

Comparative histology of the femur between mouflon (*Ovis aries musimon*) and sheep (*Ovis aries aries*)

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Abstract

Mouflon (*Ovis aries musimon*) and sheep (*Ovis aries aries*) are considered as the wild and domestic subspecies of the same species. A comparative study on the microstructure of mouflon and sheep femoral bone diaphysis is here reported. Bone microstructure is described for the first time in the mouflon. More than 200 secondary osteons from both subspecies were analyzed and qualitative evaluation was followed by quantitative determination of perimeter, area, minimum and maximum diameters of secondary osteons and Haversian canals. The basic structural patterns observed in both subspecies can be classified as plexiform and irregular Haversian tissue, in accordance with what reported in the literature for most ruminants. The presence of many secondary osteons in the mouflon means that the bone also consists of dense Haversian bone tissue. Statistical analysis

demonstrated that mouflon secondary osteons are larger than in the sheep and made of a greater number of lamellae. Since mouflon and sheep are taxonomically closely related and their body size is very similar, the qualitative and quantitative differences here reported could be primarily explained on account of their different lifestyle. Indeed, the habits of wildlife typical of mouflons may lead to the presence of wide areas of dense Haversian tissue in that subspecies, as mechanical stresses are known to be related to number and size of secondary osteons. Finally, this analysis could provide a useful tool to recognize bones from different species, in forensic exam and archaeozoological studies as well.

Introduction

Mouflon is a wild sheep native to the Near East. In prehistoric age it was introduced to few Mediterranean islands, such as Sardinia, Corsica, Cyprus and Rhodes.¹ In the last centuries, it was widely spread in many mountain regions of Europe. Nowadays mouflon populations are subjected to local restrictions on hunting since it has been listed as vulnerable by the World Conservation Union (IUCN). Hence, any possible difference in bone microstructure between mouflon and sheep may be of interest in the forensic field, especially in those geographic areas where mouflons are legally protected.

In the past, mouflon taxonomy was rather confusing,¹ until a final classification² indicated that mouflon can be considered as a wild sheep (*Ovis aries musimon*) different to the domestic sheep (*Ovis aries aries*), so they are seen as two subspecies of the same species. Despite some different phenotypic features, such as coat colour and horn morphology, their skeletons are very similar. Thus, distinguishing bones between these two subspecies is very hard and depends on different interpretations among osteologists. Regarding bone structure, it is interesting to highlight that despite the fact that mouflon and sheep have the same body size, they live in habitats exposed to different biomechanical stresses according to wild or domestic lifestyle, respectively. In Sardinia, mouflons live in rocky hilly regions mainly covered by Mediterranean scrub, frequently jumping, whereas domestic sheep are usually less exposed to uneven grounds. Indeed, it is ascertained that the presence, size and morphology of secondary osteons in each bone depend on biomechanical stress.³

The histological structure of bones of ruminants such as the elk (*Cervus canadensis*), red deer (*Cervus elaphus*), and roe deer (*Capreolus capreolus*), where domestic individuals do not exist, was described in previous studies.⁴⁻⁶ In the present work, the results obtained from a wild subspecies are compared for the first time with those from the corresponding domestic subspecies in order to high-

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light the role played by the lifestyle on bone structure and secondary osteon morphology.

Moreover, the femur of sheep brings on some interest because it represents an excellent experimental model to better understand the development and evolution of some human bone diseases, such as osteoporosis and fracture.⁷⁻¹⁰ Finally, the comparison between mouflon and sheep here proposed may represent an example of recognizing bones from different animals, which may be useful in forensic exam and archaeozoological studies.

Materials and Methods

Bone specimens consisted of 4 femoral diaphyses from mouflons and 4 from sheep from both sides. All the femora came from the osteological collection of the Department of Veterinary Medicine, University of Sassari (Italy). Mouflon bones belonged to adult male animals that died of natural causes in the protected natural area of Capo Figari, Isola di Figarolo, in North-Eastern Sardinia, a site of EU Community importance (SIC ITB 01009). Sheep belonged to the Sarda breed and bones came from adult male animals regularly slaughtered in Sardinian abattoirs. No evidence of skeletal pathology was detected in both subspecies and the age range, estimated on the basis of skeleton ossification and horn morphology,¹¹ was 4-6 years in all animals. The femur was chosen as is the longest bone of the skeleton and is subjected to very heavy stress loads. Femora were crosscut at the level of the smallest breadth of their diaphyses (midshaft) using an electrical saw to obtain 2 mm thick sections. The rings were ground and thinned using either a fine sandpapering machine or handily processed by emery paper to obtain 50 μm thick sections. After thorough washing to remove debris, transverse sections from periosteal, mesosteal and endosteal zones were mounted onto glass slides with Eukitt (Merck, Darmstadt, Germany) and coverslipped. Afterwards, sections were observed and photographed by means of a Zeiss Axiophoto microscope using $\times 2.5$, $\times 10$ and $\times 20$ objectives. Bone patterns were classified in agreement with the bone tissue classification proposed by Enlow and Brown.¹² About 200 secondary osteons from each subspecies were examined, that is well-defined osteons surrounded by an evident cement line. The number of lamellae was counted based on the position of osteocyte lacunae. The following parameters were measured by means of *Scion Image* software (Scion Corporation, Frederick, MD, USA): perimeter, area, minimum and maximum diameters of secondary osteons and Haversian canals. The secondary osteon area included the entire osteon with its Haversian canal. Only intact osteons were considered for statistical analyses. For quantitative comparison, the values obtained for each parameter were compared between mouflon and sheep considering the criterion for statistical significance $P < 0.05$, and variability was expressed as the standard deviation.

Results

The qualitative observations of the femoral sections belonging to mouflon and sheep indicate that the basic structural patterns can be classified as plexiform and irregular Haversian tissues (Figures 1A, 2), following the bone tissue classification proposed by Enlow and Brown.¹² In the majority of the sections studied, such tissues change one to the other mainly starting from mesosteal zone. The main difference between these two subspecies is related to secondary osteons. In the mouflon, dense Haversian tissue is also present in many areas. Indeed, a high number of secondary osteons are found. They are mainly elliptical in shape and clustered together in small groups (Figures 1B, 3). On the contrary,

sheep secondary osteons are small and isolated (Figure 2). No differences in bone patterns are found among anterior, posterior, medial and lateral sides in both subspecies.

Regarding the morphology of secondary osteons, they are constituted of 5-7 lamellae or more in the mouflon (Figure 3), whereas those of sheep are formed of 4-5 lamellae only (Figure 2).

The quantitative investigation (Table 1) shows that secondary osteons are larger in the mouflon (mean maximum diameter is 214 μm) than in sheep (164 μm), and Haversian canals are wider in mouflon (mean maximum diameter 27 μm) than in sheep (24 μm). On account of that, values obtained from area and perimeter of secondary osteons and Haversian canals are consequently larger in the mouflon. The comparison between mouflon and sheep demonstrates that all

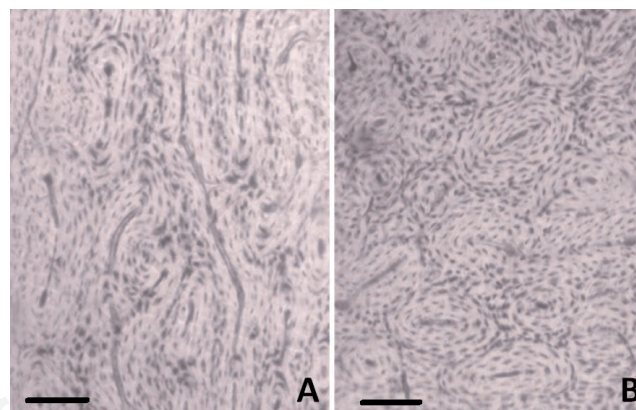


Figure 1. Different types of bone tissues in the femur of the mouflon. A) irregular Haversian tissue; B) dense Haversian tissue. Bars=150 μm .

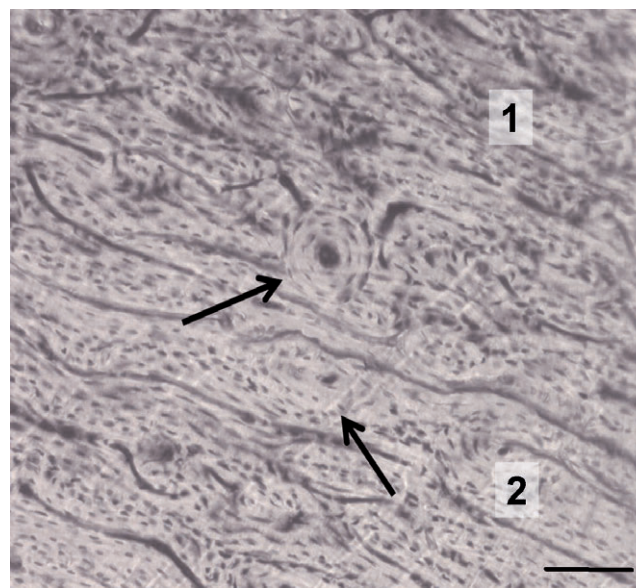


Figure 2. Sheep. Irregular Haversian tissue (1) gradually changes to plexiform tissue (2). The arrows indicate small and isolated secondary osteons formed by 4-5 lamellae. Bar=100 μm .

morphometrical values (maximum and minimum diameter, perimeter and area) of secondary osteons are significantly different ($P < 0.01$). As to Haversian canals, significant differences are present between the two subspecies for maximum diameter ($P < 0.01$), area ($P < 0.01$) and perimeter ($P < 0.05$). In contrast, values of minimum diameter of Haversian canals do not show significant difference ($P > 0.05$).

Discussion

In this work, the microscopic structure of the femoral diaphysis is described in the mouflon for the first time. A comparison of the mouflon with the sheep was thought of some interest, especially in forensic and archaeozoological investigations, since it is possible to highlight some differences in histological features. The basic structural pattern observed in both subspecies can be classified as plexiform and irregular Haversian tissues. The literature reports that plexiform tissue, also called fibro lamellar system, consists of primary vascular canals organized into a regular, well-defined plexus. Moreover, as described by Enlow and Brown,¹² irregular Haversian tissue is characterized by isolated secondary osteons, generally having a direction perpendicular to the primary vascular canals.

Our results are mostly in agreement with Skedros and colleagues⁴

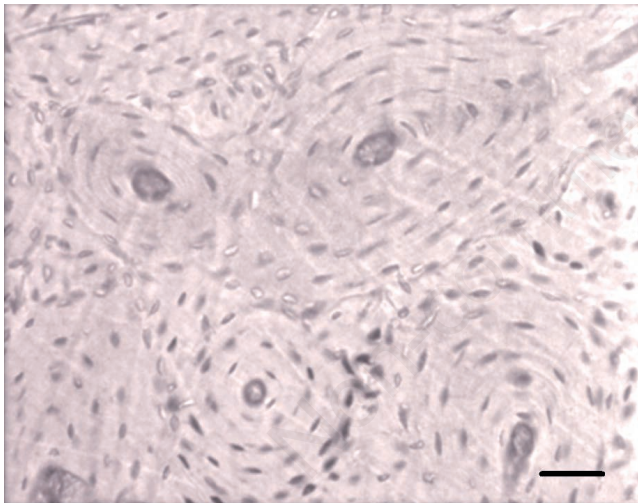


Figure 3. Mouflon. A group of well-defined secondary osteons is present in dense Haversian tissue. These secondary osteons are formed by 5-7 lamellae. Bar=50 μm .

for sheep and deer calcaneus, Metz and colleagues¹³ for sheep ulna, Mori and colleagues¹⁴ for calf tibia and radius, Skedros⁵ for sheep and elk calcaneus, Martiniaková and colleagues^{15,16} for sheep femur, Zedda and colleagues¹⁷ for bovine femur and humerus, Gudea and Stefan⁶ for sheep, goat and roe deer humerus and metapodial bones. All descriptions made by those authors are rather overlapping, although few differences are present, probably related to the type of bone (depending on main direction and extent of mechanical loads), or species taken into account and are confirmed by the present work. In accordance with Brits and colleagues¹⁸ and unlike what reported by Martiniaková and others,¹⁶ no dense Haversian bone tissue characterized by a dense concentration of secondary osteons is detected here in sheep femora. Such difference could be related to both diverse age of specimens¹⁹ and to different sheep breeds. Indeed, Martiniaková and colleagues¹⁶ studied Merino sheep, Brits and colleagues¹⁸ Merino and Dorper breeds, Gudea and Stefan⁶ a Zackel breed, whereas the present work refers to Sarda sheep.

Moreover, it is well-known that bone microstructure and in particular the presence, shape and size of secondary osteons, also depend on the biomechanical stress loading on the bone.²⁰ Mouflon secondary osteons are elliptical in shape, which provides better resistance to biomechanical stresses. Indeed, breaking of elliptical osteons at their major axis requires higher strains than those needed for circular osteons.²¹

Our quantitative data concerning osteons and Haversian canals from sheep are similar to those reported by others, with some differences. Indeed, our values of minimum and maximum diameter of Haversian canals (24 ± 3 and 22 ± 2 μm , respectively) are higher than those reported by Martiniaková and colleagues^{15,16} in femora (34 ± 9 and 12 ± 3 μm , respectively). The secondary osteons described by these authors in sheep femora are more elliptical in shape as the values of their minimum and maximum diameter are smaller (67 ± 17 μm) and larger (208 ± 70 μm) respectively than those reported here in the same species (min diameter 135 ± 21 μm , max diameter 164 ± 29 μm). In addition, our data are very similar to what reported by Gudea and Stefan⁶ in sheep humerus. Their values often differ from ours by 10% only (Haversian canals: min diameter 19 ± 4 μm , max diameter 25 ± 7 μm , perimeter 70 ± 17 μm , area 364 ± 136 μm^2 ; secondary osteons: min diameter 127 ± 22 μm , max diameter 164 ± 26 μm , perimeter 464 ± 77 μm , area 16.514 ± 6542 μm^2).

Conclusions

In the present investigation, bone microstructure is described for the first time in the mouflon. Since mouflon and sheep belong to the same species and their body size is quite similar, the qualitative and quantitative differences in femoral microstructure here reported could be explained primarily on account of their different lifestyles. The basic structural pattern observed in both subspecies can be classified as plex-

Table 1. Mean morphometrical values from secondary osteons and Haversian canals of mouflon and sheep.

Subspecies	Osteons				Haversian canals			
	Minimum diameter (μm)	Maximum diameter (μm)	Perimeter (μm)	Area (μm^2)	Minimum diameter (μm)	Maximum diameter (μm)	Perimeter (μm)	Area (μm^2)
Mouflon (<i>Ovis aries musimon</i>)	163 ± 37	214 ± 42	597 ± 58	27.382 ± 2.945	23 ± 3	27 ± 4	79 ± 8	487 ± 82
Domestic sheep (<i>Ovis aries aries</i>)	135 ± 21	164 ± 29	472 ± 36	17.380 ± 1.540	22 ± 2	24 ± 3	72 ± 9	414 ± 63

iform and irregular Haversian tissues. In the mouflon, dense Haversian tissue is also present in many areas. The main difference between these two subspecies is related to secondary osteons. Indeed, in the mouflon a high number of secondary osteons are found, which are mainly elliptical in shape and clustered together in small groups, whereas in the sheep secondary osteons are small and isolated. The quantitative results show that secondary osteons and Haversian canals are larger in the mouflon than in sheep. Finally, the analysis here reported could provide a useful tool to recognize bones from different species, in forensic exam and archaeozoological studies as well.

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