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The consequences of living longer—Effects of an experimentally extended velvet antler phase on the histomorphology of antler bone in fallow deer (*Dama dama*)

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Abstract

Antlers are periodically regenerated paired cranial appendages of male deer (both sexes in reindeer) that constitute the fastest-growing bones in the animal kingdom. The annual antler cycle of male deer is linked to testicular activity and largely controlled by seasonal fluctuations of testosterone concentrations in their blood. We studied the effects of an experimental doubling (to eight months) of the velvet antler phase, during which the antlers are covered by skin (velvet), on the histomorphology of antler bone in three adult fallow bucks. Extension of the velvet antler phase in the experimental animals had been caused by administration of the antiandrogen cyproterone acetate (CPA). The distal portions of the antlers from two of the CPA-treated bucks exhibited partial sequestration of the antler cortex, with the separation plane typically located along the border between cortex and spongiosa. It is hypothesized that this was caused by cortical necrosis due to severe ischemia during later stages of the extended velvet antler phase. In places, new cancellous bone had been deposited on the resorption surface of the spongiosa, indicating a regeneration process. Normal fallow deer antlers (“controls”) from this and a previous study, that is, antlers with a timespan of about four months between onset of new antler growth and velvet shedding, exhibited no or only minor bone remodeling and still contained remnants of calcified cartilage in their distal portions. In contrast, the antlers of the three CPA-treated bucks showed evidence (secondary osteons and resorption cavities) of marked bone remodeling along their entire length and lacked remnants of calcified cartilage. Our results underscore that the typical histological features of antler bone reflect its short-lived nature. Antlers are not mechanically loaded during the velvet stage, and it is presently unclear what triggered remodeling activity in the antlers whose lifespan had been experimentally extended.

KEYWORDS

antler cortex, bone remodeling, bone sequestration, cyproterone acetate, ischemic necrosis, primary and secondary osteons

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1 | INTRODUCTION

Antlers are the annually cast and regenerated bony cranial appendages of cervids and constitute the fastest-forming bones in the animal kingdom (Goss, 1983; Landete-Castillejos et al., 2019). Except for the reindeer (*Rangifer tarandus*), antlers are normally produced only by males and thus constitute secondary sex characteristics (Goss, 1983; Landete-Castillejos et al., 2019).

Antlers are grown and cast from the tips of permanent frontal protuberances, known as pedicles (Goss, 1983; Landete-Castillejos et al., 2019). The antler growth process is appositional and has been characterized as a special form of endochondral (and perichondral) ossification (Banks & Newbrey, 1983; Kierdorf et al., 1995a; Li et al., 2005; Price et al., 1996). The apically produced cartilage forms a longitudinal tubular framework or scaffold (Clark et al., 2006; Kierdorf et al., 1995a; Krauss et al., 2011; Muir et al., 1988). Further proximally, the chondrocytes become hypertrophic and the cartilage matrix is mineralized, followed by the replacement of the cartilaginous by a bony scaffold (Banks & Newbrey, 1983; Facheux et al., 2001; Gomez et al., 2013; Kierdorf et al., 1995a; Krauss et al., 2011; Price et al., 1996). The soft tissue spaces between the trabeculae of the bony scaffold are then reduced in size by infilling with primary osteons (Gomez et al., 2013; Kierdorf et al., 2013; Krauss et al., 2011; Skedros et al., 2014). There is only limited circumferential growth of antlers by perichondral/periosteal ossification, which is most prominent in their basal portions (Banks & Newbrey, 1983; Kierdorf et al., 1995a, 2007).

In the periphery of the growing antler, primary osteon formation causes the development of a dense antler cortex (Banks & Newbrey, 1983; Gomez et al., 2013; Kierdorf et al., 2013; Skedros et al., 2014). Towards the center of the antler, the degree of apposition of lamellar bone on the scaffold diminishes, and the central portion of the antler therefore consists of cancellous bone, the antler spongiosa (Banks & Newbrey, 1983; Kierdorf et al., 2013). In the basal antler portion, an outer cortical zone, also referred to as the subvelvet zone (Rolf & Enderle, 1999), can be found that is less densely structured than the main cortex. This peripheral zone is formed late during antler growth (Gomez et al., 2013).

Prior to the infilling of the intertrabecular spaces with lamellar (primary osteonal) bone, resorption occurs at the scaffold, which in places can be reduced to thin streaks (Gomez et al., 2013; Kierdorf et al., 2013). Some studies have reported the presence of highly mineralized seams along the periphery of primary osteons in antler bone (Kierdorf et al., 2013; Krauss et al., 2011; Skedros et al., 1995). These seams have been interpreted as reversal lines (Kierdorf et al., 2013). Their presence complicates the distinction between primary and secondary osteons in antler bone, as reversal lines are generally considered a distinctive feature of secondary osteons (Burr & Akkus, 2014; Francillon-Vieillot et al., 1990).

Antler bone typically contains only few secondary osteons that are mostly present in the more basal antler portions (Chapman, 1975; Gomez et al., 2013; Kierdorf et al., 2013; Krauss et al., 2011; Launey et al., 2010; Skedros et al., 2014; Wislocki, 1942). The scarcity of

secondary osteons in antlers reflects their short lifespan that does not allow for any significant remodeling activity. The short-lived nature of antlers is also considered the reason for the persistence of calcified cartilage in more distal portions of hard antlers (Kierdorf et al., 2013; Landete-Castillejos et al., 2012, 2019).

During growth, antlers are invested in a special type of hairy skin, referred to as velvet (Goss, 1983). The arteries supplying the growing antlers with blood are located in the vascular layer at the base of the velvet (Clark et al., 2006; Rhumbler, 1929; Waldo et al., 1949).

The annual antler cycle is closely linked to the seasonal changes of blood testosterone levels (Bubenik, 1990b; Lincoln, 1992; Suttie et al., 1995). The old antlers are cast when testosterone levels drop below a threshold, and antler regrowth occurs while testosterone concentrations are low. Rising testosterone levels at the end of the antler growth phase cause velvet shedding, by which the bare bony (hard) antlers are exposed. As long as testosterone concentrations in blood remain high, the hard antlers are retained (Bubenik, 1990b; Lincoln, 1992). Following the drop of testosterone levels after the rut, intense osteoclastic resorption then leads to antler casting.

The crucial role of androgens in the antler cycle has been studied in vivo mainly by castration of male deer (Goss et al., 1992; Kierdorf et al., 1995b; Wislocki et al., 1947) and the administration of sex hormones or antiandrogens to intact or castrated males (Bartoš et al., 2000; Bubenik, 1990b; Bubenik et al., 2002; Goss, 1968; Jaczewski, 1979; Jaczewski et al., 2004; Kierdorf et al., 1993; Kolle et al., 1993; Lincoln, 1992; Schams et al., 1987; Suttie et al., 1995; Wislocki et al., 1947). Most of the antiandrogen studies used cyproterone acetate (CPA), which is both an androgen receptor blocker, inhibiting androgenic activity at the cellular level, and a highly effective progestin that suppresses gonadotropin secretion and, in consequence, androgen biosynthesis by the Leydig cells of the testes (Neumann, 1994).

Thus far, only little information on the histological structure of antler bone in castrated or antiandrogen-treated male deer is available (Kierdorf et al., 1993, 2004). The present study reports our findings on the histomorphology of hard antler bone from fallow bucks (*Dama dama*) whose velvet antler phase had been markedly extended by CPA treatment. Specifically, we tested the hypothesis that the extended antler lifespan had caused a more extensive remodeling of the antler bone compared to short-lived "normal" antlers.

2 | MATERIALS AND METHODS

The present study was conducted on the antlers of three adult fallow bucks (animals H, I and K) from a previous study (Kolle et al., 1993) whose velvet antler phase had been extended by CPA treatment. The original experimental study had been conducted in accordance with all animal care regulations in Germany effective at the time and had been approved by the responsible animal care authorities. No additional ethical approval was required for the present study.

The experimental animals, which were four years of age at the start of the experiment, were kept outdoors in an enclosure near

Göttingen (Germany). Adult fallow bucks from this experimental herd normally cast their antlers in late April/early May, while velvet shedding occurred in late August/early September (Figure 1). The antlers of adult fallow bucks are typically palmate with a number of backward pointing projections (spellers) at the palm (Chapman & Chapman, 1997).

Over the period from 15 April to 26 November, the three experimental animals were repeatedly ($n = 36$) injected intramuscularly with CPA (Schering AG, Berlin, Germany). CPA administration started about 2 weeks before the normal date of antler casting and extended past the end of the rutting period in untreated males (Figure 1). The CPA dose per injection was increased from 600 mg per buck (15 April to 8 July, 13 injections) over 800 mg (15 July to 9 September, 9 injections) to 1000 mg (16 September to 26 November, 14 injections). Over the course of CPA administration, plasma testosterone concentrations of the bucks were very low (individual maximum value of 0.37 ng/ml), and no rutting behavior was observed in the CPA-treated bucks during the normal rutting period from mid-October to mid-November. For further details of animal handling, experimental setup, and the morphological and physiological parameters measured in the fallow bucks, the reader is referred to the paper by Kolle et al., (1993).

Antler casting occurred between 22 and 26 April in bucks H and I, while buck K had lost its antlers during handling already on 9 April (indicated by the red asterisk in Figure 1), that is, prior to the onset of CPA-application on 15 April. Antler casting was followed by the growth of a new set of antlers in all three animals. These antlers ceased growing in mid-August, around the normal time for the species. Velvet shedding in the experimental animals did, however, not take place at the normal time but was delayed for several months. Thus, velvet shedding in the three bucks occurred between 31 December and 8 January, about five to six weeks after the last CPA administration. This means that the duration of the velvet antler phase in the experimental animals had approximately been doubled (to eight months) compared to normal fallow bucks (Figure 1). The antlers from which the velvet had been belatedly shed were cast between 14 and 20 April. These three pairs of cast antlers were analyzed in the present study. For comparison, we also analyzed the hard antlers (still attached to the pedicle) of a three-year old wild

fallow buck (control) that had been culled during normal hunting operations.

The cast antlers of the three experimental bucks were photographed and weighed. Subsequently, full-thickness cross-sectional discs (basal and antler tip portions) as well as segments of antler bone from the palms were cut from the (experimental and control) antlers with a bone saw and embedded in epoxy resin. For inspection in the scanning electron microscope, one of the cut surfaces of each embedded sample was smoothed and polished as previously described (Kierdorf et al., 2013). For light microscopy, thin ground sections (thicknesses of about 50 and 70 μm) were prepared following an established protocol (Schultz, 1988).

The polished block surfaces of the antler bone samples were examined in a scanning electron microscope (SEM, Zeiss EVO 15 MA) operated in the backscattered electron (BSE) mode at 20 kV. Gray-level variation in the SEM-BSE images reflects differences in mineralization, with brighter gray levels indicating a relatively higher mineral content (Skedros et al., 1993). Low-power overview photos of the thin ground sections were obtained with a Keyence VHX 500F digital microscope and converted to grayscale images (Adobe Photoshop). Light microscopic observations at higher magnifications were made with a Zeiss Axioskop 2 Plus microscope and a Zeiss Axio Imager 2 microscope, each equipped with a digital color camera (Zeiss AxioCam 503 color). The sections were viewed and photographed using different imaging modalities, and the captured images were processed using Adobe Photoshop.

3 | RESULTS

In two of the experimental animals (bucks H and I), the antlers grown during CPA-treatment were of a relatively normal shape with moderately to well-developed palms (Figure 2a,b). In contrast, the antlers of the third buck (K), which were also lighter than those of the other two bucks (Table 1), possessed only weakly developed palms, and the right antler exhibited an abnormal curvature. In addition, the tip of the left antler of buck K exhibited a V-shaped furrow (Figure 3a) that had probably originally been occupied by cartilage. The bone lining this furrow consisted of a system of woven bone trabeculae

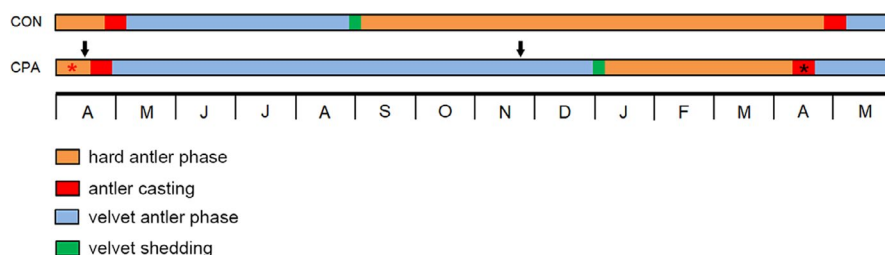


FIGURE 1 Schematic illustration of the antler cycle in an untreated (control) fallow buck (CON) and in the CPA-treated bucks (CPA). Arrows indicate dates of first and last CPA administration, respectively. The red asterisk indicates the date of antler loss in buck K prior to the onset of CPA-administration, the black asterisk identifies the casting period of the antlers analyzed in the present study. Letters identify the months of the year

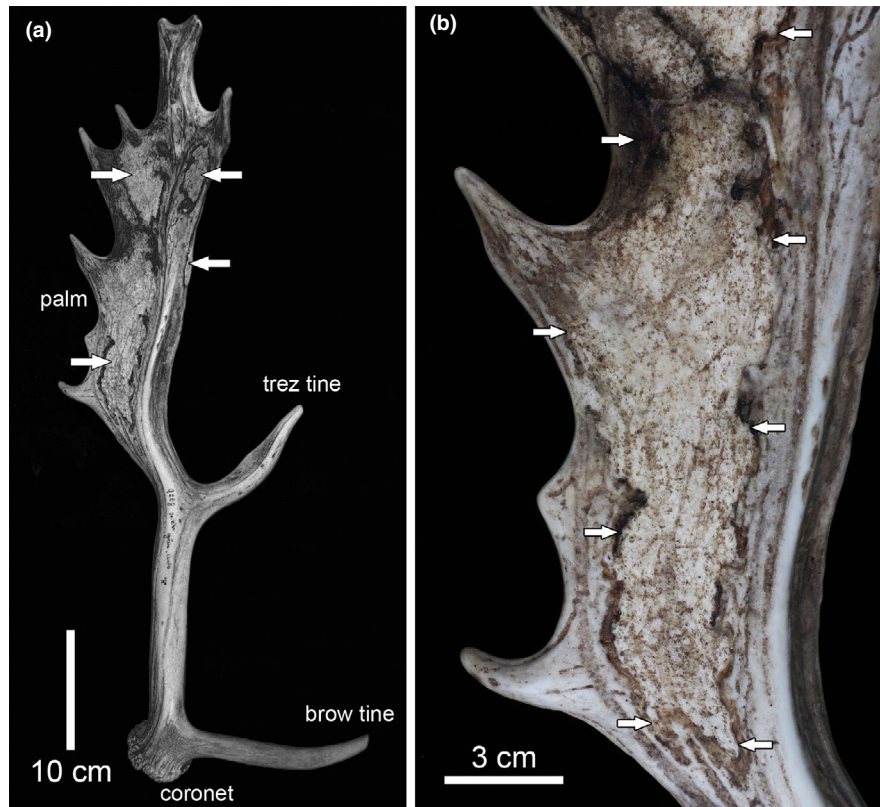


FIGURE 2 Left antler of fallow buck H, medial views. (a) Overview of antler with basal coronet, brow tine, trez tine and palm. Note demarcated, partially sequestered necrotic cortical portions (arrows) in the distal half of the antler. (b) Higher magnification of extended demarcated cortical bone area on the palm. Arrows: distinct groove separating a large area of the antler cortex with a coarse surface from the adjacent bone exhibiting a smoother surface

TABLE 1 Weight, palm development, and abnormalities of the fallow deer antlers cast following the experimentally extended velvet phase

Animal ID	Antler	Antler weight (g)	Palm	Abnormalities
H	Left	920	well-developed	cortical sequestration
	Right	850	moderately developed	cortical sequestration
I	Left	820	moderately developed	cortical sequestration
	Right	1110	well-developed	cortical sequestration
K	Left	755	weakly developed	notch at palm tip
	Right	740	weakly developed	abnormal antler curvature

and primary osteons that were filling up the intertrabecular cavities (Figure 3b).

On macroscopic inspection, pathological changes of the cortex were apparent in the antlers of bucks H and I. Thus, in the distal antler portions, cortical areas of variable size were demarcated from the surrounding bone by distinct grooves (Figure 2a,b). In places, remnants of dried soft tissue had been retained in these grooves. The surface of the demarcated cortical portions was coarser than that of the adjacent bone, indicative of resorption at the bone surface (Figure 2b). The demarcated cortical areas were partly undercut and separated from the more central bone areas by distinct clefts (Figure 4a,c). These clefts had typically been eroded along the border between the antler cortex and the

underlying spongiosa. The bone bordering the clefts exhibited an uneven surface with signs of intense osteoclastic resorption (Figure 4b). Locally, portions of cortical bone had been lost from the antlers (Figure 4a).

In addition to bone resorption, apposition of new bone onto the former resorption surface of the spongiosa bordering the cleft was observed in the right antler of fallow buck I (Figure 4c,d). Due to its fine cancellous structure, the newly deposited bone was clearly distinguishable from the original spongiosa. The histological appearance of the regenerated bone indicated that it had been formed in a two-step process with initial formation of woven bone and subsequent addition of lamellar bone onto the woven bone scaffold in a process of primary osteon formation (Figure 4d). The presence of

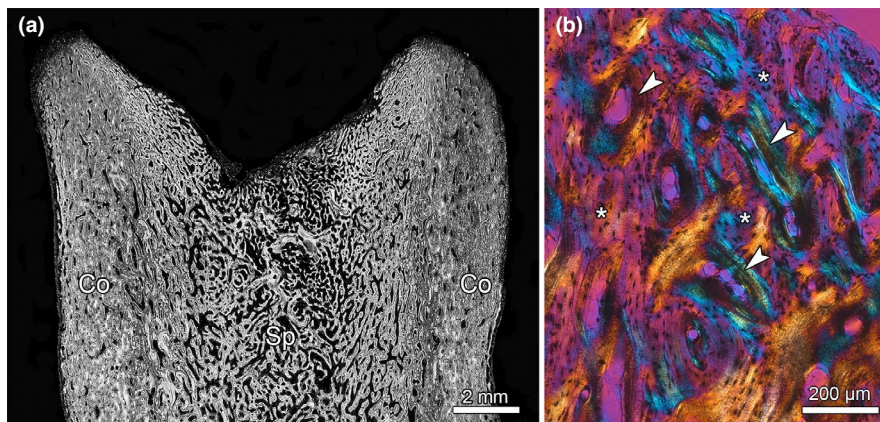


FIGURE 3 Tip of weakly developed palm of the left antler of fallow buck K. (a) Low-power light micrograph of longitudinal section (mediolateral plane, lateral to the right) showing V-shaped furrow at the antler tip; Co: cortex, Sp: spongiosa; darkfield reflected light. (b) Higher magnification of the bone lining the furrow. Note formation of primary osteons (arrowheads) within intertrabecular spaces bordered by woven bone (asterisks); linearly polarized light plus 1λ -plate

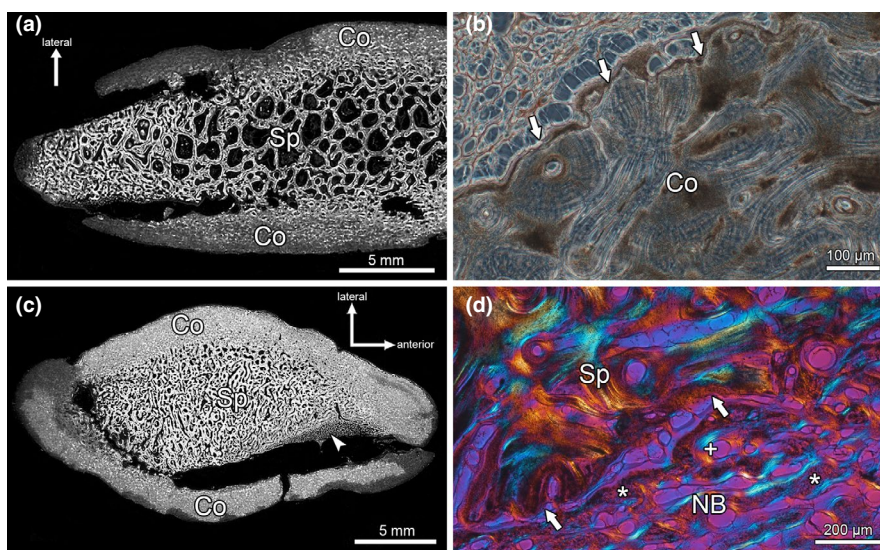


FIGURE 4 Light micrographs of antler sections showing partial sequestration of cortical portions. (a) Low-power micrograph of full-thickness transverse section of palm segment of left antler of fallow buck H (approximate position indicated by upper right arrow in Figure 2a). Note partial separation of the medial and lateral cortices (Co) from the spongiosa (Sp) by distinct clefts, and partial loss of cortical portions; darkfield reflected light. (b) Higher magnification showing scalloped surface (arrows) from osteoclastic resorption and dense structure of the medial cortex (Co) bordering on the cleft, left antler of fallow buck H; transmitted light with phase contrast. (c) Low-power micrograph of transverse section through a speller of the right antler of fallow buck I. Note cleft separating the medial cortex (Co) and a small portion of spongy bone from the bulk of the spongiosa (Sp); arrowhead: fine cancellous bone deposited onto the resorption surface of the spongiosa; darkfield reflected light. (d) Higher magnification showing the original spongiosa (Sp) and the newly deposited fine cancellous bone (NB) in the right antler of fallow buck I. The NB consists of initially formed woven bone (asterisks) and subsequently deposited lamellar bone (cross); arrows: former resorption surface of the spongiosa; linearly polarized light plus 1λ -plate

these (incomplete) primary osteons was therefore restricted to the oldest (inner) portions of the newly apposed bone.

The antlers of the control buck and the CPA-treated bucks consisted of a dense cortex and an internal spongiosa. There were, however, marked differences in the histological structure between the antlers of the control buck and those of the experimental bucks (Figure 5). Thus, the cortex of the control antlers was composed almost exclusively of relatively large osteons, with

often more than one vascular canal, and portions of the scaffold (Figure 5a,c). In SEM-BSE images, these osteons frequently exhibited bright seams along their periphery (Figure 5a), denoting a higher mineral content compared to the adjacent bone. These hypermineralized seams were diagnosed as reversal lines. The large osteons, which had primarily filled up non-bone compartments but had also replaced portions of the scaffold, were classified as primary osteons. In the tip regions of the control antlers, larger

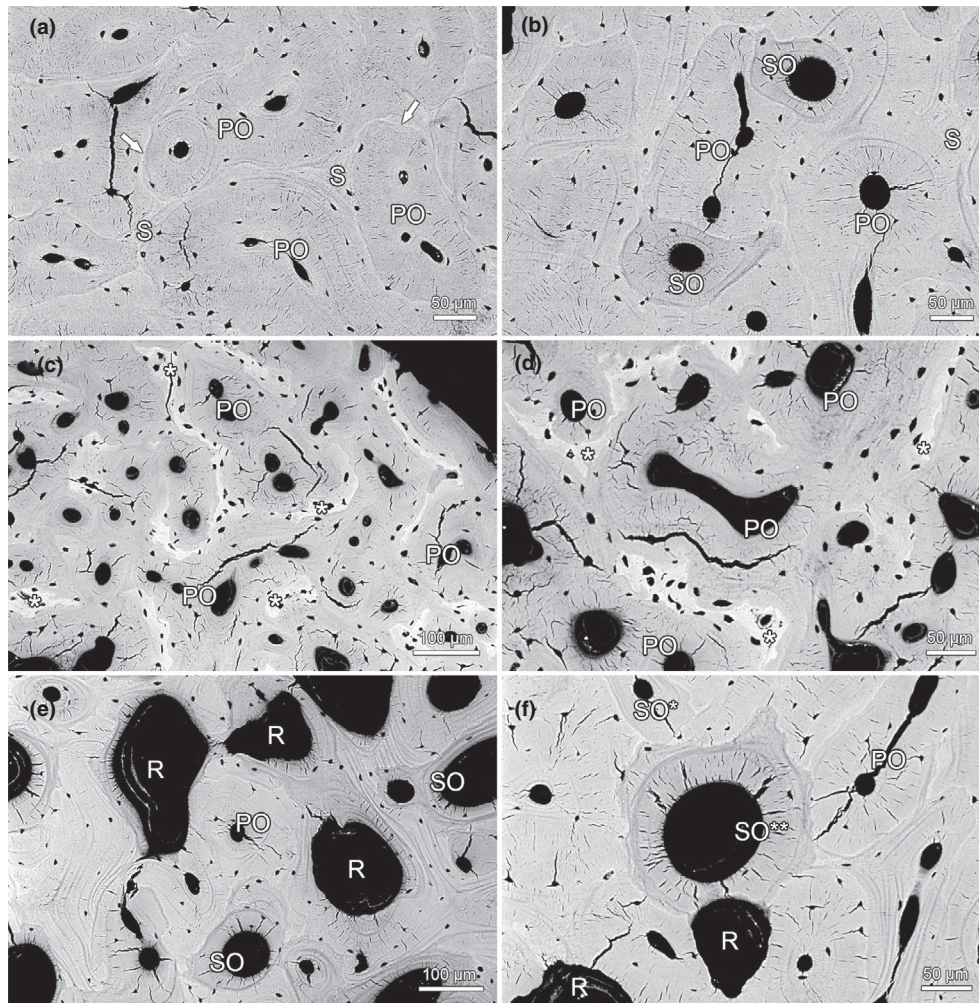


FIGURE 5 SEM-BSE micrographs of polished transverse sections through different areas of the antlers of the control fallow buck (a, c, d) and the CPA-treated fallow bucks (b, e, f). (a) Central cortex of basal portion (approximately 5 cm above brow tine) of main beam from left antler of the control buck; PO: primary osteons, S: scaffold. Note bright seams (arrows) along the periphery of the primary osteons. (b) Central cortex of basal portion (about 4 cm above brow tine) of main beam from left antler of fallow buck K; PO: primary osteons, SO: secondary osteons, S: remnants of scaffold. Note the smaller size and lower mineral content of the two secondary osteons that have partly replaced a larger and more mineralized (brighter) primary osteon. (c) Overview of cortex in the distal portion of the right antler from the control buck about 3 cm below the antler tip. Primary osteons (PO) are bordered by a scaffold that is partly composed of remnants of calcified cartilage (asterisks). The latter extends up to the antler surface and is more mineralized (brighter) than the bone. (d) Higher magnification of antler cortex in the tip region of the right antler from the control buck: Note wide vascular canals of the primary osteons (PO) and signs of resorption at the surface of the calcified cartilage (asterisks). (e) Overview image of the cortex in the distal portion of the right antler of fallow buck K about 3 cm below the antler tip. Resorption cavities (R) and secondary osteons (SO) in different stages of formation indicate marked remodeling activity. Note lower mineral content of secondary compared to primary osteons and presence of a forming secondary osteon with two vascular canals in the right half of the image. (f) Higher magnification of antler cortex in the tip region of the right antler of fallow buck K. A completed (SO*) and a (less mineralized) forming secondary osteon (SO**) are identified. The latter has already been partly eroded due to formation of a resorption cavity (R). Note the irregular border of the resorption cavity compared to the smooth internal border of the forming secondary osteon

portions of the scaffold still consisted of calcified cartilage that was more highly mineralized than the bone (Figure 5c,d). The surface of the calcified cartilage exhibited distinct signs of resorption, indicating that it had been partly eroded prior to the infilling of the intertrabecular spaces with primary osteons.

In stark contrast to the control antlers, the antler cortices of the CPA-treated bucks exhibited histological evidence of marked remodeling activity in both the basal (Figure 5b) and the distal antler

portions (Figures 5e,f and 6a,b). This included resorption cavities as well as forming (infilling) secondary osteons (with wide vascular canals) and completed secondary osteons (with narrow vascular canals). These three structures were also present in the partially sequestered cortical portions. Contrary to the controls, no remnants of calcified cartilage were observed in the antlers of the CPA-treated bucks.

Freshly formed resorption cavities were characterized by uneven, scalloped walls, while forming secondary osteons exhibited smooth

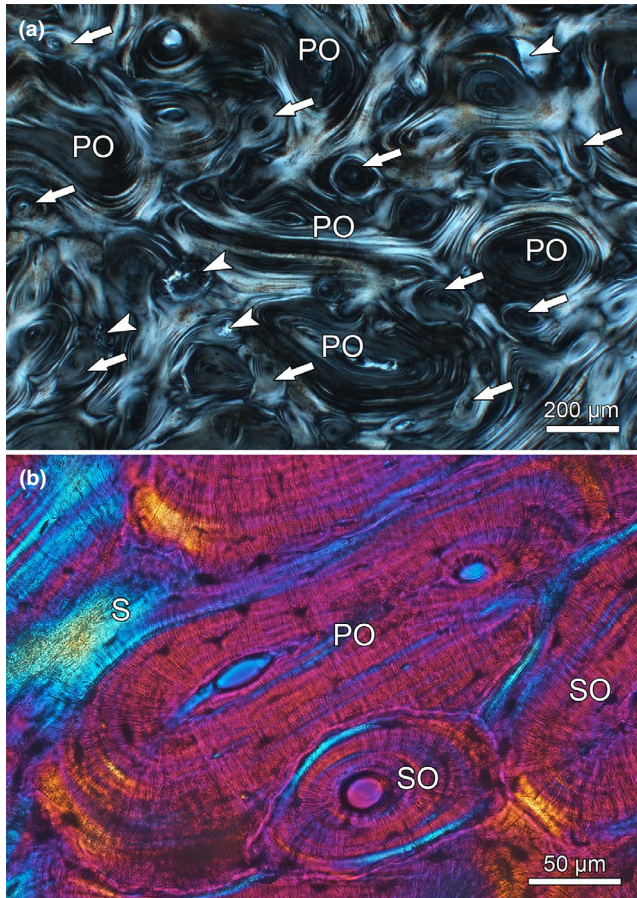


FIGURE 6 Light micrographs of transverse sections through distal antler portions of CPA-treated fallow bucks, showing signs of marked bone remodeling. (a) Medial cortex of the palm of the left antler of fallow buck H showing numerous secondary osteons (arrows) and resorption cavities (arrowheads). The secondary osteons are typically smaller than the primary osteons (PO); circularly polarized light. (b) Lateral cortex of the palm of the left antler of fallow buck I. A primary osteon (PO) has been partially replaced by two secondary osteons (SO); S: scaffold; linearly polarized light plus 1λ -plate

walls along their wide vascular canals (Figure 5e,f). A definite diagnosis of secondary osteons was possible when they infringed on and had thus (partly) replaced previously formed osteons (Figures 5b,e,f and 6a,b). The secondary osteons of the antler cortex were typically smaller than the primary osteons and, in contrast to the latter, mostly (but not invariably, for an exception, see Figure 5e) contained only a single vascular canal. Furthermore, on SEM-BSE images, the (younger) secondary osteons could be distinguished from primary osteons by their lower degree of mineralization (Figure 5e,f). Completed secondary osteons were typically more mineralized than forming ones (Figure 5f).

4 | DISCUSSION

Administration of CPA to the fallow bucks had approximately doubled the lifespan of their antlers, which lost their velvet between 31

December and 8 January. Velvet shedding about five weeks after termination of CPA treatment is indicative of an initial recovery of androgen production by the testes that was also detected by an increase in plasma testosterone levels (Kolle et al., 1993). Recently, a similar initial resumption of testosterone production four to five weeks after termination of CPA administration was observed in male sheep and goats (Flores-Gil et al., 2020).

The extended antler lifespan in the experimental bucks was associated with the occurrence of pathological changes in parts of the antler cortex. The presence of demarcated areas of compact cortical bone that had become partly separated from the underlying spongiosa by osteoclastic resorption indicates that portions of the cortex were in the process of being sequestered at the time of velvet shedding. In the affected areas, distinct clefts had been eroded along the border between cortex and spongiosa. It is suggested that in these areas the antler cortex had become necrotic and that osteoclastic resorption had occurred along the border between dead (outer) and living (inner) bone. The view that the antler core had remained viable is supported by the observation that in places new cancellous bone had been deposited onto the resorption surface of the spongiosa. No such deposition, which constitutes a bone regeneration process, had occurred at the cortical resorption surfaces. Portions of the necrotic cortex that had become completely separated from the spongiosa were apparently lost from the antlers at velvet shedding. Osteoclastic resorption within the antler was terminated when the velvet was belatedly shed and the entire antlers died off.

The death of portions of the antler cortex in two of the three CPA-treated bucks was mostly likely caused by ischemia. It is hypothesized that ongoing cortical compaction in association with an overall diminished blood flow to the antlers following the cessation of antler growth resulted in a progressive reduction of their blood supply. Due to the CPA-application, this condition extended over a protracted period and eventually led to ischemic necrosis of portions of the cortex. Since the experimental animals were kept outdoors, it seems possible that cold-induced vasoconstriction (Alba et al., 2019) had aggravated the ischemic conditions in the antlers. The reason why apparently only cortical antler areas became necrotic while the spongiosa remained viable remains speculative. It might be assumed that the dense structure of the cortex and/or certain characteristics of its vascular architecture, particularly in the antler palms, rendered it especially susceptible to ischemia.

The morphological findings in the antlers of buck K (lower antler weight, only weak palm formation) suggest a reduced intensity of antler growth compared to the other two CPA-treated bucks. The finding that at the tip of the left antler of buck K, the ossification process had apparently not been completed is in line with these observations. Impaired antler growth in buck K was probably related to the CPA treatment, as it was previously shown that castrated fallow bucks treated with high doses of CPA produced smaller antlers than castrates receiving no CPA (Bartoš et al., 2000). It is presently unclear why such marked growth impairment occurred only in one of the three CPA-treated fallow bucks from the present study.

The histological findings in the basal antler portions of the control buck match those previously obtained for the corresponding region of a hard antler (grown and cast on time) from an adult fallow buck (Kierdorf et al., 1993). Studies in other extant deer species likewise demonstrated that their hard antlers basically consist of primary osteons and scaffold and that antler bone does not undergo any significant remodeling (Gomez et al., 2013; Kierdorf et al., 2013; Krauss et al., 2011; Launey et al., 2010; Skedros et al., 2014; Wislocki, 1942). In a study on red deer, Gomez et al., (2013) found a few secondary osteons in the lower and middle thirds of the antlers, but none in the upper third, apparently because there had not been enough time for remodeling in this late-formed antler region prior to velvet shedding.

The presence of remnants of calcified cartilage in the distal portions of the antlers of the control fallow buck observed in the present study matches previous findings in antlers of the European roe deer (*Capreolus capreolus*) (Kierdorf et al., 2013). In the distal antler regions, the replacement of the cartilaginous by a bony scaffold had thus not been completed prior to the onset of primary osteon formation. Due to its partial resorption, the calcified cartilage had become reduced to irregularly shaped streaks, and the scaffold consisted of a mixture of calcified cartilage and bone.

In contrast to the control antlers, those of the CPA-treated bucks (with extended velvet antler phase) exhibited numerous secondary osteons and resorption cavities, indicative of a marked remodeling activity. This process had also led to the complete removal of calcified cartilage in the antlers of the CPA-treated bucks.

The present study has again demonstrated that the histological features of normal hard antlers reflect the short antler lifespan. We further demonstrated that antlers undergo marked remodeling along their entire length if their lifespan is experimentally extended. It has more recently been concluded that our understanding of the role of remodeling for bone maintenance and the drivers of the remodeling process is inadequate and that more research involving a variety of organisms and bone types is needed to improve this situation (Currey et al., 2017). Antlers are an interesting subject in this respect because during their velvet phase, they are not exposed to any major mechanical loading (Goss, 1983). It is currently unknown which factors trigger the remodeling activity in antlers that occurs when their lifespan is experimentally extended.

Some investigators have assumed that the cranial appendages of early members of the cervid lineage were permanent structures (Bubenik, 1990a). However, morphological studies later provided strong evidence for a regular cycle of death, casting and regeneration of the cranial appendages in Miocene deer species (Heckeberg, 2017; Rössner et al., 2021). In consequence, the notion that true (deciduous) antlers were preceded by nondeciduous "protoantlers" in the evolution of the Cervidae has been rejected (Heckeberg, 2017; Rössner et al., 2021).

A recent histological study of antlers from various Miocene deer species described the widespread occurrence of secondary osteons, especially in the basal antler portions (Rössner et al., 2021). These

authors, however, also reported the presence of remnants of (calcified) cartilage in the tip regions of the antlers. Based on the findings of the present study, the latter finding would indicate a short lifespan of the Miocene antlers, comparable to the situation in the control fallow buck, while the occurrence of many secondary osteons in the Miocene antlers would, in contrast, point to a more extended lifespan.

This apparent inconsistency may be partly attributable to varying opinions about what defines a secondary osteon in antler bone between the present study and that of Rössner et al., (2021). In our view, reversal lines cannot be used as an unequivocal identifier of secondary osteons in antler bone, and their identification must therefore be based on other criteria. A clear diagnosis of secondary osteons is possible when they have partially replaced earlier formed (primary or secondary) osteons. On SEM-BSE images, the younger secondary osteons were moreover mostly distinguishable from the older primary osteons by their lower degree of mineralization. However, this criterion is of little help when studying fossil antlers. Osteonal size and morphology are further potential criteria, as secondary osteons are typically (but not always) smaller than primary ones and mostly possess only a single vascular canal. To further elucidate the relationships between antler growth rates, duration of the velvet antler phase and the extent of bone remodeling, additional histological studies on the antlers of early and modern deer are recommended.

Most authors agree that antlers die from ischemia following velvet shedding (Currey et al., 2009; Gomez et al., 2013; Rhumbler, 1929; Waldo & Wislocki, 1951; Wislocki, 1942). Based on studies in fallow deer, Rolf and Enderle (1999) have, however, claimed that antlers survive velvet shedding for longer periods of time. Summarizing their findings, these authors stated that "our present observations point to a continuous bone remodeling in different regions of hard fallow deer antlers possibly persisting till shortly before antler separation. These findings lead to the conclusion that from an osteological point of view the polished fallow deer antler doubtless represents living bone" (Rolf & Enderle, 1999, p. 76). The results of the present study and those of our previous investigation (Kierdorf et al., 1993) are inconsistent with the views expressed by Rolf and Enderle (1999), but completely in line with the notion that hard antlers are dead bony structures. Signs of marked remodeling activity in fallow deer antlers were only observed when their lifespan (velvet phase) was experimentally prolonged.

In summary, the present study has demonstrated that the experimental extension (doubling to eight months) of the velvet antler phase of adult fallow bucks was associated with marked remodeling of antler bone and ischemic necrosis of portions of the antler cortex. It is presently unclear what had triggered remodeling activity in the longer lived antlers, as antlers are not loaded to any larger extent while being covered in velvet. Antlers with an extended lifespan thus constitute suitable model structures for analyzing bone remodeling in the virtual absence of mechanical loading.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

U. K. and H. K. designed the study; U.K. drafted the manuscript; U.K., H.K., and M.S. acquired, analyzed, interpreted the data, and critically revised the manuscript and approved its final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of the present study are available from the corresponding author upon reasonable request.

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