Reproduction of the red fox in western France: does staining improve estimation of litter size from placental scar counts?

Sandrine Ruette, Michel Albaret

Eur J Wildl Res (2011) 57:555–564
DOI 10.1007/s10344-010-0465-y

ORIGINAL PAPER

Reproduction of the red fox *Vulpes vulpes* in western France: does staining improve estimation of litter size from placental scar counts?

Sandrine Ruette · Michel Albaret

Received: 1 June 2010 / Revised: 22 October 2010 / Accepted: 25 October 2010 / Published online: 12 November 2010

Abstract We tested a staining method on uteri for counting placental scars on red fox. We estimated reproduction parameters on 358 females collected in three study areas in western France from 1st February 2002 to 31st January 2005. Placental scars (n=103) were described by macroscopic examinations using the following variables: (1) the width and (2) the aspect of placental scars, (3) the abundance of macrophages or the presence of blood, (4) the presence of swellings, (5) the presence and colour of a central band and (6) the presence and colour of lateral bands. A factorial correspondence analysis showed strong associations between the month when scars were examined and categories of variables. Staining on placental scars made macrophages more visible, facilitating identification of 'active' placental scars, i.e. related to the last pregnancy. However, distinction between placental scars due to earlier pregnancies and resorptions was not possible. The staining method used provides a standard that could be useful for obtaining comparable and repeatable results. The mean number of placental scars was 4.85±1.46 (n=103) per vixen. The mean number of embryos per vixen was 4.66± 1.35 (n=68) for yearlings and 5.53±1.50 (n=96) for older females. Including percentages of barren vixens, the total population productivity was significantly smaller for yearlings (3.62±1.86, n=158) than for older females (4.28± 1.75, n=186). We discuss these results in relation to fox densities, culling and food availability.

Keywords *Vulpes vulpes* · Placental scar counts · Litter size · Embryos counts · Productivity · Reproductive performance

Communicated by C. Gortázar

S. Ruette (✉)
Office National de la Chasse et de la Faune Sauvage,
CNERA Prédateurs Animaux déprédateurs,
Montfort 01330 Birieux, France
e-mail: sandrine.ruette@oncfs.gouv.fr

M. Albaret
Office National de la Chasse et de la Faune Sauvage,
CNERA Prédateurs Animaux déprédateurs,
63000, Clermont-Ferrand, France

Introduction

Determining the reproductive performance of fox (*Vulpes vulpes*) is of great interest because such data are needed for management decisions and population modelling (Llyod et al. 1976; Vos 1994; Villafuerte et al. 1996; Pech et al. 1997; Chautan et al. 2000; Marlow et al. 2000; Harding et al. 2001; McIlroy et al. 2001). Since uteri from all categories of females are readily obtained from hunters and trappers; placental scars are often the best source of information on fox reproduction. Counting of placental scars and embryos in the uterus has been widely used in a number of mammalian species, including several rodents (Martin et al. 1976), lagomorphs (Bray et al. 2003) and carnivores (Lindström 1994; Helle and Kauhala 1995; Mowat et al. 1996; Asano et al. 2003; Elmeros et al. 2003; Elmeros and Hammershøj 2006; Kristiansen et al. 2007) to estimate female fecundity in free-ranging populations. Indeed, in mammals with a zonary endoepitheliochorial or discoid hemochorial type of placenta, a distinct implantation site is formed for each foetus in the uterus. At the time of parturition, the separation of the placenta from the tissues of the uterus generates an imprint at each implantation site, which becomes pigmented due to the phagocytosis of placental and blood remains by the macrophages.

Placental scar counts (PSC) have been used to determine litter size and pregnancy rate in red foxes since the late 1940s (Lindström 1981), and the persistence of placental scars in foxes has been evaluated (Strand et al. 1995; Elmeros and Hammershøj 2006). In foxes, most workers have assumed that scars persist to the next oestrus. However, the reliability of placental scar counts has been thoroughly assessed only for relatively few species, either directly by comparing estimated litter size with known litter size using captive individuals (Bray et al. 2003 on hare; Strand et al. 1995 on Artic fox; Fournier-Chambrillon et al. 2010, Lindström 1981 and Elmeros and Hammershøj 2006 on red fox) or indirectly by comparing estimated litter size with embryo counts (Allen 1983).

Moreover, placental scar counts can either overestimate the litter size due to embryo resorptions, prenatal mortality and stillborn litters or underestimate it due to the regeneration of the uterine tissues a certain time postpartum.

There has been increasing concern that some scars of light shade might represent abortions, resorptions or be persisting from earlier pregnancies (Lindström 1981), fading of scars being due to macrophage migration and deterioration (Martin et al. 1976). However, large variation in the intensity of pigmentation in placental scars of similar age has also been observed (Englund 1970; Lindström 1981). The use of a grey scale (with six shades) to distinguish 'active' placental scars from placental scars due to earlier pregnancies or resorptions was first proposed by Englund (1970). Lindström (1994) suggested including successively scars of lighter shades in the estimated placental scars counts. Most authors recommend only the counts of dark placental scars to estimate litter size (Elmeros et al. 2003; Heydon and Reynolds 2000; Harris and Smith 1987). Heydon and Reynolds (2000) suggest grading individual scars by using a Kodak Reflection Density Guide with 22 shades of grey between white and black. Most authors agree that differentiation of scars according to shading for indicating either successful full-term pregnancies or post-implantation loss of embryos is not possible. Identifying 'active' placental scars, i.e. that correspond to live embryos from the last pregnancy, is thus still subject to interpretation and related to observer experience.

A staining method of the uterus was first applied on European hares (*Lepus europaeus*) by Bray et al. (2003), who showed that the reliability of the placental scar counts at the end of the persistency period could be improved by using this method. In this study, we apply this staining method to placental scars of red foxes to determine whether the method could be helpful in identifying 'active' placental scars. We also compare estimates of PSC with embryos counts, differentiating yearlings (first-time breeders) from older females in three sites of western France.

Materials and methods

Study area and sample collection

Foxes were obtained from three study areas in western France (Fig. 1) from 1st February 2002 to 31st January 2005. The three areas were 366, 238 and 248 km^2 for respectively sites A, B and C and less than 10 km apart from each other but separated by highways. The landscape was predominated by farming and arable lands, forested areas being present in less than 10% of the total areas. Farming predominated, especially chicken, cattle and pig farming. Arable lands predominated at site A, whereas

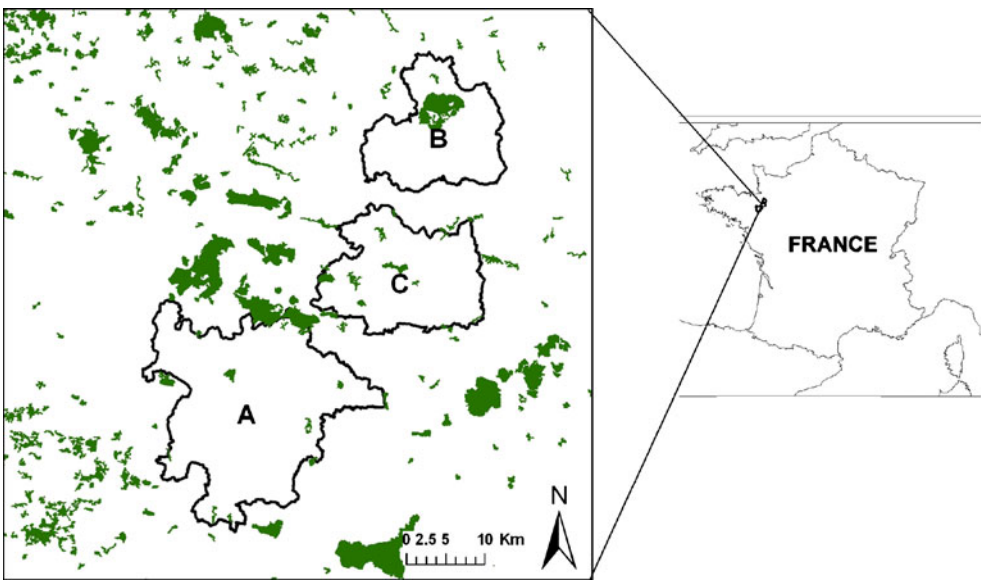

Fig. 1 Location of the three study areas. *Light grey patches* represent forests and wooded patches

pasture predominated in sites B and C. In the three study areas, fox culling took place throughout the year by various methods: hunting between October and February and unearthing, trapping and shooting in winter and spring. The fox culling effort has increased since winter 2000–2001 in site A and since winter 2001–2002 in sites B and C. We registered all foxes killed and estimated fox culling at around 1.3 foxes/km^2/year (adults and young of the year) in sites A and C and 2.5 foxes/km^2/year in site B during the study period. In the three study areas, densities were estimated in winter each year, applying the distance sampling methodology (Buckland et al. 1993) to spotlight counts of red fox (Heydon et al. 2000; Ruette et al. 2003). Density estimates were stable at 1.0 foxes/km^2 (CV=6.7%) in site A from winter 2002 to winter 2005, 2.2 in site B (CV=6.8%) and 0.9 foxes/km^2 in site C (CV=7.5%) from winter 2003 to winter 2005 (unpublished data). We collected foxes all the year round in the three sites from hunters and trappers. Females were necropsied and embryos were counted when visible or uteri were collected, 12–48 h after the death of the animal, and soaked in water before freezing and stored at −20°C until examination. We evaluated the total number of females culled at 1,285 in the three study areas between 01 February 2002 and 31 January 2005. Reproductive status could be determined on 358 adult females (more than 10 months old at death) corresponding to 53.5% of adult females collected.

Preparation of the reproductive tracts

A sample of uteri with placental scars was stained to test the method. Following Bray et al. (2003), before staining, we removed ovaries, oviducts, mesometrium and connective tissues and cut the entire horns lengthwise. The method was based on the Turnbull reaction, first developed by Salewski (1964) for rats and then used by Bray et al. (2003) for European hares. We soaked the uteri 10 min in a fresh 10% solution of ammonium sulphide (H_8N_2S) and rinsed them thoroughly in tap water. We then immersed the tracts for 10 min into a solution made of equal parts of 1% chlorhydric acid and of a 20% solution of potassium hexacyanoferrate ($K_4[Fe(CN)_6]$, $3H_2O$). As a result, macrophages filled with hemosiderin had a blue-black coloration. The analysis of scars should be made soon after staining (<2 h) to ensure that their characteristics are not modified.

Reproductive parameters

PSC, including a precise macroscopic description of placental scars, were performed before and after staining. Six variables were defined using a camera connected to a ×7–30 zoom binocular to code: (1) the width of the placental scar (millimetres), (2) the abundance of macrophages and the presence of blood, (3) the aspect of the scar, (4) the presence of swellings, (5) the presence and colour of a central band and (6) the presence and colour of lateral bands, surrounding the central band (see Table 1 for coding).

Adult females that should either have been pregnant or should have displayed placental scars but did not were considered as not reproductively active and are hereafter called barren vixens. Productivity was calculated as the mean number of placental scars, for all females including barren vixens.

Tooth sectioning procedures for ageing

The age of foxes at death was determined from the number of annual growth lines visible in the tooth cementum and the date of death. Canine teeth, or premolar teeth when canines were unavailable or damaged, were extracted from the lower jaw following Matson's laboratories (Milltown, MT, USA) procedure (Harris 1978). Foxes were assigned to age-classes based on their recruitment into the adult population on 1 February of the year following birth (i.e. at the age of 10 months). Animals between 10 and

Table 1 Categories of variables used for the FCA on placental scars in red foxes according to month after parturition

Variable	Categories			
	1	2	3	4
WD	≤5 mm	5.5 to 9.5 mm	≥10 mm	Not measured (n=4)
MA	Presence of blood and very few macrophages	Absence or few and isolated macrophages	Aggregates of macrophages	Large and numerous aggregates of macrophages
AS	Homogeneous aspect with no white points nor alveoli	Small white points or small white alveoli	Presence of white and well delimited alveoli	Presence of large white alveoli, without precise delimitation
RL	Very flat	Fairly flat	Pronounced swellings	
CB	Absent or light-coloured	Visible CB	Pronounced black CB	
LB	Absent or light-coloured	Visible LB	Pronounced black LB	

WD width, *MA* macrophages, *AS* aspect, *RL* relief, *CB* central band, *LB* lateral bands

22 months of age were classified as age-class 1 (yearlings) whereas older ones were classified as age-class 2.

Data analysis

We used a factorial correspondence analysis (FCA) to describe the evolution of macroscopic criteria of placental scars following parturition (n=328) and especially associations between these macroscopic criteria and the month of death. Since foxes are mono-oestrous, with a distinct breeding season, all foxes could be placed into an annual cohort, and date of death was simply related to the date after parturition.

Differences between mean PSC, mean embryos and productivity were compared by age-class and by period of death using an analysis of variance (after testing that residuals were normally distributed). Proportions of barren vixens were analysed by logistic regression, using a binomial error distribution and logit link function. Analyses were performed using the statistical software R 2.7.1 (R Development Core Team 2009) with the package Ade4 (Chessel et al. 2004).

Results

Sample collection

The age distribution of the 358 females examined was 46% of yearlings, 17% of 2 years old, 13% of 3 years old, 13% of 4 years old and 11% of older females. From these adult females, 164 uteri had visible embryos and were collected from February 1st to March 20th. In February, we observed the uterus at the beginning of pregnancy on 38 females. At this stage, uterine horns are pale pink, thick and rounded, and the membrane becomes thick with small swellings breaking the lengthwise structures. Due to these important modifications, placental scars from the previous reproductive season could no longer be counted, while embryos from the current reproductive season were too small to be detected. We examined 156 uteri of which 53 had neither placental scar nor embryo at all. Uterine horns were small, pale pink and the membrane was thin with lengthwise lines, and we concluded that these vixens were not reproductively active. The proportion of barren vixens was significantly higher in yearling females (19.0%) when compared to older ones (11.8%, Wald test for age effect, p=0.06), without significant difference between sites (Wald test for site effect, p=0.64; Table 2). A total 103 uteri had placental scars, of which 75 were stained to test the method.

Macroscopic description of placental scars before staining

The placental scar appeared most often as a large band that broke the lengthwise line of the uterine horn, delimited by two thicker and darker lateral bands, corresponding to macrophage bands. However, we observed large variation among uteri, depending on the date of examination. Lateral bands were not always visible, and their colour varied from black to light grey. Adjacent to this band, some irregularly distributed aggregates of macrophages could be found. These aggregates could also be observed isolated. Among the 103 uteri examined, atypical placental scars were observed on 25 uteri, i.e. some scars (n=30) presented features different from all other scars present in the same uterus.

Macroscopic description of placental scars after staining

Staining on 75 uteri corresponding to 328 placental scars made macrophages more visible and placental scars easier to detect. When staining the uteri, placental scars appeared

Table 2 Mean PSC, embryo counts, proportion of barren vixens and productivity by age-class in red foxes from three sites in France (from 2002 to 2005)

	Embryo counts[a]								Placental scar counts[c]		Percent barren vixens	Productivity	
	Site A		Site B		Site C		Total						
Age-class	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n		Mean ± SD	n
1	4.81±1.57	27	4.52±1.12	31	4.70±1.42	10	4.66±1.35	68	4.47±1.31	34	19.0	3.62±1.86	158
≥2	5.58±1.37	33	5.21±1.46	47	6.38±1.63	16	5.53±1.59	96	4.86±1.39	56	11.8	4.28±1.75	186
Total	5.23±1.50	60	4.94±1.37[b]	78	5.73±1.73[b]	26	5.17±1.50	164	4.85±1.46	90[d]	14.8	3.98±1.83	344

[a] Mean embryo counts increase (p<0.001) with increasing age-class and significant difference between sites (p=0.04)

[b] Significant post hoc Bonferroni tests (p=0.04)

[c] Mean placental scar counts not related to increasing age-class (p>0.05)

[d] Age could not be determined on 13 vixens due to jaw damage

most often as a large black band across the horn, but we observed large variation among uteri, depending on the date of examination. In three uteri, placental scars that had been classified as atypical before staining appeared after staining. All other atypical placental scars were confirmed as atypical. Thus, 24% of uteri presented resorptions or placental scars from earlier pregnancies, without significant difference between age-class 1 and older vixens (Fisher exact test, $p=0.36$). For two uteri, an 'active' placental scar appeared that had been missed prior to staining. Thus, staining increased the PSC in 2.5% of cases, by adding one placental scar. However, it was not possible to distinguish old placental scars from resorptions. Atypical scars, possibly due to resorption of embryos or old placental scars from earlier pregnancies, were not included in the estimated mean PSC per vixen.

Concordance between period of collection and categories of variables

FCA confirmed the evolution of placental scars after parturition regarding the coded criteria. Variables were projected on the plane of the first two axes of the FCA (Fig. 2a). The spatial distribution of the month when placental scars were examined fits well the distribution of the different categories of variables. The youngest placental

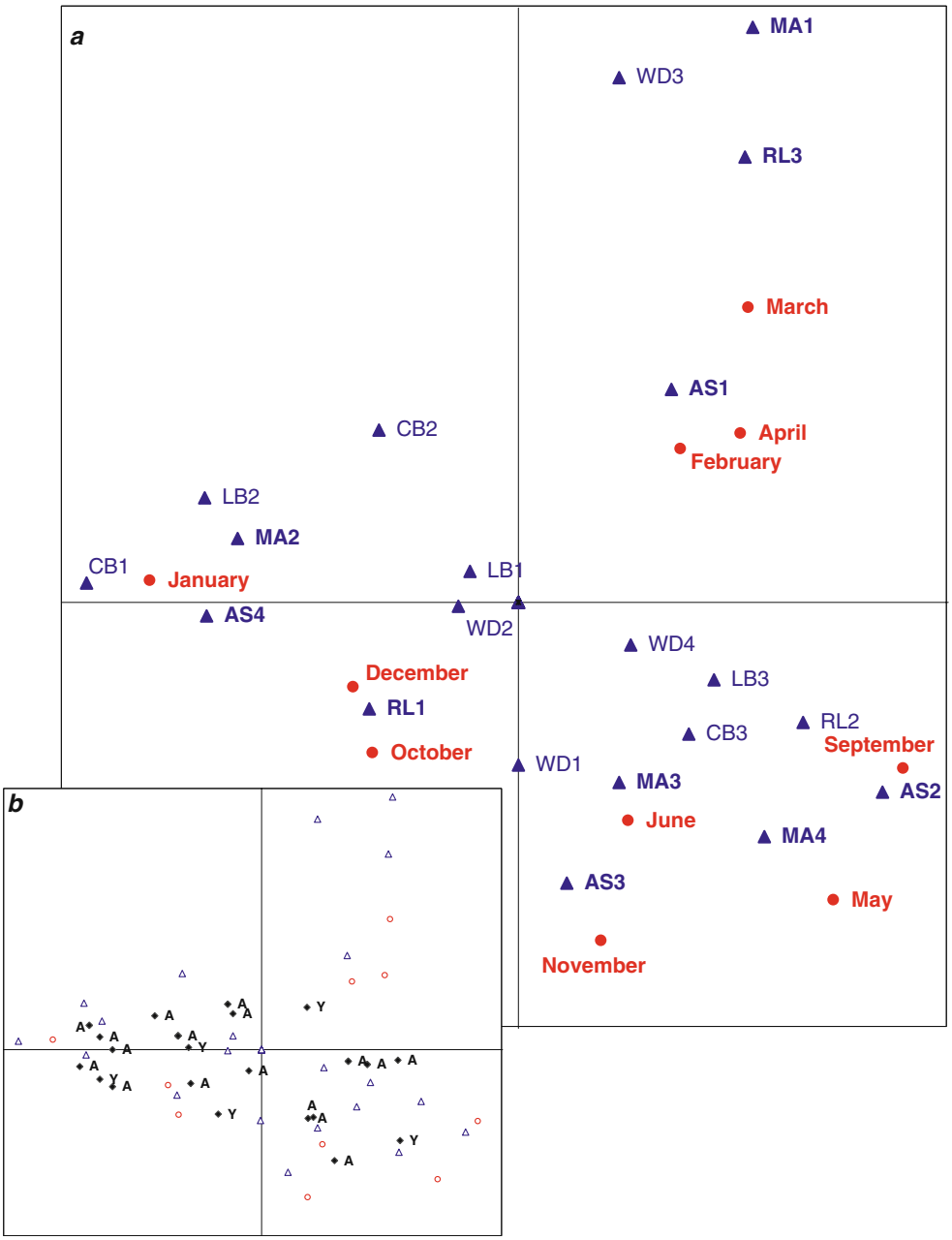

Fig. 2 Factorial correspondence analysis of associations between variable categories and month of examination of 328 scars of red foxes. **a** All categories in *bold* are best represented in the F1–F2 plane (based on the values of scores from FCA). **b** Supplementary units, i.e. atypical scars, are in *black* on the F1–F2 plane: *Y* from yearlings ($n=5$), *A* from older females ($n=25$). *WD* width, four categories; *MA* macrophages, four categories; *AS* aspect, four categories; *RL* relief, three categories; *CB* central band, three categories; *LB* lateral bands, three categories

scars, examined from February to April (months 2 to 4), were closely related to the presence of blood (MA-1), had the largest width (WD-3 ≥10 mm) and a homogeneous aspect with a pronounced imprint (AS-1 and RL-3). Those placental scars were characterized by pools of blood and formed large black bands. The placental scars collected from May to September (months 5 to 9) formed a second group on the F1–F2 plane of FCA (Fig. 2a) and were characterized by aggregates of black macrophages (MA-3 and MA-4), white points (AS-2 and AS-3) and showed a fairly flat aspect (RL-2), a pronounced black central band (CB3) delimited by darker lateral bands (LB3). From October to December, placental scars were completely flat (RL-1), white alveoli were very visible (AS-3 and AS-4) and central and lateral bands became light-coloured. In the oldest placental scars (January), coloration of the different variables faded out: Central and lateral bands were light-coloured (LB-1 and LB-2, CB-1), white alveoli were very visible (AS-4) and macrophages were isolated (MA-2).

Atypical scars were included in this analysis as supplementary units. Those scars were well distributed over the F1–F2 plane and were not related to particular categories (Fig. 2b). Atypical scars from yearlings, which could only be related to resorptions or abortions, did not present particular features.

Estimation of reproductive parameters

The mean PSC and the mean embryo counts were calculated taking into account only females having at least one scar or one embryo. Using FCA results, four periods were defined: January (period 1, n=67), February to April (period 2, n=113), May to September (period 3, n=53) and October to December (period 4, n=95).

Mean placental scars counts

The mean number of placental scars was 4.85±1.46 (n=103) per vixen, with a maximum observed of nine placental scars per uterus. There was no difference between examination periods ($F_3^{85} = 0.22$, p=0.88), so we pooled data over the periods. The mean number of placental scars was not significantly different between yearlings (4.47±1.31, n=34) and older females (4.86±1.39, n=56; $F_1^{86} = 1.47$, p=0.23, Table 2). There was no significant difference between sites ($F_1^{86} = 1.79$, p=0.17).

Mean embryo counts

Eight vixens (5%) collected in February and March showed evidence of embryo losses, i.e. placental remnants with one

Table 3 Comparison of reproductive parameters of the red fox in Europe (mean is given for each variable; sample size in brackets)

Country	Mean placental scars	Mean embryos counts	Mean number of cubs per den	% barren vixens	Reference
Britain	4.9 to 6.4 (133)			0 to 19	Heydon and Reynolds 2000
England (London)	4.6 (pooled)			24 to 52 (192)	Harris 1979
England (Bristol and London)	4.7–4.8			15.4–20.3 (444)	Harris and Smith 1987
England and Wales		4.2–5.4		8.6–25	Llyod 1968
Finland	5.2 (31)	5.1 (16)			Kauhala 1996
France	4.3 (183)	4.6 (67)		3.8 (185)	Artois et al. 1982
Germany	4.8 (112)	5.8 (108)	4.6	15.3 (170)	Vos 1994; Vos 1995
Germany	6.7	6.3			Ansorge 1990 in Vos 1994
Germany (East)		6.36 (67)			Pitzschke 1972 in Lindström 1981
Germany (East)			4.76 (108)		Stubbe and Stubbe 1977 in Lindström 1981
Ireland	5.4 (114)	5.4 (73)		10	Fairley 1970
Italy (Pisa)	3.95 (37)	3.88 (42)		20	Cavallini and Santini 1996
Poland	5.5		2.7–4.5 (10)		Goszczynski 1989
Spain	3.9 (25)			19.3 (31)	Villafuerte et al. 1996
	3.3 (114)			1.7 (116)	Gortazar et al. 2003
Scotland	5.9 (143)	5.0			Kolb and Hewson 1980
Spain (protected area)	3.3 (31, pooled)		3.1		Zapata et al. 1997
Sweden	4.3–4.9 (179)	4.6–5.1 (75)			Englund 1970
Sweden	5.2 (30)			40	Lindström 1981; Lindström 1994
Switzerland	5.1–5.2 (388)	5.1–5.2 (126)	4.7		Wandeler et al. 1974 in Vos 1994

to five visible placental scars besides embryos. The mean embryo counts was 5.17±1.50 (n=164) per vixen with a maximum observed of ten embryos per uterus. The mean embryo counts was significantly lower for yearlings ($F_1^{160} = 14.99$, $p<0.001$) than for older females, with respectively 4.66±1.35 (n=68) and 5.53±1.50 embryos per vixen (n=96). There was also a significant difference between sites ($F_2^{160} = 3.18$, p=0.04, Table 2), mean embryo counts being higher in site C than in site B (post hoc Bonferroni test, p=0.04, Table 2).

Productivity

The mean number of placental scars was significantly lower than the mean number of embryos for each age-class (age effect $F_1^{251} = 30.03$, $p<0.01$; method effect $F_1^{251} = 6.84$, $p<0.01$). Including percentages of barren vixens by age-class, the total population productivity using only PSC was significantly lower for yearlings (3.62±1.86, n=158) than for older females (4.28±1.75, n=186; $F_1^{340} = 11.65$, $p<0.01$), and there was no significant difference between sites ($F_2^{340} = 0.07$, p=0.94).

Discussion

Identification of atypical placental scars

Macroscopic description of placental scars enables us to get a precise description of the evolution of placental scars after parturition. It clearly facilitates identification of atypical scars, i.e. with a singular aspect compared to others from the same uterus or from other uteri at the same period of examination. During our study, atypical scars were detected rather frequently (24%) and not related to the period of examination. Staining in these cases allowed for a quick identification. However, it was impossible to distinguish between scars that could have persisted from earlier pregnancies or have been due to resorption or abortion. The staining method is time-consuming, and very few new 'active' placental scars (2.5%) were detected after staining so that it could be preferentially applied when atypical scars are present. However, the staining method used here provides a standard that could be useful for obtaining more comparable and repeatable results, when interpreting placental scars. Even if some scars might persist to the next oestrus, we conclude that it is impossible to estimate previous litter size with this method.

Mean PSC and mean embryo counts

The mean PSC was slightly lower than mean number of embryos, in each age-class. These differences might have been due to the period in which the samples were collected. Indeed, when counting embryos, intra-uterine mortality in the second half of gestation is not considered (Llyod 1968; Lindström 1981; Englund 1970; Heydon and Reynolds 2000; Elmeros et al. 2003). In contrast, when counting the placental scars, all visible losses between implantation and birth were taken into account (Vos 1994). However, we could not exclude that some scars may have vanished rapidly or been wrongly classified as atypical scars, due to a rapid physiological evolution. Since PSC might reflect more accurately litter size at birth, we only took into account PSC to estimate productivity. However, the sample size of uteri with detectable placental scars was rather small when compared to the total adult vixens collected (29%), whereas females with embryos represented 46%, so the sample size limited statistical analyses. Differences between PSC and embryo counts were also rather small when compared to other sources of biases in estimating true litter size, e.g. the non-evaluation of perinatal mortality. Indeed, Vos (1994) indicated a loss of around 20% between the number of embryos in early pregnancy stages and the number of cubs observed at den sites in early summer. This should be kept in mind when estimating litter size for modelling.

Age-class effect

Mean embryos counts and percentage of barren vixens varied by age-class, with yearlings being less productive than older females. The difference was not significant for mean PSC. Our results are in accordance with other studies from northern Europe and the British Isles (Allen 1983; Englund 1970; Kolb and Hewson 1980) where yearling females produced significantly fewer cubs than older adults. This could be partly explained by physiological 'immaturity' of yearling vixens and partly by a behavioural factor at high densities (Harris 1979). However, age-class differences have been observed in areas where food availability was a limiting factor (Englund 1970; Lindström 1988; Kolb and Hewson 1980) or at high densities, in sub-urban populations (Harris 1979) or in areas where human-induced fox mortality was low (Zapata et al. 1997). Moreover, Harris and Smith (1987) observed a decrease in litter size only in the oldest vixens (for fifth or sixth breeding season) in two urban fox populations. In contrast, no age-class differences were observed in other studies (Artois et al. 1982; Martorell and Gortazar Schmidt 1993; Gortazar et al. 2003; Vos 1994; Cavallini and Santini 1996; Elmeros et al. 2003; Marlow et al. 2000), but sample size might have been an important limitation. We conclude that stratification between yearlings and older adults should be applied when estimating reproduction parameters in red fox.

Reproductive parameters

In accordance with other authors (Englund 1970; Elmeros et al. 2003), we found that PSC did not vary with the period of examination, so that the method could be used from February to November. However, the number of uteri with placental scars was rather low in February, when compared to the number of females collected because many vixens were at the beginning of a new pregnancy and placental scars could not be counted.

Both estimates, mean PSC and mean embryo counts, obtained in this study are within the range of those reported in other studies (see Table 3 for a review in Europe). In most situations in Europe, the mean PSC was between 4.3 and 5.2, and the mean number of embryos was between 4.2 and 6.4. However, red fox populations show high spatial and temporal variability in reproduction parameters, which appear to be dependant on food availability, social constraints and mortality rate.

Influence of food availability (small- and medium-sized vertebrates) on reproductive parameters has been shown not only at high latitudes (Englund 1970; Kolb and Hewson 1980; Von Schantz 1981; Angerbjorn et al. 1991; Goszczynski 1989; Lindström 1988, 1989) but also at lower latitudes (Gortazar et al. 2003; Villafuerte et al. 1996), values being lower in poor habitats (semi-arid steppe). In our study, the three sites were dominated by agricultural habitat, with rather high estimated densities when compared to rural densities estimated in Europe (Heydon and Reynolds 2000), and it is likely that food availability and food resource diversity were high. Surprisingly, we observed a higher mean embryo counts and a higher (but not significantly so) mean PSC, in site C than in site B. This result is in contrast with other studies in central Europe, where variations in litter size between regions and between years were generally narrow (Artois et al. 1982; Weber et al. 1999).

Reproduction parameters also seem to be limited by social constraints, especially at lower latitudes or when food availability is high (Lindström 1989; Harris and Smith 1987). Macdonald (1977, 1981) suggested that the red fox might live in social groups, including one dog fox and several vixens, only one of which is reproductive. Among reproductive parameters, the percentage of breeding females might be the most important source of variation in productivity (Cavallini and Santini 1996). Up to 52% of yearlings failed to breed (Harris 1979) in sub-urban fox populations, but most values were around 10% (Table 3). Comparing two urban fox populations, Harris and Smith (1987) found that despite fox culling, a stable population was maintained by increased productivity, i.e. by reducing the proportion of non-breeding vixens but not by increasing litter size. Diseases, such as sarcoptic mange or rabies, also affected the proportion of reproductive females but not the litter size (Soulsbury et al. 2007; Lindström and Mörner 1985; Vos 1995). Papers on moderately to strongly controlled fox populations report a percentage of barren vixens, ranging from 0% to 25% (Artois et al. 1982; Vos 1994; Marlow et al. 2000), with more persecuted populations having a lower proportion of barren vixens (Harris and Smith 1987; Heydon and Reynolds 2000). Our results, i.e. the lower productivity in yearlings and the relative large proportion of barren vixens, may be related to relatively high densities, which match well the estimated densities obtained from spotlight counts, when compared to other rural areas in Europe (Heydon and Reynolds 2000). Estimated density was higher in site B when compared to sites A and C but was also associated with higher fox culling so that the impact of culling on fox populations in the three sites may be similar.

Despite increased fox culling effort, no variation in estimated densities in winter was observed during the study period. At the same time, productivity was rather moderate and the proportion of barren vixens was rather large. Therefore, it is unlikely that fox populations in the three sites were limited by the level of culling. The impact of culling on fox populations is much debated, especially on large geographical scales (Hewson 1986; Baker et al. 2004; Heydon and Reynolds 2000; Baker et al. 2002; Aebischer et al. 2003), and conclusions of those studies are contrasted. One of the key points explaining these contrasting results may be the various levels of culling effort applied in relation to fox densities. Further studies are needed in common agricultural landscapes to better understand the impact of culling on fox populations, especially on reproduction parameters and densities.

Acknowledgements We are grateful to Yves Desmidt, Director, Jean-Louis Pilard, President of the Hunting Association of Ille-et-Vilaine and Joseph Bouvier, responsible of hunting in the Domagné study area. We also thank Franck Drouyer, Nicolas Haigron and Cyril Mangeard for efficient support in the fieldwork and all local hunters and trappers who helped in collecting foxes. Many thanks also to Catherine Carter who kindly edited the English.

References

Aebischer NJ, Baker SE, Johnson PJ, Macdonald DW, Reynolds JC (2003) Hunting and fox numbers in the United Kingdom. Nature 423:400

Allen SH (1983) Comparison of red fox litter sizes determined from counts of embryos and placental scars. J Wildl Manage 47 (3):860–863

Angerbjorn A, Arvidson B, Noren E, Stromgren L (1991) The effect of winter food on reproduction in the arctic fox, *Alopex lagopus*: a field experiment. J Anim Ecol 60:705–714

Artois M, Aubert MFA, Gerard Y (1982) Reproduction du renard roux (*Vulpes vulpes*) en France. Rythme saisonnier et fécondité des femelles. Acta Oecol 3(2):205–216

Asano M, Matoba Y, Ikeda T, Suzuki M, Asakawa M, Ohtaishi N (2003) Reproductive characteristics of the feral raccoon (*Procyon lotor*) in Hokkaido, Japan. J Vet Med Sci 65 (3):369–373

Baker PJ, Harris S, Webbon CC (2002) Effect of British hunting ban on fox numbers. Nature 419:34

Baker PJ, Funk SM, Harris S, Bruford MW (2004) Polygynandry in a red fox population: implications for the evolution of group living in canids? Behav Ecol 15:766–778

Bray Y, Marboutin E, Péroux R, Ferron J (2003) Reliability of stained placental scars counts in European hares. Wildl Soc Bull 31 (1):237–246

Buckland ST, Anderson DR, Burnham KP, Laake JL (1993) Distance sampling. Estimating abundance of biological populations. Chapman & Hall, London

Cavallini P, Santini S (1996) Reproduction of the red fox *Vulpes vulpes* in central Italy. Ann Zool Fenn 33:267–274

Chautan M, Pontier D, Artois M (2000) Role of rabies in recent demographic changes in red fox (*Vulpes vulpes*) populations in Europe. Mammalia 64:391–410

Chessel D, Dufour AB, Thioulouse J (2004) The ade4 package—I: one-table methods. R News 4:5–10

Elmeros M, Hammershøj M (2006) Experimental evaluation of the reliability of placental scar counts in American mink (*Mustela vison*). Eur J Wildl Res 52:132–135

Elmeros M, Pedersen V, Wincentz TL (2003) Placental scar counts and litter size estimations in ranched red foxes (*Vulpes vulpes*). Mammalian Biology 68:391–393

Englund JS (1970) Some aspects of reproduction and mortality rates in Swedish foxes (*Vulpes vulpes*), 1961–63 and 1966–96. Swed Wildlife 8:1–82

Fairley JS (1970) The food, reproduction, form, growth and development of the fox *Vulpes vulpes* (L.) in north-east Ireland. Proc R Ir Acad 69(B 5):103–137

Fournier-Chambrillon C, Bifolchi A, Mazzola-Rossi E, Sourice S, Albaret M, Bary Y, Cena J-C, Urra Maya F, Agraffel T, Fournier P (2010) Reliability of stained placental scar counts in farmed American mink and application to free-ranging mustelids. J Mammal 91:818–826

Gortazar C, Ferreras P, Villafuerte R, Martin M, Blanco JC (2003) Habitat related differences in age structure and reproductive parameters of red foxes. Acta Theriol 48:93–100

Goszczynski J (1989) Population dynamics of the red fox in central Poland. Acta Theriol 34(10):141–154

Harding EK, Doak DF, Albertson JD (2001) Evaluation the effectiveness of predator control: the non native red fox as a case study. Conserv Biol 15(4):1114–1122

Harris S (1978) Age determination in the red fox (*Vulpes vulpes*)—an evaluation of technique efficiency as applied to as sample of suburban fixes. J Zool 184:91–117

Harris S (1979) Age-related fertility and productivity in red foxes, *Vulpes vulpes*, in suburban London. J Zool Lond 187:195–199

Harris S, Smith GC (1987) Demography of two urban fox (*Vulpes vulpes*) populations. J Appl Ecol 24:75–86

Helle E, Kauhala K (1995) Reproduction in the raccoon dog in Finland. J Mammal 76:1036–1046

Hewson R (1986) Distribution and density of fox breeding dens and the effects of management. J Appl Ecol 23:531–538

Heydon MJ, Reynolds JC (2000) Demography of rural foxes (*Vulpes vulpes*) in relation to cull intensity in three contrasting regions of Britain. J Zool Lond 251:265–276

Heydon MJ, Reynolds JC, Short MJ (2000) Variation in abundance of foxes (*Vulpes vulpes*) between three regions of rural Britain, in relation to landscape and other. J Zool Lond 251:253–264

Kauhala K (1996) Reproductive strategies of the raccoon dog and the red fox in Finland. Acta Theriol 41(1):51–58

Kolb HH, Hewson R (1980) A study of fox populations in Scotland from 1971 to 1976. J Appl Ecol 17:7–19

Kristiansen LV, Sunde P, Nachman G, Madsen AB (2007) Mortality and reproductive patterns of wild European polecats *Mustela putorius* in Denmark. Acta Theriol 52 (4):371–378

Lindström E (1981) Reliability of placental scar counts in the red fox (*Vulpes vulpes* L.) with special reference to fading of the scars. Mamm Rev 11(4):137–148

Lindström E (1988) Reproductive effort in the red fox, *Vulpes vulpes*, and future supply of a fluctuating prey. Oikos 52 (1):115–119

Lindström E (1989) Food limitation and social regulation in a red fox population. Holarct Ecol 12:70–79

Lindström ER (1994) Placental scar in the red fox (*Vulpes vulpes* L.) revisited. Z Saugetierkd 59:169–173

Lindström ER, Mörner T (1985) The spreading of sarcoptic mange among Swedish red foxes (*Vulpes vulpes*) in relation to fox population dynamics. Rev Ecol (Terre et Vie) 40:211–216

Llyod HG (1968) The control of foxes (*Vulpes vulpes* L.). Proc Association of Applied Biologists 61:334–349

Llyod HG, Jensen B, Van Haaften JL, Niewold FJJ, Wandeler A, Bögel K, Arata AA (1976) Annual turnover of fox populations in Europe. Zbl Vet Med B 23:580–589

Macdonald DW (1977) The behavioural ecology of the red fox. In: Kaplan C (ed) Rabies—the facts. Oxford University Press, Oxford, pp 70–90

Marlow NJ, Thomson PC, Algar D, Rose K, Kok NE, Sinagra JA (2000) Demographic characteristics and social organisation of a population of red foxes in a rangeland area in Western Australia. Wildl Res 27:457–464

Martin KH, Stehn RA, Richmond ME (1976) Reliability of placental scar counts in the prairie vole. J Wildl Manage 40 (2):264–271

Martorell JI, Gortazar Schmidt C (1993) Reproduction of red foxes (*Vulpes vulpes* Linnaeus, 1758) in north-eastern Spain: a preliminary report. OIE Revue Scientifique et Technique 12:19–22

McIlroy J, Saunders G, Hinds LA (2001) The reproductive performance of female red foxes, *Vulpes vulpes*, in central-western New South Wales during and after a drought. Can J Zool 79(4):545–553

Mowat G, Boutin S, Slough BG (1996) Using placental scar counts to estimate litter size and pregnancy rate in lynx. J Wildl Manage 60:430–440

Pech R, Hood GM, McIlroy J, Saunders G (1997) Can foxes be controlled by reducing their fertility? Reprod Fertil Dev 9:41–50

Ruette S, Stahl P, Albaret M (2003) Applying distance-sampling methods to spotlight counts of red foxes. J Appl Ecol 40:32–43

Salewski E (1964) Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. Naunyn-Schmiedebergs Archiv für experimentelle Pathologie und Pharmakologie 247:367

Soulsbury CD, Iossa G, Baker PJ, Cole NC, Funk SM, Harris S (2007) The impact of sarcoptic mange *Sarcoptes scabiei* on the British fox *Vulpes vulpes* population. Mamm Rev 37 (4):278–296

Strand O, Skogland T, Kvam T (1995) Placental scars and estimation of litter size: an experimental test in the Arctic fox. J Mammal 76:1220–1225

Team RDC (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 3-900051-07-0, http://www.R-project.org

Villafuerte R, Luco DF, Gortazer C, Blanco JC (1996) Effect on red fox litter size and diet after haemorrhagic disease in north-eastern Spain. J Zool Lond 240:764–767

Von Schantz T (1981) Red fox numbers, reproduction and social organization at different prey densities. Ph.D. thesis, Department of Animal Ecology, University of Lund, Sweden, 112 pp

Vos AC (1994) Reproductive performance of the red fox, *Vulpes vulpes*, in Garmish-Partenkirchen, Germany, 1987–1992. Z Saugetierkd 59:326–331

Vos A (1995) Population dynamics of the red fox (*Vulpes vulpes*) after the disappearance of rabies in county Garmisch-Partenkirchen, Germany, 1987–1992. Ann Zool Fenn 32:93–97

Weber JM, Meia JM, Meyer S (1999) Breeding success of the red fox *Vulpes vulpes* in relation to fluctuating prey in central Europe. Wildlife Biol 5(4):241–244

Zapata SC, Travaini A, Delibes M (1997) Reproduction of the red fox, *Vulpes vulpes*, in Donana, southern Spain. Mammalia 61(4):628–631