Development and Clinical Significance of Side Bones in Cold-blooded Trotters

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Cover: Equine pedal bone >1.2 million years old with ossified ungular cartilages (Photo courtesy of Dr. Ove Wattle, SLU)

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Abstract

Ossification of ungular cartilages (OUC) is apparent on fossil P3 bones and seems to be as old as the history of Equus. OUC has been scientifically studied at least since the 18th century. Traditional diagnostic techniques, palpation and pathology, have more recently been replaced by radiographic techniques, scintigraphy and magnetic resonance imaging. However, these new techniques have not yet been fully validated for OUC and have added little to the current understanding of how the ossification process arises, develops and influences performance in a population perspective. Based on results from studies using palpation as a diagnostic tool, stallions with a high degree of OUC are not allowed to breed and material from horses with OUC cannot be stored in gene banks. In this thesis, different grades of OUC in 649 cold-blooded trotters, with performance parameters from 23,556 races, were studied and compared. The results revealed significant relationships between gender and performance, but not between different grades of OUC and performance. The proximal ossification process of OUC decreased significantly after three years of age, when only a few of 2,591 cartilages examined changed their grade of OUC. An improved grading system to achieve more consistent and reliable assessment of radiological findings was devised. This system, encompassing four grades instead of six, does not use navicular bone and palmar level of distal interphalangeal joint as reference points. It is thus more forgiving of individual anatomical variations in P3 and of slightly uneven positioning/loading of hooves at time of exposure. Overall, the results suggest that OUC is either a physiological variation or an adaptation to unknown stimuli early in life, as many years of intense training and racing appeared not to affect the presence or development of OUC, confirming previous reports of moderate to high heritability. Based on these findings, excluding stallions with high OUC grades will not improve equine welfare. However, the influence of environmental factors before three years of age on the extent of OUC warrants future study.

Keywords:

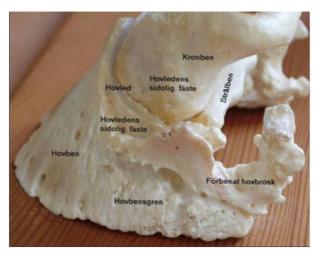
Side bones; sidebones; radiology; equine, horse; ossification; performance; ungular cartilage; collateral cartilages; statistical methods

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Dedication

To previous, present and future professional equine practitioners struggling to evaluate clinical findings and their correlation to diagnostic imaging.



Bones of the equine hoof (anatomical structures labelled in Swedish). (Photo courtesy of Dr. Ove Wattle, SLU.)

Contents

Ackr	nowledgements	47
Refe	erences	39
8	Future prospects	37
7.3	Methodology perspective	35
7.2	Clinical and performance perspective	33
7.1	Development perspective	31
7	Results and discussion	31
6	Statistical methods	29
5	General methods	27
4	Material	23
3	Aims of the thesis	21
2	Hypothesis	19
1	Introduction	13

List of Publications

This thesis is based on the work contained in the following papers, referred to in the text as Paper I and Paper II.

- I Ulf O Hedenström, Ulf Olsson, Arne W Holm, Ove S Wattle. Ossification of ungular cartilages in front feet of cold-blooded trotters – a clinical radiographic evaluation of development over time (2014). Acta Veterinaria Scandinavica 2014, 56:73.
- II Ulf O Hedenström, Ove S Wattle. Significance of ossified ungular cartilages regarding the performance of cold-blooded trotters. *Acta Veterinaria Scandinavica* 2014, 56:74.

Papers I and II are reproduced with the permission of the publishers. The contribution of the author (UOH) to the papers included in this thesis was as follows:

- I OSW designed the work. UOH and OSW collected data and UOH wrote the manuscript with the help of the co-authors.
- II OSW and UOH designed the work. UOH collected data and wrote the manuscript with help of OSW.

Abbreviations

AIC: Akaike information criterion BLUP: best linear unbiased prediction value BPM: beats per minute BSS: body size score CT: computed tomography DNA: deoxyribonucleic acid kV: kilovolt mAs: milliampere second MRI: magnetic resonance imaging M/S: metres per second, trotting pace NS: new scale OUC: ossification of cartilages in distal phalanx PSO: possible significant ossification RS: Ruohoniemi scale SE: standard error

1 Introduction

Horses are continuously being selected and rejected for training, racing and breeding. These decisions can be subjective or objective and may or may not be based on evidence-based knowledge. Practical and clinical aspects of different conditions in distal limbs of athletic or working horses have always been of vital importance for equine welfare and the horse industry. In modern veterinary equine practice, symptoms are often subtle. Reduced performance potential is often the only obvious clinical sign present to correlate to physical examination and diagnostic imaging. Other aggravating circumstances are protracted physiological adaptations coexisting with possibly significant pathological lesions, making correct diagnosis difficult or sometimes even impossible. Looking back at the history of veterinary medicine in general and the history of OUC in particular, the literature contains a certain amount of inherited opinions and anecdotes, so humbleness is a good starting point for the reader.

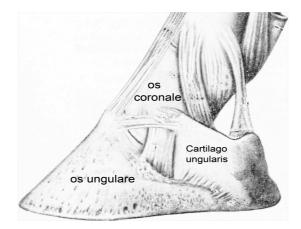


Figure 1. The five ligaments (*l. chondrocompedalia, l. chondrocoronalia, l. chondroungularia collateralis, l. chondroungularia cruciata* and *l. chondrosesamoidea*) that attach each ungular cartilage to nearby bony structures.

The collateral cartilages of the foot are C-shaped structures attached to the bony structures of distal phalanx (Figure 1). The cartilages extend proximally to the coronary band and in palmar direction along and sometimes beyond the distal phalanx. They vary in size and are associated with the digital cushion and thereby theoretically playing a role to reduce concussion to structures within the foot (Bowker 1998). At birth cartilages are hyaline (Bragulla 1999) and during the postnatal period they, like all tissues of the hoof, adapt to workload, i.e. bodyweight and environmental factors. The hyaline cartilage gradually becomes fibrous and sometimes this process transitions into ossification.

The history of side bones or ossification of ungular cartilages (OUC) seems to be as old as the history of horses, since OUC can be seen on Equus fossil P3 bones >1.2 million years old, found in North America (see cover picture). OUC has been discussed in classic veterinary books such as "Cours d'hippiatrique" (Lafosse 1772). Palpation was once the only existing diagnostic tool and diagnosis could possibly be confirmed upon examination of tissue by the naked eye or light microscopy (Lungwitz 1889). Seminal works reported that OUC could cause lameness and correlated pathological findings to sidebone prevalence (Witte 1906; Eberlein 1908). Lafosse (1772) stated that the ossification process starts at the base of the cartilage, *i.e.* at the interface between the third phalanx and ungular cartilage, and proceeds in the proximal direction. At that time, OUC was considered to be the result of hard work on hard surfaces early in life and lameness, while abscesses and fistulation were clinical signs frequently reported in connection with OUC (Mangin 1841). Furthermore, methods such as cauterization and surgical removal of ossified cartilages were used when trying to treat distal limb lameness until just a few decades ago.

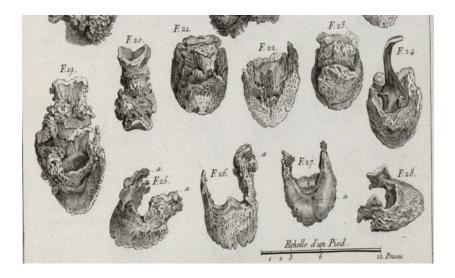


Figure 2. Illustration from "*Cours d'hippiatrique*" showing different degrees and appearances of side bones (Lafosse, 1772).

Witte (1906) performed light microscopy studies on ungular cartilages from 130 horses and reported different stages of the ossification process. The first in-depth study of the development of the ungular cartilages themselves was performed some 93 years later, by Bragulla (1999).

Witte (1906) argued that OUC is a condition that develops secondary to osteitis of the distal phalanx (P3) and to joints of the middle and distal phalanxes. However, the connection between OUC and lameness was questioned already then and Eberlein (1908) suggested that only some 5% of horses with OUC showed clinical signs of lameness and that the signs of lameness in these individuals mainly originated from inflammation of the solar dermis. In the early literature, it was common to study OUC in connection with lameness and other maladies such as osteitis, 'ring bone' and dermal inflammation.



Figure 3. Collection of coffin bones with OUC. Palpation and examination of tissue by the naked eye or microscopy were the only available diagnostic methods until the arrival of radiology in 1959. (Photo courtesy of Daniel van der Blij.)

From the mid-20th century onwards, it became more common to study OUC as a separate condition with the focus on prevalence and heritability. Protocols from pre-breeding evaluations of stallions were used in several studies when calculating hereditary as a component of OUC (Robertson 1932; Wirstad 1951; Waxberg 1953; Bengtsson 1983). Based on an order from the Swedish parliament, from 1939 onwards stallions of Swedish Ardennes draught horses had to be examined and excluded if OUC was found at pre-breeding evaluations. However, this breeding measure had very little effect on its prevalence, since 95% of 60 randomly selected Swedish Ardennes had OUC 70 years later (Tullberg and Wattle, 2008).

Gösta Bengtsson was one of the first in Sweden to use radiography when examining horses with OUC, at National Equine Centre Wången back in 1959 (Jämthagen 2003). When radiology was introduced, it became obvious that palpation was an imprecise diagnostic method, so radiographs became the new gold standard for studying OUC in living horses (Figure 4).



Figure 4. Radiograph from a clinical case with worried owners seeking information and support before buying, breeding or racing horses.

Since 1982, radiographs of front hooves are mandatory in pre-breeding evaluation of stallions. In Bengtsson (1983), heritability was estimated at 0.68. This is a remarkably high and today somewhat questionable value, which indicates that more than two-thirds of OUC variation in a population of horses is inherited and less than one-third can be attributed to environmental factors influencing the ossification process at an unknown time during life. Similar findings of moderate to high heritability have been reported for other breeds (Sergio *et al*, 2002). Recent genetic studies in cold-blooded trotters propose the use of new and more reliable DNA- and calculation methods to estimate the potential threat of increasing inbreeding (Klementsdal 1993 and 1999; Olsen *et al*. 2012). However, heritability and inbreeding are not covered in the present work.

Based on two interesting previous studies (Bragulla 1999; Lejeune *et al.* 2006), the main hypothesis in this thesis is that OUC is a physiological condition that mainly develops during the first years of life, at the same time as the development, maturation and adaptation of bone and other tissues in the third phalanx. Although early hoof development is discussed and described in the literature (Colles 1983; Becht *et al.* 2001; Redden 2003; Kummer *et al.* 2004), there is a lack of scientific data on normal development of the third phalanx in different horse breeds, making this an interesting field for research.

A second hypothesis was formulated based on several studies suggesting a clinical impact of OUC (Honnas *et al.* 1988; Körber 1991; Dyson and Marks 2003; Ruohoniemi *et al.* 2004; Lejeune *et al.* 2006; Mair and Sherlock 2008; Dyson *et al.* 2010; Dyson and Nagy 2011). A common explanation offered for clinical impact/lameness is that OUC causes reduced plasticity in proximal parts of the hoof capsule, theoretically causing higher force amplitude in the supportive tissues when working. Mair and Sherlock (2008) and Dyson and Nagy (2011) suggest that OUC plays a clinically significant role when collateral or other nearby ligaments are injured from a modern diagnostic imaging, rather than clinical, point of view. The questionable term 'possible significant ossification' (PSO) is established in the literature (Ruohoniemi *et al.* 1993; Dyson and Nagy 2011), but has never been further specified or evaluated.

One suggestion based on radiology and performance in 37 draught horses goes against the current stream of opinions and maintains that OUC is a non-relevant finding from a clinical and locomotion point of view (Fiorentino 2003). Verschooten *et al* (1996) drew similar conclusions using radiology and computed tomography in warmbloods.

The special cases of occasional OUC with a clearly traumatic origin (Dakin *et al.* 2006) are not covered in our present work.

The aim of the present work was to study putative clinical manifestation of OUC in correlation with official sports data. Swedish and Norwegian sports data are easily accessed and collected and are considered very reliable because of stringent betting and breeding regulations. Officials at the race track make few mistakes and minor human errors in protocols are usually corrected immediately afterwards by employees at trotting associations. The use of official sports data is well established in equine research (Jörgensen *et al.* 1997; Cheetham *et al.* 2010).

2 Hypotheses

Two hypotheses were tested in this thesis work:

Hypothesis 1: OUC is a condition arising early in life, synchronous to development and adaptation of the third phalanx.

Hypothesis 2: OUC of some degree causes low-grade clinical problems and thereby affects the performance of racing cold-blooded trotters.

3 Aims of the thesis

The aims of the work presented in Papers I and II were as follows:

Aim 1: To investigate development of OUC in cold-blooded trotters using follow-up radiographs from studies in Norway (Holm *et al.* 2000) and consecutive radiographic examinations of cold-blooded trotters in Sweden performed 2005-2009.

Aim 2: To correlate official sports data to different degrees of OUC in order to determine the clinical significance of this condition.

Aim 3: To improve currently used methods of evaluation and, if supported by the results, propose an update of breeding regulations for horses with OUC.

4 Material

Racing with trotting horses dates back to 1555, when the first official sledge race on ice was described (Breeding Plan 2001; Uhlin 2007). In 1778 the Russian Orlov trotter was established as a breed. Over the years, standard-bred and cold-blooded trotting racing horses were developed and later became separate breeds in both breeding and racing. In the early history of cold-blooded trotting, a pure breed horse was not guaranteed at the race track. From 1965, the Swedish cold-blooded trotter has its own studbook, separating it from warm-bloods and draught horses (Uhlin 2007; Breeding Plan 2011). Blood typing and later DNA testing have been used for over 40 years to guarantee the purity of breed in order to ensure official racing under fair conditions. Today, there are about 10,000 cold-blooded trotters in Sweden and in total about 40,000 very similar horses in a unique population only existing in Scandinavia (Official Swedish and Norwegian Sports Data 2010-2015). Weight and height have changed over the years and today an average cold-blooded trotter is about 150-155 cm over the withers and weighs in at about 500 kilograms.

The cold-blooded trotter is under target-orientated selection, training hard early in life, often racing for many years and thereafter often kept as a pleasure horse. There are two kinds of cold-blooded trotters in Scandinavia (Figure 5). Sweden and Norway have a common breeding plan for cold-blooded trotters since the year 2000, while Finland has had a very similar breed of its own for a millennium. These two breeds sometimes race against each other in competitions, but have not yet officially interbred. Today, cold-blooded trotters make up about 10% of the multi-billion SEK, the top three in the world, Swedish trotting industry (www.travsport.se).

Unlike in thoroughbred racing, the speed of racing trotters has increased notably over the years. In 1934, the best cold-blooded horses could trot at 10m/s, in 1965 at 11 m/s and today at over 12 m/s over a distance of 2000 m. Selective breeding, improved training methods and better medical treatment contribute to an improvement in average racing speed in young horses of 1-1.5 seconds/km every five years (Uhlin 2007; Official Swedish and Norwegian Sports Data 2015).

The cold-blooded trotter normally starts training at 1-1.5 years of age and today eight out of 10 horses start racing at sub-maximal levels when they are 2 years old. These 'baby' races provide a premium to all owners of sufficiently trained and educated horses maintaining a speed of 8 m/s and keeping their trotting gait over a 2140 m long distance on a 1000 m race track oval. For horses between 3-15 years of age, there are about 1000 races with organised betting every year, many of which take place in northern Sweden. For horses within this age interval, racing means a maximal physical performance event with heart rate well over 200 beats per minute (BPM) for several minutes on outdoor tracks with curves often designed for the faster warm-blooded trotter.

Many aggravating circumstances are present for the equine veterinarian trying to exclude OUC in relation to other sport-related orthopaedic problems. Horses often appear healthy, sound and without symptoms that can be palpated or provoked. In these patients, lameness, if present at all, is often low grade. Clinical signs are absent from a general medical point of view, but appear obvious and real to owners, trainers and equine professionals from a sports medicine perspective. Diagnostic anaesthesias are too non-specific and subjective to separate possible effects of OUC from other orthopaedic conditions in nearby tissues. Signs of lameness, regardless of diagnosis, may occur only close to fatigue or may show a non-consistent pattern because of speed or race track surfaces.

In all Scandinavian cold-blooded trotters, OUC is considered a problem and is mentioned as one of the top 10 reasons for lameness in cold-blooded horses (Ertola and Houttu 2011). Therefore, since the 1980s stallions with highly ossified cartilages have been disqualified in pre-breeding evaluations. This measure has been debated and appealed against, since a good number of disqualified horses have had long and successful careers before receiving this verdict (Figures 5 A-B). In evaluation of Finnhorses, with OUC breeding regulations give a small opening for discussions about future breeding if overall breeding value is considered to be high.

In Sweden, any grade of OUC is disqualifying for horses of all breeds when evaluated for storage of semen, eggs or other tissues used for official gene banks (Official guidelines for frozen semen used in gene banks). A Swedish Court of Appeal has established that OUC is a hidden defect and a likely cause of lameness (Svea Hovrätt 2011). This verdict may now be used as a precedent in Swedish courts of law. Between 2002-2009, the Swedish courts heard 22 cases of compensation claims for hoof-related hidden defects, mostly OUC or laminitis (Horses and the Consumer Purchases Act 2010).





Figure 5. A) Famous Swedish stallion and B) famous Norwegian stallion that both have been rejected from breeding, solely or partly, because of high-grade OUC. C) An approved Finnhorse stallion representing a very similar breed with a different genetic origin. (Photos courtesy of Erik Widén.)



5 General material and methods

Due to good availability of sports data and the high number of hardworking horses within the breed, the cold-blooded trotter was considered an excellent model for evaluating OUC in this thesis. Furthermore, any inability to maintain gait or speed causes trainers to seek help from equine professionals, usually veterinarians or farriers.

In the present work, a Swedish-Norwegian population of cold-blooded trotters, with a total of 649 horses, was examined. Of these horses, 147 were examined on two occasions with a nine-year mean and median interval. The detailed age distribution of these horses is shown in Table 1 in Paper I.

Because of strict betting regulations, reliable official identification and detailed racing data for the last 40 years are available in Sweden and Norway. These data were used in the present work.

For many years, palpation was the only tool available when evaluating OUC in living horses. However, this method is subjective and has not been evaluated in scientific studies, so its use has been questioned (Attrell *et al.* 1983). For that reason, the experienced equine practitioners involved in the present research tried to palpate and subjectively classify OUC immediately before making radiological examinations.

The re-examination was performed in standardised ways both under field conditions and at equine clinics, as described in Papers I and II. Radiographs were taken by the Swedish research team and by Norwegian colleagues, in the field and at different equine clinics. Mud was brushed off the hooves prior to examination in order to improve picture quality and prevent false positive kernels. Registration of kernels was only made in pictures of sufficient quality. However, subjectivity became an issue again when the radiographs were being interpreted and classified. Clinically relevant diagnosis was difficult because of variations in examination techniques between the clinicians supplying the radiographs. When evaluating radiographs taken on the same individual, but on different occasions, it became clear that less varying landmarks were needed, since the extent of ungular ossification should not diminish with time. Every radiograph was presented blind to the interpreter (Dr. Ove Wattle) and read on two separate occasions when evaluating the grading systems (Figure 6), as described in Papers I and II.

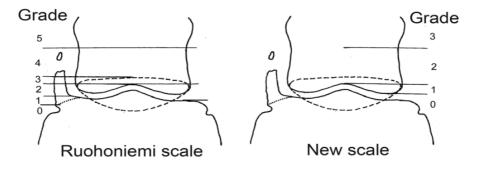


Figure 6. The new proposed grading system for OUS, which allows normal variation in bone mass in the third phalanx and is not dependent on the current position of the navicular bone.

Comparability of the two populations was monitored through three measures:

1) The re-examined population was found to be spread geographically over a central third of Scandinavia, often kept as pleasure horses, making them likely to be without any connection to OUC.

2) The prevalence of OUC was continuously monitored as the number of horses increased.

3) The breeding index (best linear unbiased prediction value, BLUP) (Arnason, 1999) was continuously compared to secure genetic and performance equality between examined and re-examined groups. BLUP is a well-established genetic evaluation tool for selection of horses and production animals.

In order to estimate body size, a score (body size score, BSS) was created and applied to 100 of the horses involved in the studies using a measuringtape/stick and adding height over the withers (cm) to chest circumference (cm).

6 Statistical methods

It was suspected that degree of OUC (an ordinal parameter) increased while the horse was young, but stopped increasing at a certain unknown age. Hence, data were analysed using piecewise regression methods (Quant, 1958) where the predictor function is linear with the slope β_1 when the age of the horse is less than *c* years and with the slope β_2 thereafter. This ordinal piecewise regression model was implemented using the NLMIXED procedure of the SAS package (SAS, 2008). Extension of the model into a mixed model for two hooves on each horse was also discussed. Four ordinal regression models with different model assumptions were tested (Akaike, 1973).

The extent of OUC was graded ordinally using two grading systems rating either from 0 to 3 or from 0 to 5 (Figure 6). Four measurements were made on each horse, at the lateral and medial positions of each front hoof. To account for this, generalised linear mixed models (Olsson, 2002; Littell *et al.*, 2006) were used for the analyses. The response variable was modelled using ordinal logistic models with a multinomial distribution and a cumulative logit link function. The horse was used as a random factor. The Glimmix procedure (SAS, 2008) was used for these analyses. A similar generalised linear mixed model was used to compare the maximum assessment between the two diagnoses for each horse. For this too, horse was used as a random factor. The model included sex, age, BLUP, BSS, time point, money gained, race results, presence of kernels (Figure 8) and total number of races completed during the career. The horses were also evaluated by number of races completed during their career without remarks about irregularities in gait.

The distribution of the variable 'amount of winnings' was rather skewed with data from a few successful horses with high-grade OUC. To reduce the effect of possible outliers, this variable was re-analysed in log-transformed form.

A value of P<0.05 was considered significant in all statistical analyses.

7 Results and discussion

7.1 Development perspective

The ordinal regression model used suggested that the degree of OUC increases until the horse is about three years old (2.83 ± 0.38) and remains rather stable thereafter (Figure 7). Before the age of three, the slope of the linear predictor was significantly greater than zero (p=0.0025). This means that the estimated level of OUC increased. After this age, the slope was close to zero, but not significantly so (p=0.3084). This suggests a correlation between age and grade of OUC that no longer exists after the calculated break-point in age.

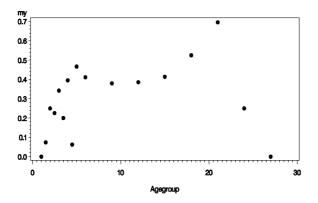


Figure 7. Illustration of the break-point (2.8 years) at which grade of OUC no longer increases significantly in cold-blooded trotters.

The extremely high prevalence of OUC in some heavy breeds (Sergio *et al.* 2002; Lejeune *et al.* 2006) is a fact that alone requires careful consideration regarding what is normal or not in a population perspective. Hoof size and ungular cartilages are normally larger in the front than in the hind hooves. This can either be caused by evolution or, as proposed by Bragulla (1999), by gradual adaptation to strain early in life. In horses three years and older studied in the present work, only 7/208 (3.4%) of the cartilages examined increased their grading of OUC according to the new scale devised. This implies a low impact of environmental factors, especially in a population of horses in full training and racing at that age.

In contrast to studies in 202 Finnhorses by Ruohoniemi *et al.* (1997), there were no correlations between grade of OUC and sex, BSS or medial/lateral positioning in the horses studied here (Papers I and II). This difference in results may be due to differing genetic origin of the breeds, or to a different population perspective.

From the work in Papers I and II, it was concluded that OUC is a physiological condition arising and stabilising before the age of three years in cold-blooded trotters. Thus, the results obtained strongly support Hypothesis (1) that OUC develops synchronously with development and adaptation of the third phalanx after birth.

7.2 Clinical and performance perspective

General sports data for the population studied in Paper II are shown in Table 1. A highly significant relationship between gender and performance was observed. This was an expected finding, since gender differences in performance are well known, although not scientifically evaluated previously. The significantly better performance of male horses indicated no animal welfare problems and is already partly compensated for by a 20-m distance handicap when different genders are raced together and by separate races only open for mares. Irregular gait at high speed is a clinical sign often reported by owners of cold-blooded trotters with OUC. This biomechanical phenomenon is rarely and somewhat subjectively registered at race tracks. In Paper II, there was a tendency (p=0.08) for horses with OUC grade 1 and 2 to receive official notification of irregular trot, but not for horses with grade 3 (NS). However, these results must be considered unreliable because of the subjective recording methods, with a potential risk of repeat notifications after an initial finding. Official notifications of irregular trot are no longer made in Sweden, having been replaced by other animal welfare regulations. Irregular gait at high speed in different horse breeds has recently been explained from a biomechanical and genetic perspective by scientific breakthroughs, which account for many gaitrelated concerns in cold-blooded trotters (Andersson et al, 2012). The existence of any genetic connection between irregular gait and OUC has not been studied.

No correlations were found between different positions or grades of OUC and number of starts, running pace, race winnings and number of races completed, or trotting or breeding index.

Proximal centres of ossification (kernels) were found in 7% of the horses examined, but these kernels had no effect on performance parameters. Regardless of grading system, the presence of kernels implies high-grade OUC, as explained in Paper I. The results obtained here suggest that no special attention should be paid to proximal origin OUC.

Possible significant ossification (PSO) is a term established by Ruohoniemi *et al.* (1993) and Dyson and Nagy (2011) based on studies using indirect means of correlation between OUC and clinical signs. However, based on the results in this thesis and from a population perspective, PSO is an unspecific and irrelevant term for scientific use.

The data indicate that OUC is not likely to cause any low-grade clinical problems in cold-blooded trotters or to have any effect on short or long-term performance potential. Hence Hypothesis (2), that OUC of some degree causes

low-grade clinical problems and thereby affects the performance of racing cold-blooded trotters, must be rejected.

Number of horses included (one horse missing due to lack of sports data)	293 Mares	355 Stallions/geldings	All 648 horses
Best linear unbiased prediction value, BLUP	104	105	105
Mean no. of races throughout the career	23	48(*)	36
Record (average per-km time in minutes and s)	1'33.2"	1'30.2"(*)	1'31.6"
Total earnings/career [SEK/NOK]	58 200	228 500 (*)	151000
Average earnings/start [SEK/NOK]	2 530	4 760 (*)	4160
% of races completed without official notification of irregular gait, gallop or pace.	54	59	58
% of races with official notification of irregular gait	1.5	1.5	1.5
Horses with separate centres of ossification	20	23	43
Average body size score (cm) for the 100 horses measured	348	349	348.5

Table 1. Summary of (significant*) findings made in Papers I and II of this thesis (*p<0.05)



Figure 8. Highly ossified ungular cartilages. On the right of the radiograph, it can be seen how ossification starts in a separate proximal centre of ossification (kernel). (Photo courtesy of Dr. Ove Wattle, SLU.)

7.3 Methodology perspective

Predicting the presence or grade of OUC by palpation proved to be highly unsuccessful. After testing about 70 horses, a decision was made to focus on making standardised radiological examinations and collection of objective and quantitative data. It was concluded that palpation is too subjective and nonspecific a method to be used for scientific purposes.

A population perspective and the more limited sports medicine perspective counterbalance each other well when studying prevalence and development of common inherited conditions such as OUC. Clinical relevance of OUC has so far been mainly been studied in individual horses referred to a clinic for investigation of lameness, sometimes combined with reduced performance as the only clinical sign. For a limited number of horses, use of advanced and expensive diagnostic tools like CT, MRI and scintigraphy is possible.

However, the history of OUC shows the need to be careful not to overinterpret clinical findings found in a few, selected individuals. Furthermore, any diagnostic method needs numerous cases and often a refined and welldescribed and defined methodology before being used in scientific studies (Spriet *et al.* 2007).

In general, after decades of selective breeding (Uhlin 2007), Norwegian cold-blooded trotters are a genetically stronger population, with an average BLUP index of 108 compared with 98 for Swedish horses. BLUP was used during collection of data in the present work to monitor equality between originally examined and re-examined groups (Table 2). These data, in addition to the great random geographical spread of re-examined horses, justifies consideration of the re-examined group as a representative population.

	BLUP index	Mean grading horses >3 yrs under the NS
Original population	105	0.4
Re-examined population	104	0.4

 Table 2. Best linear unbiased prediction value (BLUP) and grade according to the NS awarded to the original and re-examined horse populations

A statistical piecewise regression model is useful to describe conditions such as OUC that develop in stages over time. Moreover, ordinal sets of data, such as OUC grades, are handled well in a generalised linear mixed model. Ordinal piecewise regression model had the lowest Akaike information criterion (AIC) and was thereby used in calculation of OUC development (Table 3).

Model	AIC value	
Ordinal piecewise regression	1071.4	
Ordinal model linear in age	1087.1	
Ordinal model quadratic in age	1082.1	
Age as 'class' variable	1091.5	

Table 3. Akaike information criterion (AIC) values for the different models tested

The new grading system proposed here worked as well as an established system in statistical analyses. Moreover, it simplifies clinical radiological classification and reduces the risk of false positive diagnoses of low grade OUC (Figure 9). Based on lack of evidence for OUC being a pathological condition, using a more forgiving grading system seems reasonable.

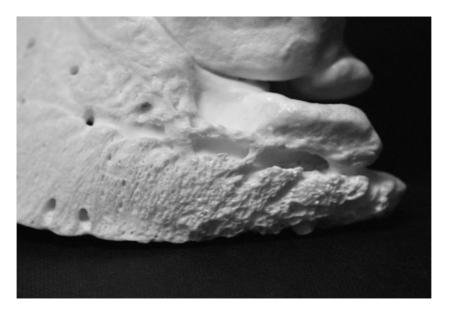


Figure 9. Palmar process of PIII without OUC. The new grading system proposed in this work allows differing appearance of P3 without necessarily being classified as OUC. (Photo courtesy of Dr. Ove Wattle, SLU.)

Excluding stallions with high-grade OUC from breeding is an inefficient measure in selective breeding (Tullberg and Wattle 2008) and does not improve equine animal welfare from a population perspective.

8 Future prospects

Inbreeding is a growing problem in cold-blooded trotters that may have an influence on future performance and health (Klementsdal 1993 and 1999). Inbreeding data are relatively easy to collect for the population studied here and a lack of correlation to presence of OUC would further strengthen the conclusion reached in this work that OUC is a physiological response rather than a defect. Possible effects of inbreeding on performance parameters are an interesting topic of study and material is available for a separate paper.

In order to separate physiological from possible pathological OUC conditions developing simultaneously in growing horses, modern non-invasive diagnostic tools can be used after appropriate validation. Conventional methods such as pathological and histological studies of legs after horses are slaughtered for other reasons may provide interesting additional information, with certain reservations about their selection as individuals. Data on training and non-training horses from foetus to three years of age can provide relevant information on whether body size or physical exertion (workload) early in life is a factor contributing to development of OUC.

Trainers often refer to gait irregularities at high speed as the only existing symptom of OUC. Modern systems for analysing gait at high speed trotting open up an interesting field of equine research applicable for horses without and with different grades of OUC.

Furthermore, there is a need for basic research on distal limb and distal phalanx development, in order to fully understand and distinguish physiological from pathological conditions in the equine foot.

The results of the present thesis indicate a need for minor updates of government and sport regulations on animal welfare concerning OUC and breeding of Scandinavian cold-blooded trotters.



A 1.5-year-old cold-blooded trotter in his first ever training session at the National Equine Centre Wången. Young equine athletes such as this are proposed as objects for future research. (Photo courtesy of Marie Hasselbom.)

References

Akaike H. (1973). Information theory and an extension of maximum likelihood principle. In: *Second International Symposium on Information Theory*. Edited by Petrov PN, Csaki F. Budapest, Hungary: Akademiai Kiado: 267–281.

Andersson LS, Larhammar M, Memic F, Wootz H, Schwochow D, Rubin CJ, Patr K, Arnason T, Wellbring L, Hjälm G, Imsland F, Petersen JL, McCue ME, Mickelson JR, Cothran G, Ahituv N, Roepstorff L, Mikko S, Vallstedt A, Lindgren G, Andersson L, Kullander K. (2012). Mutations in DMRT3 affect locomotion in horses and spinal circuit function in mice. *Nature. Aug* 30:488(7413):642-6.

Arnason T. (1999). Genetic evaluation of Swedish standardbred trotters for racing performance traits and racing status. *J Anim Breed Genet*, 116:387-398.

Attrell B, Graaf K, Magnusson L-E. (1983). OUC in Swedish Ardenner Horses. [In Swedish]. *Svensk Vettidn*, 35(suppl.3):35-37

Becht JL, Park RD, Kraft SL, Steyn PF, Wrigley RH. (2001). Radiographic interpretation of normal skeletal variations and pseudo lesions in the equine foot. *Vet Clin North Am Equine Pract.* Apr; 17(1):1-18. Review.

Bengtsson G. (1983). Side bones in Swedish cold-blooded horse breeds. [In Swedish]. *Svensk Vettidn*, 35(suppl. 3):35–37.

Bowker RM. (1998). Functional anatomy of the cartilage of the distal phalanx and digital cushion in the equine foot and a hemodynamic flow hypothesis of energy dissipation. *J Vet Res*, 59:961-968.

Bragulla H. (1999). Development of hoof cartilage with special considerations of its ossification. *Dtsch Tierarztl Wochenschr*, 106:87–93.

Breeding Plan (2001). *Breeding Plan for Cold-blooded Trotters* [In Swedish]. Available at: <u>https://www.travsport.se/artikel/avelsvardering_av_kallblod</u>

Changes in Swedish Official Animal Welfare Regulations (DFS 2004:22) concerning breeding. *SJVFS*,2009:28, L 115. Available at: *https://www.jordbruksverket.se*. (Accessed June 27 2012)

Cheetham J, Riordan AS, Mohammed HO, McIlwraith CW, Fortier LA. (2010). Relationships between race earnings and horse age, sex, gait, track surface and number of race starts for Thoroughbred and Standardbred racehorses in North America. *Equine Vet J.* May; 42(4):346-50.

Colles CM. (1983). Interpreting radiographs 1: the foot. *Equine Vet J*. Oct; 15(4):297-303.

Dakin S, Robson K, Dyson S. (2006). Fractures of ossified cartilages of the foot. *Eq Vet Educ*, 18:120-138.

Directions of changes in regulations of animal welfare concerning breeding. [In Swedish]. (2009). *SJVFS*, 28:115. Saknr.L. Available at: *https://www.jordbruksverket.se*

Dyson S, Marks D. (2003). Foot pain and the elusive diagnosis. *Vet Clin North Am Equine Pract*. Aug; 19(2):531-65, viii. Review.

Dyson S, Murray R, Schramme M, Branch M. (2003). Magnetic resonance imaging of the equine foot: 15 horses. *Eq Vet J.* 35:18–26.

Dyson S, Brown V, Collins S, Murray R. (2010) Is there an association between ossification of the cartilages of the foot and collateral desmopathy of the distal interphalangeal joint or distal phalanx injury? *Equine Vet J.* Sep; 42(6):504-11.

Dyson S, Nagy A. (2011). Injuries associated with the cartilages of the foot. *Eq Vet Educ*, 23:581-593.

Eberlein, (1908). Die Hufkrankheiten des Pferdes. In: J. Bayer und E. Fröhner (Hrsg.): *Handbuch der Tierärztlichen Chirurgie und Geburtshilfe*. Bd. 4, Teil 2, 274-328. Verlag Wilhelm Braumüller, Wien, Leipzig.

Ertola K, Houttu J. (2011). The Finnish horse and other Scandinavian Coldblooded trotters. In: *Diagnoses and Management of Lameness in the Horse*. 2nd edition. Edited by Dyson SJ, Ross.MW. Saunders, St Louis: Elsevier: 1076– 1081. Fiorentino, L. (2003). *Ossification des cartilages ungulares chez le cheval de trait sportif-incidence et influence sur la clinique*. Diss. Ecole nationale veterinaire d'Alfort, Paris.

Hedenström UO, Olsson U, Holm AW, Wattle OS. (2014). Ossification of ungular cartilages in front feet of cold-blooded trotters--a clinical radiographic evaluation of development over time. *Acta Vet Scand*: 56:73.

Hedenström UO, Wattle OS. (2014). Significance of ossificated ungular cartilages regarding the performance of cold-blooded trotters. *Acta Vet Scand*: 56:74

Henderson CR. (1976). Multiple trait evaluation using relatives' records. *J Anim Sci*, 43:1188-1197.

Historical currency of Swedish and Norwegian crown. Available at: <u>http://www.riksbank.se/sv/Rantor-och-valutakurser/Aldre-valutakurser</u> <u>forskningsprojekt/Manatliga-valutakurser-1913-2006</u> (Accessed Dec.15 2011).

Holm AW, Bjørnstad G, Ruohoniemi M. (2000). Ossification of the cartilages in the front feet of young Norwegian coldblooded horses. *Equine Vet J.* Mar; 32(2):156-60.

Honnas CM, Ragle CA, Meagher DM. (1988). Necrosis of the collateral cartilage of the distal phalanx in horses: 16 cases (1970-1985). *J Am Vet Med Assoc*. Nov 15; 193(10):1303-7.

Horses and the Consumer Purchases Act (2010). [In Swedish] Available at: <u>https://www.jordbruksverket.se</u>

Jørgensen HS, Proschowsky H, Falk-Rønne J, Willeberg P, Hesselholt M. (1997). The significance of routine radiographic findings with respect to subsequent racing performance and longevity in standardbred trotters. *Equine Vet J*. Jan; 29(1):55-9.

Jämthagen S. (2003). *Jubilee Book of Wången 1903-2003*.[In Swedish] Östersund, DAUS Tryck och Media.

Klemetsdal, G. (1993). Demographic parameters and inbreeding in Norwegian trotter. *Acta Agric Scand A Anim Sci*, 43:1-8.

Klemetsdal G. (1999). Stochastic simulation of sire selection strategies in North-Swedish and Norwegian cold-blooded trotters. *Livest Prod Sci*, 57:219-229.

Kummer M, Lischer C, Ohlerth S, Vargas J, Auer J. (2004). Evaluation of a standardised radiographic technique of the equine hoof. *Schweiz Arch Tierheilkd*. Nov; 146(11):507-14.

Körber H.D. (1991). Radiographic studies of ossification of the hoof cartilage of horses and its clinical evaluation. *Berl Munch Tierarztl Wochenschr*. Oct 1; 104(10):334-40.

Lafosse PE. (1772). *Cours d'hippiatrique ou Traité complet de la médecine des chevaux*. College de Pefle, Paris.

Lejeune JP, Schneider N, Caudron I, Duvivier DH, Serteyn D. (2006). Radiographic evolution of the forelimb digit in the Ardenner horses from weaning to 28 months of age and its clinical significance. *J Vet Med A*, 53:364–370.

Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O. (2006) *SAS for Mixed Models*. Second edition. Cary N C: SAS Institute Inc.

Lungwitz A. (1889). Beitrag zur Verknöcherung der Hufknorpel beim Pferde. *Osterr. Mschr.fur Tierheilkunde*, 32:529-536

Mangin M. (1841). Sidebone in front hooves of draught horses [in French] *Rec Méd Vét Pra.* 1:303.

Mair TS, Sherlock CE. (2008). Collateral desmitis of the distal interphalangeal joint in conjunction with concurrent ossification of the cartilages of the foot in nine horses. *Eq Vet Educ*, 20 (9): 485-492.

Official guidelines for frozen semen used in gene banks. (2014) [In Swedish]. *SJV* Dnr36. 12905/07. Available at: <u>https://www.jordbruksverket.se</u>

Official Swedish and Norwegian Sports Data. (2012, 2015) [http://www.travsport.se] and[http://www.travsport.no] (Accessed Oct. 2012 and August 2015).

Olsen HF, Klemetsdal G, Odegård J, Arnason T. (2012). Validation of alternative models in genetic evaluation of racing performance in North Swedish and Norwegian cold-blooded trotters. *J Anim Breed Genet*. Apr; 129(2):164-70.

Olsson U. (2002). *Generalized Linear Models: an Applied Approach*. Lund: Studentlitterature AB.

Quandt RE. (1958). The estimation of the parameter of a linear regression system obeying two separate regimes. *J Am Stat Assoc*, 53:873–880.

Redden RF. (2003). Radiographic imaging of the equine foot. *Vet Clin North Am Equine Pract*. Aug; 19(2):379-92, VI. Review.

Robertsson, W.A.N. (1932). *The hereditary character of sidebone*. Vet Rec.; 12, 83-90.

Ruohoniemi M, Tulamo RM, Hackzell M. (1993), Radiographic evaluation of ossification of the collateral cartilages of the third phalanx in Finnhorses. *Equine Vet J.* Sep; 25(5):453-5.

Ruohoniemi M, Raekallio M, Tulamo RM, Salonius K. (1997). Relationship between ossification of the cartilages of the foot and conformation and radiographic measurements of the front feet in Finnhorses. *Equine Vet J*. Jan; 29(1):44-8.

Ruohoniemi M, Laukkanen H, Ojala M, Kangasniemi A, Tulamo RM (1997). Effects of sex and age on the ossification of the collateral cartilages of the distal phalanx of the Finnhorse and the relationships between ossification and body size and type of horse. *Res Vet Sci.* Jan-Feb; 62(1):34-8.

Ruohoniemi M, Kärkkäinen M, Tervahartiala P. (1997). Evaluation of the variably ossified collateral cartilages of the distal phalanx and adjacent anatomic structures in the Finnhorse with computed tomography and magnetic resonance imaging. *Vet Radiol Ultrasound* Sep-Oct; 38(5):344-51.

Ruohoniemi M, Ryhänen V, Tulamo RM. (1998). Radiographic appearance of the navicular bone and distal interphalangeal joint and their relationship with ossification of the collateral cartilages of the distal phalanx in Finnhorse cadaver forefeet. *Vet Radiol Ultrasound*. Mar-Apr; 39(2):125-32.

Ruohoniemi M, Ahtiainen H, Ojala M. (2003) Estimates of heritability for ossification of the cartilages of the front feet in the Finnhorse. *Eq Vet J*, 35:55–59.

Ruohoniemi M, Mäkelä O, Eskonen T. (2004). Clinical significance of ossification of the cartilages of the front feet based on nuclear bone scintigraphy, radiography and lameness examinations in 21 Finnhorses. *Eq Vet J.* Mar; 36(2):143-8.

SAS Institute Inc. (2008). SAS/Stat 9.2 User's Guide. Cary N C: SAS Institute Inc.

Sergio RA, Silva ME, Vulcano LC. (2002). Collateral cartilage ossification of the distal phalanx in the Brazilian jumper horse. *Vet Radiol Ultras.*, 43:461–463.

Spriet M, Mai W, McKnight A. (2007) Asymmetric signal intensity in normal collateral ligaments of the distal interphalangeal joint in horses with a low-field MRI system due to the magic angle effect. *Vet Radiol Ultrasound*. Mar-Apr; 48(2):95-100.

Svea Hovrätt (2011). *Dom T-5745-10*. Available at: <u>http://www.domstol.se/Ladda-ner-bestall/Domar-beslut-och-handlingar/</u>

Tullberg M, Wattle O. (2008). Hovbroskförbening hos den svenska Ardennerhästen. *Svensk Veterinärtidning*, 13:13-19.

Uhlin H-E. (2007). Vår kallblodstravare. Available at: http://www.ulleriks.se/

Verschooten F, Van Waerebeek B, Verbeeck J. (1996). The ossification of cartilages of the distal phalanx in the horse: an anatomical, experimental, radiographic and clinical study. *J Eq Vet Sci*, 16:291–305.

Waxberg G. (1953). *Report of examinations of OUC etc. in Ardenner horses within the county of Skaraborg* [In Swedish]. Stockholm, Emil Kihlströms tryckeri AB.

Wirstad HF. (1951). Radiographic findings in 185 "Döle" stallions at prebreeding evaluation [in Norwegian]. *In Proceedings of the 6th Nordic Veterinary Congress*; Oslo. Edited by Sorensen Moller A. Copenhagen:59–69.

Witte K. (1906). Sidebone in horses [In German]. *Monatsh Prakt Tierheilkd*, 18:241–284.

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