuations in prey (Sadlier 1969; Gittleman 1987). The form of parental care has relevance to the rate of growth of a litter post partum; young in a biparental or communal caring system have a higher relative growth rate than those in a maternal system (Oftedal and Gittleman 1989). Biparental and communal caring has been well documented in wolf packs (Rabb et al. 1967; Woolpy 1968; Harrington et al. 1983; Mech 1980, 1988; Moehlman 1989); the cooperation of mates and helpers in acquiring prey may increase the energy that can be directed to offspring and reduce the risk of injury to a pregnant female. More information is necessary, however, to determine the role of other pack members in relation to preferential treatment of the gestating female.

Reabsorptions were indicated in some females at various stages of gestation and may have been influenced by the physical condition of the female before and during the pregnancy. Haber (1977) hypothesized that a female's physical condition may result from the interaction of physical and social environmental factors prior to mating. Observations have shown that breeding females frequently dominate other females in the pack (Woolpy 1968; Mech 1980) and should have greater access to food, ensuring better condition at parturition. However, considerable agonistic interaction among wolves may occur as a result of increased density. The high incidence and numbers of reabsorptions in yearling and adult females during this study, when primary prey species appeared to be readily available, put in question the relationship between food, condition, and reproductive success of wolves in this region. The reabsorption rate may therefore be largely due to social environmental factors (aggressive interaction, etc.) rather than to a shortage of food. Food shortage cannot be totally ruled out, however, as the high abundance of caribou and muskoxen may not be a good indication of food availability, if the prey populations are composed mainly of adult animals in their prime.

The presence of reabsorptions at most stages of development may indicate prepartum developmental "checks," which may also be a reproductive adaptation to reduce vulnerability to fluctuations in prey, and may indicate critical periods for reabsorptions to occur, although this remains to be tested. Variation in gestation times in wild canids does occur (Wayne, 1986); generally, breeding in wolves occurs in February and the first weeks of March, but times may vary according to the position of the female in the hierarchy, her age, and her condition (Mech 1980). These developmental checks in fetuses may reduce the differences in the time of breeding and thereby synchronize births at a time when prey are available.

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District exhibited significant changes in all morphological parameters throughout gestation. Variation in breeding times, and thus gestation lengths, may also explain differences in fetal growth. Further studies are needed to determine the effects of condition (metabolic body size) of a female during gestation and the role of other pack members in relation to the gestating female.

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