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REVIEW

Methods for estimating the age of roe deer (*Capreolus capreolus*): a review

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Abstract
Accurate age estimation is essential for understanding roe deer (*Capreolus capreolus*) population dynamics and implementing effective wildlife management. This review systematically evaluates existing age estimation methods, categorizing them into tooth-based approaches (cementum and dentin incremental lines, tooth metrics, occlusal surface morphology, dental development), non-tooth-based methods (bone fusion status, eye lens weight, pedicle morphometry), molecular techniques (epigenetic clocks), and combined criteria. Validation studies using marked individuals reveal considerable discrepancies between methodological prevalence and actual accuracy. Cementum annuli analysis, often regarded as the reference standard, achieves only 46% accuracy in the largest direct validation. Dental development provides monthly precision but is limited to individuals under 15 months. Occlusal surface wear assessment, the most widely applied field method, shows high accuracy for young animals but declines markedly beyond 24 months. Eye lens weight and pedicle morphometry exhibit curvilinear relationships with age, losing precision after four years. Epigenetic clocks demonstrate superior accuracy (mean absolute difference ± 0.558 years) but require blood sampling and incur higher costs. Combined morphological criteria achieve 68–82% accuracy for animals up to three years. No single method satisfies all requirements of accuracy, practicality, cost-effectiveness, and applicability to live animals. Individual methods should be considered complementary rather than exclusive, with the choice of method depending on the research objectives, available resources, and the condition of the study material. Future research priorities include validating epigenetic clocks across populations, developing non-invasive techniques such as dental radiographic pulp measurements, and establishing standardized protocols using marked individuals of known age.

Keywords *Capreolus capreolus* · Age estimation methods · Wildlife management · Wildlife biology

Introduction

Accurate age estimation represents a fundamental cornerstone in wildlife biology, however it remains a central challenge in research (Morris 1972). Estimating the age of individuals is essential for understanding demographic structures and ecological characteristics. These insights are critical for effective conservation and sustainable management (Kierdorf and Kierdorf 1989; Gaillard et al. 1992; Flajšman et al. 2017; Wilbourn et al. 2017; Chirichella et al. 2021; Melletti and Focardi 2025; Paniccià et al. 2025).

A prime example for this need is the roe deer (*Capreolus capreolus*). As the most widespread cervid in Europe, it acts as an ecologically significant selective browsers, shaping vegetation structure in forests and agricultural landscapes (Apollonio et al. 2010; Burbaitė and Csányi 2010). Roe deer are small- to medium-sized herbivores, feeding primarily on leaves, shoots, herbs, and buds, with their diet changing seasonally (Tixier and Duncan 1996; Cornelis et al. 1999; Hirst et al. 2026). Their digestive physiology includes a multi-chambered stomach with a rumen adapted to ferment fibrous plant material with the help of microorganisms, which also changes with seasonal diet shifts, impacting nutrient intake and growth (Deutsch et al. 1998; Dahl et al. 2020).

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The life cycle of the roe deer is characterized by high reproductive synchrony, with around 80% births occurring between mid-April and mid-June (Linnell 1998). These are typically divided into fawns (0–1 year), yearlings (1–2 years), young adults (2 years), prime-age individuals (3–7 years), and senescent individuals (>7 years) (Hewison et al. 2025). The maximum documented ages for roe deer are 14 years for males and 18 years for females (Hewison et al. 2025).

However, assigning an individual to a specific age remains a complex task, therefore a variety of methods based on different traits have been developed. Many of them were originally developed for other mammal species, especially larger cervids, before being adapted to roe deer (Severinghaus 1949; Robinette et al. 1957; Sergeant and Pimlott 1959). These include tooth-based methods that rely on predictable age-related changes of the teeth, such as the timing and order of tooth eruption, patterns of tooth wear, or the analysis of growth lines in the tooth cementum (Severinghaus 1949; Robinette et al. 1957; Sergeant and Pimlott 1959; Mitchell 1963, 1967; Brown and Chapman 1991b; Tomé and Vigne 2003). Beyond dentition, morphological and skeletal traits provide further indicators of age (Longhurst 1964; Morris 1972; Stubbe et al. 1987). For example the weight of the eye lens, the length of certain bones, and the closure of growth plates change in predeterminable ways over the course of an individual's lifetime (Lord 1959; Longhurst 1964; Morris 1972; Tomé and Vigne 2003). Most recently, molecular approaches have emerged as significant advancements, utilizing biological markers such as DNA methylation patterns or telomere length to estimate chronological age (Olovnikov 1971; Olsen et al. 2010; Horvath 2013; Meng et al. 2019; Lemaître et al. 2022; Yagi et al. 2024).

Despite this bewildering variety of criteria, validation studies for roe deer remain scarce, with few having systematically evaluated their applicability and reliability for age estimation. In this review, the methods for roe deer described so far are compared and the underlying descriptions are analyzed with the aim of developing recommendations for standardized, reliable age estimation protocols.

Literature search approach

To identify relevant literature, a systematic search was conducted in the PubMed and Google Scholar databases between May 2024 and May 2026. The search string included combinations of the terms "age estimation", "age determination", "*Capreolus capreolus*," and "roe deer" in both English and German. Additionally, a recursive search of the reference lists of selected publications was performed to identify further pertinent sources.

In addition, the scope of the search included scientifically reliable sources such as peer-reviewed articles, academic books, monographs, and historical management manuals, if they provided substantial methodological insights. To ensure a broad historical and regional perspective, no language restrictions were applied. Emphasis was placed on studies involving animals of known age by marking and those providing high procedural transparency and clarity.

Methods for estimating the age of roe deer

The age-estimation methods for roe deer can be categorized into tooth-based methods, non-tooth-based methods, molecular approaches, and combinations of different examination criteria. A schematic overview is illustrated in Fig. 1. A total of 25 publications addressed methods for estimating age in roe deer. Since many of these studies examined several techniques simultaneously, the number of individual method descriptions exceeds the number of publications. Of the 25 publications, 23 involved tooth-based approaches. Among these, 15 publications dealt with incremental lines in dental cementum, 1 with incremental lines in dental dentin, 10 with cheek tooth metrics, 13 with occlusal surface morphology of cheek teeth, and 1 with dental development. 7 publications examined non-tooth-based methods, including 1 on bone fusion status, 6 on eye lens weight, and 2 on pedicle morphometry. 1 publication addressed a molecular approach, specifically epigenetic clocks. 3 of the 25 publications investigated combinations of multiple criteria.

Tooth-based methods

Incremental lines in dental cementum

Cementum formation already starts during dental development and cementoblasts deposit a layer of primary cementum on the surface of the developing root prior to dental eruption (Grue and Jensen 1979; Lieberman 1994; Klevezal 1996; Nanci 2013). Secondary cementum is discontinuously formed in a periodic rhythm with the highest production rates during the vegetation period and almost no production during vegetation rest (Lieberman 1994; Klevezal 1996; Nanci 2013). The periodically alternating deposition of cementum creates layers of dental cementum, which are separated by so-called incremental lines (Lieberman 1994; Klevezal 1996; Nanci 2013). Following the biological understanding of these structures, their potential for age estimation was first explored in the early 1950s (Laws 1952). The count of cementum annuli was pioneered in elephant seals (*Mirounga*

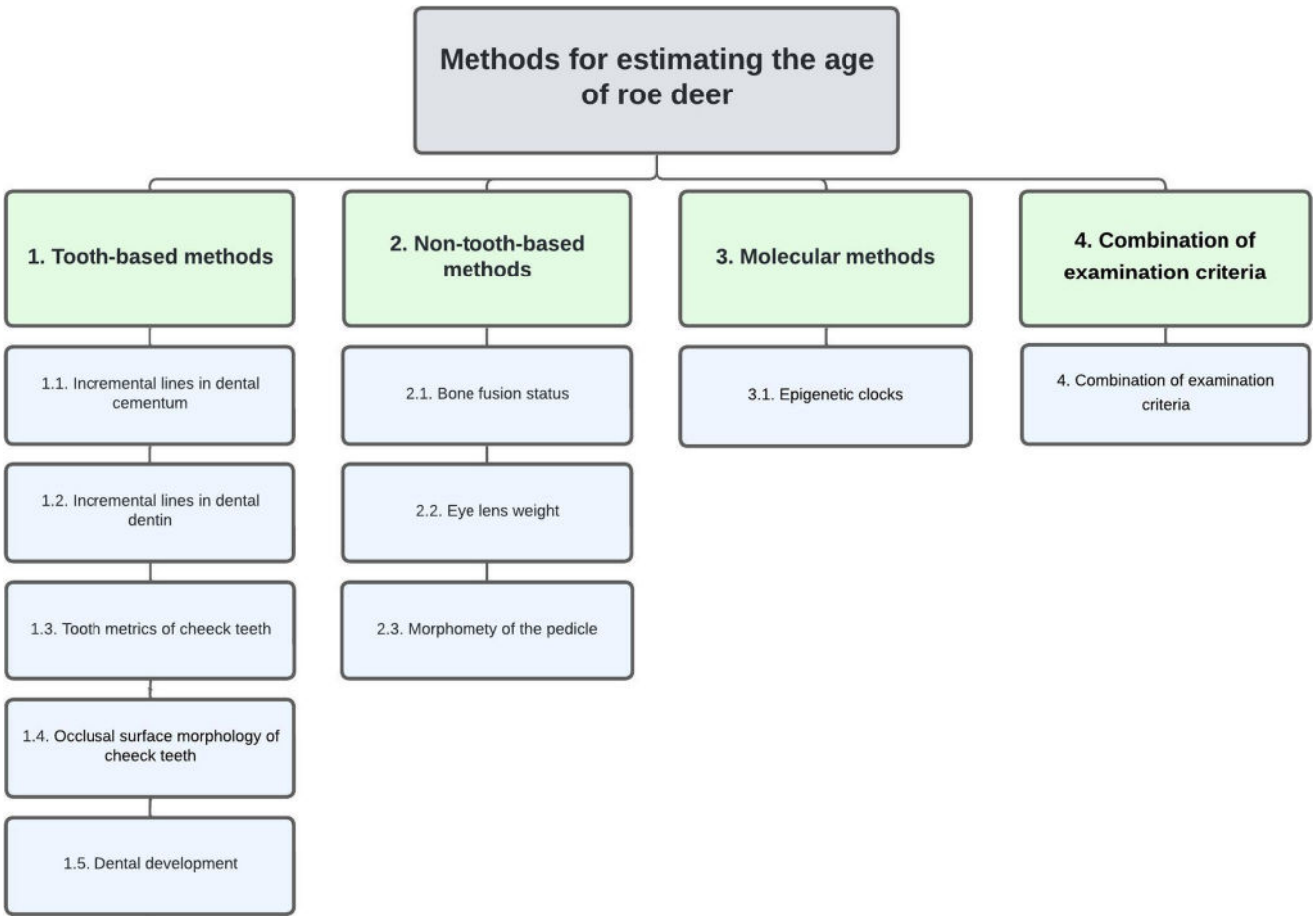


Fig. 1 Overview of age estimation methods for roe deer (*Capreolus capreolus*). The methods are categorized into (1) Tooth-based methods, (2) Non-tooth-based methods, (3) Molecular methods and (4) Combination of examination criteria

leonina) (Laws 1952) and subsequently applied to large terrestrial mammals, including moose (*Alces alces*) (Sergeant and Pimlott 1959) and red deer (*Cervus elaphus*) (Mitchell 1963, 1967), before being adapted for roe deer.

Various techniques have been developed to visualize incremental lines for age estimation in roe deer, primarily differing in tissue preparation, the choice of tooth (e.g., incisor versus molar) and the microscopy method applied. The first approach involves the mechanical grinding of molars without prior decalcification (Almasan 1972; White 1974; Aitken 1975; Briel 1978; Ashby and Henry 1979; Ueckermann and Scholz 1986; Hrabe and Koubek 1987; Cederlund et al. 1991; Gačić et al. 2007). The ground sections are typically evaluated using reflected light microscopy and can be prepared in a few minutes. Alternatively, the histological approach utilizes incisors and molars processed into thin sections of some µm (Szabik 1973; Ashby and Henry 1979; Krupka et al. 1984; Zalewski et al. 2009; Manton et al. 2026). This technique requires several weeks of preparation but produces durable samples on object carriers that are suitable for re-examination. Unlike ground preparations, the histological sections are analyzed via transmitted light microscopy. A third, hybrid method attempts to optimize contrast by decalcifying previously ground molars in order to enhance the visibility of the incremental lines in dental cementum (Almasan 1972; Ashby and Henry 1979).

Validations of cementum incremental lines counts against marked animals are scarce and contradictory. The largest direct validation on 74 mandibles from marked individuals, found cementum analysis achieved only 46% correct age assignment (Cederlund et al. 1991). Smaller direct validations on fewer than ten marked animals reported higher success rates, reaching up to 100% agreement in individual cases (Aitken 1975; Ashby and Henry 1979; Ueckermann and Scholz 1986).

Several authors have noted that incremental lines and cementum layers are influenced by intrinsic factors, such as the animal's health, and extrinsic factors, such as climatic fluctuations or nutritional composition (Almasan 1972; White 1974; Ashby and Henry 1979; Ueckermann and Scholz 1986; Cederlund et al. 1991; Klevezal 1996). They conclude that these factors lead to morphological

irregularities in the structure of the cementum, such as incomplete, indistinct, or indiscernible layers, which can result in misinterpretations of the growth lines when examining microscopic images (Almasan 1972; White 1974; Aitken 1975; Ashby and Henry 1979; Ueckermann and Scholz 1986; Cederlund et al. 1991; Klevezal 1996). Especially in southern Europe sometimes the layers are even completely illegible or absent (Klevezal 1996). This last phenomenon could be related to mild winters with relatively abundant food resources (Klevezal 1996).

Despite significant variations in accuracy and factors affecting age estimates based on cementum, incremental line counts were often used as a reference standard for evaluating several other methods (Szabik 1973; Aitken 1975; Ashby and Henry 1979; Mysterud and Østbye 2006; Gačić et al. 2007; Manton et al. 2026).

Incremental lines in dental dentin

The formation of secondary dentine follows biological principles comparable to those of cementum (Klevezal 1996; Nanci 2013). Unlike primary dentine, which forms the main body of the tooth prior to eruption, secondary dentine is deposited rhythmically on the pulpal wall throughout the animal's life (Klevezal 1996; Nanci 2013). Similar to cementum, the periodic deposition of secondary dentine results in the formation of incremental layers, which are assumed to represent annual growth cycles, with a broad light and a sharp dark band corresponding to one year of life (Klevezal 1996).

For age estimation based on secondary dentine, the first molar (M1) was extracted from the mandible (Zalewski et al. 2009). The tooth was decalcified, cross-sectioned using a freezing microtome, and stained for microscopic examination (Zalewski et al. 2009). Cross-sections of the root of the first molar (M1) have been shown to provide the best visualization of annual layers (Zalewski et al. 2009).

Zalewski et al. (2009), who did not have access to marked individuals, reported 91.1% agreement between dentine-based age estimates and tooth wear assessment across all age classes, reaching 94.6% for age groups relevant to selective hunting management. The highest agreement (100%) was observed in two-year-old individuals, however the accuracy decreased in older animals, with the lowest agreement (83.3%) found in five- and six-year-old bucks (Zalewski et al. 2009). According to Zalewski et al. (2009), the growth lines are better visible in dentin than in cementum for roe deer.

Tooth metrics of cheek teeth

As in all ruminants, the teeth of roe deer are exposed to continuous occlusal wear by attrition (wear due to contact between teeth) and abrasion (wear due to tooth to forage contact) (Fortelius 1985). Although the loss of occlusal substance can be partially compensated by the continual eruption of the tooth from the alveolus, the crown height defined as the – vertical dimension of the tooth crown visible above the bone or gum line – shortens progressively over time (Aitken 1975; Stubbe et al. 1987; Cederlund et al. 1991).

Methodological approaches to measuring this decline vary significantly. While some authors averaged the height of both buccal cusps of the first molar (M1) (White 1974; Ashby and Henry 1979; Cederlund et al. 1991), others recorded the height of the buccal and lingual cusps of the first molar (M1) separately (Aitken 1975), measured only a the highest cusp of the second molar (M2) (Manton et al. 2026), or utilized the entire height of different teeth (Stubbe et al. 1987; Mysterud and Østbye 2006). Despite these differing protocols, all share the objective of capturing the progressive decrease in crown height, representing the distance from a fixed reference point to the chewing surface. In additional approaches the ratio of crown height to crown length was also calculated based on a single tooth or an entire row of teeth, in order to consider allometric size differences (Martens 1920; Tomé and Vigne 2003).

Validations on marked animals demonstrate significant limitations. Correlations between crown height and age varied strongly, with correlation coefficients ranging from r=-0,27 to r=-0,86 depending on the tooth (Stubbe et al. 1987; Cederlund et al. 1991). Due to this variation and overlapping ranges within the age classes, crown height was not recommended as a standalone age marker (Stubbe et al. 1987; Cederlund et al. 1991; Hewison et al. 1999). However, crown height was subsequently included in an optimal multivariate age estimation model (see 4. Combination of examination criteria) (Stubbe et al. 1987). Studies based on other age reference, such as cementum annuli counts, yielded these same restrictive results (White 1974; Aitken 1975; Ashby and Henry 1979; Mysterud and Østbye 2006; Manton et al. 2026). Expressing height as a ratio to tooth length did not overcome these limitations (Martens 1920; Tomé and Vigne 2003).

Occlusal surface morphology of cheek teeth

The cheek teeth of roe deer show a discontinuous, complex, three-dimensional pattern of enamel folds (Tomé and Vigne 2003; Høye 2006). Continuous wear on the occlusal surface leads to changes in the appearance of the enamel folds and the emergence of secondary dentin (Tomé and Vigne 2003; Høye 2006). The crests and infoldings of the three premolars and the cusps and infundibula of the three molars were out in a progressive and relatively predictive way (Tomé and Vigne 2003; Høye 2006).

Age estimation based on the occlusal surface morphology of cheek teeth were first tested on white-tailed deer (*Odocoileus virginianus)* (Severinghaus 1949) and mule deer (*Odocoileus hemionus)* (Robinette et al. 1957). In Central Europe, illustrated manuals and scoring systems have trained generations of wildlife managers by providing schematic drawings or photographs of mandibular wear stages in different cervids (Rieck 1940, 1958; Wagenknecht 1984; Habermehl 1985; Brown and Chapman 1991a, b; De Marinis 2015; Bowen et al. 2016; Kohut 2022).

Existing ageing protocols for roe deer focus exclusively on the mandibular cheek teeth and differ how wear patterns are translated into an age estimate. Some protocols contain reference materials such as image tables or jaw models (Rieck 1970; Aitken 1975; Stubbe et al. 1987; Cederlund et al. 1991; Hewison et al. 1999), while others rely exclusively on the examiner's expertise, which is categorized into different levels of qualification (Szabik 1973; Ashby and Henry 1979; Krupka et al. 1984; Hrabe and Koubek 1987; Stubbe et al. 1987; Hewison et al. 1999; Reimoser et al. 2004; Manton et al. 2026). In contrast, more recent studies have developed objective scoring systems that decompose wear into defined ordinal stages, integrating data, e.g. dental eruption and replacement (Tomé and Vigne 2003; Høye 2006).

Validation studies using marked individuals show for both approaches that accuracy rates vary considerably across age groups and protocols. With reference materials, accuracy for one- to three-year-olds ranges from 9 to 92%, while for animals aged four years and older it spans from 0 to 80% (Rieck 1970; Stubbe et al. 1987; Cederlund et al. 1991; Hewison et al. 1999). Without reference materials, relying solely on examiner experience, accuracy ranges for one- to three-year-olds ranges from 30 to 79%, while for animals aged four years and older it spans from 10 to 34% (Stubbe et al. 1987; Hewison et al. 1999; Reimoser et al. 2004). U to three years of age, experienced observers and reference tables ouperform less qualified observers, but from four years onward, no differences between groups remain (Stubbe et al. 1987).

The objective scoring system developed by Høye (2006) was validated on marked individuals and achieved 100% accuracy for roe deer younger than 13 months and around 80% accuracy for individuals between 13 and 24 months. Beyond 24 months of age, accuracy decreases, making accurate age estimation difficult (Høye 2006).

Dental development

Age estimation based on dental development is frequently used in wildlife management for several species (Brown and Chapman 1991a, b; Bowen et al. 2016; Kohut et al. 2025). The underlying principle is the species-specific and temporally constrained sequence of tooth eruption and replacement, which follows a predictable pattern from the emergence of deciduous teeth through their replacement by permanent teeth (Stubbe 1967; Kierdorf and Kierdorf 1989).

From a methodological perspective, two main approaches can be distinguished. The first is purely macroscopic, involving visual inspection of the mandible or skull to assess which teeth have erupted or been replaced (Stubbe 1967). The second relies on radiographic imaging, which allows the visualization of tooth crypts, crown formation, and root development even before teeth become visible above the gumline (Carter 1997).

Validation on marked roe deer demonstrated that the radiographic approach enables age estimation accurate to the month (Carter 1997). For the macroscopic approach, no validation using marked individuals is available. However, it has been used in combination with tooth wear analysis in other studies (see 1.4 Occlusal surface morphology of cheek teeth). The method is no longer applicable after the age of about 15 months when tooth development is complete (Stubbe 1967; Carter 1997).

Non-tooth-based methods

Bone fusion status

The growth of so-called long bones is facilitated by cartilaginous growth plates that are located between the metaphysis and the epiphysis of a bone (Nickel et al. 2003). With increasing age, the growth plates ossify continuously until the bony metaphysis is fused to the bony epiphysis (Nickel et al. 2003). The ossification status of the growth plate can be determined by radiographs in the living animal or by examination of macerated bones (Tomé and Vigne 2003).

Tomé and Vigne (2003) documented the temporal progression of bone ossification at various sites on 196 complete roe deer skeletons, whose ages they estimated using a scoring system based on tooth wear. A distinction was made between three different fusion stages: (1) not fused, (2) fusion in progress, and (3) fused (Tomé and Vigne 2003).

According to Tomé and Vigne (2003), the most accurate age estimates with an accuracy of ± 1–2 months are based on the completion of the fusion of certain bony structures within the limbs: the proximal and distal epiphyses of the radius and ulna, the distal epiphyses of the metapodials, the acetabulum, the distal epiphysis of the femur and the proximal epiphysis of the first phalanx. A fusion site between the scapula and the coracoid process was also mentioned, but according to veterinary anatomical nomenclature it is likely that the fusion site between the scapula and the supraglenoid tubercle was intended (Tomé and Vigne 2003). In addition,

Tomé and Vigne (2003) described fusion times for bones within the skull base (ali- and basisphenoid) and within the spine (intervertebral disc and center of the lumbar vertebrae). However, these fusion sites do not correspond to veterinary anatomical descriptions. As there are several fusion areas in the regions mentioned, one can only speculate which fusion sites are being referred to. For the skull base, the fusion site between the pre- and basisphenoid or between the presphenoid and the ethmoidal bone might have been described. For the lumbar vertebral column, it remains unclear whether the fusion side between the metaphysis of the lumbar vertebra and its cranial or caudal epiphysis was addressed.

Eye lens weight

Various processes in the eye lens continuously change its structure and composition, resulting in changes of lens weight (Lord 1959; Longhurst 1964; Augusteyn 2010). First examined for mule deer, it has been proposed for estimating the age of roe deer (Longhurst 1964; Maringgele 1978; Ashby and Henry 1979; Stubbe et al. 1987; Mysterud and Østbye 2006; Gačić et al. 2007).

Preparation protocols are very labor-intensive and varied considerably among studies in fixation medium, fixation duration, and drying temperature, producing systematic differences in absolute lens weight that prevent direct transferability of regression equations between laboratories (Maringgele 1978; Ashby and Henry 1979; Stubbe et al. 1987; Mysterud and Østbye 2006; Gačić et al. 2007; Manton et al. 2026).

Validations of the eye lens method on marked individuals are scarce. One of the few exceptions is Stubbe et al. (1987), who validated the eye lens method on marked roe deer skulls. They reported a strong correlation between eye lens weight and age ($r = 0.82$ for males, 0.73 for females) but did not provide further details (Stubbe et al. 1987). However, despite this correlation, the authors did not include eye lens weight in their optimal multivariate age estimation model, selecting instead other morphological traits as better predictors (Stubbe et al. 1987). Most studies have relied on cementum layer analysis as reference rather than using marked animals (Maringgele 1978; Ashby and Henry 1979; Mysterud and Østbye 2006; Gačić et al. 2007). These studies consistently describe a curvilinear relationship, with lens weight increasing steeply during the first two to three years before declining or plateauing between seven and nine years (Maringgele 1978; Ashby and Henry 1979; Mysterud and Østbye 2006; Gačić et al. 2007). Beyond four years of age, accuracy drops markedly: correctly estimated age classes fell below 30% from age class four onwards, with considerable overlap in lens weight ranges across all age classes (Gačić et al. 2007).

Morphometry of the pedicle

The male roe deer carries antlers that are shed in fall and regrown within spring (Bubenik 1990). The renewal of the antlers starts from a process or apophysis of the frontal bone, which is called the pedicle (Bubenik 1990). Given that the size and branching pattern of the antlers tend to increase from year to year until the animal reaches its physiological peak, the pedicles continue to shorten and widen throughout the buck's life. (Stubbe et al. 1987; Mysterud and Østbye 2006). The use of this development for age estimation was first pioneered by Harke (1952) for red deer and has since been extended to roe deer populations (Harke 1952; Stubbe et al. 1987; Mysterud and Østbye 2006; Stubbe 2008).

Protocols for measuring pedicle diameter and height were not described in detail (Stubbe et al. 1987; Mysterud and Østbye 2006).

Validation studies of the pedicle method on marked individuals are limited. Stubbe et al. (1987) examined pedicle diameter and height on marked roe deer and reported correlation coefficients with age of $r = 0.53$ for pedicle height, $r = 0.69$ for pedicle diameter, and $r = -0.67$ for the height-to-diameter ratio. These variables were subsequently included in their optimal multivariate age estimation model (see 4. Combination of examination criteria) (Stubbe et al. 1987). Examination based on cementum layer analysis as a reference instead of marked animals, describes a curvilinear relationship between pedicle diameter and age, with pedicle diameter increasing rapidly during the first four to five years before reaching a plateau, however beyond four years of age, correct age assignment declines considerably (Mysterud and Østbye 2006). However, age-specific accuracy rates weren`t provided (Mysterud and Østbye 2006).

Molecular methods

Epigenetic clocks

Epigenetic clocks are a relatively new tool for estimating age (Horvath 2013; Lemaître et al. 2022; Barratclough et al. 2024). They are based on DNA methylation patterns that change continuously with age (Horvath 2013). These clocks have been explored in various species, including mice (*Mus musculus*) (Petkovich et al. 2017; Stubbs et al. 2017), roe deer (Lemaître et al. 2022), wild house mice (*Mus musculus domesticus*) (Hanski et al. 2024), brown bears (*Ursus arctos*) (Nakamura et al. 2023), and bottlenose dolphins (*Tursiops truncatus*) (Barratclough et al. 2024; Yagi et al. 2024) with promising results.

Lemaître et al. (2022) developed an epigenetic clock analysis for roe deer using blood samples from marked roe

deer, ranging from 8 months to 13.5 years old. Blood samples were taken from the jugular vein and genomic DNA was then isolated from the leukocytes and analyzed using a methylation array (Lemaître et al. 2022). The epigenetic clocks proved to be highly accurate, with a mean absolute difference of ± 0.558 years (Lemaître et al. 2022).

Combination of examination criteria

The potential benefit of combining multiple age criteria for roe deer has been addressed repeatedly, yet the corresponding investigations differ considerably in their experimental design and the specific question they address.

Stubbe et al. (1987) examined the accuracy of age estimation in roe deer by combining criteria that did not require elaborate and time-consuming microscopic analysis of teeth, using roe deer of known age as a reference. The most accurate age estimation for male roe deer was achieved by a combination pedicle diameter, eye lens weight, clinical crown height of second premolar (P2), and the ossification stage of the intersphenoidal junction (Stubbe et al. 1987). Using this approach, 68% accuracy was shown for male roe deer up to 3 years old, with a decrease in accuracy for older male animals (Stubbe et al. 1987). For female roe deer, the most accurate age estimation was obtained by combining skull width, lower jaw length, the morphology of the sutura frontalis, the ossification stage of the intersphenoidal junction, clinical crown heights of first molar (M1) and third molar (M3) and the average height of all mandibular cheek teeth (Stubbe et al. 1987). This combination resulted in 82% accuracy for females up to 3 years old (Stubbe et al. 1987). As with males, accuracy decreased significantly from the age of three years onwards (Stubbe et al. 1987). However, the study did not explicitly examine whether this combination outperformed the best single criterion, as the analysis was designed to maximize overall classification accuracy using multiple features (Stubbe et al. 1987).

In contrast, Mysterud and Østbye (2006) explicitly tested whether combining two criteria, molar height of second molar (M2) and pedicle diameter, improved accuracy compared to each criterion alone. They found only a slight improvement, however the authors did not report the exact magnitude of the improved fit (Mysterud and Østbye 2006). Their combination analysis did not include eye lens weight, which was the strongest single predictor in their dataset (Mysterud and Østbye 2006). The age of the specimen was estimated based on counts of dental cementum incremental lines (Mysterud and Østbye 2006).

Manton et al. (2026) evaluated whether a combined field approach, consisting of visual observation of body traits (including head coloration, neck thickness, body shape and behavior in males, and abdominal profile, skeletal prominence and neck form in females), supplemented by post-mortem examination of tooth wear, performs better than selected laboratory methods. The assessment followed no standardized protocol, and hunters weighted the criteria intuitively, reflecting routine field practice (Manton et al. 2026). When compared with counting cementum incremental line as the reference, the field approach showed only moderate agreement (Manton et al. 2026). Whether a standardized combination of objective laboratory criteria would outperform single indicators was not tested (Manton et al. 2026).

Discussion

The methodological analysis reveals a heterogeneous picture. Despite the existence of numerous approaches for age estimation in roe deer, all procedures show considerable limitations in terms of accuracy, methodological effort or practical applicability. Remarkable is the discrepancy between the presumed established status of certain methods and their actual accuracy under validation conditions.

Analysis of the growth lines in dental cementum is currently considered as the method that best meets scientific standards, a classification that was likely adopted from other species of deer (Pérez-Barbería et al. 2014; Veiberg et al. 2020; Hinton et al. 2023). In roe deer, however, this method is associated with several problems. First, direct calibration using marked individuals was performed only on a small group of marked animals, with accuracy in correctly assigning ages ranging from 46% to 100% (Ueckermann and Scholz 1986; Cederlund et al. 1991). This raises fundamental questions about the method's reliability as a reference standard for this species. Second, incremental lines are frequently reported as poorly defined, irregular, or entirely absent, with pronounced variation in clarity among populations/individuals (Szabik 1973; Aitken 1975; Ashby and Henry 1979; Zalewski et al. 2009). Third, the method carries substantial practical disadvantages compared to alternative approaches: it requires the extraction, often elaborate preparation, and time-consuming microscopic analysis of at least one tooth, and the interpretation of the resulting images depends on the experience of the investigator (Szabik 1973; Klevezal 1996). A potential solution may lie in analyzing incremental lines in secondary dentine rather than cementum, since dentine layers have been argued to offer superior visibility (Klevezal 1996; Zalewski et al. 2009). However, the dentine-based approach has been explored in only a single study on roe deer, and further investigation on larger samples of marked animals would be required to substantiate this claim (Zalewski et al. 2009).

Tooth wear assessment represents the oldest and widely applied method in hunting practice (Nehring 1903; Brandt 1907; Rieck 1940, 1958; Wagenknecht 1984; Habermehl 1985). The analysis demonstrates that practical prevalence and scientific accuracy are not congruent. The systematic decline in accuracy and the high variation within age classes is particularly problematic (Rieck 1970; Aitken 1975; Stubbe et al. 1987; Cederlund et al. 1991; Reimoser et al. 2004). Providing reference materials such as jaw models slightly improves accuracy in the first three years but it doesn't make any difference after that (Stubbe et al. 1987; Hewison et al. 1999). This suggests that the limitation lies in the inherent variability of the trait itself, a finding that carries significant implications for the development of training programs (Szabik 1973; Hewison et al. 1999; Reimoser et al. 2004). The objective scoring systems developed by Tomé and Vigne (2003) and Høye (2006) offer higher precision for young animals, because they include data on dental eruption and shedding. However, discriminative power declines beyond an age of 24 months significantly, because indices reached a plateau what makes age estimation insecure (Tomé and Vigne 2003; Høye 2006).

The dental development represents a rare example of a method with documented monthly accuracy (Stubbe 1967; Carter 1997). However, its applicability is strictly limited to individuals under approximately 15 months of age (Carter 1997). While this makes it valuable for fawn age estimation, it addresses only a narrow temporal window of the roe deer lifespan.

The analysis of crown height shows high variation between age classes and correlation (Cederlund et al. 1991; Hewison et al. 1999). A certain variability in the degree of tooth wear is quite understandable, given that this depends both on the texture of food (softer or tougher) and on the varying hardness of the enamel and dentin. However, when used in combination, it works very well, which makes it useful (Stubbe et al. 1987).

Bone ossification analysis claims an accuracy of ± 1–2 months, but its findings were only validated using tooth wear rather than marked individuals (Tomé and Vigne 2003). Despite this validation problem, the method also suffers from the same fundamental limitation as dental development: it is only applicable to juvenile animals. Furthermore, the described fusion sites within the skull base and spine do not correspond to standard veterinary anatomical nomenclature, making independent replication difficult (Tomé and Vigne 2003).

Eye lens weight and pedicle morphometry share fundamental limitations that harms their routine application for age estimation in roe deer. Both suffer from methodological deficiencies that compromise their scientific validity. Preparation protocols for lens weight vary considerably among studies in fixation medium, duration, and drying temperature, preventing direct transferability of regression equations between laboratories. For pedicle morphometry, measurement protocols for diameter and height were not described in detail, and since the edge of the pedicle forms an irregular ring and pedicle height is a poorly defined measurement point, a clear and unambiguous measurement protocol would be an essential prerequisite for using pedicle dimensions as an age indicator. Eye lens weight and pedicle morphometry show a curvilinear relationship with age, characterized by rapid early growth followed by a plateau – around six to seven years for lens weight and four to five years for pedicle diameter (Mysterud and Østbye 2006; Gačić et al. 2007). Individual variation within age classes is substantial for both methods, with considerable overlap between adjacent age groups (Ashby and Henry 1979; Mysterud and Østbye 2006; Gačić et al. 2007).

The epigenetic clock analysis represents a methodological advancement. This precision surpasses all conventional methods by a substantial margin, with a mean absolute difference of ± 0.558 years (Lemaître et al. 2022). Several considerations warrant attention. The method requires currently blood sampling, which limits applicability to live-captured animals or recently deceased specimens with preserved blood (Lemaître et al. 2022). It remains unclear whether DNA can also be extracted from other parts of the body (Lu et al. 2023). The cost of methylation array analysis is substantially higher than traditional methods (Lemaître et al. 2022). Validation on independent populations outside the original study sample has not yet been reported. Nonetheless, epigenetic clocks currently represent the most accurate available method for roe deer age estimation where sample collection is feasible.

The combination of multiple age criteria has produced inconsistent findings across studies (Stubbe et al. 1987; Mysterud and Østbye 2006; Manton et al. 2026). Some multivariate approaches achieved substantial accuracy by integrating complementary morphological traits, yet often without explicitly testing whether the combined model outperformed the best individual criterion (Stubbe et al. 1987). Other investigations that directly compared combined against single predictors found only marginal improvements (Mysterud and Østbye 2006). Furthermore, non-standardized field applications combining visual and dental traits showed only moderate agreement with reference methods, suggesting that intuitive weighting does not necessarily enhance accuracy (Manton et al. 2026). Overall, the combination of selected parameters can improve age estimation when based on careful statistical analysis, but this improvement is not guaranteed. It remains essential to

examine whether a single method performs equally well or even better, as without such comparison the added value of combining criteria cannot be properly assessed.

Based on the analysis, recommendations can be made for age estimation across different contexts. For fawns and juveniles under 15 months, dental development analysis is recommended, as it provides documented monthly accuracy and does not require lethal sampling, with eruption status assessable either visually or radiologically (Stubbe 1967; Carter 1997). For live-captured adults where maximum accuracy is required, epigenetic clocks should be considered despite their higher cost and logistical requirements, as no other method approaches their precision and blood sampling can be integrated into routine capture protocols (Lemaître et al. 2022).

When selecting a method, the criteria of accuracy, reproducibility, methodological effort, and intra vitam or post-mortem use must be considered and weighed against each other. However, non-molecular methods still offer important advantages that make them difficult to replace. Tooth-based methods, especially the analysis of the occlusal surface, are widely used in wildlife management and research because they are practical, fast, well established, and relatively cheap compared to genetic analysis (Rieck 1940, 1958; Wagenknecht 1984; Habermehl 1985). Tooth wear examination can be used on large samples, such as the analysis of tens of thousands of harvested animals, when a certain approximation is more acceptable, while for scientific research on dozen specimens, the use of more accurate methods or combination of methods would be highly desirable (Kamieniarz and Panek 2026). Morphological approaches can also be applied directly in the field or retrospectively to skeletal material when molecular data are not always available due to DNA degradation (Allentoft et al. 2012). In addition, many long-term observation programs rely on morphological data sets, ensuring comparability over decades (Tomé and Vigne 2003).

For future research applications, several methodological standards are essential. Validation should use marked individuals with known dates of birth from long-term monitoring programs in different habitats, when available (Cederlund et al. 1991; Hewison et al. 1999). Preparation protocols must be described in detail to permit independent replication, including all steps from tissue collection and storage to sectioning, staining, and measurement, with measurement points defined unambiguously with reference to anatomical landmarks (Szabik 1973; Reimoser et al. 2004; Kohut et al. 2025).

Further validation and investigation of all methods is required, with special attention to molecular approaches such as epigenetic clocks, which remain insufficiently tested across different populations and environmental conditions (Lemaître et al. 2022). However, from the perspectives of animal welfare and wildlife management, methods applicable to live animals are of primary importance (Festa-Bianchet et al. 2003). Non-invasive or minimally invasive techniques that can be performed on live animals without tooth extraction, lethal sampling, or euthanasia are urgently needed (Nelson 2001; Festa-Bianchet et al. 2003). Radiological examination, for instance, offers a promising avenue. The measurement of pulp cavity size reduction on dental radiographs, which reflects progressive secondary dentin deposition throughout life, deserves systematic investigation in roe deer. Studies in other species, such as humans (*Homo sapiens*) (Cameriere et al. 2007), African wild dogs (*Lycaon pictus*) (Mbizah et al. 2016), bottlenose dolphins (*Tursiops truncatus*) (Herrman et al. 2020), Arctic Fox (*Vulpes lagopus*) (Czajkowska and Pokojska 2024), and cats (*Felis catus*) (Park et al. 2014) suggest potential use. Such a method would directly address the fundamental gap identified throughout this review: the need for a reliable, practical, and animal friendly technique for age estimation in live roe deer.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval This article is a review of existing literature and does not contain any new studies with human participants or animals performed by the authors.

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