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Characteristics of reproductive organs and reproductive potential in Scandinavian female grey wolves (*Canis lupus*).

Eva Axnér[a,*], Eric Cederlund[a,1], Erik O. Ågren[b], Anne-Marie Dalin[a]

[a] *Department of Clinical Sciences, Division of Reproduction, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, P.O. Box 7054, SE-75007 Uppsala, Sweden*
[b] *National Veterinary Institute, SVA, SE-75189 Uppsala, Sweden*

A R T I C L E I N F O

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A B S T R A C T

The Swedish wolf population is closely monitored and managed to keep the population at a sustainable level while avoiding conflicts. Detailed knowledge about reproduction is crucial for estimates of population size and the reproductive potential of a population. Post-mortem evaluation of reproductive organs can be used as a complementary tool to field monitoring for evaluation of cyclicity and previous pregnancy, including litter size. Therefore, we evaluated reproductive organs from 154 female wolves that were necropsied during the period 2007–2018. The reproductive organs were weighed, measured, and inspected according to a standardised protocol. Presence of placental scars was evaluated for estimates of previous pregnancy and litter size. Data about individual wolves were also obtained from national carnivore databases. Body weight increased during the first year of life before levelling out. There was evidence of cyclicity the first season after birth in 16.3 % of the 1-year-old females. No females < 2 years had evidence of a previous pregnancy. Pregnancy rates were significantly lower in 2- and 3-year old females than in older females. Mean uterine litter size was 4.9 ± 2.3, and did not differ significantly between age groups. Our data supports earlier field data that female wolves usually start to reproduce at the earliest at 2-years of age but that they occasionally start to cycle one season earlier. All females ≥ 4 years of age had reproduced. Pathological findings of the reproductive organs were rare, indicating that reproductive health of female wolves is not a limiting factor for population growth.

1. Introduction

The grey wolf (*Canis lupus*) was regarded as functionally extinct in the Scandinavian Peninsula (Norway and Sweden) about 50 years ago, and therefore became protected around this time (Wabakken et al., 2001). The present population is based on only a few immigrants. This has led to several documented cases of inbreeding depression, expressed as reduced reproduction and offspring with reduced fitness (Liberg et al., 2005; Åkesson et al., 2016). The wolves in Sweden are part of a Scandinavian population that is monitored continuously in a joint Norwegian-Swedish program. The monitoring includes snow tracking, radio collaring, observations, and DNA analyses of biological material found during tracking (Wabakken et al., 2001; Milleret et al., 2017; Åkesson and Svensson,

* Corresponding author.
E-mail address: Eva.Axner@slu.se (E. Axnér).
[1] Present address: Växa Sverige, Gotland Grönt Centrum, 622 54 Romakloster, Sweden.

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2021). The number of wolves is estimated from results of yearly surveys (Åkesson et al., 2022). Yearly decisions on culling of a specific number of wolves (licensed hunting), are made by the responsible authority, based on the estimates of these yearly surveys (Ekblom, 2022). In addition, permission is provided for protective hunting, mainly when wolves kill, or are likely to kill domestic animals.

Successful reproduction is fundamental for the growth of a population. Canids have unique reproductive cycles, characterized by a long period of heat (pro-oestrus and oestrus combined), preovulatory luteinisation, and similar lengths of the luteal phase in non-pregnant and pregnant females (Seal et al., 1979; Packard, 2003; Nagashima and Songsasen, 2021). The breeding season in Scandinavian wolves is from early February to mid-March, with birth of pups from mid-April to mid-May (Seal et al., 1979; Schmidt et al., 2008). The estimated whelping of GPS-collared female wolves in Scandinavian peninsula occurred during the period between 24 April and 20 May with an average of 1 May (Nonaka, 2011) or 5 May (Alfredéen, 2006) respectively.

Knowledge about age of first reproduction and the proportion of females that produce offspring is crucial for management of wild populations (Wikenros et al., 2021). Age at first reproduction in wild animals is related to both physical maturation and social factors (Geiger et al., 2016; Wikenros et al., 2021). Wolves usually do not reproduce until approximately two years of age, the second season after birth, although there are some very rare exceptions with both male and female wolves in captivity reproducing in the first season after birth (Medjo and Mech, 1976). Median age of first reproduction in 60 Scandinavian female wolves was found to be 3 years (Wikenros et al., 2021), with the earliest age being 2 years. Male wolves were, in rare occasions, found to reproduce already at their first season after birth (Wikenros et al., 2021).

Although field data is essential in the management of wild populations, it has some limitations associated with difficulties in collecting accurate data. Snow tracking is for example dependent on weather conditions (Wabakken et al., 2022). Even though field monitoring has been shown to have a high level of accuracy, some reproductive events are missed (Åkesson et al., 2022). According to the Swedish Environmental Protection Agency's regulations on wildlife (Swedish Environmental Protection Agency, NFS, 2019:5), all dead wolves (culled by licensed or protective hunting, died in accidents, or found dead) have to be sent to the National Veterinary Institute (SVA) for post mortem examination. A complementary method to field observations to study wolf reproduction is to examine the reproductive organs of dead wolves. The ovaries and tubular genitalia can be evaluated to determine cyclicity. The placental scars can be evaluated to document a previous pregnancy, and to determine litter size the previous season (Rausch, 1967; Mech et al., 2016).

Therefore, the aim of this study was to examine the reproductive organs to evaluate the reproductive pattern and potential, i.e. ovarian activity, pregnancy rates and litter sizes correlated to BW, age and season. Our hypotheses were: 1) puberty and age at first reproduction are related to growth; 2) pregnancy rates and litter size are affected by age; 4) season and age affect ovarian structures; 5) uterine size is affected by age and reproductive cycle. In addition, we aimed to evaluate the presence of pathological conditions affecting reproductive performance.

Fig. 1. Uterus with five placental scars. Photo: Anne-Marie Dalin.

2. Material and methods

2.1. Animals and source of organs for evaluation

Reproductive tracts from 154 wolves necropsied at SVA, with dates of death from January 2007 to April 2018, were examined. The organs were stored frozen (stored at −20 °C) at SVA, and were examined after thawing at room temperature. The time from the death of an individual to necropsy varied depending on whether the animals had been culled or found dead. For the former, the time to necropsy was about one to two days, whereas for the latter it was very variable time. The organs were marked with the individual animal identity number according to the record system at SVA. This study did not require official or institutional ethical approval as the studied organs originated from the regulated submission of wolf carcasses to SVA.

2.2. Evaluation of reproductive organs

2.2.1. Evaluation of tubular reproductive organs

Most of the reproductive organs had been removed from the carcasses at the level of the cranial vagina at routine necropsy. Therefore, the vestibulum-vagina were usually not available for evaluation. The *Ligamentum mesometrium* was removed from the uterus. The uterus (excluding cervix) was weighed, the length of the uterine horns (from tip to bifurcation), and the uterine body (from bifurcation to cervix), as well as the width of the uterine horns were measured. The uterus was cut open and the endometrium evaluated (colour, thickness, scars after pregnancy and presence of pathological changes). Dark placental scars, presumed to be from a pregnancy in the last breeding season, were counted if clearly visible (Fig. 1. Irregular, narrower and/or more faded regions (faded scars) were presumed to be scars from earlier seasons (McNay et al., 2006). Faded scars were noted but not used to estimate litter size. Presence or absence of dark placental scars could not be evaluated in 14 females (9 %) because of a high degree of decomposition. The cranial vagina was macroscopically inspected for evidence of oestrogen influence (thickened epithelium and keratinization of the outer layer) when possible (Rehm et al., 2007). A vaginal neoplasia found in one wolf was evaluated histologically.

2.2.2. Evaluation of ovaries

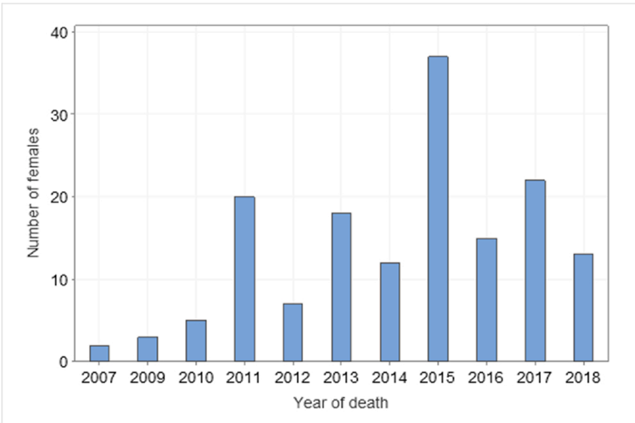
The ovarian bursa was cut open on both the right and left side, and the ovaries removed. The ovaries were weighed, measured in length, height and width, and macroscopically investigated for presence of follicles, corpora lutea (CL, Fig. 2) and corpora albicans (CA). Thereafter, the ovaries were fixed in 10 % neutral buffered formalin. After fixation, the ovaries were sectioned in approximately 1 mm thick slices and again macroscopically examined for the presences of follicles, (CL) and (CA, Fig. 3). Ovarian follicles ≥ 4 mm were classified as active follicles (England et al., 2009; Groppetti et al., 2015) indicating that the female was in prooestrus/oestrus. Luteal structures ≥ 6 mm were classified as active CL (Groppetti et al., 2015) while smaller pale luteal structures were classified as CA. Twenty-two wolves had incomplete ovaries (missing ovary or part of ovary), or ovaries that could not be evaluated because of decomposition. Incomplete ovaries were caused by loss of tissue during necropsy and retrieval of reproductive tracts from the carcasses. Cycle stage could generally not be confirmed in these individuals, as we could not exclude the presence of structures in the missing parts. One exception was a wolf with shredded ovaries but presence of six corpora lutea, 7–11 mm in diameter, in the remaining tissue, classified as being in the luteal phase.

2.3. Age determination

Juveniles (< 1 year) were categorized at necropsy by macroscopic inspection of the juvenile dentition, or presence of epiphyseal growth plates in the larger bones of the limbs. The age of older wolves had been determined by tooth root sectioning and counting cementum annulation at Matson's Laboratory, USA (Landon et al., 1998; Gipson et al., 2000). Wolves were aged according to the number of birthdays. With May 1 as the estimated date of birth, juveniles were < 11 months, 1-year-old females were 12–23 months, 2-year-old females were 24–32 months (32 months being the oldest female in this category), and so on. Although exact birth dates were

Fig. 2. Ovaries with CL. Photo: Anne-Marie Dalin.

Fig. 3. Fixed ovary with CA. Photo: Anne-Marie Dalin.



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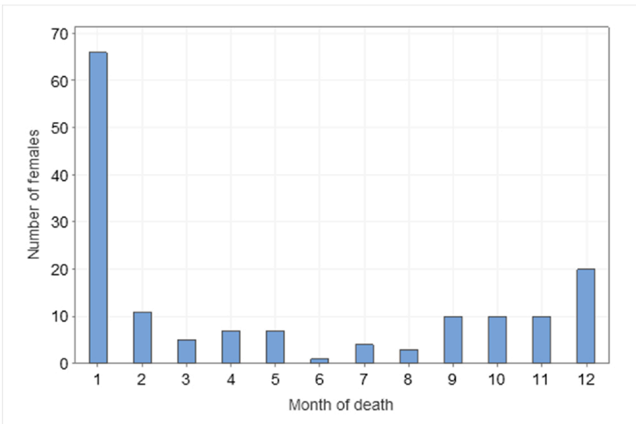

b

Fig. 4. a. Distribution of year of death of the female wolves included in the study. b. Distribution of month of death of the female wolves included in the study.

not known, the difference from exact age should not differ more than approximately half a month. Wolves ≤ 2-years were analysed according to estimated age in months as well as age in years, while older wolves were only analysed according to age in years.

2.4. Information about individual wolves from databases

Information about individual wolves, i.e. identity, date of death, date of necropsy, BW, age, cause of death (protection, licensed or illegal culling; road/train accidents; disease, killed by other animal, other causes), and pathological findings were obtained from SVA's data base SVALA and the national database for large carnivores (Naturvårdsverket, 2023). Our data were also compared with yearly published reports from field monitoring of the wolf population, and the known pedigrees of Scandinavian wolves (Åkesson and Svensson, 2021).

2.5. Statistical analyses

Body weight in relation to age in months was evaluated with a linear regression analysis. For 1-year-old wolves, the analysis was repeated without wolves that died in May, as there could be some uncertainty if these individuals were 12 or 24 months of age. Linear regression was also used to evaluate the number of placental scars in relation to BW. Body weight in different age classes were compared with a one-way Anova and with Tukey's pairwise comparisons to compare age groups (juvenile, 1, 2, 3, and ≥ 4 years). Normal distribution of the residuals were confirmed with the Ryan-Joiner's test. Homogeneity of variances were tested with Levene's test. The number of dark placental scars in different age classes was compared with the non-parametric Kruskal-Wallis test, and pairwise with Mann-Whitney's test. The probability of having dark placental scars and evidence of previous reproduction (old or fresh placental scars and information from field data) were compared with Fisher's exact test. All statistical calculations were performed with Minitab® 19.2020 (Minitab, LLC, 2020. Available at: https://www.minitab.com). P-values < 0.05 were considered significant.

a

b

Fig. 5. a. Linear regression for age in months against body weight in juvenile females, $P < 0.001$. b. Linear regression for age in months against body weight in 1–2year old females, $P = 0.3$.

3. Results

3.1. Basic data

The causes of death were protective culling (37.7 %), licensed culling (33.8 %), road/railway accidents (15.6 %) and miscellaneous (13.0 %). The distributions over years and different months are summarized in Fig. 4a and b. Ages ranged between 3 months and 7 years, and were skewed towards the younger ages. The proportions of juveniles (< 1 year), sub-adults (1 years) and adults (> 2 years) were 35.7 % ($n = 55$), 37.7 % ($n = 58$) and 26.6 % ($n = 41$), respectively.

Age determination overlapped somewhat for young wolves that died in May. Two wolves, estimated to be juveniles, and five 1-year old wolves were classified as being approximately 12 months old. As age in wolves was estimated based on passed birthdays, and BW did not continue to increase significantly in wolves > 1-year of age, 1-year old wolves that died in May were 12 or 24 months of age. The juveniles that died in May were, however, likely to be close to 12 months, as they could not have been born the same season based on their BW. No wolves ≥ 2-years died in May (i.e. the estimated birthday month).

3.2. Body weight

Body weights in all wolves ranged between 16.0 and 43.0 kg, and increased significantly ($P < 0.001$) during the first year of life (Fig. 5a). Linear regression of BW in the 12–23 months old females indicated no further linear growth ($P = 0.3$, Fig. 5b and Table 1, $P = 0.55$ without 1-year-old females that died in May). Body weight was significantly lower in the juvenile females than in the other age groups and no differences were between the other ages (Table 1).

3.3. Uterine weights

The uterine weights ranged between 0.44 and 41.31 g. Uterine measurements grouped according to ovarian structures, with juvenile wolves in a separate group, are shown in Table 2. Data are descriptive only due to a low number of animals with active structures in the ovaries.

3.4. Ovaries and cycle stage

Ovarian structures according to age are shown in Table 3 and according to month in Fig. 6. Only four animals had active structures (follicles ≥ 4 mm, or CL ≥ 6 mm) in the ovaries. One female had seven follicles 3–7 mm and three other females had CL 7–11 mm in diameter. In addition, a juvenile female that was found dead in February (approximately 9 months old) had keratinised vaginal epithelium, indicating that the female was in oestrus. One ovary of this wolf was missing and the other had no structures. As only one ovary was available, cycle stage could not be confirmed by presence of ovarian follicles. Thus, of the 58 1-year-old females, eight (14.5 %) had ovarian structures or vaginal keratinisation indicating current or previous ovarian activity. If the nine 1-year-old females in which ovaries could not be evaluated are excluded, 16.3 % (8/49) had started to cycle before the second season after birth. Few adult females died during the estimated reproductive season. Only four wolves ≥ 2-years died in February, of which two only had one ovary availalbe. No active ovarian structures were found in any these four females. Two adult females died in March, of which one had CL and one had CA. Small follicles < 3 mm in diameter could be observed throughout the year, with no evidence of seasonal variation.

3.5. Placental scars and litter size

Evidence of previous reproduction, i.e. faded and/or dark placental scars, was found in 29 females, of which 28 had dark scars. The number of dark scars, presumed to reflect the previous season's number of implanted foetuses, ranged between 1 and 9 with a mean ± SD of 4.9 ± 2.3 and median of 5 ($n = 28$, Table 1). No females < 2 years of age had placental scars (dark or faded).

The proportion of females with dark placental scars was significantly lower for the 2- and 3- year-olds than for those ≥ 4 years of age ($P < 0.05$). Litter size, as evaluated by the number of dark placental scars, did not differ with age (Table 1). In females with dark placental scars, the number of dark scars was not related to BW ($P = 0.31$).

Table 1
Proportion of females with placental scars, number of implantations and means±SD of body weight in females of different age classes.

	Body weight (*n*)	Females with dark placental scars*	Females that had reproduced#	Number of implantations in females with fresh scars (*n*)*
Juvenile	27.0 ± 5.2 (55)[a]	0/52	0/52	-
1 year	32.9 ± 3.6 (58)[b]	0/49	0/49	-
2 years	34.9 ± 2.8 (10)[b]	5/10 (50.0 %)[a]	5/10 (50 %)[a]	4.6 ± 1.9 (5)
3 years	34.3 ± 3.7 (12)[b]	6/11 (54.5 %)[a]	9/12 (75 %)[a]	4.5 ± 1.8 (6)
≥4 years	35.5 ± 4.1 (19)[b]	17/18 (94.4 %)[b]	19/19 (100 %)[b]	5.1 ± 2.6 (17)

*Only females that could be evaluated included. #Females with dark or faded scars, and including females with reported reproduction in field data. [ab]Different letters = significant difference within column. Except for body weight, comparisons between age groups did not include juvenile and 1-year old females as none of them had evidence of reproduction.

Table 2
Uterine weight, mean diameter of uterine horns and mean length of uterine horns in juvenile females and in relation to ovarian structure in females ≥ 1 year. Descriptive data only because of few individuals with active ovaries (CL or follicles). Means ± SD.

	Uterine weight g (*n*)	Mean diameter (mm) of uterine horns (*n*)#	Mean length (mm) of uterine horns (*n*)#
Juvenile	1.4 ± 0.9 (47)	2.0 ± 0.8 (49)	126.6 ± 14.1(48)
Inactive	3.8 ± 3.4 (50)	3.1 ± 1.5 (49)	146.5 ± 18.2 (49)
CA	7.6 ± 4.3 (29)	4.9 ± 2.0 (29)	182.7 ± 28.0 (30)
Follicles	8.3 (1)	5.0 (1)	182.5 (1)
CL	26.6 ± 13.8 (3)	8.7 ± 4.6 (3)	224.2 ± 57.8 (3)
CA/ND*	8.0 ± 4.6 (3)	3.8 ± 1.1 (3)	183.8 ± 26.5 (2)
ND	7.3 ± 7.8 (7)	3.6 ± 2.0 (7)	167.4 ± 38.1 (7)

*Females with data from one ovary missing but with CA #Only females with data from both sides included. ND=structures could not be determined, Inactive = no structures were found, CA = only presence of corpora albicans/corpora lutea < 6 mm, CA/ND = only one ovary could be evaluated and this had CA, Follicles = presence of ovarian follicles > 3 mm, CL = presence of corpora lutea > 6 mm

Table 3
Number of females with different categories of ovarian structures according to age.

	Juvenile *n*= 55	1-year *n*= 58	2-year *n*= 10	3-year *n*= 12	≥ 4 years *n*= 19
ND*	6	9	1	1	1
Inactive	49	42	2	3	1
CA	0	4	6	7	13
CA/ND*	0	1	1	1	2
Follicles	0	0	0	0	1
CL	0	2	0	0	1

*ND=Cycle stage could not be determined because ovaries were incomplete and no structures were found. CA/ND = Ovaries were incomplete but CA were found. Other structures cannot be excluded.

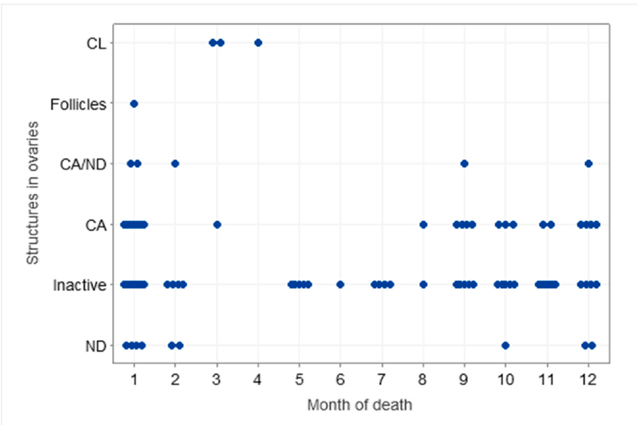

Fig. 6. Ovarian structures in relation to month of death in females ≥ 1 year. ND=structures could not be determined, Inactive = no structures were found, CA = only presence of corpora albicans/corpora lutea < 6 mm, CA/ND = only one ovary could be evaluated and this had CA, Follicles = presence of ovarian follicles > 3 mm, CL = presence of corpora lutea > 6 mm.

There was no evidence for presence or the number of scars differing with month of death or cycle stage (visual inspection, data not shown).

3.6. Relation between field monitoring data and results from evaluation or reproductive organs

In 11/12 females ≥ 2-years of age, in which we did not observe dark placental scars, there were no field reports of a reproductive event the previous season, thus corresponding with our findings. One exception was a 3-year old female without placental scars but a field report of reproduction the previous season. In 28 females with dark placental scars, field data confirmed reproduction the previous season in 21 cases. In two females ≥ 2-years of age that could not be evaluated for presence of dark placental scars, there was no field report of reproduction in a 3-year-old female, while a 7-year-old female had reproduced the previous season according to field data. Combining females with faded and/or dark scars, and females with field data of a previous reproduction, all females ≥ 4-years of age had reproduced (Table 1). All females with dark placental scars also had CA in their ovaries, with two exceptions. These females, without CA but with dark placental scars, had reproduced the previous season according to field observations.

3.7. Pathological findings

One female had a fibroma in the cranial vagina. This was the only abnormality found in the organs. She had eight dark placental scars and confirmed reproduction the previous season, according to field data.

4. Discussion

The data in our study support earlier findings (Rausch, 1967; Fuller et al., 2003), that most female wolves are physically and sexually mature at 2-years of age. At this age, 7/9 specimens, that could be evaluated, had evidence of a previous ovulation (CA), and from 2-years of age, females had dark placental scars, indicating a pregnancy the previous season. No females $<$ 2 years had been pregnant, as indicated by the absence of dark placental scars, although a low proportion of females $<$ 2 years of age had started to cycle the first season after birth.

Body weight increased rapidly and significantly, the first year of life as expected. Regression analysis showed no significant further growth in females from the age of 12 months. Of the 1-year-old females that could be evaluated, 16.3 % had evidence of previous or current ovarian activity (CA, CL or vaginal keratinisation). This indicates that cyclicity may start already the first season after birth, as also shown in previous publications (Medjo and Mech, 1976; Mech and Seal, 1987). At this age skeletal maturity is achieved (Geiger et al., 2016), and there was no further growth after the first season after birth in our data. Although somatic maturity and start of cyclicity usually are related (Geiger et al., 2016), the majority of females did not start to cycle before the second season after birth, and no females had conceived the first season after birth. The growth curve in our data is similar to other published reports with a rapid growth the first year of life followed by levelling off (Stahler et al., 2013). Our data are also in accordance with Scandinavian field monitoring data that female wolves may start to reproduce in the second reproductive season after birth i.e. may have their first litter when they reach the age of 2 years old (Wikenros et al., 2021). Although most wolves at 2-years of age had evidence of a previous ovulation, pregnancy rates, based on placental scars, in 2- and 3-year old wolves were lower than in older females. This is likely due to social factors, such as population size, rather than physiological maturation (Wikenros et al., 2021). Sub-dominant females in a pack usually do not reproduce even if they show signs of oestrus (Zimen, 1976). Interestingly, male wolves seems to reach sexual maturation earlier than females. A large proportion of male wolves produce spermatozoa $<$ 1 year of age (Petersen et al., 2021), and data from the field confirm that the average age of first reproduction is lower in males, and that occasionally males may reproduce the first season after birth (Wikenros et al., 2021).

There is a paucity of information about the morphometrics of ovarian structures in canids. Interpretation of published data is further complicated by the effect of body size on the size of ovarian structures (Groppetti et al., 2015). The domestic dog, often used as a model species for other canids, differs widely in size, which is why data extrapolated from domestic dogs may not be accurate for wolves. *Corpora lutea* $\geq$ 6 mm were only observed in females that died in March or April, i.e. during the expected period of pregnancy or metoestrus/dioestrus, while smaller luteal structures (CAs) up to 5 mm in diameter could be observed between throughout the year (Fig. 6). It is not known how long CA may persist in the ovaries of the wolf (Rausch, 1967). According to Rehm et al. (Rehm et al., 2007), CA in the domestic bitch may remain for several months, and may still be noticeable well into the next oestrus cycle. Therefore, the number of CA cannot be used as an estimate of the ovulation rate (Rausch, 1967). Ovarian follicles $\geq$ 4 mm, indicating prooestrus/oestrus were found only in one individual, which was culled at license hunting 19 January. Considering that the birth dates for Swedish wolves have been shown to be between 24 April and 20 May (Alfredéen, 2006; Nonaka, 2011), and that the length of pregnancy is similar to the domestic bitch, maximum follicle size at ovulation would be expected approximately 63 days earlier i.e. mid-February to mid-March. Three of the four wolves $\geq$ 1-year of age that died in March-April, i.e the expected period of luteal activity, had CL. The relatively low number of females with active ovarian structures was probably because of the skewed months of death, with most of the females being culled at licensed or protective hunting, outside the breeding season.

Absence of dark placental scars corresponded with absence of reported reproductive events in 11/12 females, while presences of dark placental scars corresponded with field reports in 21/28 females. Reproductive events may be missed in field data (Åkesson et al., 2022), and all puppies in a litter may die before being observed. Dark placental scars were usually easy to evaluate and to count. McNay et al. (2006) found 100 % agreement between pregnancy diagnosis by ultrasound, and post-mortem evaluation of placental scars within 10 months, confirming the reliability of the method. Unlike McNay et al. (2006) who concluded that placental scars may not be visible in the reproductive season because of a thickened endometrium, we observed dark scars in all death months included, also in the female with ovarian follicles and the adult female with CL. In autolysed material or during the reproductive season, presence of scars may nevertheless be missed. It is not known how long placental scars remain visible. Observation of faded scars is a likely an indication of a previous pregnancy while absence probably should be interpreted with caution. Discrepancies between our data and field data is thus most likely due to the possibility that reproductive events may be missed in field data, and that not all organs are suitable for post-mortem evaluation because of decomposition. A combination of both methods is therefore likely to be beneficial for a more accurate evaluation of reproduction in the wolf.

The mean $\pm$ SD and median number of scars from fresh implantation sites in 28 females (4.9 $\pm$ 2.3 and 5.0, respectively) was similar to the average number of pups/den of 4.7 $\pm$ 0.21 observed by Stahler et al. (2013) but somewhat lower than the average of 5.5 foetuses reported by Fuller et al. (2003). It was also lower than the average number of 5.6 pups counted in 20 dens in Scandinavia (Sand et al., 2014). Similarly Webb et al. (2011) reported an average of 5.6 pups (range 4–7) in 24 litters in dens, while Rausch (1967) reported a higher average of 6.7 placental sites in 79 females. Considering the large variation in litter size and usually a relatively low number of observations / study, a single litter may have a large effect on the average litter size. Similar to Stahler et al. (2013), but in contrast to Rausch (1967), 2- and 3-year old females in our study did not produce fewer pups/litter than older females, as judged by the number of dark placental scars. In contrast to the hypothesis by Stahler et al. (2013), there was no correlation between BW and litter size in our data.

The only pathology in the reproductive organs in this study was a vaginal fibroma. This is a relatively common condition in ageing domestic bitches (Kydd and Burnie, 1986). In contrast to the domestic bitch, which often develop cystic endometrial hyperplasia with advancing age (Moxon et al., 2016), we could not find any uterine pathologies. This could be due to a low mean age and few old individuals in our material. It is also possible that the strict seasonality of female wolves, and usually yearly pregnancies in adults, is less predisposing to pathological conditions related to repeated hormonal cycles compared with the domestic dog. We could not evaluate pathologies of the vestibulum-caudal vagina as these parts were generally missing. Defects in these parts, incompatible with normal mating and parturition, are not rare in the domestic dog (Mathews, 2001). The high pregnancy rates in the adult wolves in this material, indicates, however, that pathological conditions and defects impairing reproduction are likely to be rare.

5. Conclusions

In conclusion, our data indicate that reproductive health of female wolves is not likely to be a limiting factor for population growth. All females $\geq$ 4 years of age had reproduced and pathology of the reproductive organs was rare. Population growth may, however, be affected by the proportion of young females that reproduce.

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CRediT authorship contribution statement

Anne-Marie Dalin: Conceptualization, Methodology, Investigation, Writing-Original Draft, Writing-review & editing, Project administration. **Eva Axnér:** Conceptualization, Methodology, Investigation, Formal analysis, Writing-Original Draft, Writing-review & editing, **Erik Cederlund:** Investigation, Writing-review & editing. **Erik Ågren:** Resources, Data curation, Writing-review & editing.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Prior publication

Parts of the data have been previously published in Swedish as a student project.

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