The Journal of Wildlife Management; DOI: 10.1002/jwmg.21623

Research Article

Factors Affecting Implantation Failure in Roe Deer

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ABSTRACT Reproductive performance is one of the most important life-history traits that should be routinely studied and considered in adaptive wildlife management. In the case of roe deer (*Capreolus capreolus*), a species with delayed implantation, which complicates studies on fetuses, corpora lutea (CL) counting is the only alternative for routine monitoring. However, because of a possible implantation failure, the reliability of this method is questionable, and factors influencing implantation success have been poorly understood so far. We analyzed 2,594 intact uteri of roe deer hunted from 2006–2015 in an Apennine population, central Italy, during winter (mid-Jan to mid-Mar). By comparing the number of CL and fetuses in the same individuals (i.e., success in blastocyst implantation), we revealed a mean implantation failure of 8.6% in a pooled sample set (regardless of the age and origin of animals), with a high inter-annual variability (range = 3.6–19.8%). Contrary to adults ($\bar{x} \pm$ SE = 11.1 ± 1.9%), the implantation failure in yearlings was low (4.4 ± 1.9%). Implantation success was affected by individual maternal characteristics (positive effect of body mass and negative effect of age), climatic condition in summer (positive effect of July temperature up to 23.4°C, and negative effect above this threshold), winter harshness (negative effect of snow cover duration), and altitude (negative relation with the elevation). Reproductive performance of adult female roe deer cannot be adequately measured by CL counts because of high inter-annual variability in implantation failure and important effects of female attributes and environmental factors. However, for yearlings, which also express the highest variability in the ovulation rates, CL counts provide important information on their reproductive outcome because they have low implantation failure.

KEY WORDS *Capreolus capreolus*, central Italy, corpus luteum, fetus, implantation failure, litter size, reproductive performance, roe deer.

Systematic, science-based monitoring of free-ranging ungulates is important for modern management and conservation of their populations (Apollonio et al. 2017). The need to assess population dynamics is a central issue in any population monitoring (Smart et al. 2004), but population estimates usually face inaccuracies and biases (Fuller 1991, Redfern et al. 2002, Gaillard et al. 2003, Campbell et al. 2004, Ward et al. 2004). Therefore, different population parameters as ecological indicators should be routinely studied and considered in decisions (Morellet et al. 2007, Maublanc et al. 2016). Among them, female reproductive success is one of the most important life-history traits that should be monitored (Vincent et al. 1995, Morellet et al. 2007). In the majority of free-ranging ungulates, the most reliable parameters predicting reproductive potential of females before calving and post-natal mortality are based on analyses of fetuses (i.e., number, sex structure, developmental phase). Roe deer (*Capreolus capreolus*), however, have a unique reproductive pattern among ungulates and implementation of such indicators would represent almost an unsolvable challenge.

Roe deer is the only artiodactyl that has developed the obligatory embryonic diapause (Ziegler 1843, Bischoff 1854, Short and Hay 1966). This strategy enables roe deer females to mate (in the mid-summer) and to give birth (in the spring) in the parts of the year with favorable nutritional conditions (Andersen et al. 1998) because as a polytocous species, roe deer females face high energy demands after implantation and during pregnancy (Hewison and Gaillard 2001). Moreover, during the rut females are still suckling the previous year's fawns and because of the costs of lactation in spring, they are likely to be in poorer body condition at that time (Hewison 1996).

Received: 2 March 2018; Accepted: 5 November 2018

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In the majority of European countries, the hunting period of roe deer females overlaps with embryonic diapause (Apollonio et al. 2010), and collecting samples of uteri with fetuses is therefore very difficult or almost impossible. Indeed, almost all yearlings and adult females are hunted in autumn (i.e., when females are in the stage of embryonic diapause; Short and Hay 1966), which complicates studies on fetuses. However, at that time, corpora lutea (CL) are present in ovaries, and could be used as a measure of potential reproductive success (Strandgaard 1972, Ratcliffe and Mayle 1992, Langvatn et al. 1994, Hewison 1996, Flajšman et al. 2017*a*), potentially enabling forecast of the increment rate (i.e., number of offspring) on a yearly basis. Considering reproductive biology of the species and management practices, CL counts seem to be the only solution that could be widely implemented in management of roe deer populations throughout Europe.

Reliability of CL counts as an indicator of reproduction is still questionable because it is measured in the early stage of pregnancy when it is still a low-cost process (i.e., prior to the substantial investment in implantation in mid-winter; Bronson and Manning 1991, Mauget et al. 1997); it does not account for implantation failure (i.e., difference between the ovulation rate [number of CL] and successful implementation of ova [number of embryos or fetuses]), which is poorly understood but can exert high spatiotemporal variability; and there are few studies on influential factors that affect implantation success. The scarce data on implantation failure in roe deer are inconsistent; in some studies the overall implantation failure was <10% (Borg 1970, Strandgaard 1972, Flajšman et al. 2017*b*), but it might be also as high as 30% and may be influenced by the female age, body mass, and weather conditions in a given year (Hewison and Gaillard 2001). Implantation failure seems to increase with senescence, and was lower in healthy and heavier individuals compared to females with low body mass (Hewison and Gaillard 2001). All these findings, however, originated from small and very specific sample sets (i.e., road-killed animals [Borg 1970]), or from specific research areas with coniferous woodlands (Hewison and Gaillard 2001). Therefore, large-scaled data on implantation failure, including different environmental and climatic contexts, would be essential to understanding implantation failure in roe deer.

Our objective was to evaluate the relation between ovulation rate and the subsequent implantation success (i.e., between the CL count and the number of fetuses in same individuals) in the roe deer population in Apennine, Tuscany, central Italy. In this area, the main hunting season for roe deer females is 1 January to 15 March, which is distinct from other European countries where roe deer hunting usually ends in December or January. This provided an opportunity to obtain unique data in the later stage of reproduction, when fetuses are present in uteri. We studied the rate of implantation success in roe deer in relation to individual characteristics of the mother, climatic conditions in summer and winter, local population density, and local elevation (Table 1).

STUDY AREA

The study took place in the Arezzo Province (3,235 km^2), Tuscany, central Italy. In the study period (2006–2015), roe deer hunting was allowed in 22 hunting districts (average size ~9,500 ha), divided in 1,910 hunting zones (average size = 109.35 ha ± 1.22 SD; Fig. 1). About 57% of the territory is >400 m above sea level (asl) and 7.4% is >1,000 m asl. The northern part of the province is mostly mountainous, including the Apennine chain and other secondary chains, with altitudes ranging from 300 m to 1,654 m; 66% was forested. The southern part of the area includes the lower course of the Arno River and Chiana Valley, the Chianti hills, and some low mountains, with elevations ranging from 120 m to 1,081 m. Approximately 50% of this area was cultivated fields, and forests covered 32% of the area. Forests were predominantly deciduous with dominant species being oaks (turkey oak [*Quercus cerris*] and downy oak [*Q. pubescens*]), beech (*Fagus sylvatica*), and sweet chestnut (*Castanea sativa*); the percentage of conifers was only 6.5%. The climate is temperate-continental, with the mean temperatures ranging from 1.4°C in January to 24.9°C in July.

The study area was inhabited by a rich wild ungulate community. Roe deer were present in 80% of the province and wild boar (*Sus scrofa*), fallow deer (*Dama dama*), red deer (*Cervus elaphus*), and mouflon (*Ovis orientalis musimon*) were also present. Wild boar was homogeneously distributed across the whole province, whereas red deer, fallow deer, and

Table 1. Hypothesized effects on the implantation success of roe deer females in the Arezzo Province, Tuscany, central Italy, 2006–2015.

Hypothesized effects	Description	Explanatory variables	Predicted direction of effect
Individual characteristics	Maternal phenotypic quality and age (Hewison and Gaillard 2001, Flajšman et al. 2017*a*)	Body mass	+
		Age class	Peak at middle-aged class
Climatic conditions in summer	Affect the chance of ovulation and might affect also the subsequent implantation (Flajšman 2017, Lombardini et al. 2017)	Jul temp	+
		Jul rainfall	−
Climatic conditions in winter	Affect implantation success and fetal resorption (Hewison and Gaillard 2001)	Jan temp	+
		Snow cover duration	−
		Snow cover extent	−
Local elevation	Increasing environmental harshness (Hewison and Gaillard 2001)	Elevation class	−
Local population density	Intra-specific competition affects reproductive performance (Hewison and Gaillard 2001, Flajšman et al. 2018)	Local population density	−

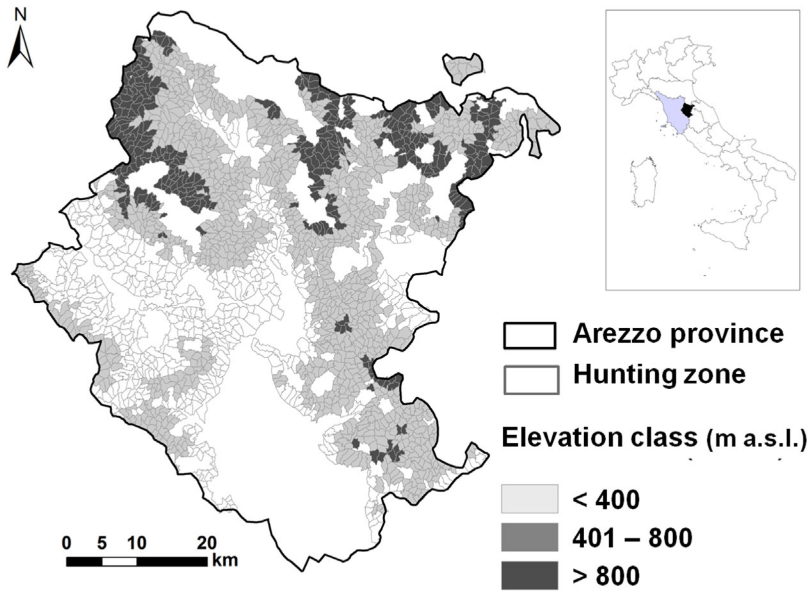

Figure 1. The study area located in the Arezzo Province, Tuscany, central Italy. Hunting zones ($n = 1{,}910$) where roe deer females were legally hunted from 2006–2015 are shown in the digital elevation model classes of the study area (darker color corresponds to higher elevation).

mouflon were localized (Apollonio and Mattioli 2006). Wolf (*Canis lupus*), with an estimated number of 25 packs (Bassi et al. 2015), and red fox (*Vulpes vulpes*) were also present in the study area.

METHODS

Data Collection

We collected 2,623 intact uteri of roe deer females that were legally hunted during the annual winter harvest (1 Jan–15 Mar), 2006–2015. To be sure all pregnant females had implanted their embryos, we set the beginning of the study period on 15 January (Hewison and Gaillard 2001). For each individual, hunters were required to recorded date of culling, hunting zone, and body mass (expressed as eviscerated body mass to the nearest 0.5 kg; all data provided in Table S1, available online in Supporting Information).

Immediately after the cull and dissection, hunters placed uteri into plastic bags and stored them frozen until collection. We defrosted samples and analyzed them in the laboratory at the Casa Stabbi field station, in Chitignano (Arezzo Province, Tuscany, central Italy). To determine the potential litter size, we counted the CL after the dissection of each ovary; moreover, we determined the number, sex, size, and weight of fetuses.

Hunters also collected mandibles of all studied individuals for age assessment made by macroscopic inspection of teeth development and tooth wear (Ratcliffe and Mayle 1992). Two authors (RC and EB) determined age using a tooth wear table developed locally, and validated age by histological examination of teeth through counting annual cementum layers in a sample set of >300 individuals from a previous data collection (Capitani et al. 2005). Because this method provides uncertain assessment of age of adult roe deer (Hewison et al. 1999), we grouped animals into yearlings (20–22 months old), 2-year-olds (32–34 months old), young adults (3–4 yr), middle-aged adults (5–7 yr), and old adults (≥8 yr).

Roe deer densities in the study area were obtained by drive censuses (Mattioli et al. 2004, Davis et al. 2012) in May and June 2005–2014 on a network of 187 permanent sample plots ($0.44\,\text{km}^2 \pm 0.26$ SD on an area of $81.16\,\text{km}^2$), which were uniformly distributed throughout the province. We calculated roe deer density at a local scale by spatial interpolation using the inverse distance weighting method (Li and Heap 2008) in ArcGIS 10.1 (Environmental Systems Research Institute, Redlands, CA, USA).

Collection of Climatic and Environmental Data

We obtained mean daily temperatures (°C) in January and July, and the rainfall (mm) in July from 9 weather stations (Centro Funzionale Regionale di Monitoraggio Meteo-Idrogeologico 2017) equally distributed in 3 elevation zones (<400 m, 400–800 m, >800 m) within the study area. We tested the possible effect of winter harshness as snow cover duration and spatial extent on mother's condition throughout the gestation period using a remotely derived index of snow cover presence (http://modis-snow-ice.gsfc.nasa.gov, accessed 15 Jun 2017; MOD10A2 on 8-day intervals at 500-m resolution). We defined the period of the snow season as 4 December to 14 March. We calculated the snow spatial extent as the mean percentage of each hunting area covered by snow during the study period. Given that the mean value was 11.3%, we decided to calculate snow cover duration as the number of days with >10% of the hunting area covered by snow. We defined a mean elevation of each hunting zone using the zonal statistic tool in ArcGIS 10.1 with the digital elevation model of the Arezzo Province (10-m resolution).

Data Analysis

We evaluated the consistency between authors (RC and EB) of the age class assessment based on the tooth wear table

Table 2. Potential predictors of the implantation success of roe deer females in the Arezzo Province, Tuscany, central Italy, 2006–2015.

Independent variable	Description	Period of data collection	Data source (origin)
Body mass	Eviscerated body mass of each individual	Hunting season (Jan–mid-Mar)	Arezzo Province official database
Age	Assessed age of each individual in 5 age classes		Assessment by tooth wear inspection with local validation
Jan temp	Mean daily temp (°C)	Jan	Nine weather stations equally distributed in 3 elevation classes
Jul temp	Mean daily temp (°C)	Jul	
Jul rain	Total rainfall (mm)	Jul	
Snow cover duration	Number of days with >10% of the hunting area covered by snow	Dec–mid-Mar	MOD10A2: http://modis-snow-ice.gsfc.nasa.gov (8-day intervals at 500-m resolution)
Snow cover extent	Mean percentage of hunting area covered by snow during the study period		
Elevation class	Class 1: <400 m above sea level (asl) Class 2: 401–800 m asl Class 3: >800 m asl		Digital elevation model: Arezzo Province official database
Population density	Number of roe deer/100 ha	May, Jun	Annual drive census data
Ordinal date	Date of the cull	Hunting season (Jan–mid-Mar)	Arezzo Province official database

using a paired-sample t-test, which revealed the absence of a data collector effect ($t_{499} = -0.72$, $P = 0.47$). We compared the number of fetuses with the number of CL (fetus/CL ratio; a measure of implantation success at the individual, population, or cohort scale) for different age classes and elevation classes using analysis of variance (ANOVA) and *post hoc* Tukey's honest significant difference (HSD) test.

We used generalized additive mixed models (GAMM; Poisson family) to identify the factors affecting implantation success (Table 2), implemented in gamm4 package in Program R (www.r-project.org, accessed 4 Jul 2018) and fitted models using maximum likelihood (Wood 2006, 2008; Wood and Scheipl 2014). We considered implantation success as the number of fetuses in relation to CL in each studied animal. We considered only females with ≥1 corpus luteum in our analysis, representing all the potentially pregnant females. We used the tracking number of each hunted female as a random intercept to account for the structure of our data (Machlis et al. 1985). We z-transformed continuous independent variables to compare the relative effects of predictors on implantation success. We modeled the effect of all continuous predictor variables as natural cubic spline functions. When the estimated degree of freedom of a predictor variable was 1 and the graphical inspection confirmed a linear relationship with the response variable, we refitted the model omitting the smoothing function. We fitted models with all possible biologically meaningful combinations of independent variables. We assessed collinearity using variance inflation factors (VIFs) and dropped any models with VIFs > 3 as suggested by Zuur et al. (2010).

We used Akaike's Information Criterion (AIC; Burnham and Anderson 2002, Symonds and Mousalli 2011) to select the best fitting models (ΔAIC ≤ 2). We refitted the final set of models obtained using the restricted maximum likelihood estimation, and obtained the effect of each variable included in this confidence set of models via model averaging (model.avg function in MuMIn package for R; Burnham and Anderson 2002, Symonds and Mousalli 2011, Barton 2015). We validated models by inspecting the residual plots as described by Zuur et al. (2009). Following Magee (1990) to describe how the models fit the data observed, we estimated R^2 as:

$$R^2 = 1 - \exp[-2/n \times (\log L_M - \log L_0)],$$

where n is the number of observations, $\log L_M$ is the standard log-likelihood of the model (which includes fixed and random effects), and $\log L_0$ is the standard log-likelihood of

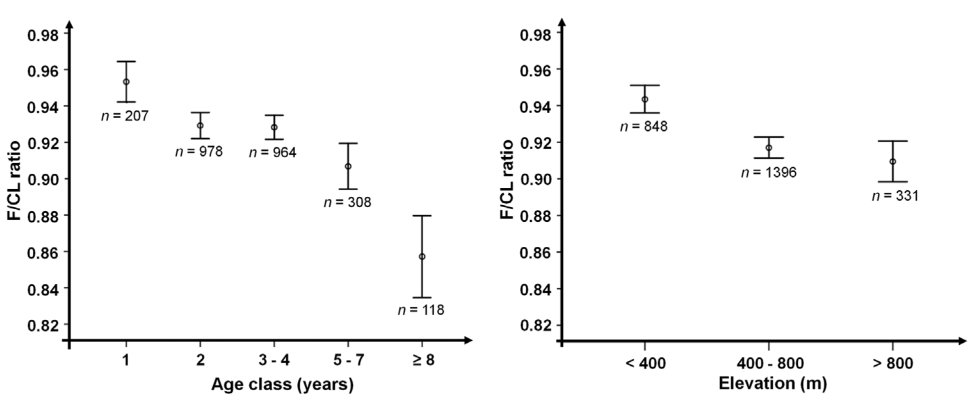

Figure 2. Comparison of the number of fetuses (F) versus the number of corpora lutea (CL; F/CL ratio; $\bar{x} \pm$ SE) in roe deer females hunted in the Arezzo Province, Tuscany, central Italy, 2006–2015.

the intercept-only model. We conducted all statistical analyses in R version 3.1.0.

The study complies with all relevant national, regional, and provincial Italian laws and with the ethical standards of scholarly research. All roe deer females used in the research were harvested during the regular hunting activity prescribed by the national authorities of Italy within the yearly hunting management plans. Therefore, no animal was shot or killed by any other means for the purposes of the research; we used reproductive organs of already dead animals.

RESULTS

In the total sample set (regardless the age and origin of animals), the mean percentage of implantation failure was 8.6%, with a high inter-annual variability, ranging from 3.6% to 19.8% (Appendix A). Contrary to adults (11.1 ± 1.6 [SE]%), particularly older ones, our data revealed a very low implantation failure in yearlings ($4.4 \pm 1.9\%$).

Comparison of fetus/CL ratios in different age classes revealed differences between yearlings, young and middle-aged (2–7 yr), and old (≥8 yr) females ($F_{2,2594} = 4.51$, $P \leq 0.01$), and comparison among different elevation zones revealed differences between low and medium-high elevations ($F_{2,2594} = 5.01$, $P \leq 0.01$; Fig. 2). Implantation success was also affected by individual characteristics of mothers; body mass had a positive effect and age had a negative effect on implantation success (Fig. 3). For years with a mean yearling eviscerated body mass of ≥17.6 kg, implantation success for yearlings approached 1 (Fig. 4).

Climatic conditions, in particular winter harshness and summer temperature, could influence the failure of implantation (Table 3; Appendix B). The fetus/CL ratio was negatively affected by snow cover and elevation. The predicted fetus/CL ratio across ages for snow cover duration of 8 days was 0.94 and 0.86 for a snow cover duration of 56 days. Implantation success was linearly related to all the variables included in the final model, except July temperature. Its effect was positive at lower temperatures, but at higher values (>23.4°C), it became negative (Fig. 5).

DISCUSSION

Reproductive performance of mammalian females depends on a broad set of factors (Clutton-Brock et al. 1985, Bronson 1989, Borowik et al. 2016, Moreira and Rodrigues 2016). In general, the most important factors that could influence roe deer reproductive potential (i.e., the number of fertilized ovulations and litter size; Flajšman et al. 2013) are female phenotype, particularly body mass and age (Focardi et al. 2002; Kjellander et al. 2004; Hamel et al. 2009; Flajšman et al. 2017*a*, 2018), weather conditions, habitat quality (Nilsen et al. 2004, Toïgo et al. 2006, Lombardini et al. 2017), population density (Andersen and Linnell 2000, Hewison and Gaillard 2001, Nilsen et al. 2009, Flajšman et al. 2018), and genetic characteristics (Hewison 1997). Within this frame, our study revealed that reproductive potential of roe deer females in the early stage of pregnancy (their ability to ovulate; Table S2) could differ from the final reproductive performance. Indeed, the mean percentage of the implantation failure (i.e., the difference between CL count, reflecting ovulation rate, and the number of fetuses, reflecting the percentage of successful implantation of fertilized ova) was 8.6%, with a high inter-annual variability (range = 3.6–19.8%). High inter-annual

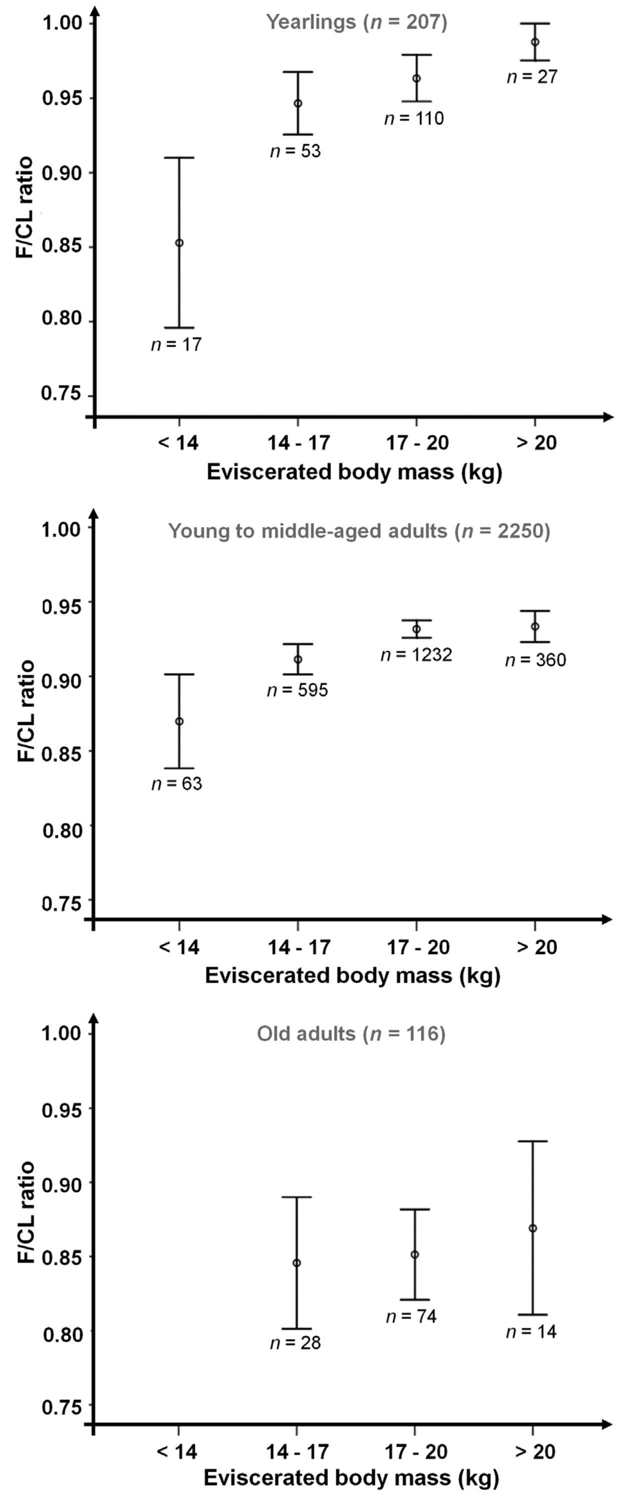

Figure 3. Comparison of the number of fetuses (F) versus the number of corpora lutea (CL; F/CL ratio; $\bar{x} \pm$ SE) in roe deer females hunted in the Arezzo Province, Tuscany, central Italy, 2006–2015, for different body mass classes and age classes (yearlings, young and middle-aged adults [2–7 yr], and old adults [≥8 yr]).

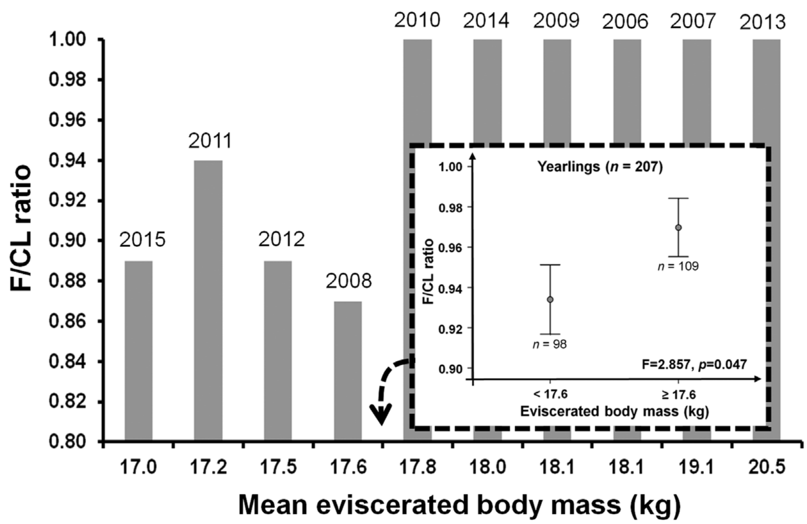

Figure 4. Inter-annual variability of the number of fetuses (F) versus the number of corpora lutea (CL; F/CL ratio) in roe deer yearlings hunted in the Arezzo Province, Tuscany, central Italy, 2006–2015, in relation to increasing mean eviscerated body mass of hunted animals (Provincial Administration of Arezzo, unpublished data). Years of data collection are reported above grey bars. In the small panel, comparison of F/CL ratio ($\bar{x} \pm$ SE) is presented according to the threshold body mass.

variability in fetus/CL ratio suggests that even if CL counts reflect reproductive potential of females at the early stage of reproduction, counting CL is not an adequate method for measuring final reproductive outcome, at least not in the age group(s) in which implantation failure is high or may express high spatiotemporal variability.

Recently, Flajšman et al. (2017*a*) demonstrated that ovulation ability of roe deer females increases with higher body mass in yearlings and adults. According to our data, body mass also plays an important role in determination of implantation success (Table 3, Fig. 3). These findings together confirm the pronounced influence of female body mass on reproductive success when considering their potential at ovulation and fertilization and when considering reproductive allocation at implantation in mid-winter. In the studied population, body masses (Table S1) of yearling females (and to a lesser extent of adult females) were higher than in other central-European populations (e.g., average body masses in Slovenia: yearlings = 15.3 kg, adult females = 16.9 kg (Flajšman 2017); in Poland: adult females = 16.7 kg (Janiszewski et al. 2016); in central-eastern Italian Alps: yearlings = 14.9 kg, adult females = 16.0 kg [Autonomous Province of Trento 2015]; this study: yearlings = 17.6 kg, adult females = 18.1 kg). These differences are primarily because we determined body mass in January–March, so younger animals developed larger body size in comparison with individuals hunted in autumn in other populations. Nevertheless, differences in average body mass among different populations indicate that some local ecological conditions within each specific population should be used (i.e., which body mass acts as a threshold body mass considering ovulation probability or implantation success) to better understand and consider parameters of reproductive performance in roe deer.

Furthermore, the positive effect of body mass on female reproductive performance, including implantation success,

Table 3. Parameter estimates of generalized additive mixed models (GAMM) predicting implantation success (i.e., number of fetuses in relation to number of corpora lutea; $n = 5{,}263$) in roe deer females in the Arezzo Province, Tuscany, central Italy, 2006–2015.

Parametric coefficients	Estimate	SE	*t*	*P*
Intercept	−0.123	0.012	10.317	≤0.001
Body mass	0.011	0.005	2.457	0.014
Age	−0.022	0.005	4.820	≤0.001
Total rainfall$_{(Jul)}$	−0.009	0.005	1.536	0.118
Snow cover duration	−0.032	0.007	4.825	≤0.001
Elevation class$_{(low)}$	0.151	0.034	4.410	≤0.001
Elevation class$_{(medium)}$	0.002	0.013	0.108	0.898
Population density	−0.001	0.004	0.161	0.872
Mean daily temperature$_{(Jul)}$[a]	–	–	–	≤0.001

[a] Coefficient was modeled using a smoothing function (estimated degrees of freedom = 4.369; $F = 23.418$).

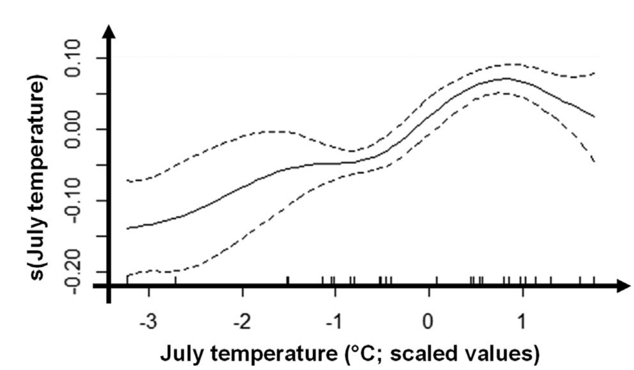

Figure 5. Estimated smoothing curve of generalized additive mixed models (GAMM) identifying factors affecting implantation success in roe deer females in the Arezzo Province,Tuscany, central Italy, 2006–2015. The *x*-axis shows the July temperature (°C; scaled values; real range: 17–25°C, negative trend for temperature >23.4°C) and the *y*-axis explains the values predicted by the GAMM in terms of the smoothing function (estimated df = 4.37). The solid line is the smoother and dotted lines are 95% confidence bands.

weakened with age (Fig. 3). Implantation failure increased with age (Table 3, Fig. 2); this finding along with the suggested trend of lower ovulation ability in very old females, revealed recently by Flajšman et al. (2017*a*), indicates the existence of reproductive senescence in roe deer females. The decrease of reproductive potential of roe deer with senescence has been rarely documented; examples include an overall decrease in fertility and smaller litters (Gaillard et al. 1998, 2003) and decrease in ovulation rates (Flajšman 2017, Flajšman et al. 2017*a*). Moreover, we confirmed higher implantation failure in senescent females reported by Borg (1970) and Hewison and Gaillard (2001). Decrease in reproductive outcome in senescent animals has been more often reported in red deer (Clutton-Brock et al. 1984, Langvatn et al. 1994, Bertouille and Crombrugghe 2002) and in fallow deer (Langbein and Putman 1992).

Implantation failure in yearlings was low ($4.4 \pm 1.9\%$; Fig. 2), indicating that contrary to adults only yearlings reaching a threshold body mass would ovulate (see also Hewison 1996, Gaillard et al. 2000, Mauget et al. 2003, Flajšman 2017). This in turn means that by counting CL in yearlings in autumn and recording their body mass (i.e., during the main hunting season when fetuses are still not present in uteri), it is possible to obtain reliable information on the ovulation rate and on their expected pregnancy rate and final reproductive outcome. Because body mass and weather condition influence reproductive potential of yearlings and to a much lesser extent of adults (Flajšman 2017), yearlings express higher variability in reproductive performance than adults (Hewison and Gaillard 2001, Flajšman et al. 2017*a*). This includes also a pronounced inter-annual variability in ovulation rates, which may differ in the same population by as much as 0.47 in ovulation rate between 2 successive years for an individual, as reported for the Slovenian roe deer population in 2013 and 2014 (Flajšman 2017).

Different environmental and climatic factors can have an influence on roe deer body condition (Toïgo et al. 2006, Douhard et al. 2013). These factors should therefore cause indirect fluctuation in the female's ability to ovulate (Flajšman 2017, Lombardini et al. 2017) and, consequently, affect recruitment in populations. Several factors, however, have shown a direct effect (i.e., not mediated by body mass) on the reproductive success of this species. Given that roe deer are income breeders (Andersen et al. 2000) and do not rely on body reserves for reproduction but more on food availability, then poor weather conditions, especially in the period of embryonic diapause, might affect the implantation of blastocyst(s) and consequently the litter size (Hewison and Gaillard 2001, Nilsen et al. 2004, Flajšman et al. 2013). In our study, favorable environmental conditions (i.e., mild winters [low snow cover], good weather conditions in summer [higher temp up to a threshold value, low rainfall quantity], low altitudes) played an important role in positively affecting implantation success of roe deer females (Table 3).

Local population density might also determine the reproductive performance of roe deer females, despite a non-significant effect on implantation success (Table 3). Nevertheless, population density is an important factor that can directly or indirectly affect the fertility or litter size of roe deer across Europe (Flajšman et al. 2013, 2018). Although roe deer are difficult to observe, particularly in forested areas, and the accuracy of population density estimates obtained from drive censuses is questionable (Cederlund et al. 1998), the obtained local densities are considered accurate enough for the purposes of our study, given its continuous application with the same monitoring standards throughout the study period (Mattioli et al. 2004, Davis et al. 2012). The recognized but non-significant density-dependent effect found in our study is related to the medium-to-high population densities of roe deer over the entire study area disregarding elevation. Indeed, negative density dependence in the reproduction of roe deer females (Gaillard et al. 1992) and in other deer species usually occurred in populations facing harsh environmental conditions (e.g., wild reindeer [*Rangifer tarandus*]: Skogland 1985, Pachkowski et al. 2013; white-tailed deer [*Odocoileus virginianus*]: Simard et al. 2010) or along a large gradient of densities as in the case of roe deer at the continental scale (Flajšman et al. 2018).

In cervids, prenatal mortality is usually associated with fetal or embryonic loss, and can be affected by different factors. For example, in red deer, post-implantation fetal mortality was affected by population density and winter rainfall (Kruuk et al. 1999); in moose (*Alces alces*), reproductive failure was related to winter nutritional conditions and body mass of pregnant females (Milner et al. 2012). However, in roe deer, also knowledge on implantation failure is relevant because of its unique delayed implantation with females adjusting their reproductive effort prior to substantial investment at implantation in mid-winter.

MANAGEMENT IMPLICATIONS

For optimal management of roe deer populations in the absence of reliable data on population size, it is important to have data on the expected number of offspring in the next year. Throughout Europe, the hunting period for roe deer females is usually in autumn, the period of embryonic diapause; therefore, only data on CL can be routinely collected in the majority of countries. To use such data as reliable indicators of the reproductive outcome, the implantation failure has to be minimal and not seriously biased by different environmental and individual factors. According to our data, CL counts negligibly to moderately differed from intra-uteri litter size only in yearlings, which are also the only age category that expresses a pronounced variability in the ovulation ability. Therefore, in roe deer females, reproductive performance based on CL counts should be routinely analyzed for management purposes only in yearlings. On the contrary, in adult females, reproductive performance based on CL counts does not have any relevant value for population management.

ACKNOWLEDGMENTS

We are grateful to the Arezzo Province for supplying data. We are indebted to M. Meacci for logistic support in collecting data. We thank all hunters who provided samples of roe deer uteri; without their collaboration, the study would not be possible. Special thanks go to E. Merli for his support in statistical analysis. We are thankful to S. J. Halvorson, who revised the English and helped improve the writing. S. Côté, J. M. Gaillard, and an anonymous reviewer provided helpful comments on earlier drafts of the manuscript. This study was partially funded by the Ministry of Agriculture, Forestry and Food (projects V4–1627, V4–1825) and the Slovenian Research Agency (research programme P4–0107, projects V4–1627, V4–1825, young researcher's PhD grant (K. Flajšman): contract no. 1000–12–0404).

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Associate Editor: Steeve Côté.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website.

APPENDIX A. SUMMARIZED DATA ON STUDIED ROE DEER FEMALES.

Table A1. Sample sizes (n), corpora lutea count (CL), number of fetuses (F), and failure rates for roe deer females in the Arezzo Province, Tuscany, central Italy, 2006–2015 (15 Jan–15 Mar).

	Summarized by year				Summarized by age class			
Year	***n***	**CL**	**F**	**Failure (%)[a]**	**Age (yr)**	***n***	**CL**	**F**
2006	8	16	14	12.5	1	2	4	4
					2	1	2	2
					3–4	2	4	3
					5–7	3	6	5
					≥8	0		
2007	12	24	22	8.3	1	2	4	4
					2	2	4	4
					3–4	7	13	12
					5–7	0		
					≥8	1	3	2
2008	170	346	303	12.4	1	44	91	78
					2	27	53	45
					3–4	51	101	90
					5–7	37	79	72
					≥8	11	22	18
2009	186	358	340	5.0	1	7	13	13
					2	72	137	137
					3–4	73	145	134
					5–7	24	48	43
					≥8	10	15	13
2010	274	554	515	7.0	1	23	44	44
					2	114	222	204
					3–4	79	166	155
					5–7	46	97	93
					≥8	12	25	19
2011	227	461	418	9.3	1	8	15	14
					2	104	208	184
					3–4	88	185	171
					5–7	20	40	37
					≥8	7	13	12
2012	183	368	295	19.8	1	37	69	61
					2	77	150	122
					3–4	44	97	75
					5–7	18	36	29
					≥8	7	16	8
2013	36	74	65	12.2	1	1	2	2
					2	11	23	19
					3–4	17	33	32
					5–7	6	13	11
					≥8	1	3	1
2014	880	1,830	1,764	3.6	1	53	103	103
					2	352	716	689
					3–4	357	754	729
					5–7	79	173	163
					≥8	39	84	80
2015	618	1,268	1,108	12.6	1	31	59	52
					2	225	454	407
					3–4	254	526	462
					5–7	77	166	137
					≥8	31	63	50
Total	2,594	5,299	4,844	8.6				

[a] The loss in number of fetuses in relation to number of CL divided by the CL count.

APPENDIX B. MODEL SELECTION RESULTS.

Table B1. Set of the most parsimonious models showing variation in implantation success of roe deer females in the Arezzo Province, Tuscany, central Italy, 2006–2015.

Component models	AIC[a]	ΔAIC[b]	w_i[c]
Body mass + age + Jul temp + Jul rain + snow cover duration + elevation class + density	−3,162.112	0.000	0.556
Body mass + age + Jul temp + snow cover duration + elevation class + density	−3,160.424	1.688	0.239
Body mass + age + Jul temp + Jul rain + snow cover duration + elevation class	−3,160.115	1.997	0.205

[a] Akaike's Information Criterion.
[b] AIC (respective model) − AIC (the best model). We present only the top model set containing models with ΔAIC ≤ 2.
[c] Akaike weights.