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**GENETICS OF OSTEOCHONDROSIS DISSECANS IN  
NORWEGIAN WARM-BLOODED HORSES**

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## **1. ABSTRACT**

The aim of the current thesis was to investigate Osteochondrosis dissecans (OCD) in Norwegian warm-blooded horses and explore potential genetic and epi-genetic influences on this disease.

A review of the literature on OCD is provided where the disease is described in a general manner, as well as the genetics, prevalence, causes and prognosis of the disease based on research done on Norwegian warm-blooded horses.

This study builds on personal research on 36 Norwegian warm-blooded trotting foals. The focus was set on the occurrence and location of OCD in two separate groups of foals, and the effect of differences in diet and living area on the prevalence of OCD was investigated. The results indicate that providing horses with larger exercising areas and an increased amount of dietary magnesium might substantially reduce incidents of OCD.

## **2. INTRODUCTION**

### **2.1 OSTEOCHONDROSIS DISSECANS IN GENERAL**

Osteochondrosis dissecans (OCD) is a common developmental orthopedic disease. It can develop in any joint, even in the vertebra, and it normally affects horses at 5–8 months of age (Bates, Jacobs, Shea & Oxford, 2014). The fetlock, tarsocrural, femoropatellar and scapulohumeral joints are most commonly involved (Butler, Colles, Dyson, Kold, & Poulos, 2011).

OCD originates from osteochondrosis (OC) which is the term of the disease before the cartilaginous flap or body can be detected. Osteochondritis is the resulting inflammation of the joint (McIlwraith, 2013). The terms osteochondritis and OC are often confused and the term OC has often been applied indiscriminately.

According to Ytrehus, Carlson and Ekman (2007), there are only certain lesions considered to be OC. The lesions listed are located at the dorsal aspect of distal metacarpus and metatarsus, the lateral trochlear ridge of talus, the intermediate ridge of distal tibia, the medial condyle of femur, the lateral trochlear ridge of femur, the medial condyle of humerus and the caudal part of the proximal articular surface of humerus (Ytrehus, Carlson & Ekman, 2007).

In many cases, the first signs of OCD is a non-painful swelling of the joint, but often there are no detectable clinical signs (Adams et al., 2012) and the disease is first diagnosed via radiography. Consequently, the disease is typically not identified until the formation of the loose bony or osteochondral fragments has occurred (Bates, Jacobs, Shea & Oxford, 2014).



**Picture 1: Severe swelling of the tarsal joint**

The most severe clinical signs, such as stiffness, flexion responses and lameness, are present when osteochondral fragments come loose within the joint. The clinical signs are often first apparent in yearlings at the time of the onset of training, but it can also appear suddenly in older horses (Adams et al., 2012).

OC lesions typically appear during the first 5 months of the foal's life. Some of the lesions will regress while others will persist, but there will be no regression after the age of approximately one year. At this age one can diagnose the disease with certainty (Donabedian et al, 2008).

### **2.1.1 The pathophysiology of OCD**

Osteochondrosis is defined as a focal disturbance of enchondrial ossification. During the early stage of the disease lesions are primarily located in the articular cartilage and later cartilage flaps or osteochondral fragments may develop (Adams et al, 2012). Most often, the fragments stay in place, loosely attached, and an inflammation in the joint is caused by the debris from the fragment (McIlwraith, 2013). In some cases weight bearing effects may lead to subchondral cysts. The initial steps of OCD, such as failure of chondrocyte differentiation, subchondral bone

necrosis, formation of fragile cartilage, and failure of blood supply to the growth cartilage has all been debated. However, recent literature suggests that the failure of blood supply to the growth cartilage is the most probable cause of the disease (Ytrehus, Carlson & Ekman, 2007).

With regards to the development of OC, it has been stated that the beginning of the disease is characterized by growth cartilage failing to undergo vascularization and matrix calcification. This means that the cartilage is not turning into bone. The earliest stage of the disease consist of a necrotized area in the growth cartilage and at that point it does not involve the subchondral bone underneath or the articular cartilage above. As the disease progresses it will be visible in a cut section as a zone of thickened cartilage together with an irregular chondro-osseous junction. If the case is more advanced the cartilage may also become red and soft with an edematous and hyperemic bone marrow. The term OCD is applied when a fissure is formed, extending through the articular cartilage from the necrotic cartilage where it originated (Ytrehus, Carlson & Ekman, 2007).

### **2.1.2 The radiology of OCD**

A review of the most commonly affected joints:

The fetlock joint: The most typical projections used on the metacarpal and metatarsal joints are the lateromedial to view the dorsal aspect of the sagittal ridge of the third metacarpal/metatarsal bone and also the dorsoproximal aspect of the proximal phalanx (which is a common location but the least severe regarding clinical signs). The dorsal 30° proximal 70° medial-plantarodistal oblique aspect and the dorsal 30° proximal 70° lateral-palmarodistal oblique aspect are often used to get the best view of the palmar/plantar fragments that are usually located at the site of attachment of the short distal sesamoidean ligaments. This type of lesions are called “Birkeland fractures” and they are most often located medially in hind limbs.

The tarsal joint: Fragments of the distal intermediate ridge of the tibia are most common. They are often bilateral, but the majority are located on the lateral aspect of the distal intermediate ridge of the tibia. The typical projections used are dorsolateral-plantaromedial to get a view of the lateral/medial malleolus of the tibia, the dorsomedial-plantarolateral to view the distal

intermediate ridge of tibia and the lateromedial to view the distal end of the medial trochlear ridge of talus.

The femoropatellar joint: Lesions commonly occurs bilaterally in both stifle joints. The projection used to get a view of the lateral trochlear ridge of the femur (which is the most commonly involved location) is the 60° lateral craniomedial oblique view. The articular surface of the patella are also sometimes involved, and a fragment occasionally occurs at the base of the patella. Less commonly involved are the medial trochlear ridge of the femur and the trochlear groove.

The scapulohumeral joint: The caudal half of the joint is most often involved and it is best viewed from a mediolateral projection. The radiographs typically shows a variable degree of lucent zones in the subchondral bone of the humerus, the scapula or both. (Butler, Colles, Dyson, Kold, & Poulos, 2011).

## **2.2 THE PROGNOSIS OF OCD**

### **2.2.1 Without surgery:**

A study investigating the prognosis of OCD was performed on 243 Danish Standardbred trotters born in 1986, 1987 and 1988 by Jørgensen, Proschowsky, Falk-Ronne, Willberg and Hesselholt (1997). Radiographs were performed routinely before the onset of training. 61% (148) of the horses had one or more abnormal findings, categorized into 5 groups where group number 5 included combined OCD and other findings, and group 1 only OCD in tibiotalar joint. Of all the 243 horses, 169 participated in at least one race. Out of the 169 horses, 107 of them had radiographic lesions, but the number of starts per year was not significantly affected by the lesions found. The average earnings per year was 10657 DKK for the horses with radiological lesions and 11568 DKK for the normal horses. There was a tendency of decreased earnings for horses with an increased number of radiological lesions. There was no increase in retirement rate among the affected horses, except in group 5 where there was a higher rate (Jørgensen, Proschowsky, Falk-Ronne, Willberg & Hesselholt, 1997).

### **2.2.2 With surgery:**

It is advised to apply arthroscopic surgery before the age of 1 year. The reason for this is that after that age there will be no more changes in the appearance of the lesions on radiographs (Van Weeren, 2006).

Studies have shown that the success of surgical treatment varies depending on the type of OCD (type I: defect without fragment, type II: fragment present within the lesion, type III: free loose body within the joint) and the degree of body (grade I: <2 cm in length, grade II: 2-4 cm, grade III: >4 cm) (McIlwraith, 2013).

The femoropatellar joint can be treated conservatively even when effusion and lameness are present, but only in the case of type I or grade I. In a study including arthroscopic surgery on the femoropatellar joint of 134 horses, 21 (16%) were unsuccessful. However, long term results from another study using polydioxanone (PDS) pins for reattachment of OCD lesions have shown a success rate of 95% (McIlwraith, 2013). In the tarsocrural joint, surgery is recommended if clinical signs are present. One study revealed that out of 183 horses operated for OCD in the tarsocrural joint, 140 (76.5%) were successful (McIlwraith, 2013). In the metacarpophalangeal joint, type II and III require surgery and a study including 42 horses showed a success rate of 60% (McIlwraith, 2013). Surgery is appropriate in the case of OCD in the shoulder joint with the exception of lesions localized to the glenoid. Results from a study of 80 horses showed a prognosis of 50% success with this form of surgery (McIlwraith, 2013).

## **2.3 THE NORWEGIAN WARM-BLOODED RIDING HORSE AND OCD**

The breed Norwegian Warmblood is based on Swedish, Danish, German and Dutch warm-blooded horses. Only about 200 foals are bred and born as Norwegian Warmblood each year, but several thousand imported warm-blooded riding horses exist in the country (Vangen, 2005: 2007). Because of the big influence of European warm-blooded horses in Norway, the prevalence of OCD and the genetic research is based on the imported breeds.



Norwegian Warmblood riding horses (Norsk Varmblods, NV) are usually checked for OCD quite randomly as their usage tend to be on a hobby basis. Regarding breeding, however, it is an obligation to check the NV stallions for OC and osteochondrosis-like conditions. The positive stallions may have permission to be used for breeding, as OC/OCD is graded with a 4 by the Norwegian Warmblood Organization. Grade 4 means that it is a very significant disorder, but permission to breed may be granted if the individual is particularly good (Styret I Norsk Hestesenter, 2012).

## **2.4 THE NORWEGIAN STANDARDBRED AND OCD**

The Norwegian Standardbred heritage comes from France and America. In recent years a higher percentage of horses with France ancestry has been born in Norway. In 1988 around 80% of the horses born in Norway were pure American Standardbred, but in 2006 and 2007 the number had decreased to 45% (Lykkjen et al., 2014). Norwegian Standardbreds are usually checked for OCD at an early age (before the onset of training). The Norwegian Trotting Organization (Det Norske Travelskap, DNT) operates with a “defect list” of the diseases that excludes the stallions from breeding. This list does not include OC or OCD (Norsk Hestesenter, 2015).

## **2.5 CAUSES OF THE DISEASE**

Potential causes of OCD include a high energy diet, trauma, dietary imbalance, not enough movement or exercise, and genetics. The following sections will discuss these causes sequentially.

### **2.5.1 High energy diet**

It is suggested by Ytrehus, Carlson and Ekman (2007) that rapid growth caused by receiving diets with high energy content, thus increasing the serum level of insulin and decreasing the serum level of thyroxine, could have a negative effect on chondrocytes. In their study, the cartilage cores in the transition zone between the cartilage and bone became necrotic because of

biomechanical stress. However, this theory failed because it suggests generalized lesions, and not focal as in the case of OC and also because the theory does not match the morphology observed in initial lesions of OC where the formation of cartilage cores is only observed after the lesions have become necrotic (Ytrehus, Carlson & Ekman, 2007).

A high growth rate is related to a high energy intake, and studies have found an increase in the number of OC lesions associated with a high growth rate, but it is suggested that a high growth rate may be caused by either a high food intake or by genetic predisposition (Van Weeren 2006).

### **2.5.2 Trauma**

It has not been proven that sudden macrotrauma can cause the onset of OC, but it has been suggested that trauma can induce OCD from OC. In the latter case the trauma may be very weak, or even just normal movement (Ytrehus, Carlson & Ekman, 2007).

### **2.5.3 Dietary imbalance**

Dietary imbalance in the form of copper deficiency or deficiencies of other minerals leading to decreased copper absorption, such as inorganic sulphates, zinc and cadmium, has been suggested as a possible cause of OC lesions. However, these theories are weakened by the fact that copper deficiency is a systemic disease and the associated lesions are often extensive and involves many joints at once (Ytrehus, Carlson & Ekman, 2007).

A study from New Zealand showed that horses that were given less copper than recommended did not develop OC lesions, but another study done on the relation between the copper content of the liver of mare and foal and the development of OC lesions showed that copper has a positive effect on the repair of lesions, but no effect on number of lesions developed (Van Weeren, 2006).

Two studies performed in Netherland on groups of 64 and 54 foals aging from 0 to 12 months both states that magnesium supplements could decrease the prevalence of OC significantly. (Counotte, Kampman & Hinnen. 2014).

#### **2.5.4 The influence of movement or exercise**

One previous study concluded that exercise and access to a large area where the foals can run freely decreased the incidents of OC lesions (Counotte, Kampman, & Hinnen. 2014). Similarly, research done on the effect of increased exercise on OC lesions has shown that the severity of the lesions tend to decrease with higher levels of exercise, but not the prevalence. This suggests that exercise may benefit the regeneration of the lesions (Van Weeren, 2006).

#### **2.5.5 The influence of genetics**

Genetic influence is believed to account for around 25% of OC lesions (van Weeren, 2006). Phenotype is defined as the observable characteristics and appearance of an individual resulting from both environmental influences and genetic makeup (Marjit, 2009). OCD is a phenotype of a quantitative trait. This means that in about 25% of the cases the disease is a result of several genes, which may also be influenced by the environment (Department for Environmental Food & Rural Affairs, 2015). The sum of the environmental changes (the changes to the chromatin template that regulates the gene expression and gene silencing from the same genome) is defined as epigenetics. Epigenetics is thus the explanation for differences in phenotypes caused by the influence of environment, nutrition and other external sources on the genome. Epigenetics is also called “non-Mendelian inheritance” because it describes a change in phenotype and not genotype (Allis, Jenuwein, Reinberg & Caparros, 2007).

Some research has been done on different horse breeds to measure the heritability of OCD. Heritability is commonly referred to as a variation in a trait caused by genetic factors. It is a population parameter and defined as the proportion of phenotypic variation ( $V_P$ ) due to variation in genetic values ( $V_G$ ). The value of heritability can be measured and lies between 0 and 1. Heritability is not constant and can change over time because of changes in genetic values, environmental factors or changes in the correlation between genes and environment (Wray & Visscher, 2008).

## **2.6 GENETICS OF OSTEOCHONDROSIS DISSECANS IN NORWEGIAN WARM-BLOODED HORSES**

### **2.6.1 The warm-blooded riding horse**

Based on the imported breeds contributing to the Norwegian Warmblood, namely the Swedish Warmblood, the German Warmblood, the Danish Warmblood and the Dutch Warmblood:

The Swedish Warmblood: In Sweden, a study including radiographs of 4518 horses showed a prevalence of OC to be 13% (stifle 9%, hock 6% and fetlock 10%). The overall heritability was 0.05 (stifle 0.03, hock 0.08 and fetlock 0.13) (Jönsson et al., 2011).

Moreover, an OCD prevalence of 15% was reported in Swedish warm-blooded horses (Vos, 2008).

The German Warmblood: The German Riding Horse has been shown to have a prevalence of OC of 11% and a heritability of 0.06-0.07. The prevalence of OC in the toe and hock was 5% and the heritability was 0.45-0.64. The Maremmano horse had a prevalence of OC of 16.6% and a heritability of 0.19-0.58 in the stifle, hock and fetlock. The Hanoverian Warmblood had a prevalence of OC of 20.8% and a heritability of 0.08-0.14 in the fetlock and a prevalence of OC of 9.6 and a heritability of 0.19 in the hock (Tetens, Wulf, Gonzales-Lopez, Kühn & Thaller, 2011). A German study of Hanoverian Warmblood from auctions showed a prevalence of OCD of 20.5% (Lewczuk & Korwin-Kossakowska, 2012).

The Danish Warmblood: Out of 574 Danish Warmblood horses examined by routine radiography for a period of 10 years, 158 (27.5%) of them was diagnosed with OCD. The Tarsus had a prevalence of 16%, the stifle 7.6% and the metacarpal/tarsal phalangeal joints 7.3% (Greve & Arnbjerg, 2002).

The Dutch Warmblood: In a Dutch study, 443 (36%) out of 1231 horses had evidence of OCD (Vos, 2008). In one radiographic research study, tarsocrural osteochondrosis was present in 30% of Dutch Warmblood horses (Lykkjen et al., 2014).

There has been selective breeding among Dutch Warblood stallions to try to decrease OC, but with little success. The reason for this lack of success are suggested to be that some stallions with OC may have a high breeding value and are therefore bred anyway (Lykkjen et al., 2014).

Country	Prevalence of OCD
Sweden	15%
Germany (The Hanoverian Warmblood)	20.5%
Denmark	27.5%
Netherlands	36%

**Table 1: The percentage of OCD in the different countries.**

This review shows that horses imported from the Netherlands may contribute to a higher prevalence of OCD in Norwegian Warmblood, versus the Swedish Warmblood with the lowest contribution.

### **2.6.2 The Standardbred**

In one Norwegian study, the prevalence of OCD was based upon examinations of the 363 Norwegian Standardbred yearlings which were reared by their breeders. The exercise levels, feeding and housing varied among horses. No lesions were diagnosed in 179 (49.3%) of the horses, whereas in 184 (50.7%) horses one or more lesions were diagnosed.

In this research, the femoropatellar, tarsocrural, fetlock and scapulohumeral joints were found to be the joints most often affected. In another study done by the same authors, 464 Norwegian Standardbred yearlings were used to find the prevalence of OCD in different joints. In the

tarsocrural joint, a prevalence of 14.3% and 10.5% were reported, in the metacarpophalangeal and the metatarsophalangeal joints the prevalence was 4.3% and 10.0% respectively. In palmar/plantar first phalanx a prevalence of 11.8% and 22.4% was found. Heritability for OC in the tarsocrural joint and in palmar/plantar first phalanx in the Norwegian Standardbreds have been estimated to 0.52 and 0.21 (Lykkjen, Roed & Dolvik, 2012).

In a study of 1217 Norwegian Standardbred trotters during their first year of life, radiographs and pedigree information was used to determine the heritability of OC and palmar/plantar first phalanx osteochondral fragments (POFs). Regarding OC, the heritability was estimated at  $0.29 \pm 0.15$  for the lateral trochlear ridge of the talus and/or the distal intermediate ridge of the tibia, but for the distal intermediate ridge of tibia alone the heritability was  $0.40 \pm 0.17$ . In all 4 legs, the POF heritability was  $0.23 \pm 0.13$ . The heritability was  $0.26 \pm 0.13$  for the metatarsophalangeal POFs and  $0.32 \pm 0.14$  for the medial metatarsophalangeal POFs. The researchers performing this study concluded that POFs and tarsocrural OC has a moderate to high heritability in Standardbred trotters, and thus that the genome-analysis studies and breeding programs should concentrate on predilection sites in the future (Lykkjen et al., 2014).

OCD in the intermediate ridge of the distal tibia was identified as a quantitative trait loci (QTL) by the usage of Illumina Equine SNP50 BeadChip whole-genome single-nucleotide polymorphism (SNP) assay in a study of radiographs and blood samples from 464 Norwegian Standardbred trotters. (Lykkjen et al., 2010). The Illumina Equine SNP50 BeadChip whole-genome single-nucleotide polymorphism (SNP) assay is a whole genome genotyping application used in equine research for identification of genes and mutations in the horse breeds Arabian, Andalusian, Akhal-teke, Icelandic, Standardbred, Thoroughbred and the Quarter horse. Probes are designed to target SNPs from the entire equine genome. (EquineSNP50 Genotyping Bead-Chip, 2008). Gene regions close to the SNPs has a possible linkage to the selected trait and are referred to as QTL (Bates, Jacobs, Shea & Oxford, 2014).

162 horses were genotyped and 80 of these had the specific case of OCD. Equus caballus chromosome (ECA) 5, 10, 27 and 28 was found to have the QTL for OCD (Lykkjen et al., 2010).

In a study of 176 Norwegian Standardbred trotter yearlings by Lykkjen and colleagues (2013), putative QTL for plantar/palmar first phalanx osteochondral fragments (POF) for the medial POF were found on ECA 1, 2, 7, 9 and 31. The trait loci for lateral POF were found on ECA7, 11, 27 and X. This indicates a complex inheritance with various genes controlling the lateral and medial POF development (Lykkjen et al., 2013).

The complexity of genetics concerning OCD is further underlined by Bates, Jacobs, Shea and Oxford (2014). They have identified several other QTL possibly causing OCD in horses.

- LOC100073151
- UDP-glucose dehydrogenase (UGDH)
- Matrilin 1 (MATN1)
- Laminin (LAMB1)
- Solute carrier family 35 (SLC35D1)
- Parathyroid hormone receptor (PTH1R),
- The hyaluronoglucosaminidase gene family
- Parathyroid hormone 2 receptor (PTH2R)
- Chloride channel calcium activated family member 4 (CLCA4)
- COL24A1
- F-box protein 25 (FBXO25)
- TBC1, domain family member 22A (TBC1D22A)
- Lectin galactoside-binding soluble 3 (LGALS3)
- Frizzled-related protein (FRZB)
- Calcitonin gene-related peptide-receptor component protein (RCP9)
- Calneuron 1 (CALN1)
- Interleukin 6 (IL6)
- AOA1 and PTH1R
- Xin actin-binding repeat containing 2 (XIRP2) and neurochondrin (NCDN) which was concluded to be the functional candidate gene on ECA2.

- Collagen type III alpha 1 (COL3A1), collagen type V alpha 2 (COL5A2), collagen type V alpha 1 (COL5A2), collagen type XXVII alpha 1 (COL27A1) and collagen type XXIV alpha 1 (COL24A1), which is described as a fibril diameter regulator with specific functions at specific sites for fibrillogenesis. Abnormal collagen fibrils are associated with OCD, with evidence supporting endoplasmic reticulum (ER) stress and an unfolded protein response (UPR) which in the cells of the growth plate may contribute to OCD (Bates, Jacobs, Shea & Oxford, 2014).

### **3. AIMS AND HYPOTHESES**

OCD is a common developmental disease in horses. However, there is little consensus regarding the causes of the disease and potential environmental influences. Consequently, the current study sought to investigate two separate groups of foals in terms of OCD prevalence in general and in each joint examined with the additional aim of identifying potential epigenetic factors that might influence the disease. Based on previous findings, I expected a high prevalence of OCD in my sample. Moreover, I expected that differences in nutritional factors and environmental conditions between the groups might have an influence on OCD occurrence and severity.

### **4. METHOD:**

Radiographs were taken of 36 Norwegian Standardbred horses over a period of two years at Stall Rishaug in Trondheim, Norway. All horses were bred and owned by Bjørn and Turid Rishaug who also owns their mothers. The fathers are mainly race horses from the United States, but also from southern Europe.

The purpose of breeding these horses is to sell them as race trotters, and most of the horses are sold within their second year of life. The horses were 1 year of age at the time of the radiological examination. Moreover, the horses were all clinically healthy and x-rays were taken as a standard



procedure because a potential operation to remove the bone fragments at this age will not interfere with any training or racing.

The first group (group 1) of 16 horses were examined in 2013, and the second group (group 2) of 20 horses were examined in 2014. There were a total of 20 stallions and 16 mares.

The radiological examinations were carried out with mobile radiological equipment at the stable by veterinarian Charlotte Ericson Elvebakk from Melhus Hesteklinikk AS and Equirad AS and me as her assistant. The radiological pictures was taken of the fetlock joints on all four legs and of the tarsal joints on both legs. The radiological projections used were the lateromedial on the anterior fetlock joints, the dorsolateral-palmaromedial oblique and the dorsomedial-palmarolateral oblique on the posterior fetlock joints, and the dorsomedial-plantarolateral on the tarsal joints. These projections are standard procedure at Melhus Hesteklinikk when checking for OCD.

The checkpoints for OCD in the fetlock and tarsal joints were as follows:

Tarsus: Mainly the distal intermediate ridge of tibia, but also the medial malleolus and the medial trochlea tali.

Fetlock: The dorsal aspect of the sagittal ridge of the third metacarpal bone, the dorsoproximal aspect of the proximal phalanx, the proximal plantar eminence of the proximal phalanx and the distal third metatarsal bone.

All of the horses were quite similar in their development and exterior. There were a few exceptions where some of the yearlings would have a slightly advanced physical development, but overall the differences were small. Before the pictures were taken, a visual and physical examination was performed in the form of examining potential abnormalities in the bone structure of the legs, as well as potential lameness or swelling of the joints. The only thing found was swelling around the tarsal joint of 3 yearlings from group 1. These horses also had OCD. Some of the bone flaps found during our examination were later removed with arthroscopy at Leangen Hesteklinikk in Trondheim, depending on the size and location of the flap.

The results from the radiological examinations of each group were carefully compared. Additionally, notes were made of any dissimilarities in feed, habitat and exercise between the groups that might explain potential differences in the prevalence of OCD.

## 5. RESULTS:

A preliminary analysis including both groups revealed a total of 15 (41.7%) incidences of OCD. At a group-level, it was found that nine out of sixteen horses (56.3%) in group 1 received a diagnosis of OCD. In group 2, six out of twenty horses (30%) received the diagnosis (refer to table 2 for descriptive statistics).

	<b>Healthy</b>	<b>Has OCD</b>	<b>Total</b>
<b>Group 1</b>	7	9	16
<b>Group 2</b>	14	6	20
<b>Total</b>	21	15	36

**Table 2. Showing descriptive statistics of OCD incidences in each group and in total.**

To investigate whether there were any significant differences between the two groups in terms of incidences of OCD, a Chi-square test was performed on the data. This test, also called the Chi-square test of association or the Pearson's Chi-square test, is used to reveal any relationship between two categorical variables. In other words, a chi-squared test can be utilized as a way of rejecting the hypothesis that the data are independent (Lund Research Ltd, 2013).

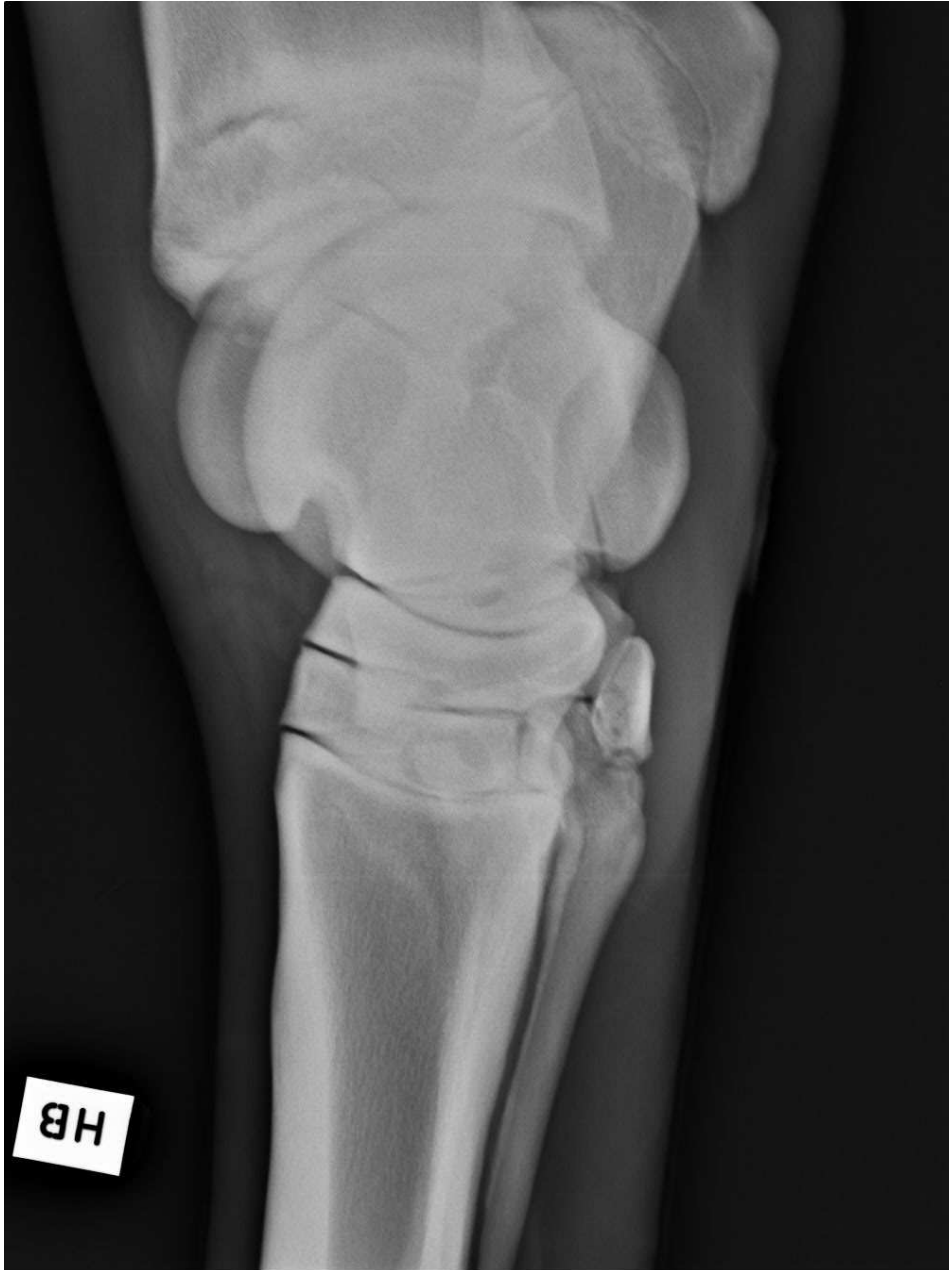
The current Chi-square analysis revealed a trending, but insignificant effect of group on OCD incidences ( $X^2(1) = 2.52, p = .112$ ). This finding indicates that the horses in group 2 showed a lower tendency towards developing OCD. A further chi-square test was performed to investigate potential sex differences in terms of ODC prevalence. A comparison of cases of OCD in stallions and mares showed no significant difference between the groups. Males: ( $X^2(1) = 1.32, p = .36$ ), Females: ( $X^2(1) = 1.00, p = .62$ ). Consequently, this suggests that there were no higher incidence of OCD in either sex. Finally, as an inspection of the data revealed a higher number of OCD in the tarsal joint in group 1 (8 cases) compared to group 2 (4 cases). A subsequent chi-square analysis revealed that this difference was closely approaching significance ( $X^2(1) = 3.60, p = .058$ ).

There were two main differences between group 1 and 2 in terms of how the yearlings were raised. Firstly, group 1 was given the same feed as their mothers during their first year of life, whereas group 2 were given feed produced specifically for foals. Secondly, the foals from group 2 had been given a larger area to move freely around on than group 1. Areas for both groups included a hall of about 20x40 meters for shelter and free access to an outside area, but group 1 had only about 30x50 meters of outside area, compared to about 30x150 meters for group 2. The foals had never lived in a closed stable, thus they had never experienced containment in boxes of a few square meters only.

Two of the radiographs taken at Stall Rishaug:



**Picture 2: The picture shows the dorsomedial-palmarolateral oblique radiographic view of the right posterior fetlock joint. A radiolucent area can be seen on the plantar proximal phalanx, together with an osteochondral fragment.**



**Picture 3: The picture shows the dorsomedial-plantarolateral oblique radiographic view of the right tarsal joint. A radiolucent area can be seen on the distal intermediate ridge of the tibia, together with an osteocondral fragment.**

## 6. DISCUSSION

The present study revealed that 41.7 % of the Standard bred yearlings had one or more lesions. The study also showed a lower number of OCD incidents in group 2 where only 30% of the horses had OCD compared to group 1 where 56.25 % had OCD. When comparing only the tarsal joints of the two groups it was found that the tarsal joints had a higher number of lesions (12 findings) compared to the fetlock joint (3 findings). The results of the chi-square analysis (although insignificant), revealed a strong trend towards a higher occurrence of OCD in group 1 compared to group 2. Moreover, an even stronger tendency was found when comparing the difference in only the tarsal joint, revealing a higher number of OCD in the tarsal joint in group 1 compared to group 2. There was no difference in the incidents of OCD between genders. None of the yearlings that had an advanced physical development compared to the others were found to have OCD.

There were two main differences between the groups that might explain the lower occurrence of OCD in group 2; a difference in feed and a difference in size of the outdoor area for the yearlings to move freely on. The yearlings from group 1 were given the same concentrate as the mares, whereas the yearlings from group 2 were given a concentrate specifically developed for foals. When comparing the two feeds, the crude protein, the crude fiber and the crude ash were found to have the biggest differences in nutrient content: the foal feed had a 1.5% increased amount of crude protein, a 4.5% decreased amount of fiber content and a 3.3% increased amount of crude ash. There was also a slight difference in the amount of crude fat, selenium, potassium, phosphorous, magnesium and sodium between the two feeds. The lists of feed content was given to me by the owners Bjørn and Turid Rishaug (refer to table 2 for food content).

<b>Content</b>	<b>Champion breeder</b>	<b>Champion foal</b>	<b>Difference regarding Champion foal</b>
<b>Crude protein</b>	17.0%	18.5%	1.5% increase
<b>Crude fiber</b>	7.9%	3.4%	4.5% decrease
<b>Crude fat</b>	3.6%	4.4%	0.8% increase
<b>Crude ash</b>	9.9%	13.2%	3.3% increase
<b>Selenium</b>	0.74 mg/kg	0.70 mg/kg	0.04 mg/kg decrease
<b>Potassium</b>	1.70%	2.57%	0.87% increase
<b>Phosphorus</b>	0.80%	1.30%	0.50% increase
<b>Magnesium</b>	0.26% (2.6g/kg)	0.30% (3g/kg)	0.04% (0.04g) increase
<b>Sodium</b>	0.41%	0.40%	0.01% decrease

**Table 2. Food content: Main differences between the concentrate given to both the mothers and the foals during the first year and the feed given to the new foals during the next year. The foals received up to 1 kg per day at the age of 0-6 months and 1.5 kg per day at 6-12 months.**

Furthermore, group 2 had an outdoor area to move freely around on that was approximately 3 times the length of group 1's area. The owners could tell us that the yearlings from group 2 seemed to be more active and also spent more time running as a herd. The ground of the outdoor and indoor areas was covered with sand to prevent mud during the wet months of the year. The outdoor area was also slightly downhill.

## 6.1 EXPLANATION

The result of the Chi-square tests when comparing the incidents of OCD in general in group 1 and group 2 was 0.112 and the result when comparing the incidents of OCD in the tarsal joint in group 1 and group 2 was 0.058. Any significant result would have had a number of 0.05 or less. Since the two results of this test were insignificant, we cannot say for sure that the decrease of incidents of OCD between group 1 and group 2 are not a coincidence.

However, the results of the Chi-square tests revealed trending effects. In the case of the general test, the result of 0.112 suggests that there is only an 11.2% chance that the lower occurrence of OCD in group 2 is a coincidence. When considering only the tarsal joints, the result of 0.058 suggests that there is only a 5.8% chance that the lower occurrence of OCD in these joints in group 2 is a coincidence. Indeed, the result from the test of the tarsal joints is very close to being significant, which requires a 5.0% or less chance of coincidence.

The number of horses with OCD in in this study was 15 (41.7%) out of 36. As OCD is a documented heritable disease and genes are responsible for about 25% of the phenotype, it seems reasonable to suggest that the genes of these horses is a main cause of the disease. However, one must not forget that OCD is a phenotype of a quantitative trait and that the genes that cause the disease may be influenced by the environment, nutrition and other external effects.

One explanation for the different results between the two groups in my study is the fact that the differences could have been caused by the changes done between the first and the second group of foals only, because these changes may have had an effect on the decrease of OCD lesions without the genetic influence.

From the prospect of epigenetics, it is possible that the trending effect in this study is caused by one or both of the two main differences in how the groups of yearlings were raised, namely the difference in feed and/or the difference in area to move freely on.

The two types of concentrate given in my study were produced by “Felleskjøpet”, which is the main food producer for horses in Norway. In the brochure from “Felleskjøpet” it is claimed that



the concentrate “Champion foal” (given to group 2) gives a 2-5% better coverage of the energy and protein demand of the foals compared to “Champion breeder” (given to group 1) (Næsset, 2011). Some studies done on the relationship between a high-energy diet and osteochondrosis claim that there is a positive relation between the two, whereas others claim the opposite (). One study even resulted in a positive relation between high growth rate and OC (Van Weeren, 2006). In my study there was no apparent increase in the number of OCD in the horses with an increased growth rate or in the horses fed with the high energy containing concentrate “Champion foal”.

In a study published by Counotte, Kampman & Hinnen (2014), one group of foals were given 4.05 grams magnesium per day from the age of 0 to 5 months and another group of foals were given 4 grams magnesium per day from the age of 5 to 12 months of age. In both groups there was a significant decrease of osteochondrotic lesions compared to the placebo groups. Group 1 from my study were at 0 to 6 months of age given 2.6 grams of magnesium per day and group 2 received 3 grams of magnesium per day. At the age of 6-12 months, group 1 received 3.9 grams of magnesium per day and group 2 received 4.5 grams of magnesium per day. Thus, at the age of 0-6 months, group 1 from my study were given about 1.4 grams less magnesium per day, and group 2 were given about 1 gram less magnesium per day than the foals from the study by Counotte, Kampman & Hinnen (2014) . At the age of 6-12 months group 1 from the present study were given about 0.1 grams less magnesium per day, and group 2 were given about 0.5 grams more magnesium per day than the foals from the study done by Counotte, Kampman & Hinnen (2014).

When comparing the two different feeds given to the two groups in the present study, the only finding that (based on previous research) could have led to a decrease in the number of OCD findings is the slight increase of 0.04 grams of magnesium that was present in the feed “Champion foal” given to group 2. The amount of magnesium given to group 2 is very similar to the amount given to the foals in the research done by Counotte, Kampman & Hinnen (2014). In their study it was also found that the more space and possibility to run outside the foals had access to, the less osteochondrotic lesions were found. Since an increased area to move

freely on was one of the two differences between group 1 and group 2 in my study, this too is a similar result to the study done by Counotte, Kampman & Hinnen Ir (2014).

## **6.2 CRITICISM**

The present study could probably have benefitted from a higher number of horses, as is the case in most other studies. For example, Lykkjen and colleagues (2012) used 464 Norwegian Standardbreds in their study, finding the prevalence of OCD to be 50.7% (Lykkjen, Roed & Dolvik, 2012). This is a big difference compared to the result from my research which was only 41.7%. When looking at the different groups of my research, group 1 in had a 56.25% prevalence of OCD and this is the closest result to the one Lykkjen had, compared to group 2 which only gave a 30% prevalence.

One can also point out that the horses in group 1 in my research was fed with “Champion breeder” which is a feed intended for mares and young horses, and thus the foals did not get the feed that is made especially for them, namely “Champion foal”, as in the case of group 2. Regarding the feed one can also state that the difference in magnesium in the feeds given to the two groups is only 0.04 grams per day and this amount may be too small to have an effect on the prevalence of OCD.

Unfortunately it was not possible to access any information on the prevalence of OCD in any of the parents of the foals in my study. Even though stallions with OCD is not excluded from breeding in Norway, that kind of information is still not given by any of the owners, in fear of ruining the reputation of the stallion or the mare.

## 7. SUMMARY:

The main goals of the study was to investigate the prevalence of OCD in general, in the different genders and in each joint examined. Because of the lack of access to any data on the parents of the yearlings in the present study, the focus in this thesis was both on the genes responsible for OCD and the environmental factors influencing these genes (epigenetics).

The choice of the particular topic "Genetics of osteochondrosis dissecans in Norwegian warm-blooded horses" was made because there has recently been done research in this area in Norway and also because of the opportunity to take part in the OCD examination of 36 Norwegian Standardbred yearlings.

Based on previous research, my initial hypothesis was that there would probably be a high prevalence of OCD among the 36 Norwegian Standardbred yearlings. OCD is a common developmental disease that, in most cases, is detected during a radiological examination after the formation of loose bony or osteochondral fragments. The fragment will most often cause an inflammation of the joint and thus the clinical signs, such as swelling, and lameness will appear. Most commonly involved are the fetlock, tarsocrural, femoropatellar and scapulohumeral joints. The initial step of OCD is thought to be the failure of blood supply to the growth cartilage. Stallions in Norway are allowed to breed even though they have OCD, but to a lesser degree in the case of the Norwegian warm-blooded riding horse.

When looking into the genetics of OCD in Norwegian warm-blooded riding horses, the available research was collected from countries contributing to the population of horses in Norway, because of the lack of research on the prevalence of OCD in Norwegian warm-blooded riding horses and the lack of Norwegian bred riding horses. The prevalence of OCD in the case of the breeds contributing to the Norwegian riding horses was between 15% and 36%. In the case of the Norwegian Standardbred horses the prevalence of OCD was 50.7%, and this indicates that there is a higher prevalence of OCD in the Norwegian Standardbred horses.

OCD is a phenotype of a quantitative trait and it is caused by genes in 25% of the cases. The genes may be influenced by the environment, for example a high energy diet or an imbalance in the diet, trauma and exercise. Concerning the diet, research has showed a link between the increase of magnesium and the decrease of osteochondrotic lesions (Counotte, Kampman & Hinnen, 2014), and also a positive link between copper and the repair of lesions (Van Weeren, 2006). A high energy diet has been both proved and disproved to have an effect on the development of osteochondrotic lesions (Van Weeren, 2006). It is stated that increased exercise contributes to the decrease of the lesions.

Genetic research has been done on OCD in Norwegian Standardbred horses and some QTL was identified by Illumina Equine SNP50 BeadChip whole-genome single-nucleotide polymorphism (SNP) assay using radiographs and blood. Some of these QTL on the chromosomes was proven to cause OCD in the plantar/palmar first phalanx and the intermediate ridge of the distal tibia.

Regarding the prognosis of OCD for horses that had not undergone surgery to remove the bony fragments from their joints, research has shown that OCD does not decrease the number of starts in trotting races but a tendency to a decrease in earnings was detected (Jørgensen, Proschowsky, Falk-Ronne, Willberg & Hesselholt, 1997).

Regarding retirement, the rate was higher in the cases where OCD was combined with other lesions.

The prognosis for horses after surgery depends on the type of joint operated on. Research has shown that in the case of the femoropatellar joint, the success rate was 95%. The tarsocrural joint and the metacarpophalangeal joint had success rates of 76.5% and 60%. The shoulder joint had the lowest success rate of only 50%.

The results from the present study on 36 Norwegian Standardbred horses revealed a prevalence of OCD of 41.7%. Importantly, a difference in the prevalence between the two groups of yearlings was detected, with group 2 having 26.25% less incidents of OCD. Even though the results did not reach statistical significance, and should thus be viewed with some caution, the

trending effects suggests a positive influence of having a larger area to move freely on, and perhaps also a benefit of increased levels of magnesium in the feed.

The present study provides a foundation for future research to further explore the benefits of exercise area and magnesium supplement in order to maximally reduce the risk of OCD in horses

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**Pictures:**

Picture 1: Osteochondritis Dissecans (OCD) in Horses (2015). *American College of Veterinary Surgeon*. <https://www.acvs.org/large-animal/osteochondritis-dissecans-horses>

Picture 2: Provided by Charlotte Ericson Elvebakk from Melhus Hesteklinikk AS and Equirad AS

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