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GENERAL ARTICLE

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Development of the blood supply to the growth cartilage of the medial femoral condyle of foals

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Abstract

Background: Growth cartilage is found in the articular-epiphyseal cartilage complex (AECC) and the physis. It has a temporary blood supply organised as end arteries. Vascular failure is associated with osteochondrosis, but infection can also obstruct vessels. The location of bacteria was recently compared to arterial perfusion, and the results indicated that they were located in the distal tips of AECC end arteries. Systematic perfusion studies were not available for comparison to the infected physes. Further studies may improve our understanding of infections and other pathologies.

Objectives: To describe development of the blood supply to the growth cartilage of the medial femoral condyle in fetuses and foals from 228 days of gestation to 62 days old.

Study design: Ex vivo arterial perfusion study.

Methods: The left medial femoral condyle of 10 Norwegian Fjord Pony fetuses and foals (228 days of gestation to 62 days old) and one Norwegian-Swedish Coldblooded Trotter foal (10 days old) was arterially perfused with barium and underwent micro-computed tomography, qualitative and quantitative description of vessels.

Results: In the fetus, the physis was supplied by metaphyseal-origin arteries. In 1-10 day-old foals, the physis was supplied by a mixture of metaphyseal- and epiphyseal-origin arteries, and from 15 days of age by epiphyseal-origin arteries only. The number of vessels increased before it decreased in both the AECC and the physis postnatally. Vessels in the cartilage showed a monopodial branching pattern, whereas vessels in epiphyseal and metaphyseal bone showed both monopodial and dichotomous branching.

Main limitations: Foals with confirmed pathologies were not examined.

Conclusions: The blood supply to growth cartilage changed with age, including the physeal supply that changed from metaphyseal- to epiphyseal-origin arteries. The number of vessels increased before it decreased postnatally, and two different branching patterns were observed. These results may improve our understanding of growth cartilage vascular failure and osteomyelitis.

The abstract is available in Portuguese in the Supporting Information section of the online version of this article.

KEYWORDS
horse, cartilage canals, foal, micro-CT, physis, stifle

1 | INTRODUCTION

Long bones grow by endochondral ossification. A growth cartilage model forms in utero and is invaded by blood vessels that run within cartilage canals.1,2 The blood vessels are organised as end arteries,3,4 defined as an artery that is the sole blood supply to a tissue. After the primary, diaphyseal centre of ossification has formed and before the secondary centre of ossification forms, the bone end consists of cartilage and is called the chondroepiphysis. Once the secondary centre has formed, growth cartilage is found in the articular-epiphyseal cartilage complex (AECC) covering the bone end, and in the metaphyseal growth plate, or physis, between the primary and secondary centres of ossification.5 The junction between growth cartilage and bone is called the osteochondral junction or ossification front, of which there are three: the epiphyseal ossification front on the superficial and abaxial periphery of the secondary centre of ossification, and the epiphyseal-side and metaphyseal-side ossification fronts of the physis.

After birth, the blood supply to growth cartilage regresses by chondrification and incorporation into bone.5,6 Failure of growth cartilage vessels during incorporation has been associated with development of heritably predisposed, articular osteochondrosis.6-8 However, growth cartilage vessels can also fail for acquired reasons including bacterial infection, as demonstrated in pigs9 and chickens.10 In chickens, bacteria were found in the distal tips of actively ingrowing canal vessels.11 The location of bacteria in histological sections from foals that were subjected to euthanasia due to septic arthritis/osteomyelitis was recently compared with arterial perfusion studies.12 The results indicate that bacteria are located in the distal tips of AECC end arteries, as previously observed in chickens.11 Some studies of the blood supply to the physis are available,13,14 but arterial perfusion of foal physes at regular age intervals is currently lacking. It is therefore unknown if the location of bacteria12 corresponds to the distal tips of end arteries in the physis. Systematic studies could improve our understanding of infections and other pathologies.

The aim of the current study was to describe the development of the blood supply to the growth cartilage of the medial femoral condyle of fetuses and foals from 228 days of gestation to 62 days old.

2 | MATERIALS AND METHODS

The ex vivo material consisted of the left femur of one Norwegian Fjord Pony fetus, nine Norwegian Fjord Pony foals (bred for an approved experimental study of osteochondrosis8), and one clinically normal Norwegian/Swedish Coldblooded Trotter (NSCT) foal, subjected to euthanasia at the owner's request after obtaining informed consent for the foal to be used for research. Included foals were free of clinical/macroscopic evidence of joint disease, systemic infections or conditions affecting the circulation. Age, breed and sex are detailed in Table 1. The Fjord Pony foals had undergone terminal femoral arterial perfusion with barium as part of the experimental study, whereas the fetus and the NSCT foal were perfused immediately post-mortem (see below).

2.1 | Arterial barium perfusion procedure

The terminal arterial perfusion procedure is described in full elsewhere,6 and the post-mortem arterial perfusion procedure was based on Hertsch and Samy.15 A catheter was placed in the left femoral artery, and the limb was flushed with saline until the effluent ran clear. The limb was then perfused with a suspension of micronised barium in saline (25% of total blood volume), followed by barium in formalin (37.5% of blood volume) delivered using a mechanical pump. Previous studies have confirmed histologically that this method results in barium filling of the arterial side of the vasculature, before the barium lodges in the capillaries.6,7

2.2 | Samples

Arterial perfusion with formalin resulted in immediate fixation, but the left femur was harvested straight away and fixed in 4% formaldehyde for a further 48 hours. A standardised sample containing the medial femoral condyle with physeal and epiphyseal growth cartilage was

TABLE 1 Age, sex, breed and body mass of nine Fjord foals, one Fjord fetus and one Norwegian-Swedish Coldblooded Trotter foal femorally perfused with barium

Age (d)	Sex	Breed	Body mass (kg)
228[a]	Not recorded	Fjord	Not recorded
1	Female	Fjord	45
10	Male	NSCT[b]	73
15	Male	Fjord	63
17	Female	Fjord	75
28	Female	Fjord	94
35	Male	Fjord	81
42	Male	Fjord	102
48	Male	Fjord	114
57	Female	Fjord	111
62	Male	Fjord	113

[a]Length of gestation.
[b]Norwegian/Swedish Coldblooded Trotter.

obtained from all femurs (Figure 1). The samples were cuboidal and measured approximately 2.5 × 2.5 × 6.5 cm. The cubes were obtained by sawing the distal femur into medial and lateral halves using a band saw (Figure 1A). The medial half was then divided into cranial and caudal quarters, and the caudal quarter (Figure 1B) was finally separated from the femur by a transverse cut immediately proximal to the physis. In the fetal femur, only the transverse cut proximal to the physis was performed as the distal femur was small enough to be scanned intact.

2.3 | Micro-CT

The surface of each sample was wrapped in sealing film (Parafilm® M) to prevent cartilage desiccation and warping during scanning. The samples were scanned using a multiscale x-ray nano-tomograph (Skyscan 2211, Bruker Corporation) equipped with an open-type X-ray tube working at 110 kV/60 µA. The standard wolfram target was used. The samples rotated at steps of 0.31°per projection around 360°, totalling 1162 projections per sample, and images were acquired using a flat panel detector, resulting in an isotropic voxel resolution of 47 µm. The exposure time was 0.370 ms, averaging four frames for each projection. A 0.5-mm Cu filter was used to remove low energy x-ray components from the beam. For each sample, a stack of 2D images was obtained and reconstructed using commercial software (NRecon, Bruker Corporation).

2.4 | Evaluation of CT-images

The 2D images were imported into a commercial software package (VGStudio Max, version 3.2.4, Volume Graphics) for evaluation in three orthogonal planes and as 3D volume-rendered models. Larger vessels in the perichondrium and bone were visualised using the software thick-slab option with up to 100 individual 2D images viewed simultaneously. Smaller vessels in the AECC and physis were visualised using the thick-slab option with a maximum of 30 slices viewed simultaneously. Qualitative description included vessel origin, course, orientation and branching. Additionally, the number of vessels entering the AECC was quantified by manual counting. All vessels were counted in all sagittal plane and all frontal plane images, and the average of these two numbers was defined as the total vessel number of the sample. Finally, the maximum medio-lateral width and cranio-caudal length of the physis included in the scanned sample blocks were measured using Vernier callipers. Width was multiplied by length to generate a measure of physeal area. The total sample vessel number was divided by physeal area to produce a relative vessel/physeal area ratio.

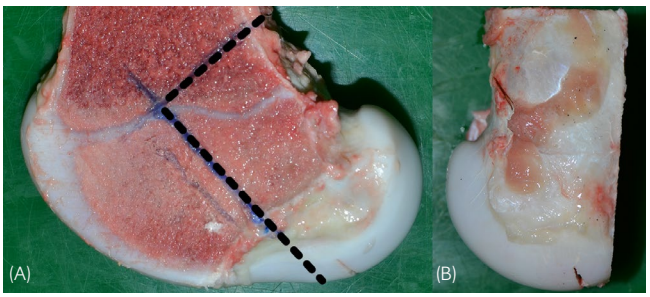

FIGURE 1 Preparation of samples for micro-CT-scanning. A, Axial view of the medial half of femur from a 10-d-old foal. Frontal and transverse cuts were made along stippled lines. B, Medial view. The cuts in (A) resulted in a cuboidal sample of the medial femoral condyle with physeal and epiphyseal growth cartilage for micro-CT-scanning

3 | RESULTS

The qualitative results were described first, but vessels were also quantified as described below and summarised in Table 2. The examined AECCs and physes did not contain any focal lesions in micro-CT scans.

3.1 | Fetus

In the fetal femur, the primary, diaphyseal ossification centre was present, but the secondary, epiphyseal ossification centre had not yet formed (Figure 2A). The fetal femur therefore contained only the metaphyseal ossification front (Figure 2B). The diaphyseal bone contained a dense vasculature towards the physis. Most branches terminated on the bone-side of the ossification front, but some branches traversed the ossification front and entered the chondroepiphysis. The location of the future physis was therefore supplied by vessels originating from metaphyseal bone, travelling perpendicular relative to the metaphyseal ossification front, from deep to superficial within the chondroepiphysis and towards the articular surface (Figure 2B).

The distal femoral epiphysis was supplied by four main arterial sources: one midline artery located at the cranio-proximal aspect of the trochlear groove, one midline artery located caudo-distally in the intercondylar fossa and a medial and lateral abaxial perichondral supply comprising more than one small artery on each side respectively. Two of these were relevant to the micro-CT-scanned blocks: the caudo-distal midline artery, which constituted the main nutrient artery to the epiphysis, and the perichondral supply on the medial abaxial aspect of the femur (Figure 2B).

The superficial part of the chondroepiphysis, including the site of the future secondary centre of ossification and AECC, was supplied by branches of the epiphyseal nutrient artery and branches of medial perichondral arteries.

3.2 | One- and 10-day-old foals

The 1- and 10-day-old foals were described together because the only difference between them was that the femur was larger and

TABLE 2 Number of vessels[a] entering the AECC[b] and the physis of the distal femur in nine Norwegian Fjord Pony foals and one Norwegian-Swedish Coldblooded Trotter aged 1-62 d

Age (d)	Vessels entering AECC	Vessels entering physis from epiphysis	Vessels entering physis from metaphysis	Physis area (cm^2)	Vessels AECC/ physis area	Vessels physis[c]/ physis area
1	145	72	68	8.6	16.9	16.3
10[d]	196	192	7	10.0	19.6	19.9
15	218	207	0	11.7	18.6	17.7
17	297	284	0	11.0	27.0	25.8
28	313	228	0	8.9	35.2	25.6
35	265	217	0	8.4	31.5	25.8
42	298	288	0	9.6	31.0	30.0
48	321	223	0	9.4	34.1	23.7
57	163	188	0	9.4	17.3	20.0
62	172	188	0	10.9	15.8	17.2

[a]Average of number of vessels identified in sagittal and frontal planes.

[b]Articular-epiphyseal cartilage complex.

[c]Sum of vessels entering from the epiphysis and metaphysis.

[d]Norwegian-Swedish Coldblooded Trotter.

contained more vessels in the 10-day-old foal than the 1-day-old foal. The principal difference between the fetus and the 1-and 10-day-old foals was that the secondary centre of ossification had formed (Figure 3A). The foal blocks therefore contained three ossification fronts: the epiphyseal ossification front on the superficial and abaxial periphery of the secondary centre of ossification, and the epiphyseal-side and metaphyseal-side ossification fronts of the physis (Figure 3A). The arterial sources and general configuration of the blood supply to the medial femoral condyle was otherwise similar to that described for the fetus, above. The secondary centre of ossification and superficial part of the epiphysis were supplied by the epiphyseal nutrient artery centrally, and by perichondral arteries peripherally. Perichondral vessels entered at 2-3 different levels from superficial to deep along the abaxial aspect of the epiphysis. Initially, all vessels coursed towards the centre of the epiphysis. After a short distance, vessels that entered superficially turned and coursed towards the superficial aspect of the epiphysis and the AECC. Likewise, vessels entering deeper on the abaxial side coursed towards the deep aspect of the epiphysis and the physis. As they followed this course, a small number of the superficial vessels coursed entirely within growth cartilage. However, the majority of the perichondral vessels coursed partly within bone as the mid-portion of the vessel was surrounded by the ossification front on both the superficial and deep sides of the secondary centre of ossification.

Vessels underwent two distinct patterns of branching. Dichotomous branching was confined to portions of arteries located within bone. This category was characterised by high numbers of small branches close together from a single or a few points along the trunk of a vessel, diverging outwards with a morphology resembling a tree crown (Figure 3B). Dichotomous branching was frequent towards the metaphyseal-side of the physis, less frequent towards the epiphyseal ossification front and did not occur towards the epiphysealside of the physis. Within bone and cartilage, vessels also underwent a monopodial branching pattern that was characterised by single branches spread out along the length of vessel trunks (Figure 3C).

Vessels entered growth cartilage via all three ossification fronts. Some vessels entering the AECC or physis from epiphyseal bone turned 90°, before continuing to course perpendicular relative to the underlying ossification front once within growth cartilage. Vessels entering the physis from metaphyseal bone did not turn, but rather continued to course straight and perpendicular to the ossification front within growth cartilage. Vessels entering the physis from the metaphyseal side typically penetrated up to 50% of the total thickness of the physis, whereas vessels entering from the epiphyseal side often extended through the entire physis, without entering metaphyseal bone. Monopodial branching in the cartilage occurred at regular intervals in epiphyseal-side vessels, but was rare in metaphyseal-side vessels. In contrast to the fetus, the physes of the 1- and 10-day-old foals had a dual supply consisting of vessels entering from both the metaphyseal and the epiphyseal sides of the physis (Figure 3D).

3.3 | 15-day-old and older foals

The arterial sources and configuration of the blood supply in the older foals were similar to that described for the fetus and younger foals, above. Subjectively, there was more extensive dichotomous branching within the metaphyseal and epiphyseal bone of foals ≥15 days than in the 1- and 10-day old foals (compare Figures 3A and 4A,B). The number and length of vessels within the AECC and physis were also reduced in the older, compared to the younger foals (see below). The frequency of monopodial branching within the physis

was lower, markedly within the axial portion of the physis. However, the most pronounced difference was a complete absence of vessels entering the physis from the metaphyseal side in foals from 15 days onwards (Table 2). From the age of 15 days, the physis therefore had a single blood supply, originating from the epiphyseal side of the physis and coursing from superficial to deep within the physis only (Figure 4B).

3.4 | Quantitative results

The absolute and relative numbers of vessels entering the AECC and the physis from different sides are presented in Table 2. The quantitative results supported the qualitative observations, above. In the 1-day-old foal, 72 (50.3%) vessels entering the physis originated from the epiphysis and 68 (49.7%) vessels originated from the metaphysis (Figure 3D). In the 10-day-old foal, only seven (3.5%) vessels entered the physis from the metaphysis, all in the abaxial region, while 192 (96.5%) entered from the epiphysis.

Both the absolute and relative number of vessels followed the same development over time in the AECC and the physis. The youngest foal had the lowest number of vessels. The numbers increased with increasing age in the next three foals before reaching a plateau stretching from the age of 17-48 days in the AECC, and from 17 to 42 days in the physis. The two oldest foals, aged 57 and 62 days, had a reduced number of vessels compared to the younger animals.

4 | DISCUSSION

In the micro-CT scans, all vessels appeared to be end arteries, corroborated by previous studies where intravascular AECC contrast columns corresponded to end arteries with stereomicroscopy.4,16 The configuration of the blood supply to the physis changed from metaphyseal-origin vessels in the fetus, via a mixture of metaphyseal- and epiphyseal-origin vessels in the 1- and 10-day-old foals, to epiphyseal-origin vessels only in ≥15 day-old foals. The observation that the blood supply to the physis goes through phases with different configuration is helpful because it unifies historical studies by suggesting that whether vessels come from one side or the other depends on maturation of the examined physis.13,14

The number of cartilage canal vessels changed with age. Several investigators have quantified vessels by slightly different methods, but all agree that the number of vessels decreases with increasing age.7,17,18 It was somewhat surprising that the number of vessels increased before it decreased during the post natal period, as this was not noticed during previous perfusion of 0- to 7-week-old foals.4,6,19 In hocks6 and fetlocks,19 vessels were few enough to count and only declined in the post natal period (unpublished data). In femurs,4 the focus was on the loss of vascularity, thus any increase before loss was missed. We were familiar with the linear decline in the vascular index of Ytrehus et al,7 but pigs were examined too late to detect any early vessel increase

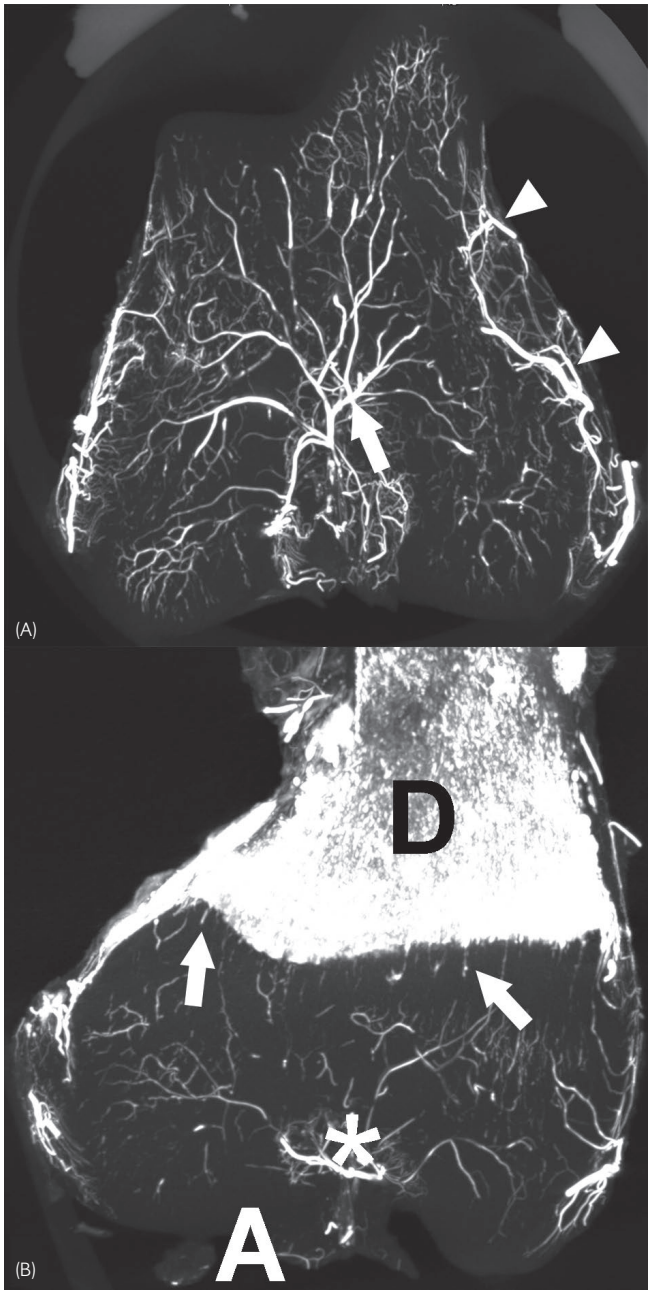

FIGURE 2 Micro-CT of barium-perfused distal femur of Fjord fetus at 228 d gestation length, voxel size 47 µm. A, Disto-proximal view, medial to the right. The origin of a large branch of the central nutrient artery (arrow) entering the medial condyle from the axial aspect is visible. Large vessels (arrowheads) originating in the perichondrium can be seen entering the medial condyle on the medial side. Thick-slab mode, 200 slices. B, Frontal view, medial to the right, D, diaphysis; A, articular surface. Branching of vessels from central nutrient artery (asterisk) and perichondral arteries towards the diaphysis and the articular surface are visible. Small vessels (arrows) are also visible entering the chondroepiphysis from the diaphysis where the primary ossification centre subsequently forms. Thick-slab mode, 100 slices

(7-15 weeks old). We believe the observed increase was genuine, and made detectable through a combination of methodology, region and age. There are at least two possible interpretations: that

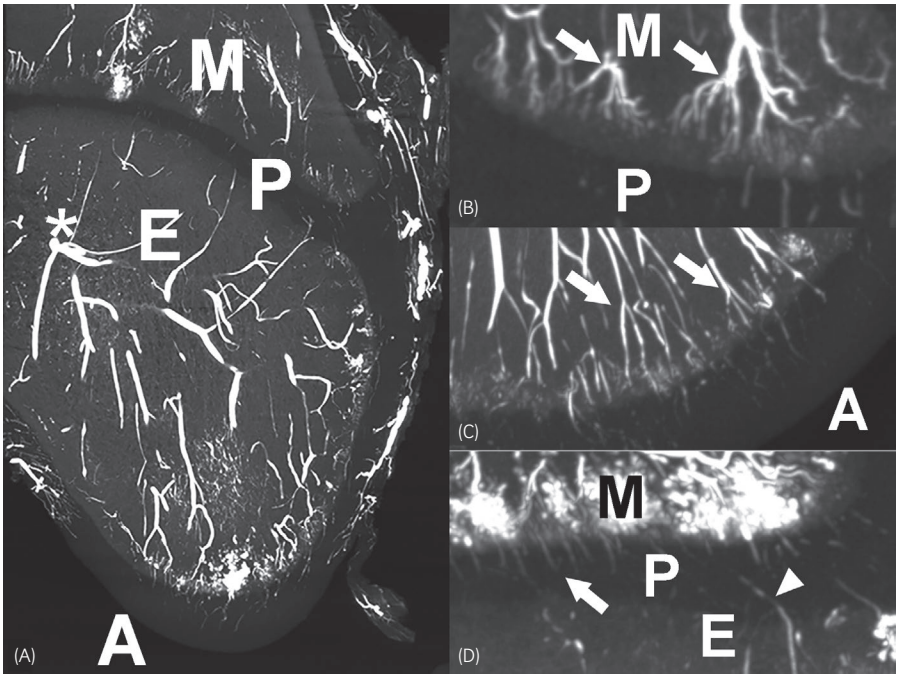

FIGURE 3 Micro-CT of barium-perfused medial femoral condyle from 1-d-old Fjord foal, voxel size 47 µm. M, metaphysis; P, physis; E, epiphysis; A, articular surface. A, Frontal view, medial to the right. Large vessels from the central nutrient artery (asterisk) are visible branching towards the physis and the articular epiphyseal cartilage complex (AECC). The secondary ossification centre has formed and three ossification fronts can be seen: the metaphyseal side (M) of the physis (P), the epiphyseal side (E) of the physis and the epiphyseal front, towards the articular surface (A). Thick-slab mode, 50 slices. B, Frontal view, metaphysis, medial to the right. Dichotomous branching of vessels is visible towards the physis (arrows). Thick-slab mode, 30 slices. C, Sagittal view, epiphysis, caudal to the right. Monopodial branching of vessels (arrows) is visible towards the AECC. Thick-slab mode, 50 slices. D, Frontal view, medial aspect of physis. Vessels entering the physis are of both metaphyseal (arrow) and epiphyseal (arrowhead) origin. Thick-slab mode, 50 slices

the vessel ingrowth that starts in utero continues in the post natal period in the distal femur (but not the hock or fetlock), that is, the increased number represents the last phase of an approximately linear decline. Alternatively, the number of vessels may fluctuate, ie increase and decrease, during the net decline. Ultimately, the numbers represent biological processes and both interpretations may be true, but they potentially explain different things. In chickens, it has been documented that vessels are discontinuous during ingrowth, and bacteria may be able to bind to cartilage matrix via such discontinuities.11 The first interpretation; active ingrowth in the post natal period could then explain increased susceptibility to infection in distal femoral cartilage canals. Vessel number has been closely linked with cartilage thickness.6,18,20 Following vascular failure, only cartilage outside diffusion distance form alternative sources undergoes ischaemic chondronecrosis.8,21 If the second interpretation is right and vessel number and cartilage thickness fluctuate, this could additionally explain periodic increase in the likelihood of vascular failure resulting in ischaemic chondronecrosis. Upper age thresholds for the development of new lesions are recognised.22,23 Longitudinal monitoring of pigs has revealed that incidence curves fluctuate up to the age thresholds.24 Fluctuation in vessel number and cartilage thickness could be at least part of the explanation for fluctuating incidence curves. Fluctuations involving ingrowth could potentially also explain early and late waves of susceptibility to orthopaedic infections in foals.25,26 Further investigation of whether increased vessel number in the post natal period represents the tail end of an approximately linear decline, fluctuations during net decline, or both, may be warranted as it could improve our understanding of temporal variations in susceptibility to disease.

In this study, the configuration of end arteries in both the AECC and the physis was described. The location of bacteria was previously recorded in 9- to 117-day-old foals subjected to euthanasia due to septic arthritis/osteomyelitis.12 Bacteria were located in necrotic cartilage canal remnants superficially within the AECC, and canals contained a consistent sequence of changes between the bacteria and the ossification front in all lesions.12 Superimposition of histological sections on perfusion images confirmed that the bacteria were located in the distal tips of AECC end arteries.12 In the physis, bacteria were located near the metaphyseal-side ossification front and canals contained the same sequence of changes towards the epiphyseal-side front as in the AECC.12 This corresponds to the distal tips of end arteries coursing from the epiphysis, towards the metaphysis,that is, one of the two configurations observed in the 1- and 10-day-old foals, and the only configuration observed in ≥15-day-old foals. Comparison of bacterial location and the current observed vessel configuration in the physis therefore agrees with the previous comparison in the AECC of foals, and the tibia of chickens.

Two patterns of monopodial branching within growth cartilage and bone, and dichotomous branching within bone were observed.

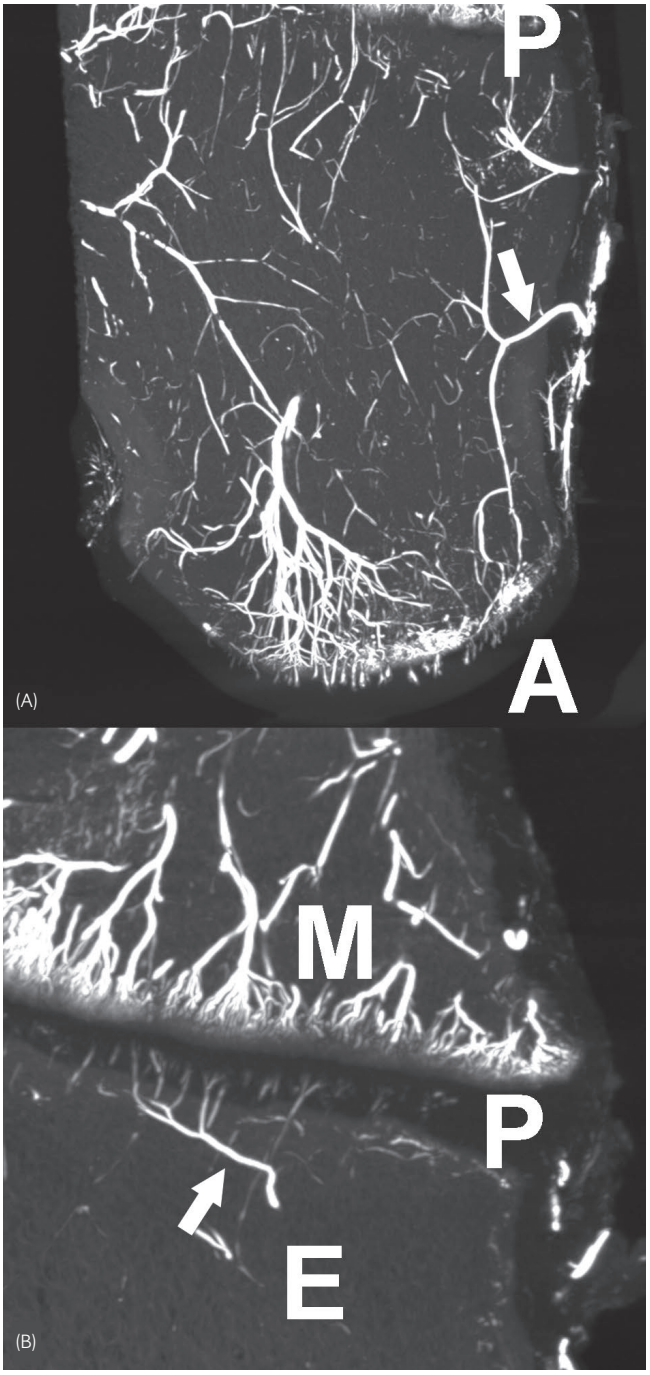

FIGURE 4 Micro-CT of barium-perfused medial femoral condyle from Fjord foal aged 62 d, voxel size 47 µm. A, Frontal view, medial to the right. P, physis; A, articular surface. A large vessel (arrow) from the perichondrium branches towards both physis and articular-epiphyseal cartilage complex (AECC). There is dichotomous branching towards the AECC and monopodial branching towards the physis. Thick-slab mode, 200 slices. B, Sagittal view, caudal to the right. A vessel (arrow) courses parallel with the physis (P) in the epiphysis (E) with monopodial branches entering the physis at a perpendicular angle and extending 2/3 of the physeal depth. In the metaphysis (M), there is extensive dichotomous branching towards, but no vessels enter the physis. Thick-slab mode, 50 slices

In foals with septic arthritis/osteomyelitis, bacteria were present in subchondral bone adjacent to some lesions.12 Osteomyelitis lesions are often found immediately deep into the osteochondral junction, and it has been suggested that this is due to slow flow in sinusoidal vessels.25 A portion of the currently observed dichotomously branching vessels in the metaphysis represented branches of the diaphyseal nutrient artery and appeared to have been there for some time. Conversely, the central part of epiphyseal bone was supplied by the nutrient artery, whereas the peripheral part was supplied by vessels that were incorporated into bone from cartilage.6,7 It is theoretically possible that some of the epiphyseal dichotomously branching vessels represent vessels recently incorporated from cartilage. As vessels branched monopodially in cartilage, this would imply that the dichotomous pattern represents newly formed branches. Dichotomous branching was also more extensive in both epiphyseal and metaphyseal bone of ≥15-day-old foals than younger foals, further suggesting active branching. It would be interesting to discover whether vessels are discontinuous during branching, in which case they may be susceptible to bacterial binding in a similar way to actively ingrowing canal vessels.11 As AECC vessels are heterogeneously distributed within cartilage,4,6,19 they will be heterogeneously incorporated into bone and this would then explain why early osteomyelitis lesions are focal, as opposed to the generally distributed ones.12,25 The dichotomous branching corresponds to the pattern described as sinusoidal filling,13 thus all agree that this vessel morphology may be particularly vulnerable to infection. We suggest that, in addition to slow flow, this morphology may be vulnerable if it represents newly formed, discontinuous branches that allow bacterial binding to matrix.

4.1 | Limitations

This study did not include foals with confirmed infections. In infected foals, one could not be sure whether contrast columns were interrupted due to technical error or disease, but this could be solved by histological validation.12 It was already confirmed that contrast columns correspond to end arteries in the AECC of the distal femur.4 With the exception of the fetus and the NSCT foal, success of the barium perfusion procedure was validated histologically during the original Fjord Pony study.27 On comparison to Firth and Poulos,13 it was considered that interrupted contrast columns in the youngest foals represented a mixture of end arteries and of column diameter dropping below scan resolution (partial volume effect). However, thin, transphyseal branches disappeared from the distal radial and distal metacarpal physes with age,13 thus in older foals, physeal contrast columns should represent only end arteries. Computed tomography is ideally suited for auto-quantification, but micronised barium in capillaries overlaps in Hounsfield units with thick trabeculae, thus samples must be decalcified before quantification. Observers have tended to

improve with practice, but bias was countered by reading scans in random order. The method does not show endothelial discontinuities. Electron microscopy shows fenestrations,28 but is not suitable for large-scale mapping. It is currently possible to purchase specific bacterial-sized particles that are observed with advanced imaging, and in future, it would be interesting to use this to map both constitutive and disease-induced discontinuities in foals.

4.2 | Conclusion

The blood supply changed with age, including that the physeal supply changed from metaphyseal- to epiphyseal-origin arteries. The number of vessels increased before it decreased post natally, and two different branching patterns were observed. These results may improve our understanding of growth cartilage vascular failure and osteomyelitis.

ETHICAL ANIMAL RESEARCH

The fetus and the nine Fjord Pony foals were from a previous study, approved by the Norwegian National Research Authority (approval number 2008-783).

OWNER INFORMED CONSENT

The Norwegian/Swedish Coldblooded Trotter foal was subjected to euthanasia at the owner's request after obtaining informed consent for the foal to be used for research.

DATA ACCESSIBILITY STATEMENT

Raw data are available from the corresponding author upon reasonable request.

ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

All authors contributed to the design of the study, acquisition and interpretation of the data. B.H. Wormstrand drafted the manuscript, and all authors critically revised and approved the final version. B.H. Wormstrand is the responsible author.

CONFLICT OF INTEREST

No competing interests have been declared.

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REFERENCES

1. Ytrehus B, Carlson CS, Ekman S. Etiology and pathogenesis of osteochondrosis. Vet Pathol. 2007;44:429–48.
2. Olstad K, Ekman S, Carlson CS. An update on the pathogenesis of osteochondrosis. Vet Pathol. 2015;52:785–802.
3. Hayashi K. Three-dimensional organization of the cartilage canal–a scanning electron-microscopic study by vascular cast of the rabbit's femoral head. Nippon Seikeigeka Gakkai Zasshi. 1992;66:548–59.
4. Olstad K, Ytrehus B, Ekman S, Carlson CS, Dolvik NI. Epiphyseal cartilage canal blood supply to the distal femur of foals. Equine Vet J. 2008;40:433–9.
5. Carlson CS, Cullins LD, Meuten DJ. Osteochondrosis of the articular-epiphyseal cartilage complex in young horses: evidence for a defect in cartilage canal blood supply. Vet Pathol. 1995;32:641–7.
6. Olstad K, Ytrehus B, Ekman S, Carlson CS, Dolvik NI. Epiphyseal cartilage canal blood supply to the tarsus of foals and relationship to osteochondrosis. Equine Vet J. 2008;40:30–9.
7. Ytrehus B, Carlson CS, Lundeheim N, Mathisen L, Reinholt FP, Teige J, et al. Vascularisation and osteochondrosis of the epiphyseal growth cartilage of the distal femur in pigs–development with age, growth rate, weight and joint shape. Bone. 2004;34:454–65.
8. Olstad K, Hendrickson EHS, Carlson CS, Ekman S, Dolvik NI. Transection of vessels in epiphyseal cartilage canals leads to osteochondrosis and osteochondrosis dissecans in the femoro-patellar joint of foals; a potential model of juvenile osteochondritis dissecans. Osteoarthr Cartil. 2013;21:730–8.
9. Denecke R, Trautwein G, Kaup FJ. The role of cartilage canals in the pathogenesis of experimentally induced polyarthritis. Rheumatol Int. 1986;6:239–43.
10. Emslie KR, Nade S. Acute hematogenous staphylococcal osteomyelitis. A description of the natural history in an avian model. Am J Pathol. 1983;110:333–45.
11. Speers DJ, Nade SM. Ultrastructural studies of adherence of Staphylococcus aureus in experimental acute hematogenous osteomyelitis. Infect Immun. 1985;49:443–6.
12. Wormstrand B, Ostevik L, Ekman S, Olstad K. Septic arthritis/osteomyelitis may lead to osteochondrosis-like lesions in foals. Vet Pathol. 2018;55:693–702.
13. Firth EC, Poulos PW. Blood vessels in the developing growth plate of the equine distal radius and metacarpus. Res Vet Sci. 1982;33:159–66.
14. Firth EC, Poulos PW. Microangiographic studies of metaphyseal vessels in young foals. Res Vet Sci. 1983;34:231–5.
15. Hertsch B, Samy MT. Arteriographic studies of the distal tibial end in relation to the pathogenesis of osteochondrosis dissecans in the horse. Zentralbl Veterinarmed A. 1980;27:469–78.
16. Olstad K, Cnudde V, Masschaele B, Thomassen R, Dolvik NI. Micro-computed tomography of early lesions of osteochondrosis in the tarsus of foals. Bone. 2008;43:574–83.
17. Shingleton WD, Mackie EJ, Cawston TE, Jeffcott LB. Cartilage canals in equine articular/epiphyseal growth cartilage and a possible association with dyschondroplasia. Equine Vet J. 1997;29:360–4.
18. Lecocq M, Girard CA, Fogarty U, Beauchamp G, Richard H, Laverty S. Cartilage matrix changes in the developing epiphysis: early events on the pathway to equine osteochondrosis? Equine Vet J. 2008;40:442–54.
19. Olstad K, Ytrehus B, Ekman S, Carlson CS, Dolvik NI. Epiphyseal cartilage canal blood supply to the metatarso-phalangeal joint of foals. Equine Vet J. 2009;41:865–71.
20. Hendrickson EHS, Olstad K, Nødtvedt A, Pauwels E, van Hoorebeke L, Dolvik NI. Comparison of the blood supply to the articular-epiphyseal growth complex in horse vs. pony foals. Equine Vet J. 2015;47:326–32.
21. Carlson CS, Meuten DJ, Richardson DC. Ischemic necrosis of cartilage in spontaneous and experimental lesions of osteochondrosis. J Orthop Res. 1991;9:317–29.
22. Dik KJ, Enzerink E, van Weeren PR. Radiographic development of osteochondral abnormalities in the hock and stifle of

Dutch Warmblood foals, from age 1 to 11 months. Equine Vet J. 1999;31(Suppl 31):9–15.
23. Carlsten J, Sandgren B, Dalin G. Development of osteochondrosis in the tarsocrural joint and osteochondral fragments in the fetlock joints of Standardbred trotters. I. A radiological survey. Equine Vet J. 1993;25(Suppl 16):42–7.
24. Olstad K, Kongsro J, Grindflek E, Dolvik NI. Consequences of the natural course of articular osteochondrosis in pigs for the suitability of computed tomography as a screening tool. BMC Vet Res. 2014;10(1):212.
25. Firth EC, Goedegebuure SA. The site of focal osteomyelitis lesions in foals. Vet Q. 1988;10:99–108.
26. Neil KM, Axon JE, Begg AP, Todhunter PG, Adams PL, Fine AE, et al. Retrospective study of 108 foals with septic osteomyelitis. Aust Vet J. 2010;88:4–12.
27. Olstad K, Hendrickson EHS, Ekman S, Carlson CS, Dolvik NI. Local morphological response of the distal femoral articular-epiphyseal cartilage complex of young foals to surgical stab incision, and potential relevance to cartilage injury and repair in children. Cartilage. 2013;4:239–48.
28. Hellings IR, Ekman S, Hultenby K, Dolvik NI, Olstad K. Discontinuities in the endothelium of epiphyseal cartilage canals and relevance to joint disease in foals. J Anat. 2016;228:162–75.

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