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Research Article

Comparative Analysis of Vascular Density in the Femoral Head Ligament of the Dog, Sheep, and Goat

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ABSTRACT

Background: The ligament of the head of the femur (ligamentum teres) is an intra-articular structure that contributes to hip joint stability and serves as a potential conduit for vascular supply to the femoral head. Despite its recognised anatomical presence across mammalian species, its vascular capacity and functional significance remain incompletely understood. This study aimed to comparatively evaluate vascular density within the ligament of the femoral head in adult dog, sheep, and goat, and to determine whether a relationship exists between ligament length and vascular characteristics. **Materials and Methods:** Nine cadavers (three specimens each of adult dog, sheep, and goat) were examined. Ligament length was measured macroscopically, and selected samples were processed for histological analysis using haematoxylin and eosin staining. High-resolution images were analysed using ImageJ software to quantify blood vessel area and vascular density. Statistical analysis was performed using one-way ANOVA followed by Bonferroni post hoc testing, with significance set at $p<0.05$. **Results:** Ligament length ranged from 13-22 mm in dogs and 20-22 mm in both sheep and goats. No correlation was identified between ligament length and blood vessel area. However, significant interspecies differences in vascularity were observed. Goats exhibited a significantly greater mean blood vessel area (47,025.58 μm^2) and higher vascular density compared to both dogs (10,289.43 μm^2) and sheep (5,806.94 μm^2) ($p<0.05$), while no significant difference was found between dogs and sheep. The ratio of vessel area to density was not significantly different among species. **Conclusion:** These findings demonstrate that vascular characteristics of the ligament of the femoral head vary between species and are not dependent on ligament length. The increased vascular density observed in goats suggests a potentially greater contribution to femoral head perfusion. This study highlights the importance of vascular density as a relevant parameter in understanding ligament function and may have implications for the pathophysiology and treatment of conditions associated with compromised femoral head blood supply.

Keywords: Comparative anatomy, Femoral head perfusion, Histological analysis, Ligament of the femoral head, Vascular density.

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INTRODUCTION

The mammalian hip joint is a highly specialised synovial ball-and-socket articulation formed by the interaction of the femoral head and the acetabulum of the pelvis.[1-3] This configuration allows for a wide range of motion while simultaneously bearing substantial mechanical loads. Within the joint, the ligament of the head of the femur (ligamentum teres) extends from the fovea capitis of the femoral head to the acetabular fossa and blends with the transverse acetabular ligament.[4,5] Positioned centrally within the joint cavity, this structure has traditionally been regarded as mechanically insignificant in adult mammals; however, increasing anatomical and clinical evidence suggests a more complex functional role encompassing both stabilisation and vascular support.[4-7]In humans, the ligamentum teres conveys a small but potentially significant arterial branch, most commonly arising from the obturator artery, which contributes to femoral head perfusion, particularly during developmental stages.[8,9] Nonetheless, the dominant blood supply to the femoral head is derived from the medial and lateral femoral circumflex arteries through retinacular vessels that ascend along the femoral neck.[9,10] Comparable vascular patterns are observed in other mammals, including ovine and caprine species, in which anterior and posterior retinacular systems constitute the principal perfusion pathways, supplemented by an acetabular branch traversing the ligament.[11,12] In canines, however, the ligament of the femoral head assumes a more prominent functional role, acting not only as a mechanical stabiliser and limiter of extreme joint movement

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but also as an important conduit for vascular structures supplying the femoral head.[4,8]

From a histological perspective, the ligament exhibits a stratified organisation consisting of a synovial lining of cuboidal cells, a sub-synovial layer rich in loose connective tissue, adipocytes, and fibroblasts, and a dense ligamentous core composed of parallel collagen fibre bundles interspersed with fibroblasts and fine elastin fibres.[4,8,13] Importantly, vascular elements are primarily located within the sub-synovial zone, suggesting that the microvascular architecture of this region may play a critical role in determining the extent of blood supply conveyed through the ligament. This structural arrangement exists in concert with the extracapsular stabilising ligaments of the hip, namely the iliofemoral, pubofemoral, and ischiofemoral ligaments, which collectively maintain joint integrity under dynamic loading conditions.[8,14] The vascular integrity of the femoral head is essential for maintaining osseous viability, and disruption of this supply is strongly associated with pathological conditions such as Legg-Calvé-Perthes disease, characterised by avascular necrosis and subsequent osteoarthrosis.[15,16] This condition has been documented across multiple species, including humans, dogs, cattle, and cats, and is particularly prevalent in small-breed dogs during periods of rapid growth.[17-19] Such pathology highlights the critical importance of understanding all potential vascular contributions to the femoral head, including those arising from the ligamentum teres.

Despite extensive anatomical descriptions of the ligament of the femoral head, there remains a notable lack in quantitative data regarding its vascular characteristics. Vascular density within the ligament has not been thoroughly investigated, nor has its relationship to ligament morphology been clearly established. This represents a significant gap in current knowledge, as vascular density may directly influence the capacity of the ligament to contribute to femoral head perfusion. Furthermore, variations in vascular density across species may reflect adaptive differences related to locomotion, biomechanical loading, and metabolic demand.[20,21] A detailed evaluation of vascular density within the ligament of the femoral head has important potential applications. From a clinical perspective, improved understanding of ligamentous vascular contribution may enhance the diagnosis, prevention, and management of avascular conditions affecting the femoral head. In veterinary medicine, this knowledge may inform breed-specific risk assessment and therapeutic strategies, particularly in species predisposed to vascular compromise. Additionally, in orthopaedic and reconstructive research, insights into ligament vascularity may guide the development of surgical techniques, graft design, and regenerative approaches aimed at preserving or restoring femoral head perfusion. From a comparative anatomical standpoint, assessing vascular density across species with differing functional demands offers an opportunity to better understand the relationship between structure, function, and vascular adaptation.

Dogs, sheep, and goats present an ideal comparative model for such investigation. Although these species differ markedly in body size, locomotor patterns, and energy expenditure, they share broadly similar hip joint anatomy and ligament structure. This makes them particularly suitable for examining whether conserved morphology is associated with comparable vascular characteristics, or whether species-specific differences in vascular density exist. Accordingly, this study, aimed to quantitatively assess vascular density within the ligament of the femoral head of the dog, sheep and goat and to evaluate its relationship with ligament length. Furthermore, this study also assessed whether similarities in ligament morphology across these species correspond to comparable vascular supply to the femoral head, thereby contributing to a more comprehensive understanding of ligament function and its clinical relevance.

MATERIALS AND METHODS

In this prospective study, three adult canine cadavers, all of which were the breed Greyhound, were obtained from the University College Dublin (UCD) Veterinary Hospital were examined. The College of Veterinary Medicine at the University of Al-Qadisiyah, Iraq (Ethics approval number P.G. No. 1890) collected three goat and sheep cadavers for analysis. Once all nine specimens were collected, both left and right ligaments of the femoral head was measured in millimetres with a ruler, and photos were taken of individual ligaments. The study was reviewed and approved by the UCD Animal Research Ethics Committee (AREC- E-21-19-Kilroy).

Following anatomical identification and documentation, the ligament of the head of the femur was carefully isolated from each specimen. A scalpel was used to sharply transect the ligament at its points of attachment to both the acetabulum and the femoral head, ensuring preservation of the ligament's intrinsic architecture. The excised ligaments were subsequently subdivided into smaller segments to facilitate optimal fixation and processing, and each segment was placed into appropriately sized tissue cassettes. Sampling varied slightly among species due to specimen availability. A consistent processing and analysis were maintained across all specimens. The tissue cassettes employed in this study were designed with a porous structure to allow efficient penetration of processing reagents while maintaining physical support for the tissue. Care was taken to avoid overfilling the cassettes, as excessive tissue volume can impede the infiltration of fixatives and processing fluids. A range of cassette sizes was therefore utilised to ensure adequate exposure of all tissue samples. All specimens were fixed in 10% neutral buffered formalin to preserve cellular and extracellular morphology. Following fixation, tissues were dehydrated through graded alcohols, cleared, and embedded in paraffin wax to produce stable tissue blocks. These blocks were then sectioned manually using a microtome to obtain thin sections of approximately 4-5 µm thickness, which is considered optimal for detailed histological assessment of soft connective tissues.[22-24]

Sectioning was performed in a vertical plane relative to the longitudinal axis of the ligament, allowing for clear visualisation of the ligament diameter as well as its internal structural organisation, including vascular elements. The resulting paraffin sections were carefully transferred onto glass slides, heat-fixed to ensure adhesion, and subsequently stained using Haematoxylin and Eosin (H&E).[25] Haematoxylin and eosin are complementary histological stains that provide a broad spectrum of contrast for tissue evaluation. Haematoxylin, a basic dye, binds preferentially to acidic components such as nuclei, DNA, RNA, and ribosomes, imparting a blue to purple coloration. In contrast, eosin is an acidic dye that binds to basic components, including cytoplasm, collagen, and extracellular matrix proteins, producing varying shades of pink to red. The combined use of these stains enabled clear differentiation of cellular and extracellular structures within the ligament of the femoral head, facilitating detailed assessment of tissue architecture and vascular components.

Each ligament sample generated multiple histological slides. These slides were examined using a light microscope equipped with digital imaging capabilities, and high-resolution images were captured for analysis. The images were stored electronically and subsequently transferred to a computer for quantitative evaluation using ImageJ, a widely utilised platform for tissue analysis in the health sciences. Within ImageJ, images were processed to identify and quantify vascular structures. Blood vessels were distinguished from surrounding connective tissue and cellular components based on morphological characteristics and staining patterns. Depending on the section, images contained either single or multiple identifiable blood vessels. The cross-sectional area of each visible blood vessel was measured in micrometres (μm^2), allowing for quantitative comparison across samples. For each ligament, measurements of individual vessel areas were recorded together with the total area of histological section and the mean density (which reflects the volume of smooth muscle cells in the blood vessel wall) of the blood vessels was also recorded. This enabled a comprehensive assessment of vascular distribution and density within the ligament tissue. All slides were systematically uploaded into ImageJ for visualisation, image processing, and subsequent quantitative analysis.

All quantitative data are expressed as Mean±Standard Error of the Mean (SEM). Statistical comparisons between species (dog, sheep, and goat) were performed using One-Way Analysis of Variance (ANOVA) to determine overall differences among groups. Where significant differences were identified, *post hoc* multiple comparisons were conducted using the Bonferroni correction to account for type I error. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of nine cadavers were examined in this study, comprising three specimens each from dog, sheep and goat. From these, ten ligaments of the femoral head were measured macroscopically using a ruler, and six ligaments were selected for subsequent histological analysis. Ligament length varied among species, with canine ligaments ranging from 13 to 22 mm, while ovine and caprine ligaments exhibited a narrower range of 20-21 mm and 20-22 mm, respectively. A representative gross anatomical image of the ligament of the femoral head alongside a corresponding histological section is presented in Figure 1. Within individual cadavers, bilateral symmetry in ligament length was generally observed. In canine specimens, left and right ligaments were comparable in length with negligible variation. In contrast, ovine and caprine specimens demonstrated a slight reduction in length of the left ligament relative to the right; however, these differences were minimal and not considered biologically significant.

Quantitative histological analysis revealed species-specific differences in vascular characteristics within the ligament of the femoral head. The mean cross-sectional area of blood vessels was 10,289.43 μm^2 in the dog, 5,806.94 μm^2 in the sheep, and markedly higher at 47,025.58 μm^2 in the goat. Statistical analysis demonstrated that the vascular area in the caprine ligament was significantly greater ($p<0.05$) than that observed in both canine and ovine ligaments (Figure 2). No statistically significant difference in vessel area was identified between dog and sheep specimens. In addition to vessel size, vascular density also differed between species. The mean vascular density was significantly higher in the goat compared to both the dog and sheep ($p<0.05$), indicating a greater abundance of smooth muscle volume within the caprine ligament. Further, sheep exhibited a higher vascular density than dogs, although this difference was less pronounced. These findings suggest interspecies variation not only in vessel calibre but also in overall vascular distribution within the ligament. Interestingly, when the relationship between vessel area and vascular density was examined, no statistically significant differences were detected among the three species. This indicates that, despite differences in absolute vessel size and number, the proportional relationship between these parameters remains relatively conserved across species. Our results demonstrate that while ligament length is broadly comparable across the species studied, the vascular architecture of the ligament of the femoral head, particularly in terms of vessel area and density varies considerably, with the goat exhibiting the most pronounced vascularisation. These findings may have important implications for understanding species-specific differences in femoral head perfusion and ligament function.

DISCUSSION

The present study provides a comparative evaluation of vascular characteristics within the ligament of the head of the femur across dogs, sheep and goats, with particular emphasis on vascular density and its relationship to ligament morphology. The ligamentum teres is increasingly recognised as a structure of both mechanical and physiological importance, contributing to hip joint stability while also serving as a conduit for vascular supply to the femoral head.[26,27] The findings of this study add to the limited body of literature addressing its vascular capacity and provide new insights into interspecies variation.Anatomically, the ligament of the head of the femur transmits vascular branches, commonly derived from the obturator artery, that traverse the ligament to

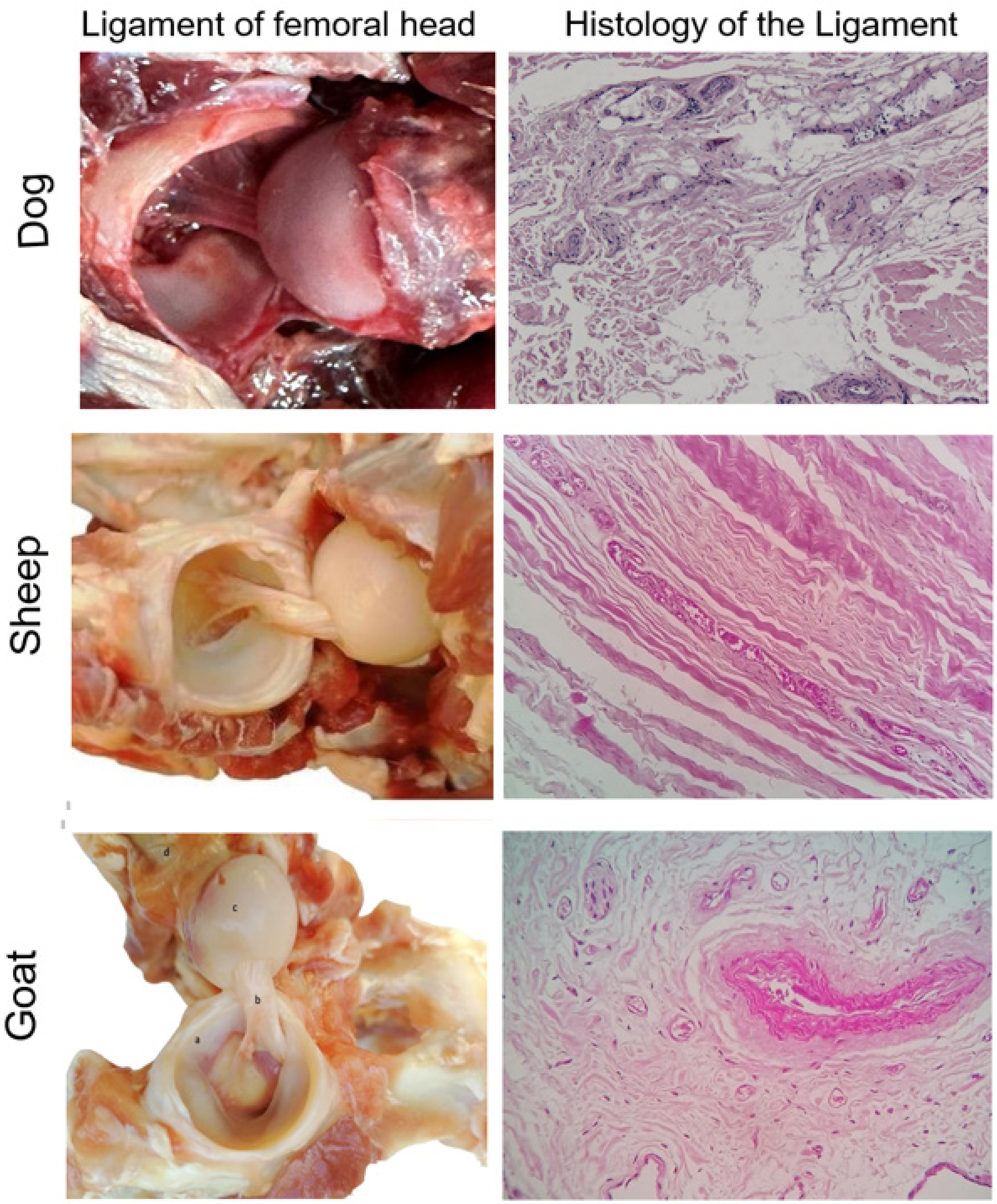

Figure 1: Morphological view of the femoral head ligament of the dog, sheep and goat together with a representative histological section of the ligament.

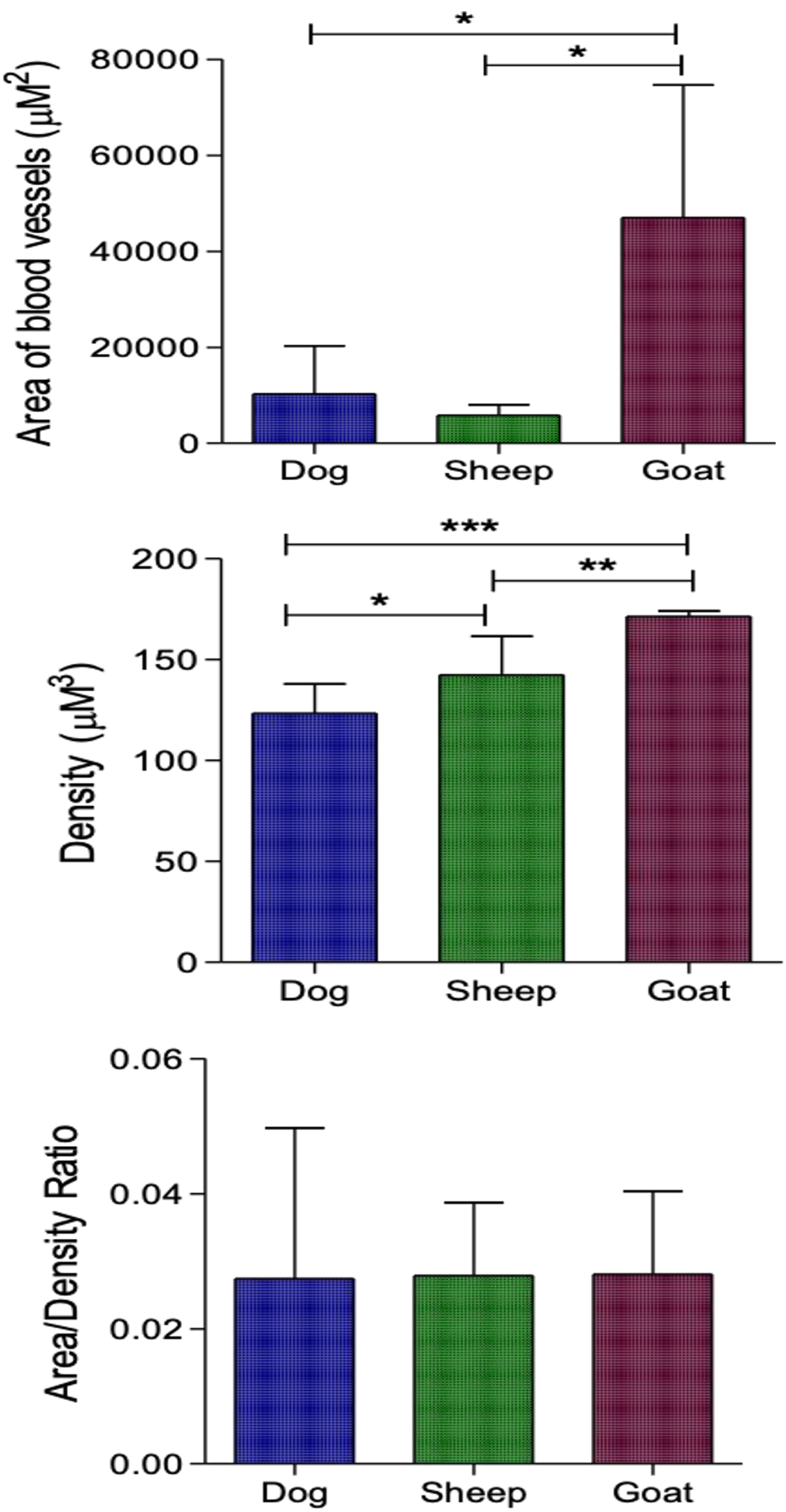

Figure 2: Graph shows the blood vessel area, density and area/density ratio in the dog, sheep and goat. Data is presented as Mean±Standard error of the mean (SEM, n=1-3). * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

reach the femoral head.[8,9,28] Although the primary blood supply to the femoral head is typically attributed to retinacular vessels arising from the femoral circumflex system, the contribution of the ligament becomes particularly important under conditions where these vessels are compromised.[4,5] Disruption of femoral head perfusion has significant clinical consequences, including decreased bone density, which is radiographically evident as increased radiolucency, and may predispose to proximal femoral fractures, delayed union or non-union, secondary osteoarthritis, and avascular necrosis.[16,29] These pathological processes highlight the potential functional importance of even secondary vascular pathways, such as those provided by the ligament. The clinical relevance of ligamentous vascularity is further underscored in conditions such as avascular necrosis and Legg-Calvé-Perthes disease.[30,31] These disorders, documented in humans, dogs, and other mammals, involve temporary or permanent loss of blood supply to the femoral head, leading to osteonecrosis and subsequent degenerative joint changes. Disease progression is commonly classified using the Ficat-Arlet system, which describes stages ranging from asymptomatic vascular compromise to advanced structural collapse.[32,33] In veterinary contexts, similar pathological processes have been observed in bovines, where compromised joint capsule integrity and growth plate damage may result in the ligament of the femoral head becoming the sole remaining vascular pathway.[34,35] Surgical interventions, such as femoral head ostectomy, aim to remove necrotic tissue while preserving surrounding structures, including the ligament, thereby emphasising its potential importance in maintaining residual vascular supply.Despite the recognised anatomical presence of vascular structures within the ligament, quantitative assessment of its blood-carrying capacity has remained limited.[8] The current study sought to address this gap by investigating whether a relationship exists between ligament length and vascular characteristics. The results demonstrate that no direct correlation exists between ligament length and the size of blood vessels within the ligament. This finding suggests that ligament morphology alone is not a reliable predictor of vascular capacity. Although a tendency was observed in canine specimens for shorter ligaments to exhibit larger vessel areas, this pattern was inconsistent. Notably, in a canine specimen where both left and right ligaments of equal length were examined, substantial differences in vessel area were identified, with one ligament exhibiting vessels approximately 5000 μm² larger than the contralateral side. This intra-individual variation further supports the conclusion that factors other than ligament length influence vascular architecture. Interspecies comparisons revealed notable differences in vascular characteristics. While sheep and goats exhibited similar ligament lengths (approximately 20-22 mm), both exceeding the average canine ligament length (approximately 17 mm), and their vascular profiles differed markedly. Goats demonstrated significantly larger blood vessel areas and higher vascular density compared to both dogs and sheep. In contrast, sheep exhibited the smallest vessel areas and comparatively lower vascular density. These findings suggest that vascularisation of the ligament is not directly proportional to its length and may instead reflect species-specific physiological or biomechanical adaptations.[28,36]

The increased vascular density observed in the caprine ligament may indicate an enhanced capacity for blood supply to the femoral head. This could be associated with differences in locomotor activity, weight-bearing patterns, or metabolic demand, although further investigation is required to substantiate these hypotheses. Conversely, the relatively lower vascular density observed in sheep may suggest a reduced reliance on ligamentous vascular supply, potentially compensated for by more robust retinacular circulation.[37,38] The canine data, which fall intermediate between these two species, may reflect a balance between these vascular contributions. Interestingly, despite significant differences in both vessel area and vascular density among species, the ratio between these parameters was not significantly different. This suggests that a proportional relationship between vessel size and number may be conserved, potentially reflecting an underlying structural or physiological constraint in ligament vascular organisation. Such conservation may indicate that while absolute vascular capacity varies, the efficiency of vascular distribution within the ligament remains relatively consistent across species.

The absence of a correlation between ligament length and vascular parameters has important implications. It indicates that gross anatomical measurements alone are insufficient to infer functional vascular capacity, emphasising the need for histological and quantitative analyses when evaluating ligament function. This is particularly relevant in clinical and surgical contexts, where preservation of vascular supply is critical.[39,40] Understanding the variability in ligament vascularity may inform surgical approaches aimed at minimising disruption to femoral head perfusion. Several limitations of this study should be acknowledged. The sample size was small, which may limit the generalisability of the findings. Additionally, variation in sampling (e.g., unilateral versus bilateral ligaments) and potential artefacts introduced during fixation and processing may have influenced measurements. Future studies should incorporate larger, more diverse sample populations and consider advanced imaging or automated quantification techniques, to improve measurement accuracy and reproducibility.

CONCLUSION

In conclusion, this study demonstrates that while the ligament of the head of the femur is consistently present across species and contributes to femoral head vascularity, its blood-carrying capacity varies significantly between species and is not directly related to ligament length. The highest vascularity observed in goats aligns with their physiological activities, while the lowest vascularity observed in sheep reflects their dependence on retinacular perfusion, with an intermediate feature in the dog (having dual functional role in joint stabilisation and vascular support). The notably higher vascular density observed in goats suggests a greater potential contribution to femoral head perfusion in this species. These findings enhance current understanding of ligament function and highlight the importance of vascular density as a key parameter in evaluating ligament physiology. Further research in this area may contribute to improved understanding of hip joint pathologies and support the development of more effective treatments for conditions such as osteonecrosis and degenerative joint disease.

ABBREVIATIONS

ANOVA: Analysis of Variance; **AREC:** Animal Research Ethics Committee; **DNA:** Deoxyribonucleic Acid; **H&E:** Haematoxylin and Eosin; **O_2:** Oxygen; **RNA:** Ribonucleic Acid; **SEM:** Standard Error of the Mean; **UCD:** University College Dublin; **μm:** Micrometre; **$μm^2$:** Square Micrometre.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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