Cartilage Canals in the Pathogenesis of Osteochondrosis in Horses

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Thesis for the degree of *Philosophiae Doctor*

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Oslo, 2008

Til Mamma

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Equine vet J. (2007)

Available online September 5th 2007, DOI: 10.2746/042516407X239836

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Olstad, K., Cnudde, V., Masschaele, B., Thomassen, R. and Dolvik, N.I.

[Submitted to *Bone*, September 2007]

Summary in English

Osteochondrosis (OC) is a common disease that arises in the developing joints of people, horses, pigs, dogs and other species. OC is defined as a disturbance in enchondral ossification, and in horses, the disturbance is due to primary disease of growth cartilage. At the epiphyseal growth cartilage, the disturbance tends to occur at specific predilection sites, and can lead to the formation of partially or completely detached fragments, or subchondral bone cysts, at these sites. The fragments consist of cartilage with or without bone. Whereas articular cartilage is avascular, growth cartilage has a rich blood supply during the early phases of development through so-called cartilage canals. Cartilage canals have been implicated in the pathogenesis of OC in pigs and horses.

Predilection sites for OC in the hock and stifle were examined in the microscope before OCD or cysts were visible. The earliest step in the pathogenesis of OC was a focal failure of the blood supply to growth cartilage. This resulted in necrosis of those chondrocytes that were dependent on the blood supply. Conversion of cartilage to bone was delayed in areas of chondronecrosis. Such areas therefore constituted a disturbance in enchondral ossification, as is characteristic of clinical OC.

The blood supply to growth cartilage in the hock and stifle was examined macroscopically. Cartilage canals became incorporated into subchondral bone during growth. Vessels in the distal portion of cartilage canals shifted from a perichondrial to a subchondral arterial source. The subchondral source was obliged to cross the ossification front in order to enter cartilage canals. Early lesions of OC were consistently found in the region where vessels crossed from bone into cartilage, prompting the conclusion that vessels were particularly vulnerable in this region.

Early lesions of OC in the hock were examined using micro-computed tomography combined with angiography and histology, enabling three-dimensional visualisation of lesions. No blood vessels were seen to exit from the ossification front deep to lesions of OC, confirming that the blood supply had been interrupted where it crossed from bone into cartilage. Indented defects in the subchondral bone plate corresponded to areas of ischaemic chondronecrosis on histological examination, and confirmed that these were capable of causing a disturbance in enchondral ossification.

We conclude that ischaemic chondronecrosis causes disturbed enchondral ossification (OC) in horses, and may result in OCD or cysts. Micro-computed tomography observations may be extrapolated to conventional computed tomography and improve understanding, early diagnosis and treatment of OC in future.

Summary in Norwegian (Sammendrag)

Osteochondrose (OC) er en vanlig sykdom som oppstår under utviklingen av ledd hos mennesker, hester, griser, hunder og andre arter. OC er definert som en forstyrrelse i den enchondrale forbeiningsprosessen, og hos hester oppstår denne forstyrrelsen som en følge av patologiske forandringer i vekstbrusken. I den epifyseale vekstbrusken oppstår forstyrrelsen på spesifikke, såkalte predileksjonssteder, og kan føre til helt eller delvis løse fragmenter (osteochondrosis dissecans, OCD), eller subchondrale bencyster, på disse stedene i leddet. Fragmentene består av brusk med eller uten benvev. Leddbrusk er avaskulær, mens vekstbrusk har en rikelig blodforsyning under de tidlige fasene av vekst gjennom såkalte karkanaler. Karkanaler har vært assosiert med patogenesen for OC hos griser og hester.

Predileksjonssteder for OC i hasen og kneleddet ble undersøkt i mikroskopet før OCD eller cyster var synlige. Det første steget i patogenesen for OC var en fokal svikt i blodtilførselen til vekstbrusken. Dette førte til nekrose av de chondrocyttene som var avhengige av denne blodtilførselen. Omdannelsen av vekstbrusk til beinvev var forsinket i områder med chondronekrose. Slike områder utgjorde dermed en forstyrrelse i enchondral forbeining, slik som er karakteristisk for klinisk OC.

Blodtilførselen til vekstbrusken i hasen og kneleddet ble undersøkt makroskopisk. Karkanalene ble omslutte av beinvev underveis i knokkelveksten. Blodårer i den distale delen av karkanalen skiftet fra en perichondral til en subchondral arteriell kilde. Den subchondrale kilden måtte krysse ossifikasjonsfronten for å entre karkanalen. Tidlige OC-lesjoner ble utelukkende funnet i den regionen der blodårene krysset fra bein til brusk. Blodårene var derfor tilsynelatende spesielt sårbare i denne regionen.

Tidlige OC-lesjoner i hasen ble undersøkt med mikro-computer tomografi kombinert med angiografi og histologi, noe som muliggjorde tredimensjonal visualisering av lesjonene. Det kom ingen blodårer ut gjennom ossifikasjonsfronten som lå direkte under OC-lesjonene, noe som bekreftet at blodtilførselen var avbrutt der den krysset fra bein til brusk. Konkaviteter i den subchondrale beinplata svarte til områder med ischemisk chondronekrose ved histologisk undersøkelse, og bekreftet at disse var i stand til å forårsake en forstyrrelse i den enchondrale forbeiningsprosessen.

Vi konkluderer med at ischemisk chondronekrose forårsaker forstyrrelse i enchondral forbeining hos hester, og kan føre til OCD eller cyster. Funn ved micro-computer tomografi kan overføres til konvensjonell computer tomografi og forbedre forståelse, tidlig diagnose og behandling av OC i fremtiden.

Acknowledgements

This PhD was funded by The Research Council of Norway and the Norwegian School of Veterinary Science, and received additional contributions from The Astri and Birger Torsted Foundation and the local Research and Ethics Committee. I am grateful to Professors Nils Ivar Dolvik and Knut Røed for applying to The Research Council of Norway and thereby making this project possible.

The experimental work was carried out at the Departments of Companion Animal Clinical Sciences, and Basic Sciences & Aquatic Medicine, of the Norwegian School of Veterinary Science in Oslo. The National Veterinary Institute of Norway, Swedish University of Agricultural Sciences and University of Minnesota, USA generously donated material for the histological studies. The micro-computed tomography scans were acquired in collaboration with Ghent University in Belgium.

I would like to thank the Head of Department, Doctor Ann Margaret Grøndahl, and Head of the Equine Section, Doctor Carl Fredrik Ihler, for their ardent support of this project. I am grateful beyond words to my supervisors Professors Nils Ivar Dolvik, Stina Ekman, Jon Teige and Doctor Bjørnar Ytrehus as well as my “honorary supervisor” in the US, Professor Cathy Carlson. Their contributions to my doctoral education are so vast they are impossible to list. Cathy, Stina and Bjørnar have spent endless hours staring into the microscope with me, and their combined internationally unsurpassed knowledge of osteochondrosis in piglets has taken this PhD onto a level that would have been otherwise unattainable. I am especially grateful to the co-authors of the papers in this thesis: to Doctor Ragnar Thomassen for helping breed the foals, and to Doctors Veerle Cnudde and Bert Masschaele for micro-computed tomography scanning and tuition in the use of post-acquisition software.

I would like to extend my thanks and apologies to the poor souls that were coerced into helping cover the mares; Veterinary Nurses or Technicians Mona Lunde, Roar Brustad, Camilla Klykken, Anne Mikkelsen, Gorm Flognes, Steinar Fjellet and Jens Røhnebæk. Thank you to Human Anaesthetic Nurse Henning Mørch, for inventing the infusion catheter, and Doctor Andreas Haga, for constructive discussion of the anaesthetic protocol. The assistance of Veterinary Surgeon Maria Kjeldaas Johannesen, Veterinary Nurse Hilde

Engeland and Veterinary Student Bjørn Wormstrand was greatly appreciated during surgeries.

I am sincerely grateful to Veterinary Techicians Tore Engen, Rolf Hautau, Anita Paasche and Rolf Isachsen for their cheerful patience with collection of material, and apparently inexhaustible tolerance of the unpleasant methyl salicylate photo sessions. Radiographer Lena Stenhaug performed conventional computed tomographic scans of several of the foals and, together with Radiographer Bernadette Helmer, helped me x-ray the femurs. The histology labs at the Norwegian School of Veterinary Science and National Veterinary Institute produced thousands of excellent quality sections from the challenging bone and cartilage samples. Scientists Josh Parker and Ann Undersander at Professor Carlson's lab enthusiastically helped with collection and conventional and immuno-histochemical staining of material during my stay in Minnesota.

Working with Professor Nils Ivar Dolvik was a pleasure and a privilege. Professor Dolvik had a unique way of issuing a compliment that drove me from resting on my laurels to strive to earn the next accolade. With his inimitable style of supervision, Professor Dolvik went way beyond the call of duty and taught me to stand up for myself.

While working at the Norwegian School of Veterinary Science, I gained an extended family with members like Tobias Revold, Maria Kjeldaas Johannesen, Ernst Otto Ropstad, Sigrid Lykkjen, Eli Hendrickson, Cathrine Fjordbakk and Sand I Love. Thank you for making sure I did not go overboard during rough weather, whether on Færder or otherwise.

I am also incredibly grateful to my other family. My parents have always showered me with their love, support and generosity, but their greatest gifts are their individual powers to help me solve problems in conversation. My Mum carried me through especially hard times in 2005. My clever sister Elisabeth inspires me by beating me to everything, including a PhD, and I am grateful to Eivind for taking such good care of her.

Finally, thank you God for creating men. Some are impossible to love, but even more impossible not to. I will remember those who were there for me when it counted.

Kristin Olstad, Oslo, November 21st 2007

Abbreviations

2D: two-dimensional

3D: three-dimensional

cn: chondrocyte necrosis

ccn: cartilage canal necrosis

DIR: distal intermediate ridge of the tibia

ICP: intermediate coronoid process; cranial part of DIR

Micro-CT: micro-computed tomography

OC: osteochondrosis

OCD: osteochondrosis (-itis) dissecans

STB: Standardbred (Norsk: varmblodstraver)

SWB: Swedish Warmblood (Norsk: svensk halvblods/varmblods ridehest)

TB: Thoroughbred (Norsk: fullblodshest)

WB: Warmblood Riding Horse (Norsk: varmblods ridehest)

Introduction

Part 1: History, Definitions, Clinical Syndrome and Diagnostic Imaging

Early Description and Definitions (1575-1979)

Paré removed lose bodies from the joints of human patients during the 1550ies and described the procedure in his "Oevres Complètes", first available in 1575, but rarely accessible today [1] (92), cited in (111). In 1870, the pathologist Paget described the loose bodies as the result of quiet necrosis of bone (91), cited in (111). König first introduced the term "osteochondritis dissecans" (OCD) for description of the loose bodies in 1888 (76).

Mineralised cartilage flaps were recognised within the joints of dogs from 1939 onwards (79). Nilsson described spontaneous separation of cartilage alone, or in combination with bone, from the lateral trochlear ridge of the distal femur as a cause of patella luxation in foals, and likened the separation to OCD in human patients (89).

Smillie (1960), and later Siffert (1981), defined OCD in human patients as the result of an anomaly of enchondral ossification, encompassing both chondrogenesis and osteogenesis (119, 120). Rooney published several papers on OCD in horses during the 1970ies, postulating that the disease related to a delay or stopping of enchondral ossification (104). Rejnö and Strömberg corroborated this postulate and emphasised that, in horses, the disturbance was due to primary disease of cartilage (101, 122). In the same journal supplement, Olsson and Reiland concluded that cartilage disease preceded disturbance of enchondral ossification in most veterinary species, and expressed disbelief at the conclusion of investigators that human OCD was a sequel to primary disease of subchondral bone (90).

Olsson, Reiland, Rejnö, Strömberg and Rooney used the term "osteochondrosis" (OC) to denote the disturbance in enchondral ossification that, if subjected to force, could lead to OCD (90, 104, 124). Enchondral ossification occurs at the metaphyseal growth plate or physis, and the epiphyseal portion of the articular-epiphyseal cartilage complex at either end of most long bones.

[1] Pages of the 4th Edition are viewable online at http://archive.nlm.nih.gov/proj/ttp/books.htm

Rooney originally considered that a disturbance in enchondral ossification would result in OCD when located at the margin, and infolding cysts when located in the weight bearing central region of the joint surface (105). Strömberg subsequently expanded the range of manifestations to include enchondral ossification at the physis, resulting in disturbed longitudinal growth (123).

Concomitant lesions in more than one site lead to the conclusion that OC in veterinary species was a generalised condition (90). As will be discussed, OC in horses can present multi-focally at predilection sites within bilaterally symmetrical pairs of joints. Use of the terms “generalised” or “atypical” have possibly done more harm than good in the investigation of naturally occurring equine OC (97, 101).

This thesis focuses on OC as a primary disease of growth cartilage causing disturbance in enchondral ossification at the epiphyses of the equine hock and stifle, and information from other joints are included sporadically rather than faithfully.

Clinical OCD in Horses (1947-1983)

The first reports of equine OCD were in the stifle and hock (5, 10, 25, 89, 127). Nilsson and Rooney claimed to have observed similar lesions in the shoulder, hip, carpus, tarsus, fetlock, pastern, coffin or articular process joints of the vertebral column (89, 105), not all of which are necessarily considered representative of OCD today (65).

The early investigators noted that individuals diagnosed with OCD tended to be of young age (10, 89, 127), male gender (**Figure 1a**, 10, 25), and sometimes descended from affected sires (89, 124). The seven cases of Birkeland and Haakenstad were Standardbreds (10), the cases of Van Pelt *et al.* and De Moor *et al.* comprised three Thoroughbreds (25, 127), and Strömberg reported the first five cases in what are now known as Swedish Warmblood Riding Horses (122). One Connemara pony was included in the subsequent publication by Strömberg and Rejnö (124), but reported incidence in pony breeds is otherwise low. The stifle was more commonly affected in Thoroughbreds and Warmbloods, whereas the hock was more commonly affected in Standardbreds (81, 124).

Presenting signs included joint effusion (**Figure 1b**, 5, 10, 25, 89, 127), and a variable degree of lameness, which sometimes worsened after flexion tests or strenuous exercise (5, 10, 25, 122, 124). As mentioned, the same individual was often affected in bilaterally symmetrical pairs of joints (10, 25, 101, 127).

Figure 1: Clinical Presentation

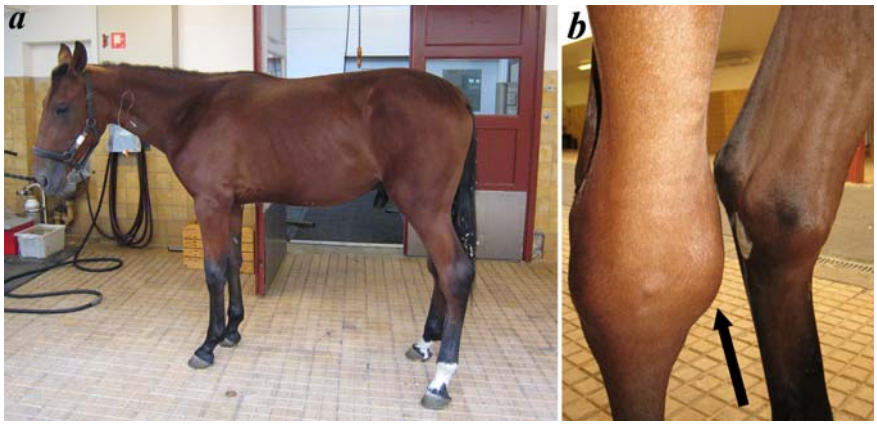

Figure 1a *shows a 2-year-old Standardbred stallion that has been sedated in preparation for surgical removal of OCD fragments from both hocks.* ***Figure 1b*** *shows effusion of the most severely affected tarso-crural joint of the same horse (arrow).*

Diagnostic Imaging (1960-1999)

Radiography of clinically suspect joints was advocated from 1960 (5). Changes of progressively smaller magnitude were recognised, from mineralised fragments (10, 122, 127) via subchondral lucency ranging from cysts down to 4 mm defects (25, 61, 122, 124) to focal, subchondral sclerosis (122). Diagnostic sensitivity improved correspondingly, and specificity increased with the realisation that OC tended to be localised to predilection sites that were specific to the joint in question, such as the cranial part of the distal intermediate ridge, medial malleolus and trochlear ridges of the talus in the hock (**Figure 2a**, 25, 61), and the trochlear ridges of the femur, medial condyle and patella in the stifle (**Figure 2b**, 81, 84, 122, 124).

Reports on the prevalence of radiographic OCD began appearing in the literature from 1980, ranging between 10-19% in different joints (21, 29, 45, 60, 103, 106). However, the size of radiographically detected fragments did not correlate perfectly with measurements at surgery or *post mortem* examination

(61), and, with the advent of arthroscopic treatment, fragments consisting of cartilage alone were identified and the relative insensitivity of radiography confirmed (85).

Longitudinal studies monitoring predilection sites in the hock and stifle revealed the dynamic nature of radiographic OCD as spontaneous resolution of lesions could occur before the ages of 5 and 8 months respectively (21, 26).

Figure 2: Radiography of Osteochondrosis Dissecans

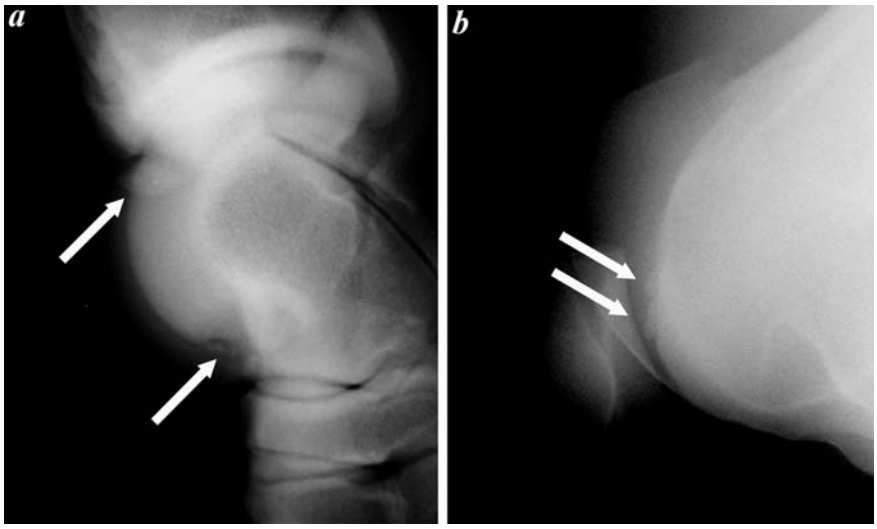

Figure 2a *shows a flexed latero-medial radiographic projection of the distal femur of a young Thoroughbred horse. Two oval radiopacities are visible in the mid-region of the lateral trochlear ridge.* ***Figure 2b*** *shows a plantaro-lateral dorso-medial oblique projection of the tarsus of a young Standardbred horse. Multiple partially or completely separate radiopacities are associated with the cranial distal intermediate ridge of the tibia and lateral trochlear ridge of the talus (arrows). (Anatomical images of predilection sites are shown in* ***Figure 5*** *in the Materials section.)*

Part 2: Hypotheses for the Pathogenesis of OC in Horses

A Disturbance in Enchondral Ossification

Osteochondrosis in horses has been defined as a disturbance in enchondral ossification (104, 124). There are many reports on the sequence of events *subsequent* to the point in time when the disturbance in enchondral ossification has become manifest. The potential for trauma or biomechanical forces to turn areas of disturbed enchondral ossification into OCD or cysts has been widely accepted (97). This stands in stark contrast to the lack of information and general confusion regarding the sequence of events *prior* to the point in time when the disturbance in enchondral ossification has become manifest. Much of the confusion has arisen due to sampling of clinical lesions, which, by definition, are chronic, in order to decipher aetiology and early pathogenesis (27). Nevertheless, hypotheses for the pathogenesis of equine OC are presented below, grouped in categories that may be helpful when considering their relationship to naturally occurring OC.

Hypotheses Based on Common Denominators of Naturally Occurring OC (1947-1999)

Early comments on the high number of affected offspring sired by particular stallions (89, 124) suggested that OC might be due to polygenic traits, and triggered investigations into heritability of the disease (62, 112), the most substantial of which comprised nearly 800 Standardbreds each (46, 95). Previous reports of higher prevalence of OC in male versus female individuals appear anecdotal by comparison (10, 25, 81, 123), and were effectively refuted in Standardbreds by the large number of horses included in the heritability investigations (46, 95).

It was considered that the higher prevalence of OC in males could be due to a more rapid rate of growth and larger ultimate body size compared to females. However, studies focussing on the rate of growth have generated inconsistent, non-reproducible results, documenting a correlation between growth rate and prevalence of OC in some, but not all joints (107, 129).

Hypotheses Based on Management Regimes Resulting in OC-Like Lesions (1947-2007)

Growth rate has been correlated with diet and the subject of several investigations into the pathogenesis of OC (39, 102). A high incidence of OC has been reported with a deficient (34, 72) or excessive plane of nutrition (39, 108). The relative influence of macronutrients such as fat (108), protein (40, 108, 113) and carbohydrates (43) on the prevalence of joint lesions have been studied without clear cut conclusions.

The influence of diet on the prevalence of OC has also been investigated in terms of micronutrients. Feeding an excess of either calcium or phosphorus increased the incidence and severity of joint lesions in one group of foals, although the effects of calcium were only seen when accompanied by excess dietary energy (109). However, deficiency of copper has received the most unrivalled attention of all micronutrients in relation to OC (15, 16, 22, 36, 37, 64, 67, 73, 93). A review by Cymbaluk and Smart explained how bivalent ions of transition metals might compete for the same intestinal absorption mechanism as copper (22), thus lesions following exposure to zinc or cadmium are probably the result of reduced availability of copper (48).

Copper is a required co-factor for the enzyme lysyl oxidase (22, 73). Lysyl oxidase plays a role in the formation of cross-links in collagen and elastin, and Hurtig *et al.* were able to demonstrate reduced collagen cross-linking in a group of foals fed a low copper diet relative to a control group (64).

An intricate hypothesis sprung from the macronutrient study involving carbohydrate, of transient post-prandial hypothyroxaemia causing asynchrony of insulin, T3 and T4, with consequences for the development of cartilage (43). One subsequent study concluded that foals diagnosed with OC demonstrated an exaggerated response to insulin (99), and insulin was found capable of promoting survival or depressing differentiation of equine chondrocytes *in vitro* (55).

Forced exercise was believed to protect against the development of OC in a 55-strong group of foals (6% incidence) compared to a 60-strong control group (20% incidence, 17), but this effect was not reproduced in a later study of a smaller group of foals (6). Exercise did, however, alter the distribution of lesions within individual joints (6).

The more extreme “management regime” of administering dexamethasone to foals has resulted in a host of growth abnormalities, some of which resemble OCD (41, 42).

Most of the factors listed are likely to affect growth cartilage throughout the body to an equal extent. Copper-induced lesions have a generalised pattern of distribution, and it remains to be seen whether the mechanism by which they arise bears any relation to naturally occurring lesions of OC, which have a different pattern of distribution entirely (97).

Hypotheses Based on Histological Examination (1972-1997)

The nature and cause of the disturbance in enchondral ossification should become apparent with strategic examination of predilection sites during the age range when lesions are initiated (26). Ideally, such sentinel studies should be carried out prior to the appearance of macroscopic signs, using techniques that are sensitive to primary disease within growth cartilage (27). Historically, this has translated to histological examination of cross-sectional collected material.

De Moor *et al*. are credited with the first histological description from 1972 of OCD fragments removed from the tibio-tarsal joint of horses as consisting of cartilage, bone and connective tissue (25), but examination of the fragments themselves is unhelpful in determining how they arose (27).

In 1975, Rooney examined the fragment site of origin and identified areas of cartilage necrosis at the chondro-osseous junction (105). This area was least accessible to diffusion, and might indirectly experience ischaemia if subjected to excessive pressure for prolonged periods of time (104). The necrotic area resisted the subchondral capillary invasion that is a step in the process of enchondral ossification, and, with continued ossification of the surrounding viable tissue, took on the appearance of a tongue of cartilage protruding into subchondral bone (105).

Rejnö and Strömberg summarised the histological characteristics of OC in 1978 as thickening, disturbance of enchondral ossification, degeneration and necrosis of cartilage (101, 122). Strömberg elaborated on the sequence of events the following year, proposing that a loss of normal differentiation of

chondrocytes precluded calcification of the matrix, causing the disturbance in enchondral ossification and retention of cartilage (123).

In 1986, Pool described arcades of vessels extending radially into cartilage from subchondral bone, and considered the histological appearance of early lesions of OC compatible with disruption of these vessels, and subsequent necrosis of the growth cartilage they supplied (96). The disruption was proposed to occur due to shear forces acting along the osteochondral junction (96). Nearly ten years later, Carlson *et al.* identified the vessels observed by Pool as arterioles within cartilage canals, which are channels of potential space within the otherwise compact structure of growth cartilage (**Figure 3**, 18, 96). Carlson *et al.* observed necrosis of vessels within cartilage canals, and believed that the resulting areas of chondronecrosis resisted conversion to bone, thus causing the disturbance in enchondral ossification (18).

In 1996, Grøndahl *et al.* suggested that fragments of OC in the hock and fetlock of Standardbreds were accessory centres of ossification and, as such, representative of normal anatomical variation (47). The following year, two publications emanating from annother research group advocated use of the term "dyschondroplasia" synonymously with OC in equine orthopaedics (56, 118). The first study defined lesions in the stifle as a disruption in chondrocyte differentiation, and separated them into two groups according to the presence of associated changes in the staining patterns of mineralised cartilage and subchondral bone (56). Henson *et al.* concluded that there was most likely more than one pathogenic mechanism behind dyschondroplasia, and, having presented the two major schools of thought of either matrix or cells being primarily responsible for the delay in enchondral ossification, proceeded to conduct further investigations of both (55-57).

Henson *et al.* interpreted vessels in the proximity of dyschondroplastic lesions as attempts to revascularise the primary lesion (56). Shingleton *et al.* signed on to the cellular school of thought, and proposed that patency, rather than necrosis (18), of vessels could lead to inappropriate exposure of chondrocytes to circulating agents including hormones such as insulin and thyroxine, with reference to the previous studies on management regimes (43, 118).

Figure 3: Enchondral Ossification and Cartilage Canals

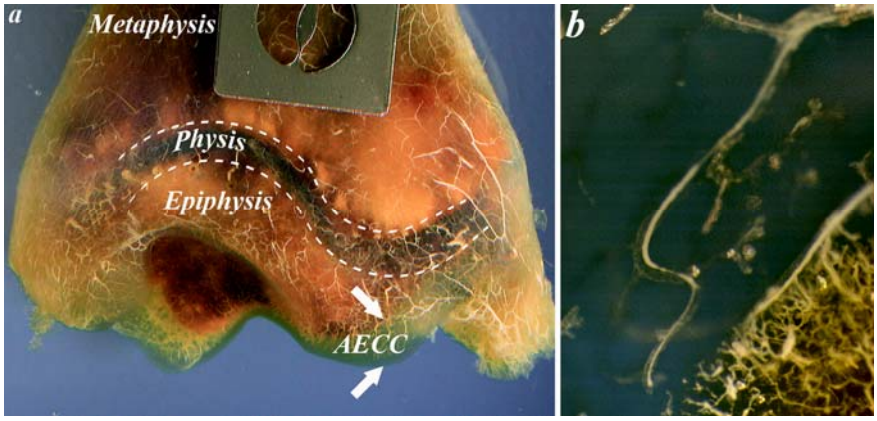

Figure 3a *is a cranial image of a distal tibia where the blood vessels have been perfused with barium, and the cartilage cleared (made translucent) with methyl salicylate. Enchondral ossification occurs at the metaphyseal growth plate or physis, and the articular-epiphyseal cartilage complex that covers the epiphyseal surface.* ***Figure 3b*** *shows a close up of a perfused arteriole within a cartilage canal. Barium is also visible within the fine capillary network surrounding the arteriole. Vessels in the lower right corner of the image were located within decalcified subchondral bone.*

Hypotheses Based on Molecular Biological Studies (1997-2007)

Lesions of equine OC or dyschondroplasia are increasingly being investigated with molecular biological techniques (1, 9, 24, 35, 57, 58, 69 (review), 77, 78, 114-116). Review of these reports is deferred until the ambiguity surrounding the question of whether they describe primary disease or secondary reparative processes is more firmly resolved (78, 115).

Hypotheses Based on Comparison to Other Veterinary Species (1933-2007)

The lack of cell differentiation observed in equine dyschondroplasia has been likened to avian tibial dyschondroplasia (125). This condition may be experimentally induced by feeding the pesticide thiram to chicks, which causes destruction of intra-cartilaginous capillaries (100). The blood supply to growth cartilage therefore merits attention in relation to both principal, histology-based hypotheses for the pathogenesis of equine OC: ischaemic chondronecrosis and chondrocyte dysplasia (18, 56, 118).

Modern day textbooks create a categorical impression of cartilage as an avascular tissue, but the temporary blood supply to growth cartilage has been

recognised since the 18th century (63), cited in (80). It runs within cartilage canals that form from the perichondrium of the primitive long bone model during gestation (11, 50, 54, 83, 133). It was suggested that cartilage canals appeared within the long bone model by passive inclusion (50), but the alternative hypothesis of active invasion appears to be rising in favour (13, 80, 83). Afferent arterioles of perichondrial origin course into a cartilage canal, branch into a capillary bed, and exit as converging efferent venules, all within the lumen of one and the same canal, and therefore represent functional end arterioles (11, 50, 133). The arrangement has been likened to the glomerulus of the kidney (54, 133). In addition to vessels, cartilage canals contain perivascular mesenchymal cells, and possibly nerves and lymphatics (133).

Cartilage canals carry an arterial supply to the growth cartilage, providing oxygen and nutrients and removing waste products (50, 51, 83, 133). The perivascular mesenchymal cells are believed capable of contributing to growth of the hyaline cartilage model (50, 83). Finally, cartilage canals are thought to initiate formation and assume responsibility for maintenance of the secondary centre of ossification (11, 13, 50, 51, 83, 132).

Cartilage canals disappear from the long bone model through physiological regression and incorporation into the secondary centre of ossification while enchondral ossification is still ongoing. The physiological regression process is known as “chondrification” (transformation into cartilage), and has not been associated with morbidity (51, 138).

The morphology of cartilage canals has been most extensively described in piglets, where focal failure of canal vessels has been associated with purported early lesions of OC (20, 70, 135, 139). One study considered involvement of cartilage canals likely due to their persistence at a predilection site for OC in the shoulder joint of dogs, but was unable to demonstrate a direct association with histological lesions (131).

The failure of cartilage canal vessels was recently linked to the process of incorporation into the ossification front in the femoral condyles of piglets (139). Chondrification occurs from distal to proximal within canals (51, 138). Incorporation into subchondral bone in porcine femoral condyles, however, was shown to occur at the midsection prior to the distal and proximal ends of canals, which remained located within cartilage for some time (139). Arterioles within

the distal terminus of the canal formed anastomoses with vessels of the subchondral bone and became dependent on these as their arterial source (139). Lesions of OC were consistently found surrounding necrotic distal portions of cartilage canals, prompting the conclusion that vessels were vulnerable to interruption in the region where they crossed the ossification front (139).

Experimental interruption of the cartilage canal blood supply to epiphyseal growth cartilage induced lesions that were morphologically identical to those of naturally occurring early lesions of OC in piglets (20, 136). Three separate morphological stages of *osteochondrosis latens* (cartilage canal and chondrocyte necrosis), *osteochondrosis manifesta* (macroscopic delayed ossification) and *osteochondrosis dissecans* were identified during the study of naturally occurring disease in piglets (140), and corroborated by experimental interruption of the blood supply (136).

The reports documenting morphological stages (140), incorporation into the ossification front (139) and the consequences of naturally occurring or experimentally induced failure in piglets (136, 140) emanated from a historical large body of literature in this species, and represent the most detailed and up-to-date information available on the role of cartilage canals in the pathogenesis of OC. By comparison, extensive literature search yielded only three reports that acknowledged the existence of cartilage canals within equine growth cartilage at the outset of this thesis (18, 56, 118). The disadvantage of the sheer bulk of work that is required in order for information on cartilage canals in foals to equal that available in piglets is easily outweighed, and most likely reduced, by the considerable potential for extrapolation of information and hypotheses between the veterinary species (reviewed in 137).

The reports on cartilage canals in piglets represent major advances in veterinary OC research. They are, however, still reliant upon cross-sectional histological examination for the diagnosis of early lesions (20, 136). The earliest, intra-cartilaginous stages of OC are radiographically silent, but recently available modalities may have improved capabilities in terms of cartilage imaging (82). State-of-the-art equine clinics currently offer computed tomography and magnetic resonance imaging. The distance between these, and their laboratory research counterparts capable of micrometer resolution, is shrinking fast (88). Alone, or in combination with physiological imaging, these modalities may

enable longitudinal imaging of ischaemia and its consequences (33, 66, 117). Essentially, the only remaining obstacle to longitudinal monitoring of experimentally induced focal cartilage canal interruption in piglets is one of resolution (86). Such monitoring has the potential to revolutionise OC research in terms of longitudinal validation of cross-sectional histological hypotheses.

Persistent Challenges in the Research of Equine OC

• The morphology, pathogenesis and aetiology of lesions of OC in horses remain undetermined. The debate over whether it is appropriate to class different clinical manifestations of joint disease as OC continues. If classification as OC is considered appropriate, the issue of whether the pathogenesis of the lesions is the same in different sites, and in different joints, is still drawn into question.

• In horses, OC was defined as a primary disease of growth cartilage causing disturbance in the process of enchondral ossification in 1978 (101). Since then, research into the aetiology and pathogenesis has proceeded without due attention to the blood supply of the tissue in which the primary disease arises.

• All current theories for the pathogenesis of OC rest on cross-sectional diagnosis and require longitudinal validation.

Aims

The primary aim of this thesis was to investigate the role of the cartilage canal blood supply in the pathogenesis of equine OC. The purpose of this aim was to improve understanding of the disease, and aid with future experimental and clinical early diagnosis of lesions. The primary aim was achieved by sequential investigation of three smaller, secondary aims as outlined below:

• To describe the morphology of early lesions of OC in horses, and determine if different morphological stages of the disease can be identified similar to those that have been observed in piglets (*osteochondrosis latens* and *manifesta*, 140).

Histologically in the tarsus, Papers (I and II)

Histologically in the distal femur (III)

Micro-computed tomographically and 3D in the tarsus (IV)

• To describe the developmental pattern of the blood supply to growth cartilage and relationship to early lesions of OC in horses, with a particular emphasis on identifying whether cartilage canals are incorporated into the ossification front during growth and whether there is any indication of an association between this process and the occurrence of lesions of OC (139).

Macro- and microscopically in the tarsus (II)

Macro- and microscopically in the distal femur (III)

Micro-computed tomographically in the tarsus (IV)

• To investigate the sensitivity of micro-computed tomography for detecting the different stages of early lesions of OC, if identified, in the tarsus of horses (IV)

Please Note: *The predilection site at the cranial part of the distal intermediate ridge of the tibia will be referred to as the intermediate coronoid process (74).*

Materials

Material from three different populations of foals was used in this thesis. Nine of the foals were purpose-bred and will be referred to as the Homebred Population. The remainder of the material was acquired from foetuses and foals submitted for routine *post mortem* examination at one of five institutions in Norway, Sweden and the USA. This latter category was subdivided into two populations: the Early Lesions Population, from which the distal tibia was collected, and the Post Mortem Population, from which the distal femur was collected.

Homebred Population (II-IV)

Six Standardbred mares and two Standardbred stallions were rented from owners with informed consent for using them to breed foals for research purposes, and permission was obtained from The National Animal Research Authority.

The first stallion used during 2003 had radiographic OC of the intermediate coronoid process, as did all except for one of the five mares used that season. At the end of the season, the stallion was castrated at the request of the owner. After foaling, the OC-negative dam was excluded and one further mare retired from breeding due to old age.

The second stallion used during 2004 had radiographic OC of the intermediate coronoid process and the lateral trochlear ridge of the talus. A new, OC-positive mare was recruited, thus all four mares used during 2004 had radiographic OC of the intermediate coronoid process.

The resulting Homebred Population comprised five colts and four fillies. Additional information on the month of birth, gestation length and body weight of these foals is given in Table 1 of Paper II. *Rhodococcus equi* was cultured from the lungs of the 6-week-old foal *post mortem*.

The foals were sacrificed at weekly intervals from newborn to seven weeks old, and assigned names of “0-” to “7-week-old” according to their respective ages at sacrifice. The foal of the OC-negative dam was sacrificed at 2 weeks of age during the first season and designated “Foal 2a”. In order to assure that each age interval was represented with at least one foal descended from two OC-positive parents, a second foal was sacrificed at 2 weeks old during the next

season and designated “Foal 2b”. The end result was that each age interval from 0 to 7 weeks old was represented by one foal, and the age of 2 weeks old was represented by two foals.

The first stallion sired the foals named 0-, 1-, 2a-, 3- and 5-weeks-old. The second stallion sired the foals named 2b-, 4-, 6- and 7-weeks-old. In summary, both parents of all but the 2a-week-old foal had radiographic OC of the intermediate coronoid process, and one parent of the 2b-, 4-, 6- and 7-week-old foals also had radiographic OC of the lateral trochlear ridge of the talus.

Early Lesions Population (I)

The Early Lesions Population comprised 55 colts, 37 fillies and 8 foals with gender unrecorded. The age range was from 191 days of gestation to 153 days old. Thirty-nine of the foals suffered a variety of systemic conditions including septicaemia and endotoxaemia, whereas the remaining 61 foals had no evidence of systemic disease. Additional information on the distribution across the age, breed and cause of death categories is given in Table 1 of Paper I.

Post Mortem Population (III)

The Post Mortem Population comprised 15 colts, 10 fillies and 2 foals with gender unrecorded. The age range was from 229 gestational days to 305 days old. Sixteen foals suffered systemic conditions such as septicaemia and endotoxaemia, whereas the remaining 11 foals had no evidence of systemic disease. The distribution across the age, breed and cause of death categories is given in Table 1 of Paper III.

Foals and Limbs Sampled

The number of foals and limbs sampled in each population is summarised in **Figure 4**.

Figure 4: Summary of Foals and Limbs Sampled

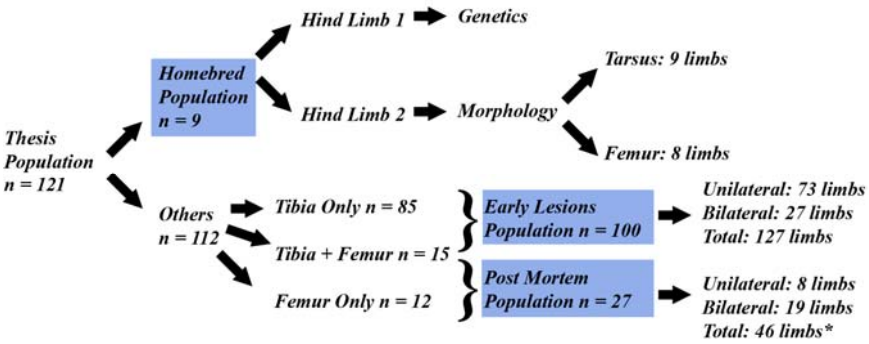

Figure 4 *summarises the number of foals and limbs sampled in this thesis. *Please note that from the 46 femora collected from the Post Mortem Population, 44 lateral trochlear ridges and 43 medial condyles were processed for histological examination.*

The total number of individual foals examined was 121. Although material was collected from both hind limbs of the Homebred Population of foals, only one hind limb was available for morphological studies as reported in this thesis, as the growth cartilage of the contra-lateral hind limb was macerated in order to study gene expression. Material was collected from the tarsus of all Homebred foals (n = 9), but the distal femur of one foal was not decalcified in time to be included in this thesis, such that only 8 femora were examined (II-IV).

A total of 112 foals were recruited from *post mortem* examination. Material was collected from the distal tibia of 85 foals, the distal femur of 15 foals and both the distal tibia and femur in 15 foals. The Early Lesions Population therefore comprised 100 foals and the Post Mortem Population 27 foals, 15 of which were common to both populations. Collection was unilateral in 73 and bilateral in 27 of the Early Lesions Population, resulting in a total of 127 distal tibiae being available for examination, all of which were processed for histology (I). In the Post Mortem Population, collection was unilateral in 8 and bilateral in 19 foals, resulting in a total of 46 distal femora being available for examination. From these, 44 lateral trochlear ridges and 43 medial condyles were processed for histology (III).

Sampling of Predilection Sites

Predilection sites and the number of limbs from which they were sampled are summarised in **Figure 5**.

Figure 5: Predilection Sites Sampled

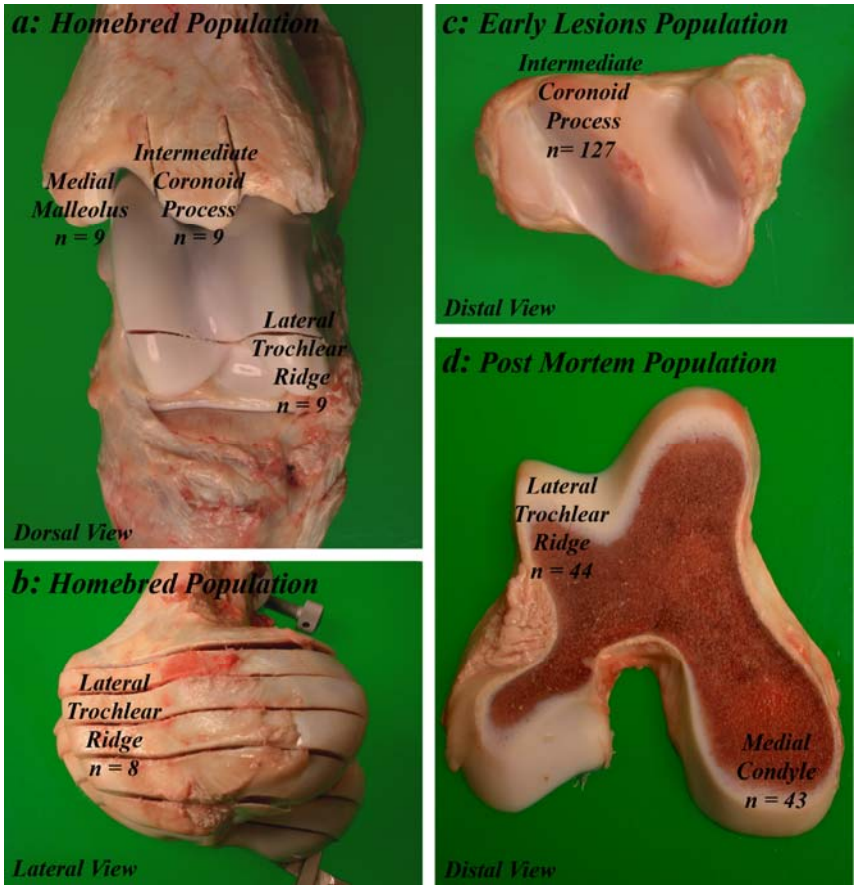

Figure 5 *summarises the predilection sites sampled. Images* ***5a*** *and* ***5c*** *are from the tarsus, whereas images* ***5b*** *and* ***5d*** *are from the distal femur. The numbers denote the total number of limbs from which the predilection sites were sampled. Please refer to* ***Figure 4*** *for the total number of individuals from which the limbs were collected.*

The distal tibia, talus and distal femur of the Homebred Population of foals were examined as intact bones (II, III). Thereafter, smaller blocks of cartilage and bone were collected from the intermediate coronoid process, medial malleolus and lateral trochlear ridge of the talus predilection sites. Images of the blocks from the intermediate coronoid process and lateral trochlear ridge of the

talus are given in Figure 1 of Paper IV. The blocks were subsequently sectioned in different planes for histological examination. The intermediate coronoid process was sectioned in a plane parallel to the intermediate ridge, the medial malleolus was sectioned in a frontal plane, and the lateral trochlear ridge of the talus in a transverse plane. Images of the planes of section are available in Figure 1 of Paper II. The distal end of the femur was sawed into slabs in the transverse plane from the articular surface distally to the proximal end of the trochlear ridges, as illustrated in Figure 1 of Paper III. The lateral trochlear ridge of all slabs was trimmed to fit standard paraffin embedding cassettes (32 x 25 x 5 mm) and processed for histological examination. The medial femoral condyles of all Homebred Population foals were macroscopically normal and were not examined histologically.

The intermediate coronoid process of 83 of the Early Lesions Population foals was sectioned in a plane parallel to the intermediate ridge of the distal tibia (I). The remaining 17 foals were a subset of the sample published by Carlson *et al.* in 1995 (18), in which the entire distal tibia was band-sawed into slabs in the frontal plane. The slab located midway between the extreme cranial and caudal slabs was processed for histological examination, as well as any region showing evidence of OC on micro-radiography, defined as focal radiolucency extending into subchondral bone (18). The two planes of section are illustrated in Figure 1 of Paper I.

The distal end of the femur of the Post Mortem Population of foals was sawed into slabs in the transverse plane (III). One slab representing the midway point between the proximal and distal extremes of each of the trochlear ridges and femoral condyles was selected, as well as any slab containing macroscopically visible irregularities. From these, 44 lateral trochlear ridges and 43 medial condyles were trimmed to fit standard paraffin embedding cassettes (32 x 25 x 5 mm) as illustrated in Figure 1 of Paper IV, and processed for histological examination.

Methods

Material from all populations of foals was examined histologically. In addition, material from the Homebred Population of foals was examined macroscopically as intact bones, blocks of cartilage and bone, and slabs. Macroscopic examination was augmented by the use of perfusion, Spalteholz clearing, micro-computed tomography, decalcification, radiography and stereomicroscopy. The methods used are summarised in **Table 1**, and described in more detail in the text following the table.

Table 1: Summary of Methods Used

Population	Bone	Site	Form	Perfusion/ Clearing	MicroCT	Radiography	Stereo-microscopy	Histology
Early Lesions	Distal tibia	Intermediate coronoid process	Section					■
Homebred	Distal tibia	Intermediate coronoid process	Intact bone	■				
			Block		■			
			Slab				■	
			Section					■
		Medial malleolus	Intact bone	■				
			Slab				■	
			Section					■
	Talus	Lateral trochlear ridge	Intact bone	■				
			Block		■			
			Slab				■	
			Section					■
	Distal femur	Lateral trochlear ridge	Intact bone	■				
			Slab			■		
			Section					■
		Medial condyle	Intact bone	■				
			Slab			■		
Post Mortem	Distal femur	Lateral trochlear ridge	Section					■
		Medial condyle	Section					■

Table 1 *summarises the methods used in the different populations of foals. The shaded boxes denote the techniques used in the given form of the sample.*

Perfusion and clearing (II, III)

The arterioles of one hind limb of the Homebred Population were perfused with optically and radiographically dense micronized barium by a protocol adapted from piglets (200 g/l w/vol, 0.7-0.8 µm median particle size, 138). The adaptations consisted of using a cuffed endotracheal tube rather than a butterfly catheter to administer the perfusion liquids, and placing it within the femoral artery instead of the abdominal descending aorta (**Figure 6a**). An electrical arthroscopy pump was used to drive the perfusion liquids, as opposed to manual pressure (Olympus Arthromat, Olympus, Winter & Ibe, Hamburg, Germany). Success of the perfusion was tested with an incision in the pastern above the coronary band to confirm bleeding of barium (**Figure 6b**).

After fixation in 4% formaldehyde, the tarsal and distal femoral epiphyseal cartilage was cleared (i.e. made translucent) using the modified Spalteholz technique in order to allow direct visualisation of barium-perfused arterioles (49) Professor Spalteholz' perfusion medium of choice was India ink, and the step of hydrogen peroxide bleaching was omitted, but the procedure otherwise appeared much as it did in 1914 (49).

Figure 6: Perfusion Procedure

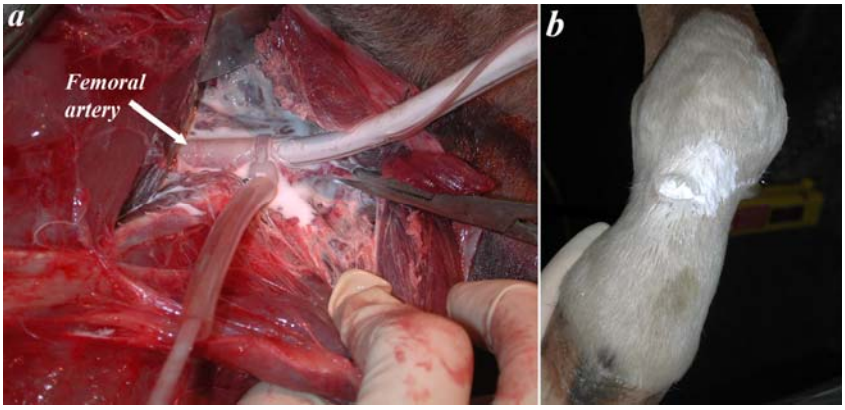

Figure 6 *illustrates the perfusion procedure.* ***6a****: Endotracheal tubes were used as catheters and placed within the femoral artery.* ***6b****: Success of the perfusion was tested with an incision at the pastern above the coronary band to confirm bleeding of barium.*

Micro-computed tomography (IV)

Prior to decalcification, tissue blocks containing barium angiograms from the intermediate coronoid process and lateral trochlear ridge of the talus of

the Homebred Population were coated in paraffin wax and scanned using routine protocols and a custom-built equipment set-up for micro-computed tomography as described in Paper IV.

Decalcification (I-IV)

Blocks or slabs consisting of cartilage and bone from all populations of foals were decalcified in 10% formic acid or 10% ethylene-diamine-tetra-acetic acid (EDTA).

Radiography (III)

After decalcification, entire transverse slabs through the distal femur of the Homebred Population were radiographed using standard digital radiographic techniques as described in Paper III.

Stereomicroscopy (II)

After decalcification and clearing for a second time, tissue blocks from the intermediate coronoid process, medial malleolus and lateral trochlear ridge of the talus of the Homebred Population were sectioned into 4-5 mm thick slabs with a razorblade. Slabs were immersed in concentrated methyl salicylate within glass Petri dishes, 10 cm in diameter, and examined under a stereomicroscope, whose field of view at the lowest magnification was approximately 15 mm.

Histology (I-IV)

Blocks or slabs of cartilage and bone from the distal tibia and talus of the Homebred and Early Lesions Populations, and the distal femur of the Homebred and Post Mortem Populations were fixed in 4% formaldehyde and decalcified in formic acid or EDTA as described above. Blocks and slabs were then cut down to fit standard histological cassettes measuring 32 x 25 x 5 mm, paraffin embedded, sectioned and stained according to routine histological protocols.

Results

The results of all papers are summarised together, and the papers that the results emanated from are given in parentheses. The individual papers represent the following bones and regions:

Paper I:	Tibia; intermediate coronoid process
Paper II:	Tibia; intermediate coronoid process and medial malleolus
	Talus; lateral trochlear ridge
Paper III:	Femur; lateral trochlear ridge and medial condyle
Paper IV:	Tibia; intermediate coronoid process
	Talus; lateral trochlear ridge

The perichondrial or subchondral end of cartilage canal vessels was referred to as proximal, whereas the end furthest from the perichondrium or subchondral bone was referred to as distal.

Examination Techniques and Normal Features

The intervening layer of opaque articular cartilage precluded direct examination of epiphyseal growth cartilage in intact, untreated bones (I-IV). In intact, cleared bones, the volume of translucent cartilage was delineated by adjacent opaque structures (II, III). Vessels within growth cartilage were only visible to the extent that they contained opaque contrast medium (II, III). Visibility of the subchondral bone plate was limited by the quality of clearing, in terms of contrast, and by the optical resolution of the human eye, in the absence of magnification (II, III).

The volume of radiolucent cartilage was once again delineated by more radiodense adjacent structures in smaller blocks of tissue scanned with micro-computed tomography (IV). Vessels were only visible to the extent that they contained radiopaque contrast medium (IV). Volume rendered 3D models of scans enabled real-size and magnified views of the subchondral bone plate with excellent contrast (IV).

The depth of cartilage, vessels in transverse and occasional longitudinal section and chondro-osseous junction profile could be examined on the cut surface of sawed, untreated bones (I, III). The same features were visible within slabs, but whereas the visibility of intact vessels relied upon the presence of contrast medium within them on radiography and stereomicroscopy, ghost

chondrifying canals were also visible directly within cleared slabs in the stereomicroscope (II, III).

Histological examination was the only technique that enabled direct identification of the resting, proliferative, hypertrophic and mineralised zones of growth cartilage, cartilage canals in physiological or pathological states, individual chondrocytes and matrix (I-IV).

The articular portion of the articular-epiphyseal cartilage complex of the distal femur, tibia and talus was avascular at all ages examined, whereas the epiphyseal growth cartilage was richly, but non-uniformly vascularised in the youngest foals (I-IV). Areas with thick cartilage contained a greater number of perfused vessels than areas with thin cartilage (II-IV). The only exception was that the lateral trochlear ridge of the distal femur retained its vascularity for a disproportionately long time relative to its thickness (III).

Figure 7: Femoral Perpendicular and Parallel Cartilage Canals

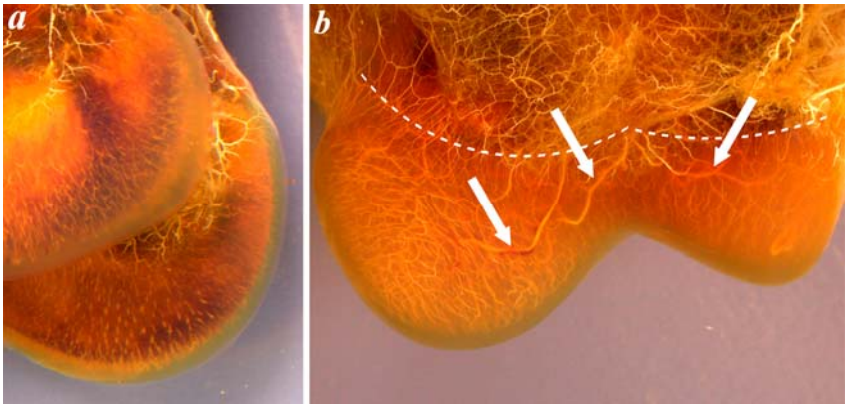

Figure 7a *is an oblique lateral view showing barium-perfused vessels travelling in a straight line perpendicular to the ossification front within the cleared cartilage of the femoral condyles of a 2-week-old foal.* ***Figure 7b*** *is a proximo-distal view showing perfused vessels (arrows) travelling roughly parallel to the ossification front (stippled line) within the femoral trochlear ridges of a 3-week-old foal.*

Cartilage canal vessels predominantly originated from arteries within the perichondrium of the distal tibia and talus (II), whereas the arterial source was either perichondrial or subchondral in the case of the distal femur (III). In some regions, such as the femoral condyles, cartilage canal vessels with a subchondral source coursed in a straight line perpendicular to the underlying

ossification front and from deep to superficial within growth cartilage (III, **Figure 7a**). In other regions, such as the intermediate coronoid process, medial malleolus and trochlear ridges of the distal femur, cartilage canal vessels with subchondral or perichondrial arterial sources changed direction within growth cartilage and travelled roughly parallel to the ossification front, often across an articulating surface of the given epiphysis (II-IV, **Figure 7b**).

The distal termini of cartilage canal vessels within the lateral trochlear ridge of the talus and the trochlear ridges and condyles of the distal femur (personal records) frequently made a 180º retrograde hook turn to travel back towards the underlying ossification front, or appeared as loops out of and into the subchondral bone in the older foals (II, III).

On histological examination, columnar arrangement of cells was rare within the intermediate coronoid process of the youngest foals, but became more prominent after 92 days of age (I). Cells within the hypertrophic zone were arranged in circles centred on vascular structures in the intermediate coronoid process (I). The same was true of the lateral trochlear ridge of the femur of the youngest foals, whereas cells within older foals and the other zones tended to be arranged in columns at this site (personal records). Cartilage canals were present within the distal tibial epiphysis prior to formation of the secondary centre of ossification (I). More than one vessel was seen in cross-section within canal lumina, which also contained ovoid- to spindle-shaped perivascular cells (I). The cartilage surrounding patent canals occasionally displayed a rim of relative hypercellularity or a halo of eosinophilic matrix (I).

In 3D volume rendered models of micro-computed tomography scans, the subchondral bone plate was highly irregular, displaying indented grooves and dimples in the intermediate coronoid process, and protruding tubes at the lateral trochlear ridge of the talus (IV). Barium angiograms revealed that these irregularities invariably were associated with perfused vessels (IV). The number of perfused vessels decreased with increasing age and the subchondral bone plate became progressively smoother (IV).

Loss of Vascularity

The vascularity of growth cartilage decreased with increasing age (I-IV). This occurred at a younger age in the tarsus compared to the distal femur (II, III). Vascularity was reduced in a regionally staggered sequence in all bones examined (II, III). The medial malleolus and distal lateral trochlear ridge of the talus were the last regions to lose vascularity within the respective bones (II). The intermediate coronoid process and lateral trochlear ridge of the femur were among the last regions, whereas the medial femoral condyle was the first region to suffer reduced vascularity within the respective bones (II, III). Loss of vascularity progressed in a particular direction within individual regions, and in a particular sequence from different aspects of each region (II). The reduction in vascularity is summarised in **Table 2**.

Loss of vascularity occurred through two processes: chondrification or incorporation of cartilage canals into subchondral bone (I-III). Chondrification progressed from superficial to deep within growth cartilage (I, III), and from distal to proximal within cartilage canals (II). On histological examination, chondrifying canals were always associated with a rim of chondrocyte hypercellularity (I). Chondrocytes adjacent to the canal impinged on the lumen, while intra-canalicular perivascular cells displayed chondrocyte-like characteristics (I).

Incorporation of cartilage canals into subchondral bone occurred at the dynamic location of the chondro-osseous junction (II-IV). Canals travelling in a straight line perpendicular to the ossification front were incorporated into subchondral bone in a proximal to distal direction (III). This contrasted with the sequence of incorporation of canals travelling roughly parallel to the ossification front (II, III). Incongruence between the course of parallel canals and the underlying ossification front resulted in the midsection of these canals becoming incorporated into subchondral bone before their proximal and distal portions (II-IV). From proximal to distal, the length of parallel canals curved concavely towards the ossification front, and the proximal and distal portions remained located within growth cartilage while the midsection was incorporated into bone (II, III). This occurred irrespective of whether the arterial source of the cartilage canal vessel was perichondrial or subchondral (III). At the site of incorporation, anastomoses formed between cartilage canal vessels and vessels of the

subchondral bone (II, III). Vessels within the distal portion of cartilage canals shifted to use subchondral vessels as their arterial source (II, III). The proportion of vessels reliant on an arterial source obliged to cross the ossification front in order to enter the cartilage canal therefore increased with increasing age (II, III).

Table 2: Loss of vascularity

	Bone, Site, Aspect	Intact Bone	Slabs	Histological Sections
Early Lesions Population Maximum age 153 days	•Tibia •Intermediate coronoid process •All aspects	Not examined	Not examined	•Resting zone avascular from 32 days •Proliferative zone avascular from 50 days •Hypertrophic zone avascular from 137 days
Homebred Population Maximum age 49 days	•Tibia •Intermediate coronoid process •All aspects	•Avascular from 35 days	•Avascular from 49 days	•Resting zone avascular from 21 days •All other zones vascular at maximum age
	•Tibia •Medial malleolus •All aspects	•Vascular at maximum age	•Vascular at maximum age	•Resting zone avascular from 35 days •All other zones vascular at maximum age
	•Talus •Lateral trochlear ridge •All aspects	•Avascular from 49 days	•Avascular from 49 days	•Resting zone avascular from 28 days
	•Femur •All sites •All aspects	•Vascular at maximum age	•Vascular at maximum age	•Vascular at maximum age
Post Mortem Population Maximum age 305 days	•Femur •Lateral trochlear ridge •Mid-cranial aspect	Not examined	Not examined	•Resting zone avascular from 142 days •All other zones vascular at maximum age
	•Femur •Medial condyle •Mid-caudal aspect	Not examined	Not examined	•Resting zone avascular from 153 days •All zones avascular from 305 days

Table 2: *Avascularity was defined as absence of macroscopically visible perfused vessels in intact bones and slabs, whereas it was defined as absence of patent and chondrifying canals in histological sections. Resolution of vessels increased from intact bones via slabs to histological examination; the age from which the growth cartilage was avascular therefore increased correspondingly. Results must be interpreted within the limits of site, aspect and age of foals available for examination (I-III).*

Lesions of OC

A total of 27 lesions of OC were found in one of the five predilection sites within 20 out of the 120 individual foals examined (I-III). The common denominator of all lesions was that they contained an area of chondrocyte necrosis. The lesions are summarised in **Table 3**.

Out of the 20 foals diagnosed with OC, six, and in retrospect possibly an additional two foals, suffered systemic conditions such as bacteraemia, septicaemia or endotoxaemia, or sequelae thereof, including polyarthritis (I-III). This applied to the 6-week-old foal of the Homebred Population (II), and three definitive as well as two potential individuals of the Early Lesions Population (listed in Table 2 of Paper I) and two individuals of the Post Mortem Population (listed in Table 2 of Paper III).

Nine of the lesions were macroscopically visible within untreated or cleared intact bones and slabs, whereas the remaining 18 lesions were detectable only with techniques capable of micrometer resolution (I-IV). Lesions in the tarsus were found in the last region and on the last aspect of each predilection site to remain vascularised (II). In the distal femur, lesions were located on aspects of each predilection site that were vascularised at the time when the lesion was initiated, but not necessarily the last aspects of each predilection site to remain vascularised on the whole (III). All lesions in all sites were found in regions of growth cartilage where cartilage canal vessels were reliant on a subchondral, rather than a perichondrial, arterial source, and this artery was obliged to cross the ossification front in order to enter the cartilage canal (I-IV).

Figure 8 illustrates two lesions and examples of secondary repair processes within cartilage and bone. Three of the areas of chondrocyte necrosis were confined to the resting and proliferative zones of growth cartilage (**Figure 8a**), whereas the remaining areas were located at the ossification front or within subchondral bone (I-III, **Figure 8d**). Lesions were located within areas of growth cartilage that were devoid of contrast-perfused vessels within cleared bones and on micro-computed tomography (II, IV). Vessels carrying a functional blood supply were seen adjacent to, but not within areas of chondrocyte necrosis in 2D or 3D (I-IV). No vessels were seen to exit from subchondral bone deep to any lesion in 3D volume rendered models (IV).

Areas of chondrocyte necrosis were associated with necrotic cartilage canals in 22 out of the 27 lesions (I-IV). Necrotic canals contained remnant outlines of dilated vessel lumina surrounded by degenerate and necrotic endothelial and perivascular cells (I).

The quality of matrix staining in areas of chondrocyte necrosis was frequently altered, consisting of eosinophilia and pallor in hematoxylin and eosin stained sections, and pallor in toluidine blue stained sections (I-III, **Figure 8d**). Within cartilage, secondary repair processes manifested as neovascularisation, i.e. sprouting of functional vessels (**Figure 8b**), and chondrocyte clusters on the margin of lesions (**Figure 8c**). A separate centre of ossification was observed around one such area of neovascularisation within the growth cartilage superficial to the lesion (IV). The neovascularisation originated from the distal terminus of a perfused cartilage canal whose perichondrial arterial source had remained patent within the growth cartilage cranial to the lesion in the intermediate coronoid process (IV). Centres of ongoing ossification were observed within lesions located below the ossification front of the intermediate coronoid process and lateral trochlear ridge of two other foals (IV).

Secondary repair processes initiated within subchondral bone were seen in association with lesions that were located at or below the ossification front (I-III). Small, viable, chondrocyte-like cells were seen within necrotic, otherwise non-functional cartilage canals within areas of chondrocyte necrosis, and interpreted as migration of stem cells from bone marrow spaces into lesions (I-III, **Figure 8e**). Multi-nucleated giant cells within marrow spaces adjacent to lesions were interpreted as chondroclasts (I-III, **Figure 8f**).

The area of chondrocyte necrosis caused focal delay in enchondral ossification in 19 out of the 27 lesions. Dissecting fissures were observed in the absence of a delay in enchondral ossification in one foal (I), and in the presence of a delay in another foal (III). Microscopic cysts were observed within the granulation tissue at the base of the dissecting fissure in the latter, which was located within the medial femoral condyle of this foal (III).

Table 3: Lesions of OC

Paper and Population (Total Number)	Bone, Number of Lesions, Number of Affected Foals	Predilection Sites Affected	Primary Cartilage Pathology		Secondary Repair Processes	
			Visible in microscope or on microCT	Macroscopically visible in intact bone or slab	Within Cartilage	Within Bone
•Paper I •Early Lesions Population (n=100)	•Distal tibia 11 lesions •9 foals	•11 lesions intermediate coronoid process	•2x cn[a] + ccn[b] •3x cn + delay[c] •4x cn + ccn + delay	•1x cn + ccn + delay •1x cn + OCD[d]	•6x chondrocyte clusters on margin •1x neovascularisation	•2x chondrocyte-like cells in canals •1x chondroclasia •2x granulation tissue
•Paper II and IV •Homebred Population (n=9)	•Distal tibia 9 lesions •Talus 3 lesions •Distal femur 0 lesions •7 foals	•5 lesions intermediate coronoid process •4 lesions medial malleolus •3 lesions lateral trochlear ridge of talus	•2x cn + ccn •6x cn + ccn + delay	•4x cn + ccn + delay	•6x chondrocyte clusters on margin •1x neovasularisation •3x ossification centres	•8x chondrocyte-like cells in canals[e] •6x chondroclasia[e] •10x granulation tissue
•Paper III •Post Mortem Population (n=27)	•Distal femur 4 lesions •4 foals	•2x lateral trochlear ridge of femur •2x medial condyle	•1x cn + ccn + delay	•1x cn + ccn + delay •1x cn + ccn + delay + OCD •1x cn + ccn + delay + OCD + micro-cysts	•3x chondrocyte clusters on margin	•3x chondrocyte-like cells in canals •3x chondroclasia •2x granulation tissue

[a] cn: chondrocyte necrosis, [b]ccn: cartilage canal necrosis, [c]delay: focal delay in enchondral ossification, [d]: OCD: osteochondrosis dissecans, [e]Variably reported separately or as part of granulation tissue in Paper II, reported separately here based on personal records.

Figure 8: Lesions of OC and Secondary Repair Processes

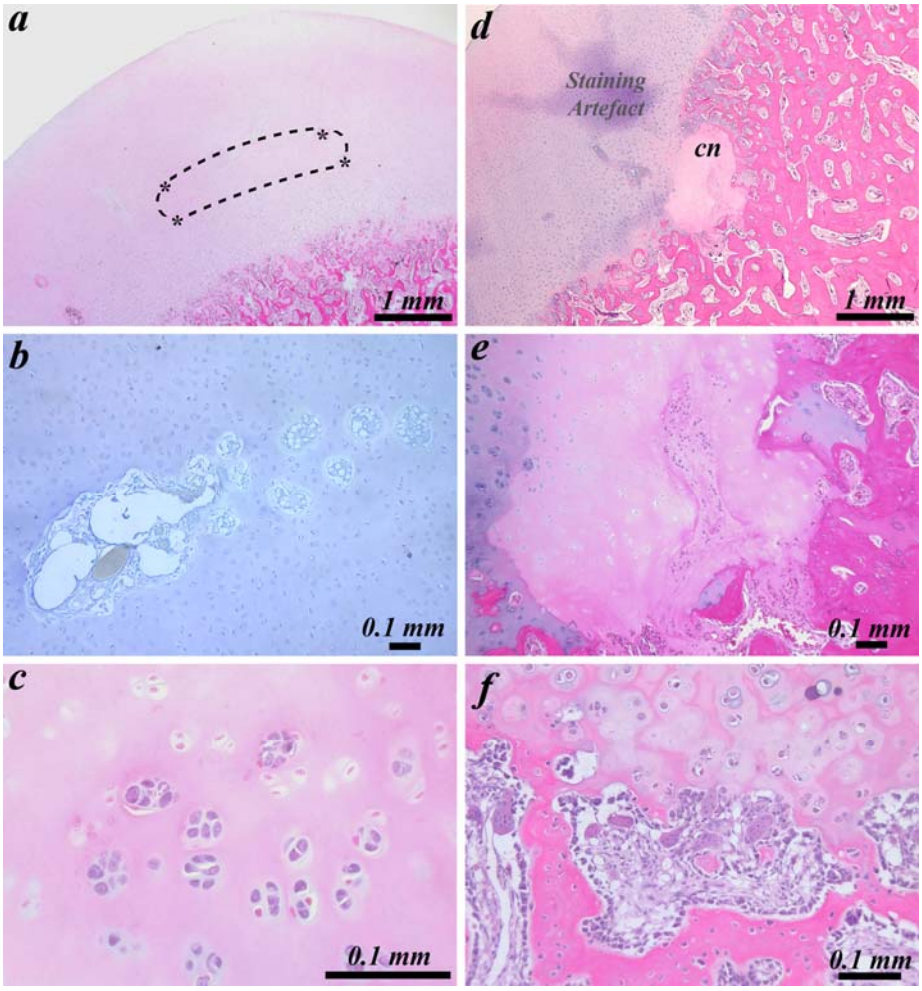

Figure 8 *illustrates lesions of OC in the intermediate coronoid process and secondary repair processes.* ***8a****: There was a lesion consisting of chondrocyte necrosis (stippled area) and necrotic cartilage canals (asterisks) within the resting and proliferative zones of this 12-day-old Standardbred filly.* ***8b****: Sprouting vessels were seen adjacent to a lesion in this 14-day-old Standardbred colt.* ***8c****: Chondrocyte clusters were seen on the margin of the lesion in this 122-day-old Standardbred colt.* ***8d****: There was a focal delay in enchondral ossification associated with an area of chondronecrosis (cn) displaying matrix eosinophilia in this 122-day-old Standardbred colt.* ***8e****: Small, viable, chondrocyte-like cells were seen within a necrotic, otherwise non-functional cartilage canal in the same foal as in* ***8d****.* ***8f****: Multinucleated giant cells interpreted as chondroclasts were seen within a marrow space adjacent to the area of chondrocyte necrosis in this 21-day-old Standardbred filly.*

Discussion

Materials

The aims of the current thesis were answered using three groups of foals. The Homebred Population of foals was bred from parents with tarsal OC to maximise incidence and thereby enable 2D and 3D microscopic description of early lesions (II-IV). Lesions of OC were found in one or more sites in the tarsus of seven out of the nine foals (II). This incidence is higher than the incidence of radiographic OC in the tarsi of the general population of Norwegian Standardbreds (14.3%, 45), but may be explained by way of heritability (46), combined with the fact that a proportion were likely to undergo spontaneous resolution without becoming clinically or radiographically detectable (21, 26). The histological incidence made the population uniquely suited for morphological description of early lesions of OC in the tarsus. The Early Lesions Population of foals, in which the parent status of OC was unknown, functioned as a control group for the Homebred foals and confirmed that early lesions were morphologically identical in these and genetically predisposed foals (I, II). The inclusion of low prevalence and pony breeds was considered especially advantageous in the Early Lesions Population, and validated the results when early lesions were confined to breeds with a high prevalence for clinical OC (I).

The stifles of the parents of the Homebred Population of foals were not examined radiographically, and lesions were not diagnosed in the distal femur of their offspring at the time of examination (III). The relative frequency of radiographic OC in the hock versus the stifle of a hospital population of Standardbreds was approximately 6 to 1, whereas it in Swedish Warmbloods was 3 to 1 (61). The Post Mortem Population comprised a number of Swedish Warmbloods, and was added to the Homebred Population to enable description of early lesions in the distal femur (III). As it were, three out of the four lesions in the Post Mortem Population were diagnosed in Standardbred foals, and only one out of the four lesions was clinically, radiographically and macroscopically silent at the time of diagnosis (III).

For the reasons discussed above, the Homebred Population of foals was uniquely suited for study of the relationship between the developmental pattern of the blood supply to growth cartilage and lesions of OC within one and the same individual (II). In the stifle, study of this relationship was limited to extrapolation of information between different individuals, and in one case, between different breeds (III). Levene observed species variations in the pattern of cartilage canals, and also claimed minor differences between mountain and lowland breeds of sheep (80), thus the appropriateness of extrapolation between different breeds of horse should be questioned. While breed differences in the pattern of cartilage canals possesses undeniable attraction in terms of the potential to explain variations in breed and joint prevalence for OC (81, 84, 124), the reasons for these may lie on a different level entirely, such as conformation (137).

At the time of the present study, perfusion of cartilage canals was limited to a terminal protocol (II). Previous studies in piglets demonstrated remarkable consistency between individuals of a similar age (20, 138), suggesting that a group size of one might still yield useful results. Perfusion should be carried out in larger groups at different ages and of different horse breeds in future. It is unknown whether the *post mortem* perfusion protocol of Hertsch and Samy is able to demonstrate arterioles within cartilage canals (59), but perfusion of large numbers of foals should probably be delayed until a reliable *post mortem* or ethically acceptable *in vivo* protocol is available.

Ethical concerns over *post mortem* collection of material are negligible, thus the sizes of the Early Lesions and Post Mortem Populations were limited purely by practical and financial constraints (I, III). Foals diagnosed with joint disease other than OC were initially excluded in order to prevent systematic errors of interpretation. A small number was subsequently included, from the point of view that they might provide useful information as long as the joint disease was recorded and disclosed.

Perfusion at regular intervals was chosen to assimilate development over time (II, III). In order to minimise systematic errors, the relationship between the developmental age (gestation + *post natal* age) of foals and the perfusion age they represented should increase linearly. Within a margin of plus or minus one day, the

chosen strategy resulted in a linear increase from conception to perfusion with the exception of one foal (**Table 4**, 2b-weeks old, II).

Table 4: Age Linearity of Homebred Population

Perfusion Age (Weeks	0	1	2a	2b	3	4	5	6	7
Total Age (Days)	344	346	345	352	347	358	364	369	370
Month of Birth	July	June	June	October	May	June	May	August	June

Table 4 *summarises the perfusion age versus total age and month of birth of the Homebred Population of foals.*

Methods

The developmental pattern of the blood supply to growth cartilage was studied using a perfusion protocol that had previously proven reliable for this purpose in piglets in the hands of two of the co-authors (II, III, 20, 138). The main methodological concern was to ensure complete filling of cartilage canal arterioles (80). Pilot studies following the piglet protocol to the letter were successful in perfusing subchondral, but failed to perfuse intra-cartilaginous vessels (138), presumably due to suboptimal perfusion pressure. The piglet protocol was modified as described in Methods, and catheter placement carried out under general anaesthesia to facilitate identification of the femoral artery and minimise the associated temporary loss of pressure (II). The foal was euthanased as soon as pressure could be maintained artificially (II).

The use of cuffed endotracheal tubes as catheters was considered beneficial in terms of minimising leaks at the catheter site (II). The most significant modification to the piglet protocol, however, was the use of an electrical pump with built-in overpressure protection to administer the perfusion liquids (II). Hertsch and Samy carried out perfusion 24 hours *post mortem* using gravitational pressure (59), and *post mortem* perfusion of intra-cartilaginous arterioles should be possible in foals (32), particularly if an electrical pump is used.

Pharmacological agents were used in support of the mechanical efforts to ensure complete perfusion (II). Foals were heparinised after induction of anaesthesia, and the first liquids administered into the perfusion catheter contained vasodilator agents (II, 98). The properties of lidocaine in this respect have been drawn into

question in horses (87), but administration of a local anaesthetic agent was nevertheless considered helpful in terms of preventing reflex constriction of vessels upon the physical stimuli of handling and catheterisation. Micronized barium was imported from the only US producer that could guarantee a median particle size of 0.7-0.8 µm, about $1/10^{th}$ of average capillary diameter in puppies (133). Data on the circulating volume of Standardbred foals did not lend itself to extrapolation by age (94) so were derived from Thoroughbred and Quarter Horse foals instead (121), and 25% of the total volume administered as a generous estimation of the volume within one hind limb.

The Spalteholz clearing technique offers excellent real-size visualisation of contrast-filled structures in cartilage, and adhering to the protocol used in piglets enabled direct comparison between the two (II, III, 138). The main drawbacks of the technique are that it is costly, time-consuming and impractical; the cleared state is volatile and presentation of results tends to be limited to 2D photographic representations of a 3D structure. Tissue clearing techniques are likely to become superseded by quicker and cheaper 3D techniques such as micro-computed tomography.

The choice to examine sample blocks with micro-computed tomography was based on its bone imaging qualities (126), ease of 3D reconstruction and accessibility (IV). Previous scans of material from three cases of OCD in human patients suggested that sample preparation was not required for micro-computed tomography (88), but in a pilot scan of an untreated foal sample block, the cartilage desiccated, shrunk and distorted during the 2-3 hour scan time. Coating with paraffin wax was chosen as this was considered unlikely to compromise subsequent processing for histological examination (IV), and some form of preservation is hereby recommended for micro-computed tomography of cartilage samples in future. Resolution was poorer in sample blocks of the given size, but the facility for 3D volume rendering and virtual sectioning of micro-computed tomography scans in any plane imaginable provided clear advantages over conventional histology (IV). The main systematic error that may have been present would be false negative diagnosis of lesions below a certain size, but the ramifications of such errors are reduced when combined with conventional histological examination (IV).

Predilection sites from the distal femur and tarsus were sawed into slabs and examined with digital radiography or in the stereomicroscope (II, III). Stereomicroscopic examination of slabs from the distal femur was abandoned when the maximal 15 mm field of view permitted visualisation of only one half of the lateral trochlear ridge and a lesser portion of the medial condyle in most foals. Tarsal slabs could, however, be examined in great detail whilst maintaining anatomical perspective due to their maximal dimension of 20 mm (II). Radiographic examination was quick, gave a representation of vessels to all aspects of the distal femur simultaneously and provided sufficient detail to describe the course of cartilage canal vessels and anastomoses with vessels of subchondral bone (III). The ossification front could be identified in spite of prior decalcification by virtue of the brush border of barium-perfused vessels within it (III). Arterioles and capillaries within tarsal cartilage canals, and anastomoses with subchondral vessels, were visualised with superior detail and resolution in the stereomicroscope (II). Continuous adjustment of focus yielded 3D information within the limitation of slab thickness (II).

There are several alternatives for decalcification of bone for histological examination, of which formic acid is considered to have the best all-round properties (3). Decalcification in EDTA takes longer time than in formic acid but may be gentler on tissues and epitopes, so is preferred at the prospect of immunohistochemical staining (3), which remains an option for the current samples. The chemicals involved in Spalteholz clearing were considered to compromise tissue and epitopes to an extent so as not to warrant decalcification in EDTA. Hematoxylin and eosin staining of tissues was considered essential to evaluate cellular viability, whereas cartilage matrix can stain heterogeneously with this technique such that toluidine blue was used for validation of suspected areas of altered matrix staining.

Blood Supply to Epiphyseal Growth Cartilage

The early descriptions of the blood supply to the growth cartilage of equine epiphyses contained herein provide valuable new information on the tissue in which OC is initiated (101). At the outset, information on equine cartilage canals was available mainly in the form of histological descriptions (18, 118). A descriptive histological Paper I therefore formed the basis for subsequent progress on to a

macroscopic level using perfusion and Spalteholz clearing in Papers II and III for the tarsus and distal femur respectively. Micro-computed tomography in the fourth and final paper enabled 2D and 3D visualisation of the relationship between perfused vessels and mineralised subchondral bone, whilst generating information that may be extrapolated to conventional computed tomography in future (Paper IV).

The blood supply to growth cartilage was studied on a global level, i.e. considering each epiphysis as an intact unit, and on a regional level, referring to smaller subunits of the epiphysis such as the predilection sites for OC (II, III). It was also examined on a local level within the different regions, on different aspects of slabs, and within different zones of epiphyseal growth cartilage (I-III).

On a global level, the blood supply to growth cartilage was temporary and disappeared through chondrification and incorporation into the secondary centre of ossification at a relatively early age (I-III), possibly explaining why it rarely features in scientific reports. Its presence for a limited period means that growth can be subdivided into vascular-dependent and –independent phases (I). The vascular-dependent phase probably ceases around 10 weeks of age in the tarsus (II, 18), and between 10-12 months in the distal femur; earlier than this on certain aspects and as early as 5 months in the resting zone (III). The separate blood supply presumably becomes redundant when the cartilage is so thin and quiescent, it may be maintained by other means, i.e. diffusion. Longitudinal radiographic studies indicate that lesions of OC are initiated before the ages of 5 and 8 months in the hock and stifle respectively, thus the vascular-dependent phase agrees with the window of susceptibility to OC (26, II).

The relationship between cartilage thickness and number of blood vessels was not quantified, but subjectively appeared relatively constant, as previously observed in the proximal humerus of dogs (133). There was considerable regional variation in cartilage thickness, resulting in a corresponding variation in the blood supply (II, III). This heterogeneity must result in part from formation of a spherical ossification centre within a rectangular epiphysis or cuboidal bone, and may influence how the consequences of pathological conditions affecting the blood supply to growth cartilage manifest (discussed in II).

The articular-epiphyseal cartilage complex can tentatively be divided into vascular-dependent and –independent zones (I-III). The articular zone contained no

evidence of vascular structures at any age, and seemed capable of survival without a dedicated blood supply (I-III, 133). The deep hypertrophic and mineralised zones appeared similarly disposed, by virtue of being located within diffusion distance of vessels in subchondral bone (II, IV, 20).

An increased proportion of distal cartilage canal termini made a 180º retrograde turn to travel back towards the underlying ossification front in the older foals (II, III). Wilsman and Van Sickle proposed that there were fields of varying metabolic intensity within the proximal humeral growth cartilage of puppies (133), and there may be zonal differences in the requirements for oxygen and nutrients in foals also. Alternatively, immuno-localisation of vascular endothelial growth factor (VEGF) to the hypertrophic zone in the distal femur of poultry was considered to stimulate directional growth of vessels into it and the centre of ossification (11). As the thickness of growth cartilage decreases with age, the relative distance between cartilage canal termini and the hypertrophic zone is reduced. Thus, vaso-proliferative factors like VEGF within the hypertrophic zone of cartilage may explain the hook morphology observed in older foals.

At least four separate morphologies of normal equine cartilage canals could be distinguished; patent, chondrifying, and surrounded by a zone of matrix eosinophilia or relative hypercellularity, respectively (I-III). The morphology of patent and chondrifying canals corresponded to previous descriptions in foals (18, 118).

Figure 9: Cartilage Canal Morphology

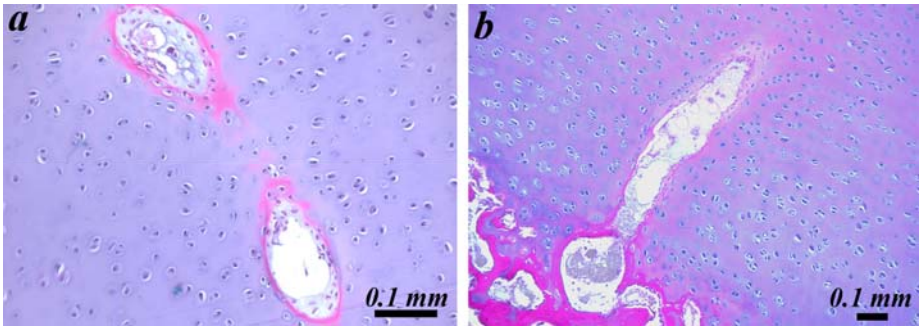

Figure 9 *illustrates morphological variation of normal cartilage canals.* ***Figure 9a*** *shows a canal surrounded by a zone of matrix eosinophilia resembling osteoid within the superficial zones of the growth cartilage of a 12-day-old Standardbred foal.* ***Figure 9b*** *shows a canal surrounded by a zone of hypercellularity at the ossification front of a 0-day-old Standardbred foal. The canal lumen contains patent vessels and does not appear to be chondrifying.*

Cartilage canals in all zones were occasionally surrounded by a halo of intensely eosinophilic matrix that resembled osteoid (**Figure 9a**, I). Canals were present within the epiphysis at a stage when it was entirely cartilaginous, and congregated at the site where the secondary centre of ossification subsequently appeared (I, 118). Early reports implied a role for cartilage canals in formation of the secondary centre of ossification (50, 83), and this role has recently been supported by more substantial evidence (11-13). Wilsman and Van Sickle observed that calcification of the hyaline cartilage model in puppies started around canals rather than in avascular matrix (132). Blumer *et al.* subsequently demonstrated collagen type I, which is typical of osteoid, in a layer around canals, and proceeded to identify bone-forming cells of presumed perichondrial origin within cartilage canals of poultry (11, 12). Enchondral ossification progressed in tubes centred on cartilage canals in the distal lateral trochlear ridge of the talus, and a separate centre of ossification had formed around a patent canal within viable cartilage adjacent to a lesion in one foal (IV). Recognition of ossifying, as well as patent and chondrifying, cartilage canal morphologies may therefore be defended in foals.

The significance of the zone of relative hypercellularity observed around some canals is more difficult to determine (**Figure 9b**, I). A correlation between this morphology and age, if demonstrated, could help clarify the likelihood of it being

associated with active canal invasion if confined to young foals (50, 83), addition of cells to the cartilage model if present in slightly older foals (132), or preparation for chondrification if confined to foals in the late stages of vascular-dependent growth (I).

In addition to the four morphologies pertaining to cartilage canals located in any zone of epiphyseal growth cartilage, above, variations were observed exclusively within the deep zone (I-IV). Volume rendering of micro-computed tomography scans revealed indented dimples or protruding tubes of the subchondral bone plate centred on perfused vessels (IV). Dimples or "gaps" were previously observed on micro-computed tomography of the ulna of puppies and tentatively correlated with sites of biomechanical stress (134). Angiograms in the current study samples afforded the benefit of confirming an association between dimples and perfused vessels, and subchondral bone plate dimples and tubes were similarly interpreted as strategies for the protection of vessels as they crossed the ossification front (IV). On histological examination, cells of the hypertrophic zone were arranged in circles centred on vascular structures in the youngest foals (II, III). Thus, it seems cartilage canals display a range of morphologies that may translate to different composition and requirements of the immediate peri-canalicular, compared to the inter-canalicular cartilage.

In summary, the cartilage canal blood supply to equine epiphyseal growth cartilage demonstrates considerable heterogeneity on global, zonal and peri-canalicular levels. Recognition of these variations is clearly important to avoid errors of interpretation, both when using 2D and 3D examination techniques. A basic understanding in the heterogeneity of the blood supply to growth cartilage may be helpful in understanding the consequences of pathological conditions affecting the patency of cartilage canals.

Osteochondrosis

The results of the current thesis indicate that necrosis of cartilage canal vessels can be the initial step in the pathogenesis of equine OC (I-III, 18). Failure occurred where vessels crossed the ossification front, and in piglets, such failure has been considered the consequence of unfortunate coincidence of incorporation of cartilage canal vessels into subchondral bone during growth, anastomoses of vessels

and bone re-modelling, or micro-fractures, during normal enchondral ossification (137, 139). Incorporation and anastomoses were demonstrated at predilection sites for OC in foals, and vessel failure may be considered a consequence of the same unfortunate sequence of events in this species as in piglets (I-IV, 137).

In contrast to failure of a subchondral arterial source in the region where it crossed the ossification front, vessels reliant upon a perichondrial arterial source were not associated with failure (II). The odds for failure of vessels that crossed the ossification front obliquely may have been marginally greater compared to vessels that crossed it perpendicularly. Vessels emerging from subchondral bone whose midsections were incorporated into the ossification front probably represented the most vulnerable configuration, as they crossed the ossification front in more than one location and did not have access to a perichondrial arterial source at any stage of development (III).

While advocating the above aetiology and pathogenesis as the true, developmental form of OC in piglets, the possibility that generalised factors such as haemodynamic disorders or dietary imbalance could secondarily induce OC-like lesions was recognised by a recent review (137). Concurrent disease only very rarely receives attention in studies of equine OC (53). Six, and a possible further two, of the foals diagnosed with OC in the current thesis suffered systemic disease or the presumed sequelae thereof, and the possibility that lesions were due to primary disease other than OC was considered (I, III). **Table 5** has been compiled listing historical purported causes of vascular failure other than biomechanical force in a variety of species, to complement the causal diagram of the cited review (137).

Table 5: Purported Causes of Vascular Failure

Site of Action	Category	Suggested Cause (Citation)
Intra-lumenal	Structural Functional	• Vascular stasis (71) • Inappropriate chondrification (19, 130, 135)
	Pathological	• Fungal emboli (4) (• Bacterial thrombi (30, 31) • Bacteraemia-associated *Staphylococcal* thrombosis (2) • Lipid emboli from circulation or bone marrow (19) • Septicaemia-associated thrombosis (8)
Mural	Structural Functional	• Vasomotor spasm, possible congenital hyper-reactivity (110) • Overstretching of arteries (38)
	Pathological	• Frostbite (52) • Endotheliotoxic hyperlipidaemia (19) • Pesticide intoxication (100)
Extra-mural	Structural Functional	• Micro-fractures/modelling during enchondral ossification (137, 139)
	Pathological	• Subchondral/intra-osseous abscess compression (28) • Subchondral/intra-osseous haematoma (7) (• Copper deficiency/reduced collagen cross-links (64)

Table 5 *lists historical purported causes of vascular failure other than biomechanical force. All references refer to failure of vessels within cartilage or bone, with the exception of (8), which refers to thrombosis in lung tissue.*

A purely intra-cartilaginous prequel to OC, defined as a delay in enchondral ossification (101), was described in three foals (I, II). This explains how dissected lesions may be observed at arthroscopy in the absence of gross cartilage thickening, and how OCD fragments may be composed entirely of cartilage (84).

Failure of cartilage canal vessels resulted in ischaemic necrosis of chondrocytes within the vascular-dependent resting and hypertrophic zones of growth cartilage (I, II). Necrotic chondrocytes were unable to maintain matrix

composition. The change in matrix staining meant that early and slightly older lesions could be differentiated (I, II). Older lesions with altered matrix staining were located at the ossification front, presumably due to continued advancement of this until it made contact with an original intra-cartilaginous area of ischaemic necrosis (I-IV). The morphology of areas of necrosis was compatible with coagulative necrosis, the form most commonly associated with ischaemia (75). Areas of coagulative necrosis within the resting and proliferative zones would possess relative inappropriate morphology after contact with the ossification front had occurred, but the term dysplasia seems inappropriate for these cells that were unable to differentiate by virtue of being necrotic.

The potential for chondrocyte necrosis centred on patent canals to occur secondary to administration of potentially chondrotoxic drugs in association with systemic disease was discussed in Paper I. The temporal heterogeneity of the blood supply to equine epiphyseal growth cartilage was not known at the time of the study published by Shingleton *et al.*, but the hypothesis that chondrocytes can be selectively exposed to circulating hormonal imbalances has been corroborated by the current thesis (II, III, 118). OC-like lesions that result from circulating pharmacological agents or hormonal imbalances are best regarded as secondary disease of growth cartilage rather than primary OC (137).

Cells and matrix displayed simultaneous pathological morphology in all areas of delayed enchondral ossification, thus the question over the two schools of thought mentioned in the Introduction, of either cells or matrix being primarily responsible for the delay, could not be addressed (56).

Clinical OC in horses has been described as the outcome of pathogenesis and repair (128). In longitudinal radiographic studies, a number of lesions underwent spontaneous resolution (21, 26), and the high prevalence of histological lesions in the Homebred Population of foals (77%) indicated that a proportion of these would have repaired before they became clinically detectable (II). In the case of ischaemic chondronecrosis, pathogenesis must be limited to the vascular-dependent phases and zones of epiphyseal growth cartilage (I-III).

Considerable efforts were made in the sentinel histological studies to differentiate between primary and secondary morphological changes in the pathogenesis of OC (summarised in **Figure 10**, I, III). Historically, it has been

difficult to differentiate the chronic stage of OCD, which qualifies as pathological fracture, from other types of fracture such as ligament avulsions (23). As our clinical ability to identify stages of OC prior to OCD increases, this issue may be resolved, or possibly replaced by difficulties in differentiating OC from other, alternative diagnoses, such as osteomyelitis (53).

Figure 10: Proposed Pathogenesis of OC

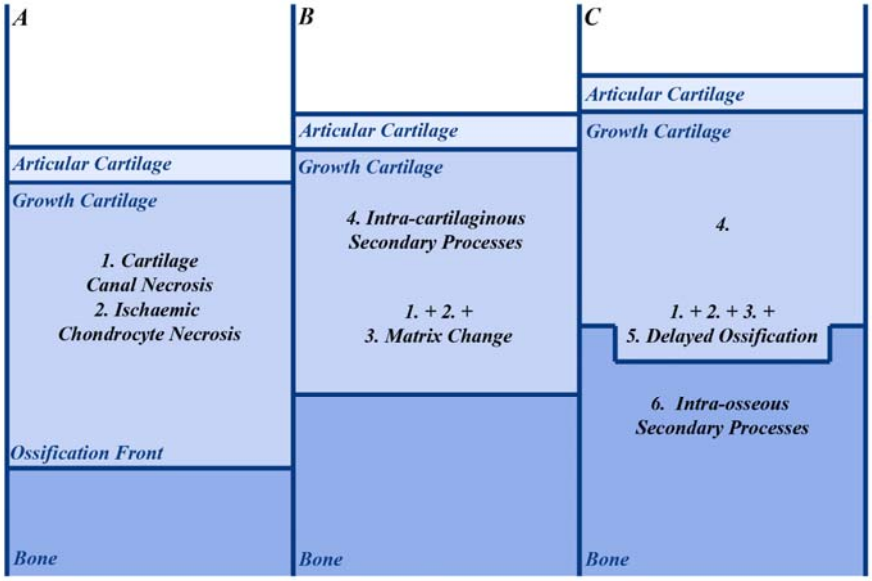

Figure 10 *illustrates the proposed sequence of events (**1.-6.**) in the pathogenesis of OC, and the location in which the events take place. Sections **A**, **B** and **C** represent different points in time, but the real time between sections is currently unknown.*

A: *The primary changes in the pathogenesis of OC are necrosis of cartilage canals (**1**), followed by ischaemic necrosis of chondrocytes (**2**) in the resting and proliferative zones of growth cartilage.*

B: *Necrotic chondrocytes are unable to maintain matrix homeostasis, thus a change in matrix composition (**3**) follows. Intra-cartilaginous secondary repair processes (**4**) such as neovascularisation, chondrocyte clusters and separate centres of ossification are seen contemporaneously with matrix change.*

C: *Upon contact with the advancing ossification front, the area of chondronecrosis (i.e. necrotic chondrocytes and altered matrix, **1.-3.**) resists conversion to bone and causes a delay in enchondral ossification (OC, **5.**). Secondary repair processes within bone (**6.**) are seen at this stage, such as chondroclasia and stem cell migration into the area of chondronecrosis.*

Distinguishing primary and secondary events in the pathogenesis is also considered to be of major importance to the interpretation of the results of future molecular biological and genetic studies. The secondary repair processes observed within growth cartilage and bone in the current thesis were similar to those of experimentally induced cartilaginous fractures in rabbits (I-IV, 14).

Repair of experimentally induced fractures depended on a wide pedicle, stable fragment and adequate collateral blood supply (14). These criteria may tentatively be extrapolated to repair of OCD lesions in foals. Vascularity decreased, and the number of vessels dependent on a subchondral arterial source increased, with age, and the potential for repair to be initiated around a collateral vessel with an alternative, preferably perichondrial arterial source, would diminish towards the late phases of vascular-dependent growth. The medial femoral condyle predilection site for OC seemed particularly vulnerable in this respect (III), and the distal lateral trochlear ridge of the talus was considered to have a lower reparative potential compared to the medial ridge by virtue of the beaked versus tapering anatomical shape (II).

Biomechanical forces were discussed for the initiation of lesions in the respective papers, and may influence the potential for repair of OCD lesions (I-III, 14). In human orthopaedics, epiphyses are subdivided into load-bearing pressure epiphyses, tendinous or ligament attachment traction epiphyses, and vestigial bone or atavistic epiphyses (44). An adaptation of this classification to fit sub-sections of a single epiphysis may facilitate description and understanding of the pathogenesis of OC in horses. Prolonged vascular dependence alone provided a satisfactory explanation for two sites (II), whereas additional factors of biomechanical trigger forces or reduced reparative potential were required in order to explain other sites as predilection sites for OC (II, III). The results of vascular dependence, epiphyseal classification, biomechanical forces and repair potential for the predilection sites examined within the tarsus and distal femur are summarised in **Table 6** (I-III).

Table 6: Predilection Sites for Osteochondrosis in the Tarsus and Distal Femur

Predilection Site	Vascular Dependence	Epiphyseal Classification	Biomechanical Forces	Reparative Potential
Intermediate Coronoid Process	Third longest in the distal tibia	Trochlear Ridge	• Central tarsal bone impingement • Lateral trochlear ridge of talus impingement	• Intermediate collateral supply • Poor stability due to continued impingement
Medial Malleolus	Longest in the distal tibia	Traction	• Traction from short collateral ligament	• Intermediate collateral supply • Poor stability due to continued traction
Lateral Trochlear Ridge of the Talus	Longest in the talus	Trochlea	• Intermediate coronoid process impingement	• Intermediate collateral supply • Poor stability due to continued impingement
Lateral Trochlear Ridge of the Distal Femur	Second longest in the distal femur	Trochlea Traction	• Patella impingement axially • Long digital extensor traction laterally	• Intermediate collateral supply • Poor stability due to continued traction
Medial femoral condyle	Shortest in the distal femur	Pressure Traction	• Weight bearing centrally • Cruciate ligament traction axially • Intercondylar eminence impingement axially • Meniscal ligament traction laterally	• Poor collateral supply • Poor stability due to continued weight bearing and traction

[a] *Classification adapted from (44),* [b] *Based on criteria of (14). Intermediate collateral supply: available from adjacent subchondral or perichondrial sources. Poor collateral supply: available from adjacent subchondral sources only.*

The vascular studies of the current thesis were focussed on predilection sites for OC (II, III), but comparison to non-predilection sites could prove rewarding. The

proximal medial trochlear ridge of the femur had the longest period of vascular-dependent growth, but is not a predilection site for clinical OC (III). It should be determined whether subclinical lesions of OC arise within such sites, and if so, what mechanisms exist to prevent them becoming clinically manifest. This may elucidate spontaneous repair mechanisms, with potential applications for future treatment and prevention of OC.

McIlwraith and Pool have implied involvement of intermittent or temporary factors in the pathogenesis in order to explain clinical features of OC (84, 97). Involvement of cartilage canals provides such intermittent or temporary factors on two separate levels. Firstly, incorporation of canals into subchondral bone, and formation of anastomoses, occurs intermittently during the vascular-dependent phase of growth (II, III). Secondly, areas of ischaemic chondronecrosis are present temporarily in the interval between their initiation and potential subsequent repair (I-IV).

On a global level, growth cartilage should be considered a functional unit comprising three interdependent components of chondrocytes, matrix and cartilage canals (**Figure 11**). Chondrocytes, matrix and subchondral bone are present throughout life, and morbidity of these components need not be confined to the period of skeletal immaturity. In contrast, the vascular supply to growth cartilage within cartilage canals is only present during the early phases of growth, and conditions affecting the patency of this blood supply may only be of significance during the early phases of development and growth.

Figure 11: The Functional Unit of Epiphyseal Growth Cartilage

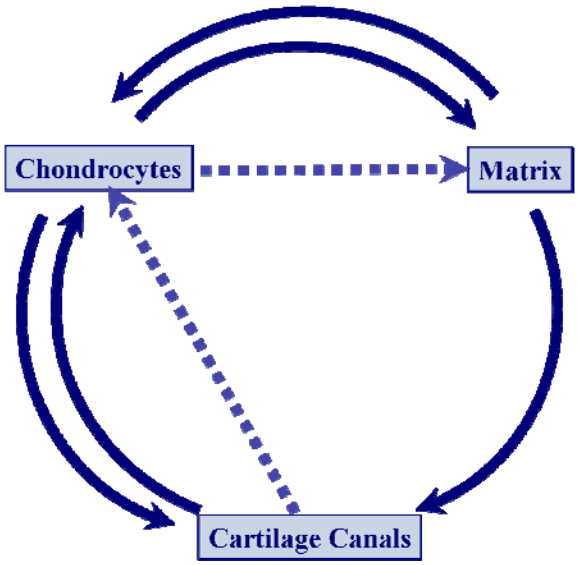

Figure 11: *Epiphyseal growth cartilage may be considered a functional unit consisting of chondrocytes, matrix and cartilage canals. Solid arrows indicate physiological relationships, whereas the stippled arrows indicate the pathogenesis for OC. Chondrocytes maintain matrix composition, and are a source of factors that are important to the survival of cartilage canal vessels such as vascular endothelial growth factor. Matrix provides structural support for chondrocytes and cartilage canals. Cartilage canals provide oxygen and nutrition to chondrocytes. Failure of cartilage canals results in ischaemic necrosis of chondrocytes, with subsequent failure to maintain matrix composition.*

The simultaneous definition of human OC as a disturbance of enchondral ossification and a sequel to primary disease of subchondral bone is slightly difficult to comprehend in view of the fact that subchondral bone is, by definition, already ossified (120). Two examples of subchondral bone disease that may affect the patency of cartilage canal vessels were listed in **Table 5**, but the resulting OC-like lesions will then inevitably be due to primary subchondral disorders that may arise at any age, rather than primary developmental OC (7, 28).

Putative aetiologies for OC should be assessed as systematically as possible in terms of their effects on chondrocytes, matrix and cartilage canals respectively, as this may help clarify their role in true developmental versus any age disease. For example: Hurtig *et al.* originally demonstrated reduced cross-linking of collagen molecules in the cartilage of copper deficient foals (64). In view of the mixed pattern

of lesions commonly seen in copper deficient foals (97), it might have made an interesting undertaking to quantify cross-links in vascular wall elastin molecules. Hurtig later concluded that the lesions observed in copper deficient foals were osteochondral fractures due to inferior quality of subchondral bone rather than developmental OC (68), and in this context, it was used purely for illustration purposes.

Although potential alternative causes of vessel failure and chondrocyte necrosis have been discussed, we maintain that true developmental OC in foals most likely occurs due to coincident unfortunate events like incorporation of cartilage canals into subchondral bone, anastomoses of vessels and modelling of the ossification front during normal enchondral ossification, resulting in failure of cartilage canal vessels at the ossification front and ischaemic necrosis of chondrocytes within the resting and proliferative zones of growth cartilage. Relative prolonged vascular-dependence alone feasibly explained some predilection sites, whereas additional factors, some of which have been suggested (**Table 6**), are required in order to explain other predilection sites for OC.

Conclusion & Future Prospects

Conclusions

The conclusions of the current thesis are as follows:

• The earliest lesions of OC in the tarsus of foals consisted of ischaemic chondrocyte necrosis within the resting and proliferative zones of growth cartilage.

• Areas of ischaemic chondronecrosis in slightly older lesions of OC in the tarsus and distal femur of foals were associated with a change in matrix staining and variable delay in enchondral ossification.

• Areas of ischaemic chondronecrosis within macroscopically visible lesions of OC in the tarsus and distal femur of foals were associated with fissures and cysts.

We conclude that ischaemic chondronecrosis is a cause of OC, OCD and cysts in foals.

• Cartilage canals arose from the perichondrium and subchondral bone during the ages examined.

• Vessels became incorporated into subchondral bone during growth, and a proportion shifted to use subchondral vessels as their arterial source.

• Areas of ischaemic chondronecrosis were not observed in regions of growth cartilage supplied by cartilage canal vessels with a perichondrial arterial source.

• Areas of ischaemic chondronecrosis were consistently found in regions of growth cartilage supplied by cartilage canal vessels obliged to cross the ossification front.

We conclude that incorporation of vessels into subchondral bone during growth results in the vessels becoming vulnerable to interruption in the region where they cross the ossification front.

• Micro-computed tomography was able to detect the earliest lesions of OC in tarsal growth cartilage in the presence of barium angiograms.

• 3D volume rendered models of micro-computed tomography scans produced high quality images of the delay in enchondral ossification associated with areas of ischaemic chondronecrosis.

• 3D volume rendered models of micro-computed tomography scans demonstrated how centres of ossification could form around patent vessels adjacent to areas of ischaemic chondronecrosis (Paper IV).

We conclude that micro-computed tomography is suitable for examination of early lesions of OC in foals and provided new information on the pathogenesis of OC that may be extrapolated to conventional computed tomography in future.

Future Prospects

In order to advance our understanding of OC, the following issues should be researched:

• Attempts should be made at inducing OC by surgical interruption of cartilage canal vessels in foals, as has been done in piglets, in order to validate the proposed sequence of events.

• Molecular biological and genetic techniques such as immunohistochemistry and *in situ* hybridisation should be employed in the investigation of OC in order to improve understanding and early diagnosis with the potential for *in vivo* application.

• The results of micro-computed tomography scans should be transferred to conventional computed tomography in live foals.

• The potential of magnetic resonance imaging for diagnosis of focal cartilage ischaemia should be investigated.

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