BREEDING AGAINST 0STEOCHONDROSIS

Phenotypic and genetic analyses in horses and pigs

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This research was conducted under the auspices of the Wageningen Institute of Animal Sciences (WIAS) graduate school

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Thesis

Submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. dr. M.J. Kropff,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Wednesday 25 May 2011
at 1.30 p.m. in the Aula

E.M. (Ilse) van Grevenhof
Breeding against osteochondrosis: Phenotypic and genetic analyses in horses and pigs
182 pages

Thesis, Wageningen University, Wageningen, NL (2011)
With references, with summaries in English and Dutch.

ISBN 978-90-8585-854-6

Abstract

Osteochondrosis (OC) is a disturbance in the process of endochondral ossification during skeletal growth. The objectives of this thesis were to assess the prevalence and genetic parameters of OC, and to facilitate optimization of breeding against OC in horses and pigs.

In the Dutch warm blood horse, 70% of individuals showed joint abnormalities in at least one of the 28 locations examined. Joint abnormalities were divided into flattened bone contours and bone fragments. Genetic parameters were estimated for flattened bone contours, fragments and overall OC. Heritability was 0.08 for flattened bone contours, 0.22 for fragments, and 0.23 for overall OC.

In fattening pigs, the prevalence of OC was 41%, and 12% of the individuals had severe OC. The prevalence was highest for individuals kept on a concrete, partially slatted floor with *ad libitum* feeding (58%), and lowest for individuals kept on a deep litter floor with restricted feeding (34%). These results demonstrate that the prevalence of OC can be reduced by applying deep litter floors and restricted feeding. The differences in growth patterns between pigs with and without OC were investigated also. After 28 days of age, piglets with severe OC at slaughter started to grow faster, and were significantly heavier after 70 days of age than pigs without OC at slaughter. These results suggest that OC might be related to high growth rates during a specific time period.

To evaluate prospects for breeding against OC, selection responses were compared between breeding schemes using phenotypic selection based on own performance or progeny testing and genomic selection. The results show that, when genomic information has an equal accuracy as phenotypic information before selection, it will have a lower equilibrium response to selection because it has a larger Bulmer effect. Especially for low heritable traits, genomic selection was of additional value over traditional selection.

The general discussion argues that OC has both economical and practical relevance for the current horse industry. Simulations showed potential for genomic selection compared to the current breeding program in the Dutch warmblood horse (KWPN), even with small reference populations. In pigs, OC is a more serious problem in sows than in fattening pigs, because sows live longer. A decrease in OC prevalence will, therefore, yield larger economic and welfare benefits then apparent from results on fattening pigs. Also for pigs, simulations indicated possibilities for genomic selection. The final section of the general discussion proposes directions for future research.

Contents

1

General introduction

Introduction

Osteochondrosis (OC) is defined as a disturbance in the process of endochondral ossification during skeletal growth (Crenshaw, 2006; Donabédian *et al.*, 2006; Grøndalen, 1974; Van Weeren, 2006; Ytrehus *et al.*, 2007). It is characterized by irregularities in the ossification process that lead to locally thickened cartilage plugs, malnutrition of the tissue, formation of focal necrotic spots, fissures, cartilage flaps and eventually loose fragments. When OC results in the formation of fragments, it is called *osteochondritis dissecans* (OCD; Figure 1.2). Clinically, the condition is in most cases characterized by joint distension and sometimes by lameness. Figure 1.1 shows a joint with the cartilage in which the disbalance in the process of ossification occurs.

Aetiology and pathogenesis

Osteochondrosis is a multifactorial disorder, of which the aetiopathogenetic factors can be divided into external environmental factors and internal factors inherent to an individual, which are entirely or partly genetically determined. In pigs, feeding level and housing are the main environmental factors, whereas gender and growth rate the most important internal factors.

Biomechanical influences play a crucial role in the development of OC, possibly because of their impact on the vulnerable micro-circulation of the ossifying epiphyses of various long bones (Ytrehus *et al.* 2007). Biomechanical influences can explain the consistent predilection sites of lesions within joints, together with the chronological differences in histomorphological ripening of joints and related time windows of vulnerability, the left-right symmetry and the joint-dependent sequence of occurrence of lesions.

Genetics

The aetiology of OC is still not fully understood, but it is agreed that the disorder is multifactorial in origin (Jeffcott, 1991; Philipsson *et al.*, 1993; Wittwer *et al.*, 2006) and that genetic influences play an important role (Schougaard *et al.*, 1990; Philipsson *et al.*, 1993; Van Weeren, 2006). Estimated heritabilities vary widely (Table 3.1), possibly due to differences in criteria, methodology and populations between studies (Ricard *et al.*, 2002). Because initial research of the genetic basis of OC indicates that the disorder is complex (Wittwer *et al.*, 2007), genetic progress can be made by using classical quantitative genetic approaches for selection. The first results using high-

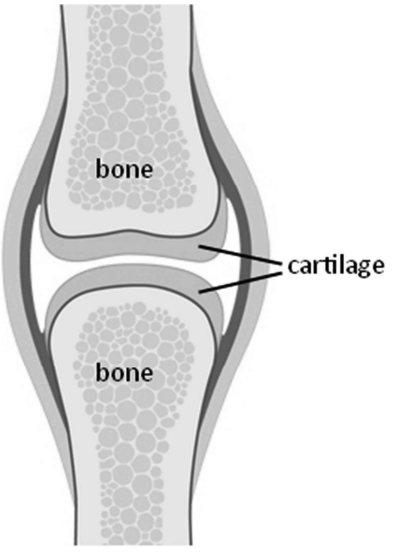

Figure 1.1 Joint with bone and cartilage.

throughput molecular genetic screening of DNA indicate that the genetic background of OC may be complicated, with different genes affecting different locations (Wittwer *et al.* 2007).

OC development

OC lesions develop in the first months after birth, much earlier than previously recognized (Carlson *et al.*, 1995; Dik *et al.*, 1999). However, this process is highly dynamic (Bittegeko *et al.*, 1994, van Weeren 2006). Lesions arise at early age, but incite a repair response immediately and most of them disappear completely during the following months. Some lesions remain and become permanent (Dik *et al.*, 1999; Carlsten *et al.*, 1993). The regenerative capacity of articular cartilage diminishes rapidly after birth, with virtually no repair capacity left in the mature individual due to the long turnover times of collagen (Maroudas, 1980; Bertone *et al.*, 2005). This means that there is a point of no return, after which existing lesions will not be repaired anymore and thus become clinically relevant. This point of no return varies among joints and species. In the horse, the point of no return occurs around 5-8 months after birth (Dik *et al.*, 1999; van Weeren, 2006).

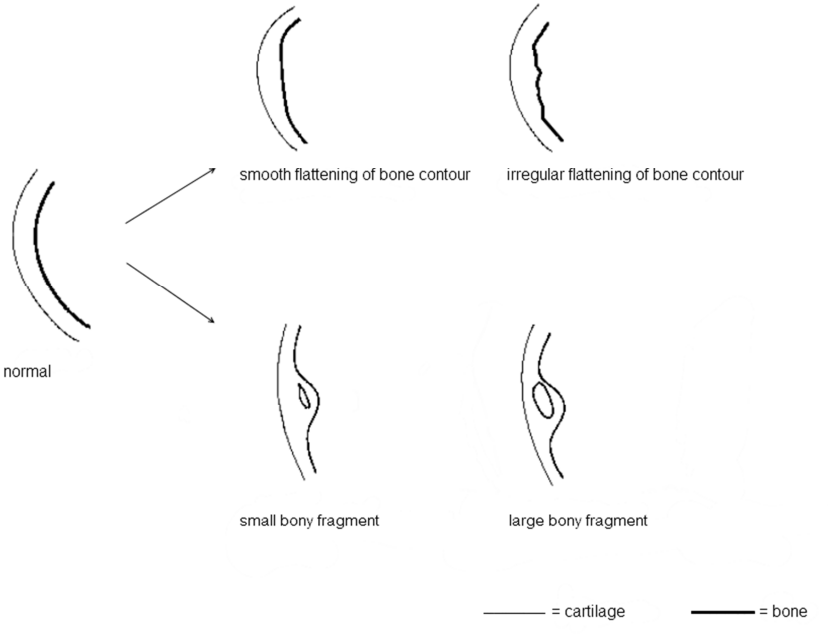

Figure 1.2 Manifestations of osteochondrosis as radiographically scored in this study in horses, distinguishing flattened bone structures and fragments (osteochondritis dissecans) (after Van den Berg *et al.*, 2001).

OC in different species

OC is common in many species, such as dogs, horses, cows, pigs and humans. In the domesticated species, the disease is most prevalent in swine and horses. Given the supposed or perceived impact on locomotion, performance, and commercial value, much research has been carried out in the horse. As the character of the disease in horses and swine is very similar, the expertise gained in horses can be used for research in swine.

In horses, environmental factors possibly affecting OC are difficult to evaluate reliably, as horses are rarely kept in large numbers under identical conditions. These limitations affect the possibilities for research, which can often more easily be performed in other species such as pigs. Besides the relevance of the results found in pigs for horses, OC is also relevant for the pig industry itself, as high prevalences of OC have been found in pigs which affect both welfare and

economics (Crenshaw, 2006; Grøndalen, 1974),. This thesis, therefore, focuses on OC in horses and pigs, and aims at gaining knowledge from both species to optimize breeding against OC.

OC in pigs

OC is the main cause of leg weakness and related lameness in pigs (Grøndalen, 1974; Jørgensen, 1995; Reiland 1978). Although culling rates due to OC are not very high in fattening pigs, OC does reduce production and welfare in fattening pigs (Yazdi *et al.*, 2000). In sows, OC is a more serious problem, and a common reason for culling (Engblom *et al.*, 2008; Yazdi *et al.*, 2000). Though no specific figures for OC are known, next to reproductive diseases, lameness is the most important cause for premature culling of sows (Gresham, 2003; Dewey, 2006). Sows have a longer life and a decrease in OC will therefore have larger economic and welfare impacts. Besides, selection in sows will also indirectly affect the prevalence of OC in fattening pigs.

The prevalence of OC found in previous studies varied from approximately 80% to 100% in pigs at slaughter (Crenshaw, 2006; Grøndalen, 1974a). In the Netherlands, direct selection against OC has not been practiced thus far, which is in contrast to the situation in Scandinavian countries, where OC has been included in the breeding goal and hence has been a selection criterion for decades already (Ytrehus *et al.*, 2007; Yazdi *et al.*, 2000).

OC in horses

For decennia, osteochondrosis has been the most important orthopaedic developmental disorder in horses. In this species, OC is mostly found in the femoropatellar and tarsocrural joints (Figure 1.3). The most common clinical sign of OC is non-painful joint distension. Other clinical signs may include a reduced range of motion in joints, a positive response to flexion tests, and varying degrees of lameness. These clinical signs are usually associated with the onset of training and therefore suggest activation of subclinical lesions through biomechanical influences (Jeffcott 1997). Osteochondrosis is common in warmblood breeds, Thoroughbreds, and Standardbreds (Jeffcott 1991; Philipsson *et al.* 1993; Stock *et al.* 2005), but a recent study also found OC in a coldblood population (Wittwer *et al.* 2006). Previous studies have reported prevalences of osteochondrotic lesions or fragments between 7% and 64% across a range of breeds (Grøndahl and Dolvik 1993; Hoppe 1984; Hoppe and Philipsson 1985; Jeffcott and Henson 1998; Philipsson *et al.* 1993; Pieramati *et*

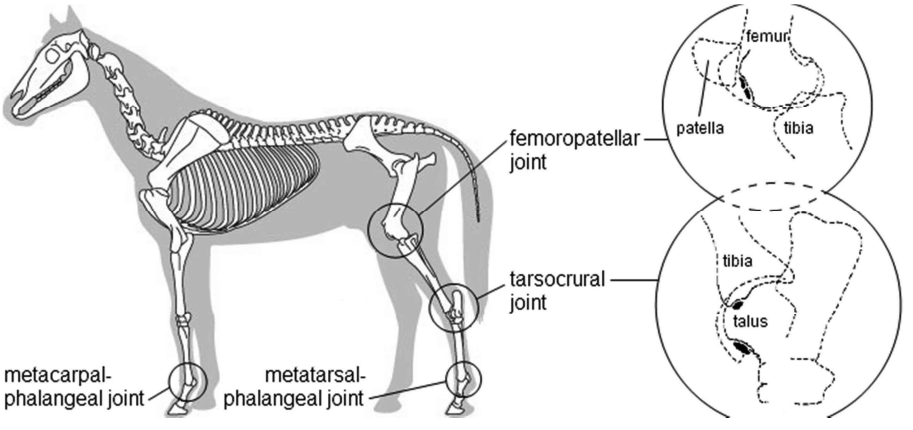

Figure 1.3 Predilection sites of osteochondrosis as radiographically found in horses, with details of the femoropatellar joint and in the tarsocrural joint (after Van den Berg *et al.*, 2001).

al. 2003; Ricard *et al.* 2002; Sandgren *et al.* 1993; Schober *et al.* 2003; Schougaard *et al.* 1990; Wittwer *et al.* 2006). The large range in prevalence may be attributed to different definitions of OC, the use of datasets from preselected populations, differences between breeds, differences scales used for OC scoring, or differences in the numbers of predilection sites screened.

Diagnosis in horses

Radiographic examination is the golden standard for the diagnosis of OC *in vivo* in the horse. However, conventional radiology cannot visualize cartilage lesions, and is not sensitive enough to detect subtle subchondral bone irregularities. Thus there is a need for alternative diagnostic methods. A possible alternative method is magnetic resonance imaging (MRI). MRI is a noninvasive method for the assessment of soft tissues including articular cartilage, and has been shown to be an excellent tool for the detection of cartilage lesions and/or the visualization of repair tissue (Recht, 2005). However, availability of MRI equipment for horses is limited and the technique may be cost-prohibitive in many cases.

History of selection in warmblood horses in the Netherlands

The Royal Warmblood Studbook of the Netherlands (KWPN) is the Dutch breeding organization that seeks to breed show jumpers, dressage horses, and

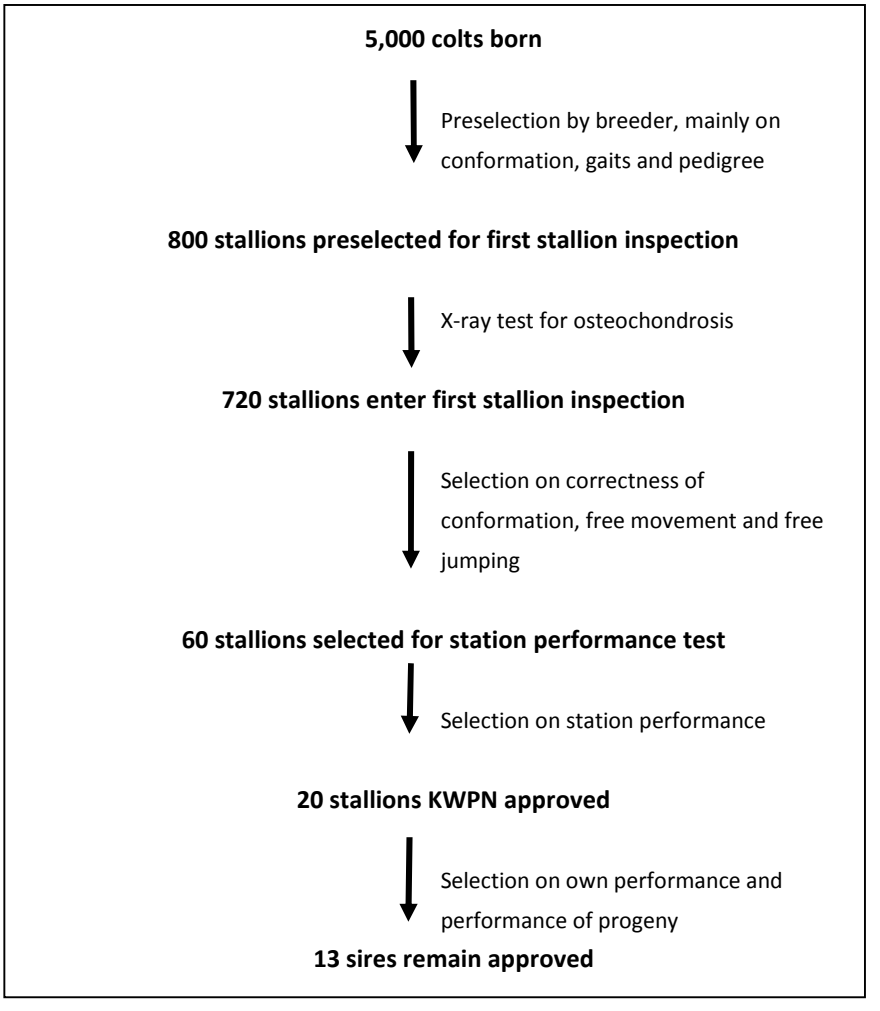

Figure 1.4 Approximate number of stallions selected at the different stages of the selection procedure by KWPN.

a few numerically minor other types of horses. With 30,000 members and approximately 12,000 foals born each year, the KWPN is one of the largest sport horse studbooks in the world. For years, the KWPN has held a top position in the studbook rankings of the international breeding organization, the World Breeding Federation for Sport Horses (WBFSH). This means that Dutch-bred horses routinely achieve success in international equestrian competition. The goal of the KWPN is to breed a performance horse that can compete at the highest level of the sport. To achieve this goal, a horse obviously must have a

healthy constitution, correct and functional conformation, and correct to superb gaits (www.kwpn.nl). Three traits constitute the main breeding goal, which are performance, health and conformation. Breeding values are estimated based on sport and test results using an animal model corrected for age and gender in sport, and for test group and thoroughbred percentage in the performance test. Performance is determined by the highest score in the branch of equestrian activity (dressage or show jumping) where a particular horse is supposed, based on its pedigree, to be best suited for. Health includes locomotion, respiration and fertility. Conformation includes functionality, correctness and attractiveness. Depending on its pedigree, conformation, and abilities, a KWPN horse is registered as a show jumper, dressage horse (together responsible for 90% of the population), carriage horse (7%), or a so-called Gelder horse (3%), which is a more basic type of horse with little Thoroughbred influence. The process of selection differs for stallions and mares. The selection in stallions is controlled by the studbook, whereas the selection of mares is only stimulated by the studbook but controlled by the breeders. Annually, about 5,000 colt foals are born, of which only approximately 13 will eventually reach full approval as a breeding sire (Figure 1.4). Prior to the start of the selection process, a stallion must meet a number of veterinary requirements. First, he must be regularly vaccinated for influenza and tetanus. Second, he must pass a radiographic exam that includes views of several bones and joints in the front and hind limbs. To avoid disappointment and unnecessary costs, most breeders have the stallion radiographed prior to registering him for the selection. In addition, stallions must undergo a respiratory exam (for laryngeal hemiplegia) as well as a clinical inspection of the heart, eyes, teeth, and reproductive organs. Stallions selected for the performance test are evaluated for semen quality. Finally, a DNA test is conducted to ensure that each stallion matches his pedigree. All examinations must be performed by veterinarians and institutions approved by the KWPN.

Outline of this thesis

The overall aim of this thesis is to generate knowledge that may facilitate optimization of breeding against osteochondrosis. The thesis contains five research chapters, and concludes with a general discussion in which three main topics emerging from the preceding chapters are discussed in depth.

In chapter 2, prevalences and severity of OC in horses were estimated at sire level, animal level, joint level, and predilection site level. The stifle, hock and

fetlock joints of 811 randomly selected horses, descending from 32 representative stallions, were radiographed and scored for the presence and severity of osteochondrotic lesions. It was shown that scoring on a detailed scale is necessary to achieve good insight into the prevalence of OC. Results showed that observations on the right and left joints can be combined in further analyses, whereas flattened bone contours and fragments should be evaluated as statistically different disorders.

In chapter 3, heritabilities and genetic correlations were estimated for various manifestations of OC in the horse. Heritabilities were low to moderate, ranging from 0.08 to 0.22. Results from this chapter suggest that selection against OC could best be performed by taking into account the OC status of all 4 joints considered (stifle, hock and fore and hind fetlock joints), and discriminating between flattened bone contours and fragments.

Pigs provide research opportunities that are often not available in (young) horses, such as repeated (bodyweight) observations and macroscopic scoring of lesions in a large population. Pigs are used as comparative species for OC in horses, but also with the aim of improving pig breeding, as OC affects both animal welfare and economics in the pig industry. In chapters 4 and 5, OC was studied in experimental pig populations where housing and feeding regimen were the main variables.

In chapter 4, the prevalence of OC in these populations was estimated. A two by two factorial design of housing system and feeding strategy was applied. At the age of 69 days, intact boars and gilts were separated and assigned to groups of five or six individuals. Pigs were slaughtered at an average age of 164 days. Five joints of the left front and hind limbs were macroscopically assessed for OC. In the total population OC had a prevalence of 41%, with 12% presenting severe lesions. The tarsocrural joint was most affected (30%).

In chapter 5, the relationship between growth and OC was investigated. For this purpose, body weights were measured ~17 times from birth until slaughter, and OC scores were used to estimate the relationship between the growth pattern and the onset of OC. Pigs were divided into three categories based on the severity of OC: no OC, minor OC, or severe OC. Growth curves were fitted for each category, aiming to detect differences in growth pattern between grades of OC or between manifestations of OC in specific joints. From weaning onwards, pigs diagnosed with minor or severe OC showed higher body weight than pigs diagnosed without OC. The higher weight was due to increased growth before the age of three months. This period might coincide with the

window of susceptibility for OC in pigs. The relationship with growth seemed to be joint-dependent.

In chapter 6, breeding programs are compared for a trait that is expensive or difficult to measure (such as OC), so that the number of phenotypic records is limited. Genomic selection was compared to phenotypic selection based either on own performance records or on progeny records. Deterministic simulations were used to calculate the break-even point at which response to genomic and phenotypic selection was equal. Results show genomic selection may yield equal response as traditional selection, even when only small reference populations are feasible.

The final chapter, the general discussion, explores possibilities for breeding against OC in horses and pigs. The first section deals with the impact of OC on locomotion and sport traits, in order to assess the relevance of OC for the breeding goal. The second section compares different breeding strategies designed for the Dutch warmblood horse population. Possibilities for genomic selection are evaluated and compared to selection strategies that have been in use until recently (own performance of stallions), and which have recently been adopted (progeny testing). The third and last section of the general discussion focuses on selection against OC in pigs, by identifying the relevance of OC in pigs, and evaluating possibilities for the implementation of genomic selection.

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2

Prevalence of various radiographic manifestations of osteochondrosis and their correlations between and within joints in Dutch Warmblood horses (KWPN)

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Equine Veterinary Journal (2009) 41(1):11-16

Abstract

Reasons for performing study: Osteochondrosis (OC) is the most important orthopaedic developmental disorder in horses and may manifest in several different forms. No detailed study on the prevalence and/or interrelation of these forms is available, even though these data are a prerequisite for conclusive genetic studies.

Objectives: To assess the prevalence of the various manifestations of OC as detected radiographically and to evaluate possible relationships between their occurrence within the same joint and between different joints.

Methods: The FP (femoropatellar), TC (tarsocrural), and MCP/MTP (metacarpophalangeal/metatarsophalangeal) joints of 811 randomly selected yearlings, descending from 32 representative stallions, were radiographed and scored for the presence and grade of osteochondrotic lesions. Results were compared at the sire level, animal level, joint level, and predilection site level.

Results: In the FP joint, the percentage of animals showing normal joint contours in all sites was 60.7%. For the TC joint and the combined MCP/MTP joints, these figures were 68.6% and 64.6%, respectively. For all joints combined, the percentage dropped to 30.5%. Sedation improved detection of OC lesions in the FP joint. There was a high correlation between the right and left joints. The correlation between flattened bone contours and fragments was considerably less.

Conclusions: Scoring on a detailed scale is necessary to achieve good insight into the prevalence of OC. Observations on the right and left joints can be combined in further analyses, whereas flattened bone contours and fragments should be evaluated as statistically different disorders.

Potential relevance: This study provides insight into the prevalences of various manifestations of OC and their relationships, within and between joints. These results form the basis for detailed quantitative and/or molecular genetic studies that may lead to the establishment of breeding indices and/or genetic marker sets for OC.

Key words: Horses, osteochondrosis, joints, radiographic findings, prevalence, fragments

2.1 Introduction

Osteochondrosis (OC) is the most important orthopaedic developmental disorder in horses.

OC is a disturbance in the physiological process of endochondral ossification, which occurs in young, growing individuals. Irregular ossification leading to the formation of thick cartilage plugs may, in combination with biomechanical influences, result in the formation of focal necrotic areas, detachment of cartilage flaps, and eventually the formation of loose fragments (Jeffcott 1997; van de Lest *et al.* 1999). When loose fragments are present, the term osteochondritis dissecans is used. The most common clinical sign of OC is non-painful joint distension. Other clinical signs can be stiffness of joints, a positive response to flexion tests, and varying degrees of lameness. These signs are usually associated with the onset of training and therefore suggest activation of subclinical lesions through biomechanical influences (Jeffcott 1997). The aetiology of OC is still not fully understood, but there is agreement that the disorder is multifactorial in origin (Jeffcott 1991; Philipsson *et al.* 1993; Wittwer *et al.* 2006).

OC is common in warmblood breeds, Thoroughbreds, and Standardbreds (Jeffcott 1991; Philipsson *et al.* 1993; Stock *et al.* 2005), but a recent study also found OC in a coldblood population (Wittwer *et al.* 2006). Previous studies across a range of breeds reported prevalences of osteochondrotic lesions or fragments in the limb joints between 7% and 64% (Grøndahl and Dolvik 1993; Hoppe 1984; Hoppe and Philipsson 1985; Jeffcott and Henson 1998; Philipsson *et al.* 1993; Pieramati *et al.* 2003; Ricard *et al.* 2002; Sandgren *et al.* 1993; Schober *et al.* 2003; Schougaard *et al.* 1990; Wittwer *et al.* 2006). The large range in prevalence may be attributed to different definitions of OC, the use of preselected datasets, differences in breeds, differences in scales used for OC scoring, or differences in the numbers of predilection sites screened.

The (radiographic) definition of OC poses a particular problem. Bony fragments seen at certain predilection sites, such as the distal tibial ridge or the lateral femoral trochlea, are with high certainty osteochondrotic in nature. Many of the loose fragments located at the palmar or plantar side of the metacarpophalangeal or metatarsophalangeal joints, however, are of traumatic origin (Dalin *et al.* 1993; Nixon and Pool 1995; Sønnichsen *et al.* 1982). Nevertheless, in some studies, all radiographically visible fragments are classified as OC, whereas other reports discriminate between fragments

probably caused by OC and fragments of other origin. Flattening of the bone contour at certain predilection sites is commonly interpreted as OC (Butler 1993). However, the relationship between flattening of the bone contour and osteochondrotic fragments has not been proven.
OC is a dynamic disorder; during the first few months of life, lesions may appear and disappear again (Dik *et al.* 1999; Van Weeren 2006b). The age at which horses are radiographed is, therefore, of great importance. Dik *et al.* (1999) showed that lesions were permanent from the age of approximately five months in the TC joint, but stability was reached later, at the age of approximately eight months in the FP joint.

The first results using high-throughput molecular genetic screening of DNA indicate that the genetic background of OC may be extremely complicated, with different genes affecting different locations (Wittwer *et al.* 2007). For a molecular genetic approach to succeed, the phenotypic definition of the disorder must be evident, with known prevalences and relationships of the various phenotypic forms. In the absence of these data, molecular genetic approaches are not yet fully applicable. Quantitative genetic approaches, such as selection using estimated breeding values for stallions based on progeny information, can also be effective, considering the heritabilities found and the heritable factors contributing to the multifactorial influences on OC (Van Weeren 2006a). However, until now, selection against OC has not been very effective in practice, possibly because of the incomplete phenotypic definition. In studies designed to fill in this gap, prerequisites are a sufficiently large population sample that is well defined for age, gender, and parentage, and a detailed and consistent radiographic scoring system.
This study used radiographic data from 811 Dutch Warmblood animals to assess the prevalence of the various radiographic manifestations of OC and to evaluate relationships between occurrences of these forms within joints, and between joints and their contralateral homologues.

Abbreviation key
OC = osteochondrosis; NJ = non-judged predilection sites; ALL = linear OC value in the animal, calculated by summing the linear OC values of all 5 joints; FLAT = continuous OC values for flattened bone contour; FRAG = continuous OC values for fragments; FP = femoropatellar; TC = tarsocrural; MCP/MTP = metacarpophalangeal and metatarsophalangeal.

2.2 Material and methods

Material

Data were collected from 811 animals of the Royal Dutch Warmblood horse population (Royal Warmblood Studbook of the Netherlands, KWPN) during 2005 (n = 593) and 2006 (n = 218). Animals were descended from 32 breeding stallions and 801 mothers, some of which had been diagnosed as OC negative; however, for most mothers the status was unknown. The breeding stallions were representative for the population of approved sires with at least 25 registered offspring in breeding seasons 2005 and 2006. For approval as a breeding stallion within the KWPN studbook, an OC-free status is a prerequisite. The number of animals per sire varied from 22 to 28. The animals varied in age with a minimum age of 9 months (mean: 12 months, sd: 2.6); in gender (47.3% males); in percentage thoroughbred in the pedigree from 0% to 58%; in withers height (mean: 149 cm, sd: 5.7); and in chest circumference (mean: 165 cm, sd: 9.3). Animals were scored from 1 to 3 for body condition: 1 being underfed, 2 being normal condition, and 3 being overweight. The horses had been reared by a large variety of breeders and therefore feeding, housing, and exercise level varied widely.

Animals were scored for OC based on radiographs from eight joints: the femoropatellar (FP), tarsocrural (TC), metacarpophalangeal (MCP), and metatarsophalangeal (MTP) joints. A total of 28 predilection sites per animal were scrutinised for the presence of OC lesions: 5 in the FP joint, 7 in the TC joint, and one in the each of the MCP and MTP joints (Table 2.1). At each site, OC was scored on a categorical scale from A through E (Table 2.2), adapted from Dik *et al.* (1999), but converting the original 0 to 4 quantitative scale to A through E to emphasize the categorical character of the trait (Table 2.2). Score A indicates 'normal joint contour', scores B and C indicate 'flattened bone contours', and scores D and E indicate 'fragments'. The radiographs were of comparable quality and taken by 15 preselected equine practices, two of which were responsible for 61% of all radiographs. Eighty-five percent of the animals were sedated for the radiographic examination. An experienced radiologist judged all radiographs.

Table 2.1 Predilection sites per joint judged for osteochondrosis on digital radiographs.

Joint	Predilection site	Judged on radiograph
FP joint[1]		
1	Lateral femoral trochlea	1
2	Medial femoral trochlea	1,2
3	Sulcus intertrochlearis	1
4	Patella	1,2
5	Other predilection sites	2
TC joint[2]		
1	Sagittal ridge of distal tibia	2,3
2	Lateral trochlea of talus	2
3	Medial trochlea of talus	2
4	Lateral malleolus of tibia	1
5	Medial malleolus of tibia	1
6	Base of talus	1,2
7	Other predilection sites	1,2,3
MCP/MTP joints[3]		
1	Proximodorsal part of the sagittal ridge of the 3rd metacarpal/metatarsal bone	1

[1]FP = femoropatellar; [2]TC = tarsocrural; [3]MCP/MTP = metacarpophalangeal and metatarsophalangeal.

Methods

Prevalences were calculated at the level of sire, animal, joint, and site. To evaluate relationships between the occurrence of the various forms of OC in different joints and between contralateral homologues, a cluster analysis (SAS, 2004) was performed. Results of the cluster analyses were used to reduce the number of variables summarizing the OC status of an animal.

A cluster analysis groups similar variables by using correlations between observations. To enable calculation of correlations, we transformed the categorical scores A through E into quantitative values on a continuous scale (see below). Subsequently, quantitative values were summed to establish three quantitative traits: the trait ALL, representing the overall OC value taking into

Table 2.2 Classification of findings of osteochondrosis (OC) on digital radiographs in 5 OC categories (Dik *et al.* 1999).

Grade	Classification	Bone contour	Subchondral bone texture	Fragment(s)
A	Normal	Rounded	Diffuse density	Absent
B	Minimal	Smoothly flattened	Obscure lucency	Absent
C	Mild	Irregularly flattened	Obvious, ill-bordered local lucency	Absent
D	Moderate	Small, rounded/irregular concavity	Obvious, well-defined local lucency	Small fragment(s)
E	Severe	Large, rounded/ irregular concavity	Obvious, well-defined extensive lucency	Large fragment(s)

account both flattened bone contours and fragments; the trait FLAT, representing only flattened bone contours; and the trait FRAG, representing only fragments. The variable ALL therefore summarizes the OC status of a joint or an animal, assuming that flattened bone contours and fragments are variants of the same disorder, which is unknown *a priori*. The variables FLAT and FRAG summarize the OC status separately for flattened bone contours and fragments. The cluster analysis showed the statistical relationship between flattened bone contours and fragments and thus indicated whether FLAT and FRAG could indeed be considered variants of the same disorder.

For the transformation from categorical observations to quantitative values, a continuous normally distributed liability (Figure 2.1) underlying the categorical observations was assumed (Falconer 1965). For example, in Figure 2.1, score A corresponds to a liability value below 0.8, and score B corresponds to a liability between 0.8 and 1.3. Categorical scores were transformed into the mean liability value for that score. For example, in Figure 2.1, score A occurred in 79% of the cases, represented by the area below a liability value of 0.8. The mean liability value of this area is -0.354. Thus, score A was transformed into a value of -0.354. Figure 2.1 indicates the remaining transformed values for B through E.

For the trait ALL, the normal distribution was split into five categories, corresponding to A through E, as visualised in Figure 2.1. For the traits FLAT and FRAG, the normal distribution was split into three categories, representing A, B, and C for FLAT, and A, D, and E for FRAG. For each predilection site, the

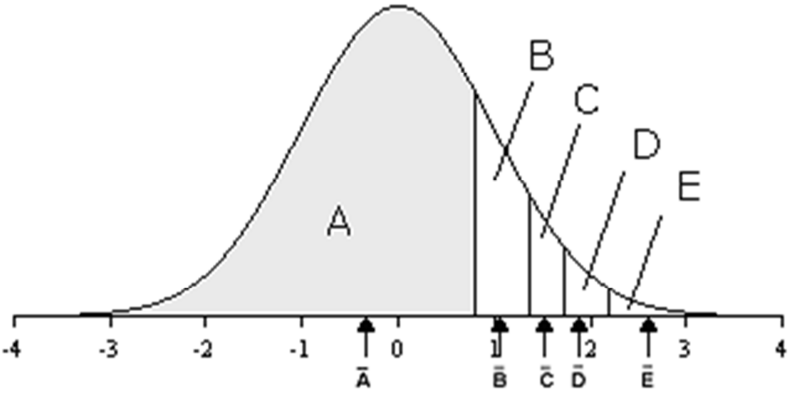

Figure 2.1 Example of a normal distribution (numbers on x-axis indicate number of standard deviations) representing the percentage of the osteochondrosis (OC) scores A to E in the metacarpophalangeal joint and the metatarsophalangeal joint to calculate the linear OC score. An animal with two predilection sites scored A, one scored B, and one scored E in the metacarpophalangeal joint and the metatarsophalangeal joint will have a linear OC value of 2*-0.354+1*1.067+1*2.618= 2.977 for these joints.

categorical score was transformed into three quantitative values, one for each trait. Subsequently, values for ALL, FLAT, and FRAG were calculated for each joint by summing the values of all sites within that joint. This approach resulted in three traits for each of the 8 joints, giving a total of 16 traits in the cluster analysis (Figure 2.2). In addition, ALL, FLAT, and FRAG were calculated at the animal level by summing trait values over all joints.

2.3 Results

The number of sites that could not be judged because of technical reasons varied among joints and among equine practices, but it was low at less than 1% for most sites. Only with respect to the patella was this figure substantially higher at 10.1%. In general, the FP joint appeared to be the most difficult joint for obtaining consistently good radiographs (Table 2.3).

Prevalences

Sire level

The prevalence of OC varied substantially among stallions ($P<0.001$), indicating a relatively high heritability for OC. The percentage of offspring with normal

Table 2.3 Osteochondrosis at the joint and site levels.

Predilection site[1]	1	2	3	4	5	6	7	Mean
FP joint[2]								
%A	88.96	98.4	78.99	88.72	99.75			90.92
%B	1.05	0.43	15.6	0.86	0.06			3.60
%C	1.48	0.49	4.93	0.31	0			1.44
%D	2.47	0.12	0	0	0			0.52
%E	5.49	0.18	0	0	0			1.13
%NJ[5]	0.55	0.37	0.68	10.11	0.18			2.38
TC joint[3]								
%A	92.23	90.44	92.66	99.45	97.41	99.51	100	95.96
%B	1.17	4.01	4.93	0	0.62	0.06	0	1.54
%C	0.31	0.99	1.79	0	0.31	0.06	0	0.49
%D	3.27	3.58	0.06	0.06	0.25	0.12	0	1.05
%E	1.54	0.55	0	0	0	0.06	0	0.31
%NJ[5]	0.06	0.31	0.55	0.43	1.42	0.18	0	0.43
MCP/MTP joint[4]								
%A	80.83	76.88						78.86
%B	12.03	10.48						11.26
%C	2.16	5.06						3.61
%D	2.90	5.18						4.04
%E	0.56	1.73						1.15
%NJ[5]	1.55	0.68						1.12

[1]For each joint, the mean of the left and right homologues is presented. In the MCP/MTP joint, site 1 represents the front limbs and site 2 the hind limbs; [2]FP = femoropatellar; [3]TC = tarsocrural; [4]MCP/MTP = metacarpophalangeal and metatarsophalangeal; [5]NJ = could not be judged.

joint contours, score A, in all predilection sites varied from 12% to 58%. The percentage of offspring with at least one score B varied among stallions from 22% to 57%. For scores C, D, and E these ranges were respectively 0–32%, 3.6–32%, and 0–32%. The breeding specialisation of the stallion, for either show jumping or dressage, did not affect the prevalence.

Table 2.4 Prevalence of osteochondrosis at the animal level.

	FP[1]	TC[2]	MCP/MTP[3]	Entire animal
=A[4]	61	69	65	30
≤B	19	13	17	24
≤C	9	5	6	14
≤D	3	10	9	17
≤E	9	4	3	14

[1]FP = femoropatellar; [2]TC = tarsocrural; [3]MCP/MTP = metacarpophalangeal and metatarsophalangeal; [4] A = % of animals with a score of A at all predilection sites; ≤B = % of animals with one or more B scores, but no C, D, or E scores; ≤C = % of animals with one or more C scores, but no D or E scores; ≤D = % of animals with one or more D scores, but no E scores; and ≤E = % of animals with one or more E scores. Results refer either to a specific joint (FP; TC; MCP/MTP) or to the entire animal. Results for joints are averages of the left and right homologues.

Animal level

For the analysis at the animal level, animals were grouped based on their worst OC score. The MCP joints will be combined with the MTP joints as suggested by the cluster analysis (see section cluster analysis below). This procedure resulted in five groups: =A, animals with A scores at *all* sites; ≤B, animals with one or more B scores, but no C, D, or E scores; ≤C, animals with one or more C scores, but no D or E scores; ≤D, animals with D scores but no E scores; and ≤E, animals with E scores. The percentage of animals that were =A was 61% for the FP joint, 69% for the TC joint, and 65% for the MCP/MTP joints (Table 2.4). At the level of the entire animal, 30% of all animals were in group =A; 24% animals were in group ≤B; 14% animals were in group ≤C; 17% animals were in group ≤D; and 14% animals were in group ≤E. At the animal level, the animal with the lowest number of A scores had 11 A scores out of 28 sites.

Joint level

For each joint, the average percentages of the left and right homologues are presented (Table 2.4). In the FP joint, 90.9% of the sites were scored A. In the TC and the MCP/MTP joints, this figure was respectively 96.0% and 79.8%. In the FP and the MCP/MTP joints, relatively more flattened bone contours than fragments were found, in contrast to the TC joint.

Table 2.4 Prevalence of osteochondrosis at the animal level.

OC[1]	FFlL	TFlR	TFlL	MCFl	MTFl	FFrR	FFrL	TFrR	TFrL	MCFr	MTFr
FFlR	0.39	0.07[2]	0.01[2]	0.05[2]	0.01[2]	0.26	0.17	0.04[2]	0.05[2]	0.03[2]	0.07
FFlL		0.08	0.07	0.05[2]	0.05[2]	0.15	0.12	0.01[2]	-.00[2]	0.03[2]	0.05[2]
TFlR			0.33	0.1	0.08	0.03[2]	0.03[2]	0.07	0.08	0.04[2]	0.01[2]
TFlL				0.11	0.04[2]	0.01[2]	0.01[2]	0.10	0.05[2]	0.01[2]	0.03[2]
MCFl					0.33	0.08	0.12	0.06[2]	0.02[2]	0.15	0.20
MTFl						0.11	0.09	0.03[2]	0.01[2]	0.13	0.15
FFrR							0.55	0.02[2]	0.08	0.04[2]	0.10
FFrL								0.05[2]	0.10	0.07	0.10
TFrR									0.49	0.02[2]	0.00[2]
TFrL										0.08	0.06[2]
MCFr											0.35

[1]The first one or two letters refer to the joint: F = FP (femoropatellar) joint, T = TC (tarsocrural) joint, MC = metacarpophalangeal joint, and MT = metatarsophalangeal joint. The next letter refers to either Fl = FLAT or Fr = FRAG (FLAT = continuous OC values for flattened bone contour; FRAG = continuous OC values for fragments). The last letter refers to R = right or L = left; [2] not significantly different from zero, with a $P > 0.05$.

Predilection site level

For the analysis at site level, we used a different scale. In the FP joint, 91.4% of flattened bone contours were found in the lateral femoral trochlea and the sulcus intertrochlearis (sites 1 and 3), and 96.3% of fragments were found at the lateral femoral trochlea (site 1) (Table 2.4). In the TC joint, 92.6% of the flattened bone contours were found at the sagittal ridge of the distal tibia, the lateral and medial trochlea of the talus (sites 1, 2, and 3), and 94.4% of fragments were found at the sagittal ridge of the distal tibia and the lateral trochlea of the talus (sites 1 and 2). In the MCP/MTP joints, prevalences were similar, varying from 12.7% to 16.1% for flattened bone contours and from 3.3% to 7.3% for fragments. Higher percentages for scores B to E were found in the MTP joints than in the MCP joints.

Relationships

Correlations within and between joints

The correlation between the overall OC scores, ALL, in the entire animal and in the FP joint was 0.60. For the TC joint and the MCP/MTP joints, these figures

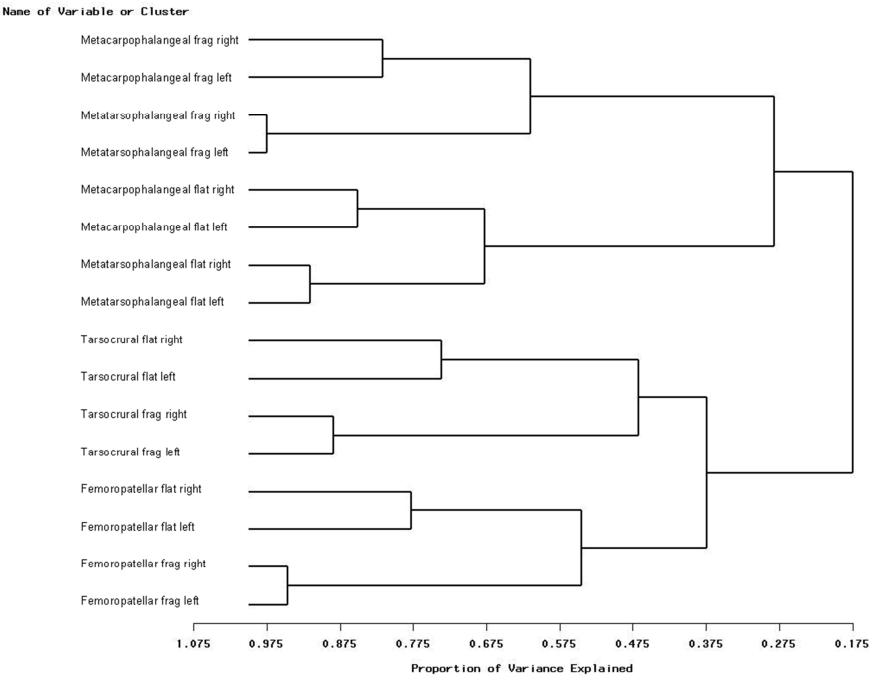

Figure 2.2 The tree from the cluster analyses visualises the associations between left and right joints and FLAT and FRAG (FLAT = continuous osteochondrosis (OC) values for flattened bone contour; FRAG = continuous OC values for fragments) based on the correlation matrix using linear OC scores. The length of the branches of the tree until the cluster with another variable represents the reduction in the proportion of variance explained by the newly created variable (two variables together) compared to the separate variables originally used.
The length of the branches until the clustering with another variable represents the reduction in the proportion of variance explained by the newly created variable (two variables clustered together) compared to the separate variables originally used.

relative to ALL were respectively 0.65 and 0.67 (not shown in the table). Therefore, all joints are approximately equally correlated with ALL at the animal level. At the joint level, the correlation between the FP joint and the TC joint was 0.10, as was also the case between the FP and MCP/MTP joints; between the MCP/MTP and TC joints, the correlation was 0.14 (not shown in the table). At joint level, the correlations between the left and right homologues, separately for FLAT and FRAG, were moderately high, varying from 0.33 to 0.55 (Table 2.5). However, the correlations between FLAT and FRAG were small, ranging from non-significant to 0.26 (Table 2.5).

Cluster analysis

The cluster analysis visualises the relationships between observations in different joints, between left and right homologues, and between flattened bone contours and fragments within joints (Figure 2.2).

The results show high similarity between the right and left homologues. Lower similarities were found between the MCP/MTP joint, and still lower similarities between flattened bone contours and fragments. The lowest degree of similarity was found when grouping different joints. Clustering left and right homologues and the MCP/MTP joints reduced the number of variables from 16 to 6, and the proportion of variance explained was reduced to 62%.

2.4 Discussion

This is the first investigation, to our knowledge, that examined in detail the prevalence of various manifestations of OC in a large population of horses selected to exclude as much as possible any bias by pre-selection. The balanced design of a representative sample of randomly drawn animals in our study was unique.

The age at which the animals were radiographed was chosen to be 12 months on average, with a minimum age of 9 months, because previous findings showed that lesions were not permanent before the age of eight months (Dik *et al.* 1999). Including older horses would have increased the risk of selection in the data because of culling or export related to OC. The percentage of animals invited for a radiographic examination that the owners declined was 45%. Refusal to participate appeared to be unrelated to OC; there was no association between the percentage of non-participating owners and the mean OC score per stallion.

OC is mostly scored as a binary trait, while the multifactorial origin of the disorder implies a continuous character (Jeffcott 1991; Philipsson *et al.* 1993; Wittwer *et al.* 2006). Scoring on a detailed scale is needed to obtain good insight into the prevalence of OC, and knowing the prevalences is a prerequisite for further genetic analysis. Our detailed method of scoring, together with the virtual absence of pre-selection, resulted in a relatively low prevalence of normal joint contours in this study compared to previous research (Grøndahl and Dolvik 1993; Philipsson *et al.* 1993; Schougaard *et al.* 1990; Stock *et al.* 2005; Wittwer *et al.* 2006). In fact, when only the FP joint was considered in our

study population, around 60% of the animals had normal joint contours. When the TC and MCP/MTJ joints were also included, this figure dropped to 45% and 30%, respectively. Therefore, in addition to the age effect and the lack of pre-selection, an important reason for the low percentages of animals with normal joint contours in this study is that many radiographs were taken and many sites were scored. Therefore, the 30% entirely OC-free animals in this study cannot be compared to figures from other studies, which are all based on less comprehensive screening within pre-selected populations.

Empirically, it has long been recognized that OC often presents bilaterally. The results of this study confirm this clinical impression; the cluster analysis showed that the right and left joints were very similar. Perhaps more unexpectedly, it became clear that this bilateralism also was true to a much lesser extent for flattened bone contours and fragments. Therefore, it can be concluded that the right and left joints can be combined in further analyses, but flattened bone contours and fragments will have to be evaluated as statistically different disorders. This necessity does not preclude, however, the possibility that associations between flattened bone contours and fragments have a genetic basis. The low correlations (0.10–0.14) between joints indicate that animals with high OC values in one of the joints did not necessarily have higher values in other joints, which again points to the probably complex genetic background of OC.

In the TC joint, three radiographs were taken, and the anterior–posterior projection was used to judge sites 4, 5, and 6. In these sites, scores B to E were very rare (0.67%). This low percentage brings into question the usefulness of this projection for detecting OC in the TC joint. Further, sedated animals had higher OC values in the FP joint (results not shown). This suggested that sedation facilitates the detection of OC in the FP joint. Given these observations, reduction of the number of projections and standard sedation might be considered as amendments to the standard protocol for OC screening that is used by the Royal Warmblood Studbook of the Netherlands (KWPN).

The outcome of the study confirms some empirically based assumptions (such as the strong left/right relationship at joint level), but calls others into question (such as the assumed relationship between flattened bony contours and fragments). A detailed phenotypic description of OC as presented in this study is a necessary basis for further quantitative and molecular genetic studies. The hope is that such molecular investigations may lead someday to the eradication of or at least a reduction in the prevalence of this disorder.

Acknowledgement

We thank the equine practices for their careful support, and all owners of the yearlings for their cooperation.

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3

Genetic parameters of various manifestations of osteochondrosis and their correlations between and within joints in Dutch warmblood horses

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Journal of Animal Science (2009) 87:1906-1912

Abstract

Osteochondrosis (OC) is an important orthopedic developmental disorder in many horse populations. A review of the literature reveals widely variable heritability estimates for the disorder. We estimated the genetic parameters (heritabilities and genetic correlations) of various manifestations of OC. Femoropatellar, tarsocrural, and metacarpophalangeal/ metatarsophalangeal joints of 811 randomly selected yearlings from the Royal Warmblood Studbook of the Netherlands (KWPN), descending from 32 representative stallions, were scored for OC at 28 predilection sites. At each site, OC was scored in 5 categories, distinguishing between flattened bone contours and fragments. At the animal level, the overall heritability of OC was 0.23, the heritability of flattened bone contours was 0.08, and the heritability of fragments was 0.22. At the joint level, heritability was highest in the tarsocrural joints, intermediate in the metacarpophalangeal/ metatarsophalangeal joints, and lowest in the femoropatellar joints. The heritability estimates for the contralateral joint homologues were very similar. The genetic correlation between the tarsocrural and femoropatellar joint was high, whereas correlations between the metacarpophalangeal/ metatarsophalangeal and other joints were moderate. The genetic correlation between flattened bone contours and fragments at the animal level was 0.80. Scoring OC on a 5-point categorical scale resulted in higher heritability on the observed scale than when analyzing OC as a binary trait. Our results suggest that selection against OC could best be performed by taking into account the OC status of all 4 joints, the femoropatellar, the tarsocrural, and the metacarpophalangeal/metatarsophalangeal joints, and discerning between flattened bone contours and fragments.

Key words: genetic correlation, heritability, horse, joints, osteochondral fragments, osteochondrosis

3.1 Introduction

Osteochondrosis (OC) is an important orthopaedic developmental disorder in many horse populations. It is an endochondral ossification disturbance that occurs in young, growing individuals. Irregular ossification leads to the formation of thick cartilage plugs and areas of focal necrosis, eventually resulting in flattened bone contours and loose fragments (Jeffcott, 1997; van de Lest *et al.*, 1999). The term manifestations is used to indicate both varieties of OC, flattened bone contours and fragments.

The etiology of OC is still not fully understood, but it is agreed that the disorder is multifactorial in origin (Jeffcott, 1991; Philipsson *et al.*, 1993; Wittwer *et al.*, 2006) and genetic influences play an important role (Schougaard *et al.*, 1990; Philipsson *et al.*, 1993; Van Weeren, 2006). Estimated heritabilities vary widely (Table 3.1), possibly due differences in materials and methods used between studies (Ricard, 2002). Since initial research into the genetic basis of OC indicates that it is complex (Wittwer *et al.*, 2007), genetic progress can be made by using classical quantitative genetic approaches for selection. Using radiographic data an individuals' OC status can be measured in different joints and flattened bone contours can be distinguished from fragments. Phenotypic results (Van Grevenhof *et al.*, 2009) have confirmed the strong similarity between OC status in contralateral homologues at joint level, but also questioned the assumed relationship of OC between flattened bone contours and fragments. It is not yet known whether the phenotypic relationships reflect the underlying genetic relationships. It is necessary to estimate the genetic parameters between joints and between manifestations to effectively select and predict selection response.

Radiographic data from 811 Dutch Warmblood yearlings was used to estimate the heritabilities of flattened bone contours and fragments, and determine genetic correlations between these manifestations among joints.

3.2 Materials and methods

Animals

Data were collected on 811 yearlings from the population of the Royal Warmblood studbook of The Netherlands (KWPN) population during 2005 (n =

Table 3.1 Prevalence and heritabilities (h^2) of osteochondrosis (OC) and fragments (OCD), by joint, for different horse populations.

Population	number of sires (animals)	OC(D)	Prevalence	h^2 (SE)	Method of analysis	Author
Femoropatellar OC/OCD						
Dutch WB stallions	(n=1965)	OC	11.50%	0.09	ATM (REML, DL)	I
French WB	103 sires (n=733)	OC	1-7 %	0.00-0.17	LSM3	II
Italian WB	75 sires (n=350)	OCD		0.09 (0.240)	ATM (AIREML)	III
Tarsocrural OC/OCD						
Dutch WB stallions	(n=1965)	OC	16.00%	0.11	ATM (REML, DL)	I
Dutch WB mares	30 sires (n=590)	OC	13.70%	0.01 (0.06)	LSM (REML)	IV
Dutch WB mares	30 sires (n=590)	OC		0.14 (0.17)	LAM (REML)	IV
French WB	103 sires (n=733)	OC	11-13 %	0.00-0.02	LSM3	II
Hanoverian WB	165 sires (n=624)	OC	10.50%	0.057 (0.058)	LAM (REML)	V
SB Trotters	39 sires (n=644)	OC	14.30%	0.52	STM (REML)	VI
SB Trotters	24 sires (n=793)	OC	10.50%	0.27 (0.08)	LSM4	VII
Hanoverian WB	(n=3725)	OCD	9.60%	0.37 (0.06)	LAM (REML, DL)	VIII
Hanoverian WB	569 sires (n=5231)	OCD	9.20%	0.282 (0.042)	LAM (REML, DL)	IX
Hanoverian WB	569 sires (n=5231)	OCD	9.20%	0.273 (0.042)	LSM (REML, DL)	IX
Hanoverian WB	569 sires (n=5231)	OCD	9.20%	0.170 (0.070)	STM (GS)	IX
Danish Trotters	9 sires (n=325)	OCD	12.00%	0.26 (0.14)	STM4	X
Metacarpophalangeal/ metatarsophalangeal OC/OCD						
French WB	103 sires (n=733)	OC	8-11 %	0.04-0.21	LSM3	II
Hanoverian WB	165 sires (n=624)	OC	18.30%	0.123 (0.097)	LAM (REML	V
Hanoverian WB	(n=3725)	OCD	20.80%	0.19 (0.03)	LAM (REML, DL)	VIII
Hanoverian WB	569 sires (n=5231)	OCD	23.50%	0.170 (0.028)	LAM (REML, DL)	IX
Hanoverian WB	569 sires (n=5231)	OCD	23.50%	0.167 (0.030)	LSM (REML, DL)	IX
Hanoverian WB	569 sires (n=5231)	OCD	23.50%	0.120 (0.049)	STM (GS)	IX
SB Trotters	39 sires (n=644)	OCD	11.80%	0.21	STM (REML)	VI
SB Trotters	24 sires (n=793)	OCD	21.50%	0.17 (0.06)	LSM4	VII
ALL						
Italian WB	75 sires (n=350)	OCD	16.60%	0.14 (0.225)	LAM (REML, DL)	III

[1] ATM, animal threshold model; LSM, linear sire model; LAM, linear animal model; STM, sire threshold model; REML, restricted maximum likelihood; DL, Dempster-Lerner transformation (Dempster and Lerner, 1950); AIREML, average information REML; GS, Gibbs Sampling; [2] I: der Kinderen, 2005; II: Ricard et al., 2002; III: Pieramati et al., 2003; IV: KWPN, 1994; V: Schober et al., 2003; VI: Grøndahl and Dolvik, 1993; VII: Philipsson et al., 1993; VIII: Stock et al., 2005; IX: Stock and Distl, 2006; X: Schougaard et al., 1990; [3] Discrete and bivariate measures; [4] χ^2 heterogeneity test, DL; [5] WB=Warmblood; SB=Standardbred;

593) and 2006 (n = 218). Animals descended from 32 breeding sires and 801 dams. The breeding sires were representative for the population of approved breeding sires and included both older and younger sires and both show-

jumping and dressage bred sires with at least 25 registered offspring in the 2005 and 2006 breeding seasons. For approval as a breeding sire within the KWPN studbook, a negative OC status is prerequisite. Until 1994, OC status was assessed using radiographs of the tarsocrural (TC) joint (hock joint) only. After that time, the femoropatellar (FP) joint (stifle joint) was included in the assessment as well. Out of 32 breeding sires, 7 had been evaluated prior to 1994. Thus, the OC status of the FP joint was unknown for those 7 sires. The number of animals per sire varied from 22 to 28. The animals had a mean (SD) age of 12 (2.6) mo and a minimum age of 9 mo and included 47.3% males. The proportion of Thoroughbred genes in the pedigree ranged from 0 to 58 percent. The animals had a mean withers height of 149 (5.7) cm, and a mean chest circumference of 165 (9.3) cm. The animals had been reared by their breeders; therefore, feeding, housing, and exercise level varied among the animals.

Radiography

Animals were scored for OC based on radiographs from 8 joints: Contralateral homologues of the FP, TC, metacarpophalangeal (MCP), and metatarsophalangeal (MTP) joints (fetlock joints). A total of 28 predilection sites were scored in each animal: 5 sites in the FP joint, 7 sites in the TC joint, and 1 site in each of the MCP and MTP joints. In the MCP and MTP joints we included only the proximodorsal part of the sagittal ridge of the 3rd metacarpal/metatarsal bone in the MCP/MTP joints. Radiographs and predilection sites were described in detail by Van Grevenhof *et al.* (2009). At each site, OC was scored on a categorical scale from A through E, which was adapted from the original scale from Dik *et al.* (1999). An A score indicates a 'normal joint contour', B score indicates 'smooth flattened bone contours' and C score indicates 'irregular flattened bone contours', and D and E scores indicate 'fragments'(see Dik *et al.*, 1999 for a detailed explanation; see also Table 2.1). Manifestations is used to indicate both varieties of OC, flattened bone contours and fragments. The mean percentage of score A in the population was 30% (Van Grevenhof *et al.*, 2009). The radiographs were taken by 15 preselected equine veterinary practices, 2 of which were responsible for 61% of all radiographs. An experienced radiologist evaluated all radiographs.

Genetic Analysis

To enable quantitative genetic analysis, the A through E categorical scores were transformed into quantitative values on a continuous scale as described in detail by Van Grevenhof *et al.* (2009). For the transformation, we assumed a

normally distributed liability underlying the categorical scores. Each categorical score was transformed into the mean liability value for that score (Falconer, 1965). This procedure resulted in 3 quantitative traits, 2 for each joint and 1 for the entire animal: the ALL trait, representing the overall OC value, including both flattened bone contours and fragments; the FLAT trait, representing only flattened bone contours; and the FRAG trait, representing only fragments. Therefore, the ALL trait summarizes the overall OC status of either a joint or the entire animal, assuming that flattened bone contours and fragments are manifestations of the same disorder, which is unknown *a priori*. The FLAT and FRAG traits summarize the OC status separately for flattened bone contours and fragments, for either a joint or the entire animal. Hence, each animal had ALL, FLAT, and FRAG-values for each of its joints, as well as an overall value for ALL, FLAT, and FRAG. Values for ALL, FLAT, and FRAG were calculated for each joint by summing the values of all sites within that joint, and the traits were calculated for each animal by summing all values in the entire animal. The value for the entire animal was obtained by summing the ALL, FLAT, and FRAG values for each of its eight joints.

The prevalence of OC for each separate predilection site is low, varying from 0 to 23 percent (Van Grevenhof *et al.*, 2009), which reduces the precision of genetic analysis for individual predilection sites. To enable a meaningful genetic analysis, we reduced the number of traits by combining highly similar traits into a single trait. For this purpose, we used results of the cluster analysis. Inputs in that analysis were the FLAT and FRAG values for each of the 8 joints, giving a total of 16 traits for each animal. The cluster analysis of Van Grevenhof *et al.* (2009) showed high similarities between the right and left homologues. Intermediate similarities were present between the MCP and MTP joint. Lower similarities were found between flattened bone contours and fragments. The lowest similarity was found between different joints, except for the combination of the MCP and MTP joints (Van Grevenhof *et al.*, 2009). Therefore, we combined contralateral homologues and the MCP/MTP joints into a single trait, but treated flattened bone contours and fragments, and the other joints, as separate traits. This resulted in the following 12 traits for the genetic analysis: ALL, FLAT, and FRAG for the animal and ALL, FLAT, and FRAG separately for the FP joint, TC joint, and the combined MCP/MTP joints.

Table 3.2 Heritabilities estimated (h^2) using continuous, binary, and '1 to 5' scales.

Level	OC[a]	σ_p	h^2 continuous scale[b]	h^2 binary scale[b]	h^2 '1 to 5' scale[b]
ALL	ALL	4.05	0.23 (0.09)	0.15	0.23
	FLAT	3.16	0.08 (0.06)	(0.08)	(0.09)
	FRAG	2.45	0.22 (0.09)		
FP	ALL	2.09	0.05 (0.05)	0.05	0.06
	FLAT	1.71	0.07 (0.06)	(0.05)	(0.05)
	FRAG	1.22	0.02 (0.04)		
TC	ALL	2.01	0.36 (0.11)	0.25	0.36
	FLAT	1.47	0.15 (0.08)	(0.10)	(0.11)
	FRAG	1.39	0.26 (0.09)		
MCP/ MTP	ALL	2.24	0.14 (0.08)	0.10	0.11
	FLAT	1.86	0.08 (0.10)	(0.07)	(0.07)
	FRAG	1.36	0.06 (0.07)		

[a] OC=osteochondrosis refer to the joint level. FP, femoropatellar joint; TC, tarsocrural joint; MCP/MTP, metacarpophalangeal/metatarsophalangeal joint; ALL, both flattened bone contours and fragments; FLAT, flattened bone contours; FRAG, fragments;
[b] Heritability values are expressed as h^2 (SE). Continuous scale refers to transforming OC scores on a continuous liability scale, bivariate scale refers to OC scored as 0 or 1, and '1 to 5' scale refers to transforming A to E scores into corresponding values ranging from 1 to 5.

Estimation of Genetic Parameters

For the estimation of genetic parameters, pedigree information on 5 generations was used. The pedigree data contained 7,799 horses. Genetic parameters were estimated univariately (heritabilities) and bivariately (genetic correlations) using Residual Maximum Likelihood (REML) with ASREML software (Gilmour *et al.*, 2006). The following linear animal model was used:

$$Y_{ijklm} = \mu + sex_i + EVP_j + age_k + year_l + animal_m + e_{ijklm},$$

where Y_{ijklm} is the continuous OC value for ALL, FLAT, or FRAG of the animal or joint; μ is the mean; sex_i is the fixed class effect of sex (i = male or female); EVP_j is the fixed class effect of the equine veterinary practice responsible for taking radiographs (j = 1, 2, 3, …, 15); age_k is the fixed class effect of age in months (k = 9, 10, 11, …, 22); $year_l$ is the fixed class effect of the year of scoring (l = 2005 or 2006); $animal_m$ is the random additive genetic effect of the m^{th} animal (m = 1, 2, 3, …, 811); and e_{ijkl} is the residual. All fixed effects in the genetic model had a significance level of $P \leq 0.10$. The month of scoring did not affect the OC score ($P > 0.2$).

To investigate the benefit of converting OC scores to a continuous liability scale, heritability was additionally estimated for OC expressed on both a binary scale and a '1 to 5' scale. On the binary scale, an individual had an OC score of zero only when all predilection sites were scored as A; otherwise, an individual had an OC score of 1. Therefore, a zero indicated complete absence of OC. On the '1 to 5' scale, OC scores A through E were expressed as values 1 through 5, respectively.

3.3 Results

Heritabilities

At the animal level, the estimated heritability was 0.23 ± 0.09 (SE) for ALL, 0.08 ± 0.06 for FLAT, and 0.22 ± 0.09 for FRAG (Table 3.2). At the joint level, the heritability was highest in the TC joints (0.36), intermediate in the MCP/MTP joints (0.14), and lowest in the FP joints (0.05) (Table 3.2). The heritabilities of the separate contralateral homologues were highly similar (results not shown), but were approximately 16% lower than estimates from the combined scores. Combination of the information from both limbs reduced the impact of measurement errors, lowered the estimate of environmental variance, and led to higher heritabilities.

Sires were compared by the means of the estimated breeding values (EBV) using the continuous OC scores. The mean EBV of the sires were standardized by the phenotypic standard deviation of OC values at the animal level. The standardized means of the sires (Figure 3.1) showed large variation between sires, varying from -0.47 to 0.33, which indicates the possibility of using quantitative genetic approaches for selection based on radiographic OC status.

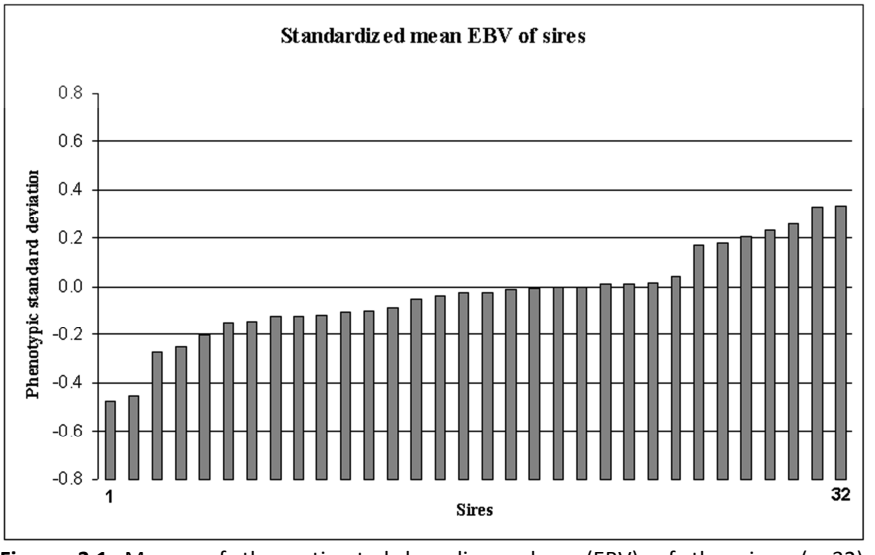

Figure 3.1 Means of the estimated breeding values (EBV) of the sires (n=32), standardized by the phenotypic standard deviation of osteochondrosis (OC) values at the animal level. The y-axis is presented on the scale of the phenotypic standard deviation.

The use of a binary scale for measuring OC resulted in lower heritabilities (decreased to between 68 and 72% of the original values, excluding the FP joint) at both the joint and animal level compared to OC on the continuous scale (Table 3.2; Note that heritabilities for the binary trait are expressed on the observed scale, not on the underlying liability scale). However, in the FP joint, the heritability showed only a limited decrease to 95% (h^2 = 0.05). In contrast, heritability estimated using a '1 to 5' scale for OC showed very similar estimates of heritability compared to OC on the continuous scale (98 to 112% of the original estimates, excluding the MCP/MTP joints). However, in the MCP/MTP joints, the heritability decreased to 80% of the original value (h^2 = 0.14).

Genetic Correlations

Genetic correlations between ALL, FLAT, and FRAG were high at the animal level (Table 3.3), although the genetic correlations of FLAT and FRAG with ALL contain a component of autocorrelation. The correlation between FLAT and FRAG at the animal level was 0.80 with large associated SE. Genetic correlations of ALL between the FP and TC joints were intermediate (0.59), but correlations with the MCP/MTP joints were low (0.09 and 0.26) (Table 3.4). Residual correlations are presented, because the selection response is also dependent on the residual correlation. Residual correlations ranged between 0.01 and 0.69

Table 3.3 Genetic correlations[1] and residual correlations[2] of the linear osteochondrosis (OC) values between manifestations within joints at the animal level, using a continuous scale.

Trait[3]	ALL	FLAT	FRAG
ALL		0.94 (0.09)	0.96 (0.05)
FLAT	0.78 (0.02)		0.80 (0.25)
FRAG	0.76 (0.03)	0.19 (0.06)	

Values in parentheses are standard errors (SE); [1] Above diagonal; [2] Below diagonal; [3] ALL, both flattened bone contours and fragments; FLAT, flattened bone contours; FRAG, fragments.

(SE=0.04 to 0.09). Genetic correlations between FLAT and FRAG within the same joints varied widely (data not shown). The standard errors of those estimates were very high, indicating low accuracy of estimates, which are therefore not shown.

3.4 Discussions

Methodology

In most studies, OC is scored as a binary trait, whereas the multifactorial origin of the disorder implies an underlying continuous character (Jeffcott, 1991; Philipsson *et al.*, 1993; Wittwer *et al.*, 2006). Indeed, our results show that defining OC as a binary trait may be one reason for the variable heritability found in previous studies. Scoring OC in 5 categories increased heritability on the observed scale compared to scoring OC as a binary trait. Therefore, OC should be scored minimally in more than 2 categories. For scoring on a categorical scale, the actual number of categories needed remains to be defined and may vary per joint. A '1 to 5' scale had similar results in most joints as OC scored on a continuous scale, but using the scale still decreased the estimated heritability in the MCP/MTP joint. Thus, detailed scoring of OC increased heritability, but the transformation to a liability scale had little impact.

The wide variability in estimated heritabilities reported in the literature is caused by differences in definitions and breeds used, varying ages at assessment, and sometimes the use of small sample sizes or progeny groups, or preselected data (Ricard, 2002). Data in this study originated from a balanced, randomly drawn sample of offspring from a representative sample of the Dutch

Table 3.4 Genetic correlations[1] and residual[2] correlations of the linear osteochondrosis (OC) values between ALL and specific joints, using a continuous scale.

Trait3	Animal	FP	TC	MCP/MTP
Animal		0.77 (0.23)	0.86 (0.10)	0.58 (0.22)
FP	0.65 (0.04)		0.59 (0.37)	0.26 (0.49)
TC	0.50 (0.07)	0.011 (0.08)		0.09 (0.31)
MCP/MTP	0.69 (0.04)	0.13 (0.06)	0.09 (0.09)	

Values in parentheses are standard errors (SE); [1] Above diagonal; [2] Below diagonal; [3] Joints: FP, femoropatellar joint; TC, tarsocrural joint; MCP/MTP, metacarpophalangeal and metatarsophalangeal joints. ALL, both flattened bone contours and fragments, were used in the calculation of the genetic correlations between these joints.

Warmblood (KWPN) breeding sire population. This design minimized the risk of selection in the data. Furthermore, yearlings that had passed the age where the radiographic OC status is still unstable due to the dynamic character of the disorder at a young age (Dik *et al.*, 1999), but before selection on OC takes place, were studied. This reduced the risk of biased estimates of the genetic parameters due to selection. The detailed scoring in our study, together with the virtual absence of pre-selection, enabled estimates of heritability for FLAT and FRAG separately and per joint.

Heritabilities

In this study, the heritability of OC at the animal level indicates substantial genetic variation of the disorder, which agrees with the large variation in the standardized means of the progeny groups per sire (Figure 3.1). Heritabilities for the TC joint (0.36) and ALL (0.23) were higher in our study compared to previous studies (0.00 to 0.27), except for those of Stock *et al.* (2005) and Grøndahl and Dolvik (1993) for the TC joint (Table 3.1). Although there will always be differences between populations, we determined these higher values to be reliable because of the balanced and well-controlled character of the sampled population and the many factors that may have affected the accuracy of the estimation of heritability for OC in some of the previous studies, in which the same population was used (Ricard, 2002). The heritability estimates in the current study confirmed previous findings (Van Grevenhof *et al.*, 2009),

including the strong relationship between the contralateral homologues at the joint level and the differences between joints. The genetic correlation between flattened bone contours and fragments was high (0.80) with a large associated SE, but the heritability of both traits was substantially different.

Which Joints Should be Taken into Account?

The heritability of the TC joint indicates that OC can accurately be scored in this joint and that genetic variation is evident. In contrast, the heritability of the FP joint is low. Improvement in the scoring of OC in the FP joint may increase the heritability estimate for that joint. The decrease in accuracy seen for the FP joint could indicate a large impact of measurement errors on the environmental variance. This results in a lower heritability estimate for the FP joint. More measurement errors are expected for the FP joint than for the other joints, also because sedation was shown to be of significant influence in diagnosing OC in the FP joint (Van Grevenhof *et al.*, 2009). Therefore, reduction of OC in the FP joint is relevant, as the prevalence of OC in this joint is high (Van Grevenhof *et al.*, 2009). Although the apparent differences in TC and the FP joint heritabilities suggest that selection on the FP joint may be of little use, there is another factor to be considered, which is the difference in the clinical relevance of OC in each joint. Osteochondrosis in the FP joint is the most important cause of lameness in the stifle, and surgical treatment results are reported as being fair to good (Stick, 2006). Conversely, OC in the TC joint rarely leads to lameness and has an excellent prognosis for return to athletic activity after surgery (Auer, 2006). Therefore, the importance of selection on the FP joint should not be underestimated. Effective breeding against OC in the FP and TC joints requires recording of both traits, as the TC joint showed a high heritability and the FP joint is clinically important. A correlated response of the FP joint when selecting for the TC joint would be uncertain given the large standard error associated with the genetic correlation estimate.

In this study, we included only the proximodorsal part of the sagittal ridge of the 3rd metacarpal/metatarsal bone in the MCP/MTP joints. In contrast to many other sites in the MCP/MTP joints, it has been shown that osteochondral abnormalities at this site belong to the OC complex (Hurtig and Pool, 1996). The heritability of OC in the MCP/MTP joints is moderate (0.14) and the genetic correlations between the MCP/MTP and FP joint (0.26), and between the MCP/MTP and TC joint (0.09) are low, and not significant. Therefore, there will only be a small correlated response in the MCP/MTP joint when selection is

performed on the FP and TC joints. Separate recording of OC lesions by joint and location in the joint is required to efficiently breed against OC in MCP/MTP, TC, and FP joints, which is in agreement with results of Stock and Distl (2006) regarding TC and MCP/MTP joints.

Physiology

A previous physiological study (Dik *et al.*, 1999) showed that, during the process of development and repair, osteochondrotic lesions were rarely found directly after birth in the FP joint, but that they generally developed during early growth, between the third and eighth month of age. This could indicate a major environmental effect rather than a large genetic component. In contrast, lesions in the TC joint were shown to generally exist from birth and recover during early growth, between the second and fifth month of age. This could indicate an underlying genetic background rather than large environmental effects during growth. These physiological results correspond to the low heritability found for the FP joint and the moderate heritability found for the TC joint in the present study.

Flattened Bone Contours and Fragments

The genetic correlation between FLAT and FRAG was high (0.80) at the animal level, though FLAT did not significantly differ from FRAG. Both FLAT and FRAG were heritable (h^2 = 0.08 and 0.22) at the animal level. Therefore, selection against FRAG (h^2 = 0.22) will also decrease FLAT in a correlated response, but the selection response in FLAT will be less compared to selection on both FLAT and FRAG. Therefore, both FLAT and FRAG should be considered separately in selection against OC.

It can be concluded at the joint level that heritability was highest in the TC joint, intermediate in the MCP/MTP joints, and lowest in the FP joints. Flattened bone contours had substantially lower heritabilities than fragments, but the genetic correlation between those manifestations was high. The heritability estimates confirmed a strong relationship between the contralateral homologues at the joint level, as well as differences between joints. Therefore, OC should be scored minimally in more than 2 categories and in all 4 joints, and take into account both flattened bone contours and fragments.

Acknowledgements

The authors thank A.J. van den Belt, of the Veterinary Faculty of Utrecht University, for scoring all radiographs. We also thank the equine practices for their careful support, and all owners of the yearlings for their cooperation

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4

The effects of housing system and feeding strategy on the joint-specific prevalence of osteochondrosis in fattening pigs

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Livestock Science (2011) 135:53-61

Abstract

Osteochondrosis (OC) is seen as the main cause of leg weakness in pigs, leading to welfare problems and economic losses. Environmental factors in pig husbandry, such as the housing system and feeding strategy are expected to influence the prevalence of OC. Therefore, this study investigated the effects of housing system and feeding strategy on the prevalence and severity of OC.

In the experiment 345 pigs were used. At an age of 69 days intact boars and gilts were separated and assigned to groups of five or six individuals. A two by two factorial design of housing system and feeding strategy was applied. The housing system was either a conventional concrete floor partial slatted, or a deep litter floor with extra space allowance. The feeding strategy was either *ad libitum* or restricted to 80% of *ad libitum*. Pigs were slaughtered at the age of 161-176 days. In total, five joints of the left front and hind limbs were macroscopically assessed for OC on a five-point scale, ranged from no OC through (semi-)loose cartilage fragments.

The prevalence of OC in the experimental population was 41.4%, and 12.4% of the individuals had severe lesions. The tarsocrural joint was most affected (30.2%) by OC. OC scores between the different joints were not correlated. Medial sections of joints were most affected (63-100%). Boars were more affected than gilts in the elbow joint. Conventionally housed pigs were more affected than deep litter housed pigs. *Ad libitum* fed pigs had more OC than restrictedly fed pigs. OC was most prevalent with 57.5% in the pigs on the conventional floor with *ad libitum* feeding. OC was least prevalent with 33.7% in pigs kept in deep litter housing with restricted feeding. The sex, housing system and feeding strategy did not affect OC in the femoropatellar, metacarpophalangeal, metatarsophalangeal joints.

Our results demonstrate that the OC prevalence can be reduced by applying deep litter floors with extra space allowance and/or restricted feeding in fattening pigs.

Key Words: feed, floor, space, joints, osteochondrosis, pigs

4.1 Introduction

Osteochondrosis (OC) is a disturbance of the endochondral ossification during skeletal growth, which occurs in many species including cattle, pigs, horses and humans (Crenshaw, 2006; Donabédian *et al.*, 2006; Grøndalen, 1974a; Van Weeren, 2006; Ytrehus *et al.*, 2007). Osteochondrosis is the main cause of leg weakness, a general term for leg problems in pigs (Grøndalen, 1974d; Jørgensen, 1995; Reiland *et al.*, 1978; Kirk *et al.*, 2008; Stern *et al.*, 1995). Leg weakness may result in lameness, which reduces welfare of fattening pigs and is an important cause for premature culling in sows, as they reach higher age (Gresham, 2003; Dewey, 2006). Reduction of the prevalence of OC could, therefore, improve wellbeing, and could reduce economic losses due to premature culling.

Environmental factors like housing system and feeding strategy affect the development of OC, but have not been studied intensively, and the outcome of studies shows substantial inconsistency (Carlson *et al.*, 1988; Goedegebuure *et al.*, 1980; Grøndalen, 1974c; Jørgensen, 1995; Nakano *et al.*, 1987; Ytrehus *et al.*, 2007). Housing systems affect leg condition and cause local overload within joints (Nakano *et al.*, 1987). Pigs housed on straw bedding showed less leg weakness (lameness, etc., with OC as a major cause) compared to pigs housed on conventional floors (Jørgensen, 2003; Scott *et al.*, 2006; Scott *et al.*, 2007).

High growth rates, either due to genetic selection or feeding, cause disturbances in bone metabolism and thereby increase the prevalence of OC (Grøndalen, 1974a; Busch, 2006; Kadarmideen *et al.*, 2004; Nakano *et al.*, 1987; Ytrehus *et al.*, 2007). High feeding levels may, therefore, increase the prevalence of OC. In contrast, a deep litter floor with additional space allowance will stimulate activity and may thereby decrease the prevalence of OC.

Public concern and legislation gradually change the housing systems in which pigs are kept (LNV, 2010). Current systems commonly have barren, partially concrete, floors, with little space per pig. The systems that will be common in the future are likely to have more space per pig and floors covered with a substrate such as straw or deep litter. An important question is whether this shift in housing systems will indeed reduce welfare problems such as OC.

This work, therefore, investigates the effects of two housing systems and feeding strategies on prevalence and severity of OC in joints of front and hind limbs of fattening pigs.

The housing system was either a concrete, partially slatted, floor, or a deep litter floor with extra space allowance, whereas feeding was either *ad libitum* or restricted.

4.2 Materials and methods

Materials

A total of 345 pigs divided in two batches were exposed to a 2 by 2 factorial design of a housing and feeding treatment. The 187 pigs from batch 1 were Tempo*Topigs40 crossbreds, descending from 23 dams. The 158 pigs from batch 2 were Piétrain*Topigs40 crossbreds, descending from 18 dams. Litters were equally divided over the treatment groups, to avoid confounding of genetic effects with treatment effects. Pigs of both batches originated from an intermitted suckling experiment, in which pigs were either weaned conventionally at three weeks of age, or weaned at varying ages (Gerritsen *et al.*, 2008). After weaning each litter was housed in a conventional pen with a 50% concrete, 50% metal slatted floor. This type of housing system together with *ad libitum* feeding is the common way to keep this genetic line during the fattening period.

Intact boars (56%) and gilts (44%) were separated at an age of 69 days. In total, 64 groups were composed of five or six individuals, based on a balanced distribution of intermitted suckling-treatment, sex, litter mates, and bodyweight at the age of 42 days. Each treatment group of the two by two factorial design contains 16 pens, of which 8 female and 8 male pens.

Experimental design

The treatments, conventional or deep litter housing, and *ad libitum* or restricted feeding, were applied to the pigs, assigned to groups of five or six individuals. A conventional pen consisted of a 50% metal slatted (ridged round bars), 50% solid concrete floor over five m^2. A deep litter pen consisted of a solid concrete floor with approximately 25-50 cm of wood shavings over 8.5 m^2. In the following, we will use the label “deep litter” to refer to the system having deep litter floors with 8.5m^2 floor area per pen. Water was available *ad libitum* for all treatment groups by two drinking nipples per pen. Pigs were fed three succeeding diets: standard pelleted, dry grower and finisher diet with decreasing protein content (respectively, 176, 160 and 152 g crude protein/kg feed). These diets are usually fed to these *ad libitum* fed, crossbred, fattening

pigs. At day 107 and 108 feed 1 was changed to feed 2, and at day 140 and 141 feed 2 was changed to feed 3. When changing feed, a mixture of 50% of each feed was supplied for two days. The *ad libitum* fed pigs had unlimited access to feed using an automatic feeding unit. The restricted fed pigs received two equal portions of feed each day per group, in a through covered by a rack ensuring individual feeding places, at 8 am and 4 pm. The amount of feed supplied to restricted fed pigs was 80% (in kg) of the *ad libitum* average daily intake in the preceding week.

During the experiment, pigs were weighed once every two weeks until slaughter. Pigs of batch 1 (n=187) were slaughtered at an age ranging from 165 to 176 days. Pigs of batch 2 (n=158) were slaughtered at an age between 161 and 165 days. The carcasses were stored at 4°C for one day. For each individual, the left limbs were dissected in the shoulder and hip joints, tagged, and stored at - 21 ºC until further dissection and scoring of the joints. Since OC prevalence in the left and right joints of pigs showed correlations close to one in a previous study (Jørgensen and Andersen, 2000), only the left limbs were used in this study.

Scoring

Pigs were scored for OC in five joints of the left front and hind limb. In the front limb, the elbow and metacarpophalangeal joint were scored. In the hind limb, the femoropatellar, the tarsocrural, and metatarsophalangeal joint were scored. In total, the five joints were scored on 24 locations (Table 4.1). The cartilage of a location was macroscopically scored on a categorical scale from A-E, as used in a previous study for macroscopic OC examination in horses (Van Weeren and Barneveld, 1999). *Score* A represented no abnormalities, *score* B flattening of cartilage, *score* C slight irregular cartilage, *score* D severe irregular cartilage, and *score* E 'classic' lesion with osteochondrotic cyst (Figure 4.1). *Score B* and *score C* are referred to as mild OC, and *score D* and *score E* are referred to as severe OC. An experienced veterinarian who is a specialist in judging OC (P.R.v.W.), scored all joints without knowing the experimental treatment.

Prevalence

The prevalence of OC was determined for each location within a joint, for each joint (joint level) and for OC within the entire pig (animal level). The prevalence of OC on location level was expressed as the frequency of *score* A, *score* B, *score*

Table 4.1 Mean slaughter weights in kg (at 163-164 days), including SD and number of animals per sex, batch, and treatment group.

	Mean final weight	number of pigs
Sex		
Boars	103 ±16	181
Gilts	101 ±15	147
Batch[*]		
1	108 ±14	173
2	95 ±14	155
Treatment[*]		
Conventional housed × *Ad libitum* fed	110 ±15	76
Conventional housed × Restrictedly fed	98 ±12	85
Deep litter housed × *Ad libitum* fed	110 ±11	79
Deep litter housed × Restrictedly	93 ±14	88

[*] Significant difference of $P<0.05$ between treatment or batch.

C, *score* D and *score* E at that location. To present prevalences at joint and animal level, animals were grouped based on their worst OC-score. The prevalences of OC on animal and joint level are presented as: =A, the percentage of pigs with only *scores* A in all joint locations; ≤B, the percentage of pigs with at least one *score* B but no *scores* C, D, or E; ≤C, the percentage of pigs with at least one *score* C, but no *score*s D or E; ≤D, the percentage of pigs with at least one *score* D, but no *score* E; ≤E, the percentage of pigs with at least one *score* E.

Data transformation

To use the OC-scores in a statistical analysis, we transformed the categorical observations into quantitative traits. Each categorical score was transformed into the mean liability value for that score (Falconer, 1965). This procedure resulted in 6 quantitative traits, one for each of the five joints and one for the entire animal. Values on animal or joint level were calculated by summing the values of all locations within that animal or joint. For example, the calculation of

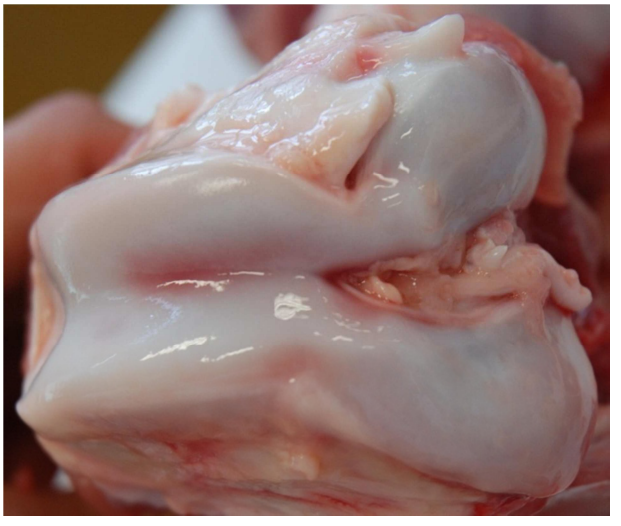
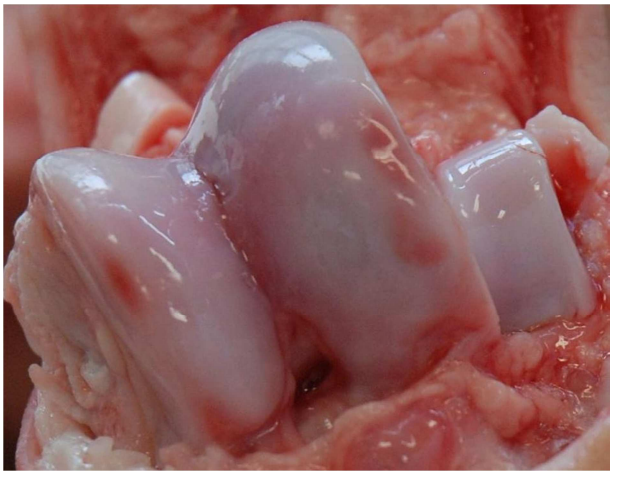
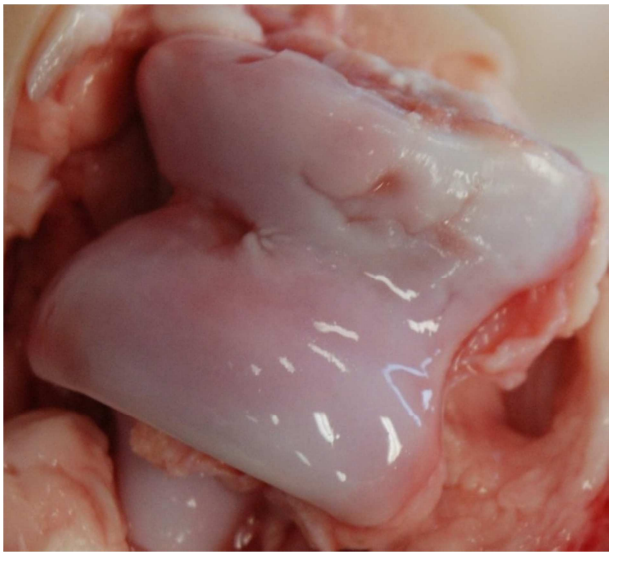


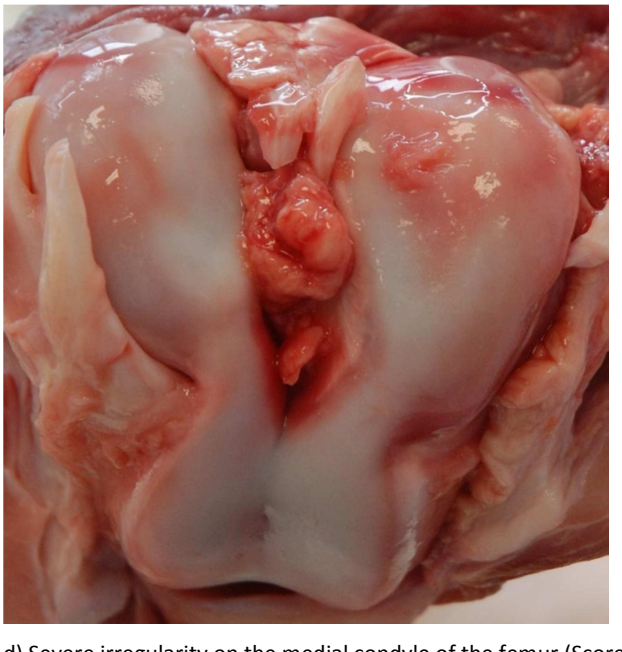
Figure 4.1 Photographs illustrating the categorical scale on which OC was scored in pigs (adapted from van Weeren and Barneveld, 1999)
a) View of the distal articular surface of the femur showing normal cartilage (score A).



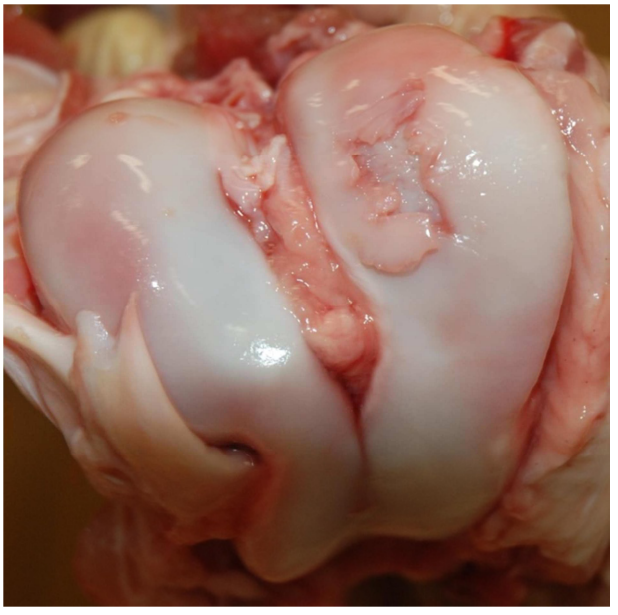
b) Flattening of the articular cartilage on the lateral and medial condyles of the humerus (Score B)



c) Irregular articular cartilage on the medial trochlea of the talus (Score C)



d) Severe irregularity on the medial condyle of the femur (Score D)



e) Severe lesion with (semi-)loose fragments and bone cyst at the medial femoral condyle (Score E)

the value for the tarsocrural joint (nine locations) is as follows: An animal was scored five times a *score* A, two times a *score* B, one time a *score* C, zero time a *score* D, and one time a *score* E, resulting in a quantitative OC value of 5(A) * -0.085 + 2(B) * 1.82 + 1(C) * 2.15 + 0(D) * 2.81 + 1(E) * 3.37 = 8.74 for the tarsocrural joint of this animal. The quantitative OC value on animal level was calculated by adding the values of the four other joints.

Statistical analysis

To investigate the relationship between OC in different locations and joints, Pearson's correlation coefficients of the quantitative OC values were estimated between joints, and between locations within a joint. To investigate the effects of the treatments, all above described parameters were tested in a linear model.

Records on pigs from the same pen may show statistical dependency, which calls for either applying the analysis at pen rather than pig level, or including pen as a random effect in the model. However, the estimate for the random effect of pen was practically zero, indicating that pen members are independent. The interest was not in resulting variance estimates, but in appropriate hypothesis testing of model terms of interest. Moreover, an

analysis using pen as the experimental unit yielded nearly identical results compared to treating pigs as the experimental unit. We, therefore, used pigs as experimental unit.
The following linear mixed model was used to estimate the treatment and animal effects on the quantitative OC values, both on animal and joint level,

$$Y_{ijkl} = \mu + Housing_i + Feeding_j + Sex_k + Housing*Feeding_{ij} + e_{ijkl},$$

where Y_{ijkl} is the quantitative OC value (animal or joint level) of pig l, with housing system i, feeding strategy j, sex k, μ is the mean; Housing i is the fixed class effect of housing system (i =conventional, deep litter); Feeding j is the fixed class effect of feeding strategy (j =*ad libitum*, restricted); Sex k is the fixed class effect of sex (k = boar, gilt); Housing*Feeding ij is the fixed interaction effect of housing system and feeding strategy; e_{ijkl} is the random residual of pig l. Statistical analysis was performed using proc GLM of SAS 9.1.3 (©2002-2003 by SAS Institute Inc.) (SAS 2005).

4.3 Results

In compliance with the regulations of the animal experimental committee, 11 of 345 pigs had to be euthanized prematurely for welfare reasons. Some of these pigs suffered from lameness, or were severely injured by pen mates. The majority of these problems appeared shortly after mixing of the animals at the start of the experiment, and seemed to be related to behavioural interactions, not to OC. Later euthanizations were due to tail biting or disease, not OC. The euthanized pigs were nearly equally distributed over the treatments.
Mean slaughter weight of the total experimental population was 102 kg (SD 15.2) (results not shown). Differences in mean slaughter weights were found between batch and treatments (Table 4.1). Conventionally (110 kg) or deep litter housed (110 kg) *ad libitum* fed pigs were significantly heavier than conventionally (98 kg) or deep litter housed (93 kg) restrictedly fed pigs.

Prevalences
Fifty-nine percent of the animals had *score A* at all locations (Table 4.2). Animals with *scores B* to *E* had either one location (33%), two locations (8%) or three locations (1%) affected, of which 12% of the animals showed severe OC (*score* D or E).

Table 4.2 Observed prevalences of OC (in % at animal level) of scores A-E in the experimental population, within treatment and sex.

Prevalence[1]	Treatment				Sex		Total
	conventional floor		*deep litter floor*				
	ad libitum	restricted	*ad libitum*	restricted	Boars	Gilts	
= A	42.5	64.8	59.3	66.3	56.6	61.1	58.6
≤ B	7.5	2.3	8.6	4.5	5.3	6.0	5.6
≤ C	36.2	22.7	18.5	16.8	23.8	22.8	23.4
≤ D	6.3	4.5	1.2	0	3.2	2.7	2.9
≤ E	7.5	5.7	12.4	12.4	11.1	7.4	9.5

[1] The prevalence of OC per treatment and sex was as follows: A=, the percentage of pigs with only scores A in all joint locations; ≤B, the percentage of pigs with at least one score B but no scores C, D, or E; ≤C, the percentage of pigs with at least one score C, but no scores D or E; ≤D, at least one score D, but no score E; ≤E, the percentage of pigs with at least one score E.

For each joint, the prevalences of OC are presented in Table 4.3. The tarsocrural joint was most frequently affected by OC, with a prevalence of 30.2% *scores* B-E. The femoropatellar joint was most severely affected with a prevalence of 7.7% *scores* D-E. The metacarpophalangeal and metatarsophalangeal joints were least affected by OC with prevalences of *scores* B-E of 1.8% and 0.3% respectively.

Results show that the medial sections of the joints were most frequently and severely affected (Table 4.3). In the elbow joint, 72% of the OC was found in the medial humeral condyle, of which most of the affected cases were classified as severe. In the femoropatellar joint, 100% of the OC was found in the medial femoral condyle, of which most of the cases were scored as severe. In the tarsocrural joint, 63% of the OC was found in the medial tibial cochlea, in most cases scored as mild.

Interactions and correlations

There were no significant effects of intermitted suckling treatment, or interaction effects of sex and feeding strategy nor sex and housing system. Random effects of mother and batch were non-significant ($p>0.2$) and therefore excluded from the model. The absence of significant effects due to intermitted

Table 4.3 Prevalences on joint location level, in %. The scores A, B, C, D and E per joint are expressed in the frequency of the total number of examined locations of the experimental population. The total joint percentages are the average of the location percentages.

Joint	Elbow	Metacarpo-phalangeal	Femoropatellar	Tarsocrural	Metatarso-phalangeal
A	93.8	97.9	89	69.2	99.4
B	1.2	0	0.9	4.4	0
C	1.5	1.5	1.8	21.9	0.3
D	0.6	0.3	1.5	3	0
E	2.3	0	6.2	0.9	0
ND[1]	0.6	0.3	0.6	0.6	0.3

[1] 'ND' means not determinable: this percentage showed the part of the joints in which OC could not be scored (for instance because of the presence of trauma).

suckling treatment and mother indicate that age at weaning did not significantly affect OC.

No significant correlations ($p<0.05$) between OC scores for different joints were found (data not shown). Within a joint, only a few significant correlations between OC scores at different locations were found. In the elbow joint, an intermediate correlation of 0.30 was found between score for the medial humeral condyle and score for the proximal edge of radius. In the tarsocrural joint, a significant correlation of 0.15 was found between the lateral trochlea of talus and the lateral tibial cochlea.

Treatments

OC was most prevalent (57.5%) in the pigs that received the treatment conventional floor with *ad libitum* feeding (Table 4.2). OC was least prevalent (33.7%) in pigs kept in deep litter floor with restricted feeding. Though overall prevalence was highest with conventional floors, score E was more frequent with deep litter floors and extra space. On average, *ad libitum* fed pigs showed the highest prevalence of OC, and restrictedly fed pigs showed least OC. For all treatments and sexes, the prevalence of severe OC was greater than 10% at animal level. The total OC prevalence (B-E) was 43.4% in boars and 38.9% in gilts. Boars had more severe OC (D-E) (14.3%) than gilts (10.1%).

Table 4.4 The effect of housing system, feeding strategy and sex on the quantitative OC values, based on all five joints on animal level, and joint level. The results from the model[1] are expressed as p-values.

Level	Animal	Joint[2]	
		Elbow	Tarsocrural
Conventional > deep litter	0.021	0.068	0.003
Ad libitum > restricted	0.003		<0.001
Gilts > boars		0.002	
Housing × Feeding[3]	0.04		0.047

[1]The model is: $Y_{ijkl} = \mu + Housing_i + Feeding_j + Sex_k + Housing*Feeding_{ij} + e_{ijkl}$; [2]Femoropatellar, metacarpophalangeal and metatarsophalangeal joints showed no significant effects of treatments (p>0.10); [3]Figure 4.3a and b.

Housing system, feeding and sex

Table 4.4 summarizes effects that were significant. Because the analysis was performed on the liability scale, absolute values of the estimated effects are difficult to interpret. We, therefore, present the estimated effects in standard deviation units and in a graphical manner in Figures 3a and b. Figures 3a and b show a clear interaction between the two treatments. The housing treatment had a large effect on OC in *ad libitum* fed pigs, but only a small effect in restricted fed pigs. Comparison of Figures 3a and b indicates that the overall OC-value on animal level is largely determined by the treatment effects on the tarsocrural joint. The influence of the treatment effects on the other joints was small.

Housing system affected on OC in the elbow and the tarsocrural joint. Conventionally housed pigs were significantly (0.19 SD units; p=0.068) more affected with OC than deep litter housed pigs in the elbow joint. Feeding strategy only had a significant effect on the tarsocrural joint and on animal level. In the tarsocrural joint and on animal level, *ad libitum* fed pigs were significantly (p=0.0004 and p=0.003 respectively) more affected with OC than restrictedly fed pigs. The combination of conventional floor with *ad libitum* feeding showed the highest OC, both at animal level and at the tarsocrural joint. In the tarsocrural joint, the change from conventional housing to deep litter when pigs are fed *ad libitum* decreased OC with more than 1 SD (p<0.05) (Figure 4.2b).

Sex affected OC only in the elbow joint. Boars were significantly (0.34 SD units; p=0.002) more affected than gilts (data not shown). Sex had no effect on animal level and on the other joints.

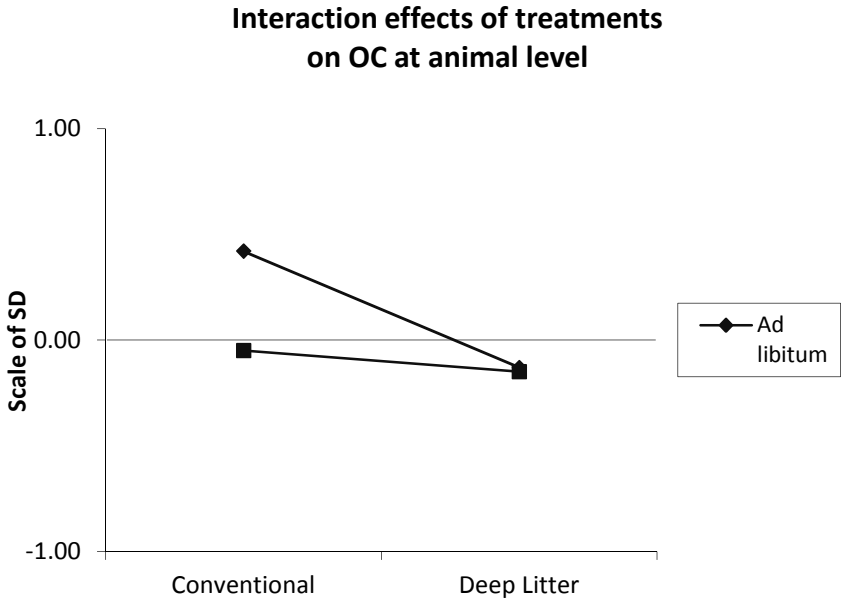


Figure 4.2a The interaction effects of treatment on the quantitative OC value at animal level. The results are expressed in SD units.

4.4 Discussion

Based on the prevalences of OC found in our study, we conclude that the elbow joint, femoropatellar joint and tarsocrural joint are most severely affected by OC, compared to the MCP and MTP joints, especially in the medial sections of the joints. These joints are, therefore, the most important joints to focus on when studying OC in pigs. Deep litter housed pigs, in combination with a higher space allowance, showed less OC than conventionally housed pigs. Restricted feeding decreased the prevalence of OC, mainly in the tarsocrural joint. Housing system and feeding strategy affected OC prevalence in pigs, but the effects differed between joints.

Prevalence

Animal

Overall, the prevalence of OC from mild to severe lesions (*score* B-E) on animal level was 41.4%. This prevalence is lower than prevalences found in previous

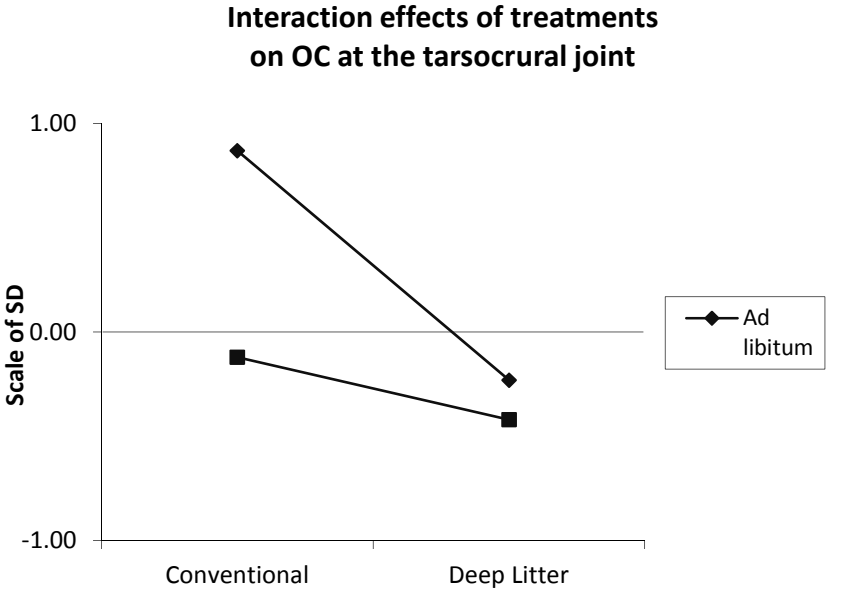


Figure 4.2b The interaction effects of treatment on the quantitative OC value at the tarsocrural joint. The results are expressed in SD units.

studies varying from approximately 80 to 100% in pigs at slaughter (Crenshaw, 2006; Grøndalen, 1974a). However, all studies varied largely in approach and results. Between studies, large differences exist in the number of examined joints and locations within the joints, breed, sex, age, and the scale on which OC is scored. Furthermore, prevalences within this study can be expected to be lower than normal, because the treatment of restricted feeding and deep litter floor decreased the prevalence of OC. The treatment conventional floor and *ad libitum* feeding, currently a common way of pig housing, had the highest prevalence of OC, 57.5%. In this study, however, the pigs housed on conventional floors had a somewhat larger floor area (0.8 - 1m^2 per pig) than common in commercial herds of fattening pigs (0.65m^2-0.8 m^2; European Food Safety Authority, 2007). This may explain some of the differences found between studies.

Joints

In our study, the prevalence of OC in the elbow was 6%, and in the femoropatellar joint 11%, which is low compared to other studies, although still within reported ranges. In literature, the prevalence of OC in the elbow joint varied between 2-56% (Grøndalen, 1974a; Jørgensen and Andersen, 2000; Jørgensen and Nielsen, 2005; Klimiene and Klimas, 2006; Luther *et al.*, 2007) and in the femoropatellar joint between 10-84% (Grøndalen, 1974a; Jørgensen and Andersen, 2000; Kadarmideen *et al.*, 2004; Klimiene and Klimas, 2006; Luther *et al.*, 2007).

In our study, the metacarpophalangeal and metatarsophalangeal joints were least affected with OC; only 1.8% and 0.3% was affected. In literature, no results were found about the prevalence of OC in these joints.

The medial sections of a joint were found to be more affected. This is possibly caused by the locally higher loading of the medial aspect of the joints in the limbs due to the closer position to the centre of gravity and the related differences in moment on the medial and lateral joint surfaces and is in agreement with findings from previous studies (Carlson *et al.*, 1988; Grøndalen, 1974b; Nakano *et al.*, 1987; Ytrehus *et al.*, 2007).

The positive correlations between locations, such as between the lateral trochlea of talus and the lateral tibial cochlea, are likely caused by physical contact between lesions, so called 'kissing lesions'.

Factors influencing OC

Sex

In our study, boars were significantly more affected by OC in the elbow joint than gilts. Based on literature, boars and barrows were often more affected than gilts and sows (Goedegebuure *et al.*, 1988; Jørgensen, 2003; Luther *et al.*, 2007; Stern *et al.*, 1995; Ytrehus *et al.*, 2004; Kadarmideen *et al.*, 2004). The higher weight of boars is considered to be related to OC (Jørgensen, 2003). Sexual behaviour, like mounting, could also cause local overloading in joints (Boyle and Bjorklund, 2007; Rydhmer *et al.*, 2006). In our study, we found no significant difference in mean weights between sexes at slaughter. Therefore, the higher weight of boars is excluded as a causal factor for OC in our study. Stern *et al.* (1995) also found a larger influence of sex on OC than could be explained by differences in growth rate among sexes.

Housing system

Conventionally housed pigs were significantly more affected with OC in the elbow and tarsocrural joint than deep litter housed pigs. In a study of young pigs walking on different wet concrete floors, front limbs of pigs slipped more and longer compared to their hind limbs (Applegate *et al.*, 1988). The effect of weight bearing in combination with a more slippery floor, gives the elbow joint extra disadvantage, leading to more cartilage damage. Slipping, correcting a slip, or more careful walking, probably can change a pig's posture and lead to local overloading of articular cartilage (Jørgensen, 2003; Nakano *et al.*, 1987). The negative effects of a conventional floor on the tarsocrural might also be due to the slippery properties. Figure 4.2b *vs.* 3a shows that the effect of housing system is greatest in the tarsocrural joint. Surprisingly, the effect of housing system on OC in the tarsocrural joint has not been investigated previously.

Pigs kept on deep litter with a higher space allowance, were found to be more active in our study (results not shown) and previous studies (Scott *et al.*, 2007; Scott *et al.*, 2006). Bone growth, strength and metabolism are positively affected by exercise or physical activity, which is reduced in situations with limited opportunities for locomotion (Jørgensen, 2003; Weiler *et al.*, 2006). Consequently for our study, the positive effects on bone development of deep litter housing and higher space allowance could possibly have decreased OC. Other studies found that pigs with more space were less aggressive and had less injuries (Randolph *et al.*, 1981; Weng *et al.*, 1998).

Feeding

Ad libitum fed pigs were significantly more affected with OC in the tarsocrural joints compared to restrictedly fed pigs. As expected, pigs that were *ad libitum* fed, regardless the housing system, were heavier at slaughter. *Ad libitum* fed pigs might have, besides a higher weight, a higher muscular growth in relation to the skeletal development, leading to an imbalanced development. This imbalanced development may cause local overload, which may result in a higher frequency of skeletal problems (Weiler *et al.*, 2006). Higher feed intake could disturb the metabolism of bone growth, because high feed intake or high carbohydrate intake is suggested to cause metabolic changes by increasing IGF-1 and negatively affecting endochondral ossification in pigs, dogs and horses (Dammrich, 1991; Nakano *et al.*, 1987; Ralston, 1996; Savage *et al.* 1993).

4.5 Conclusion

The OC prevalence of the total population was 41.4%, of which 12.4% was severely affected. Based on the prevalences of OC found in our study, and the findings in literature, it can be concluded that the elbow, femoropatellar and tarsocrural joints are most affected by OC, especially in the medial sections of the joints. Therefore, the elbow, femoropatellar and tarsocrural joints are considered to be the most important joints to focus on when studying OC in pigs. OC was rare in the metacarpophalangeal and metatarsophalangeal joints.
Deep litter housed pigs, in combination with a higher space allowance, showed less OC than conventionally housed pigs. Restricted feeding reduced the prevalence of OC, mainly in the tarsocrural joint. The prevalence of OC in conventionally housed *ad libitum* fed animals was 57.5%. Prevalence reduced to 33.7% when applying deep litter housing with more space and restricted feeding. The housing system, feeding strategy and sex affected OC in different joints. The results at animal level were largely determined by the treatment effects on the tarsocrural joint. The influence on the other joints is small. The results show that OC in pigs can be reduced by applying a housing system with deep litter floors and more space and/or restricted feeding.

Acknowledgements

The authors thank the Dutch Product Board of Animal Feed for financial support in this project. The Institutional Animal Care and User Committee of Wageningen University approved the experimental protocol.

Appendix

For the transformation of categorical scores into quantitative values, we assumed a continuous normally distributed liability underlying the categorical scores (Figure 4.3). This does not mean that we assumed the categorical scores to follow a normal distribution, but rather that the observed categorical scores depend on an unobservable normally distributed liability underlying the categorical observations. Such a liability model is a common way to analyse polygenic traits showing discrete phenotypic categories (Falconer, 1965). In this model, liability values within a certain range correspond to a particular phenotypic category of an OC score, the range being determined by the

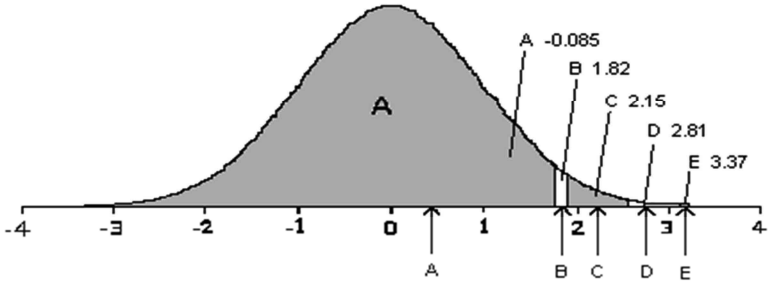


Figure 4.3 Example of a normal distribution (numbers on x-axis indicate number of standard deviations) expressed on the liability scale (Falconer, 1965), representing the transformed OC categories (scores A to E) to their linear OC value of the tarsocrural joint (Van Grevenhof *et al.* 2009).

incidence of that category. In Figure 4.3, for example, a score A of the tarsocrural joint corresponds to a liability value below 1.78, and score B corresponds to a liability between 1.78 and 1.87. Observed categorical scores were transformed into the mean liability value for that score. For example, in Figure 4.3, score A occurred in 96.23% of the cases, represented by the area below a liability value of 1.78. The mean liability value of this area is -0.085. Thus, score A was transformed into a value of -0.085. Figure 4.3 indicates the remaining

transformed values for B through E Note that Figure 4.3 depicts an example for the tarsocrural joint; liability values will differ for other joints. (Using transformation to a liability scale, rather than transforming A through E simply into 1 through 5, has the advantage that the differences between the resulting values follows from the observed incidence in the data. A transformation into 1 through 5 would imply that the difference between, e.g., B and C equals the difference between, e.g., D and E). The incidence at specific locations within a joint was too low to enable reliable transformation to a liability scale for each of the locations separately.

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5

The relationship between osteochondrosis and growth in pigs

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Submitted to Livestock Science (2010)

Abstract

Osteochondrosis (OC) is a disturbance of the process of endochondral ossification during skeletal growth. Osteochondrosis is considered the main reason for leg weakness in pigs, which is the second reason for culling in sows, after fertility problems. Previous studies suggest there is a relationship between OC and growth. However, little is known about this relationship, in particular about the timeframe in which growth influences ossification. To understand the relationship between the growth pattern and the onset of OC, repeated body weight (BW) measurements and OC scores of 345 pigs were collected. An average of 17 body weight measurements from birth until slaughter at 6 months of age was used. OC was scored macroscopically after slaughter in 24 locations of five joints. Pigs were divided in three defined groups based on the severity of OC; no OC, minor, or severe OC.

Until weaning at day 21 no differences in weight and gain were found between the three defined groups. From weaning onwards, pigs diagnosed with minor or severe OC showed higher BWs than pigs diagnosed without OC. The higher weights were due to increased growth before the age of three months. This period might coincide with the window of susceptibility for OC in pigs. The relationship with growth seems to be joint-dependent. Pigs with OC in the elbow joints or with OC in two joints have high BWs, whereas pigs with OC in the femoropatellar joints have low BWs compared to mean BW. Determination of the window of susceptibility and of the relationship between weight gain and OC may help in developing strategies to reduce OC in pig populations.

Key words: body weight, growth, joint, osteochondrosis, pigs, window of susceptibility.

5.1 Introduction

Osteochondrosis (OC) is a disturbance of the process of endochondral ossification during skeletal growth and affects growing pigs in many commercial breeds (Crenshaw, 2006; Grøndalen, 1974a; Grøndalen, 1974b; Uhlhorn *et al.*, 1995; Ytrehus *et al.*, 2007). Osteochondrosis can be seen as a biomechanically induced disease, influenced by the combination of rapid skeletal growth and increasing muscle and body mass, which increases the pressure on joint surfaces while they are still developing and structurally weak. Osteochondrosis is considered the main cause of leg weakness in pigs, which is the second reason for involuntary culling sows, next to fertility problems (Yazdi *et al.*, 2000). Therefore, it is both for economic and welfare reasons important to reduce OC in pig populations.

Osteochondrosis is claimed to be associated with high BW or growth rate (GR), and not to occur or to be milder in slow growing or wild populations (Uhlhorn, 1995). A high GR, either due to genetic selection or feeding, causes disturbances in bone metabolism, which may increase the prevalence of OC (Grøndalen, 1974; Grøndalen and Grøndalen, 1974; Kadarmideen *et al.*, 2004). In slowly growing animals, either on a nutritional or genetic basis, very little OC has been found (Reiland, 1978). However, Ytrehus (2007) found no relationship between GR and OC in pigs, suggesting that the relationship between GR and OC requires further investigation.

The period of growth in which OC develops is unknown and has not been studied previously in pigs. In the horse, van Weeren *et al.* (1999) found a positive relationship between OC prevalence in the stifle joint and weight gain of foals in the third and fifth month of life suggesting a 'window of susceptibility' for the development of OC. Therefore, the hypothesis of this study is that there is also in the pig a 'window of susceptibility' in which animals develop OC and that growth pattern differ between pigs with OC in different joints. To test these hypothesis, we repeatedly measured body weight of pigs and recorded their OC status after slaughter.

5.2 Materials and methods

The Institutional Animal Care and User Committee of Wageningen University approved the experimental protocol.

Materials

We used 345 intact boars (56%) and gilts (44%) from two batches; 187 Tempo*Topigs40 crossbreds from 23 dams, and 158 Piétrain*Topigs40 (Topigs bv., The Netherlands) crossbreds from 18 dams. All pigs had participated in an intermittent suckling experiment until they were 69 days old, in which they were either weaned intermittently from day 14 of age and completely weaned at the age of 18 days (IS treatment 1) or 40 days (IS treatment 2), or weaned intermittently from day 21 of age and completely weaned at the age of 26 days (IS treatment 3) or 46 days (IS treatment 4), or weaned conventionally at 21 days of age (IS treatment 5) (Gerritsen *et al.*, 2007). When the pigs were 69 days old, they were stratified into groups of five or six individuals based on intermittent suckling treatment, sex, litter mates, and BW at 42 days of age.

These groups were then exposed to a two by two factorial design of a housing system and feeding strategy to investigate the effects on the prevalence and severity of OC (Van Grevenhof *et al.*, in press). The treatments, conventional or deep litter housing, and *ad libitum* or restricted feeding, were applied to the pigs. A conventional pen consisted of a 50% metal slatted (ridged round bars) and 50% solid concrete floor over five m¬2. A deep litter pen consisted of a solid concrete floor with approximately 25-50 cm of wood shavings over 8.5 m2. The *ad libitum* fed pigs had unlimited access to feed using a feeding unit. The restrictedly fed pigs had access to 80% (in kg) of the average daily intake of the *ad libitum* group in the preceding week. All pigs were fed standard pelleted, dry grower and finisher diets. The restrictedly fed pigs received two equal portions of feed each day per group, in a trough with individual feeding places at 8 am and 4 pm. Water was available *ad libitum* for all treatment groups via two drinking nipples per pen.

In total, 6,596 weight measurements of the 345 pigs were used, corresponding to an average of 17 weight measurements per pig. Pigs from batch 1 (n=187) were slaughtered at an age ranging from 165 to 176 days. Pigs from batch 2 (n=158) were slaughtered at an age ranging from 161 to 165 days. During the experiment, pigs were weighed at regular intervals from birth until slaughter. The carcasses were stored at 4°C for one day. Since OC prevalence in the left

and right joints of pigs have a phenotypic correlation close to one (Jørgensen and Andersen, 2000), only the left limbs were scored in this study. These were dissected in the shoulder and hip joints, tagged, and stored at -21^{o}C until further dissection and scoring of the joints.

Scoring

Pigs were scored for OC in five joints of the left front and hind limbs. In the front limb, the elbow and metacarpophalangeal joints were scored. In the hind limb, the femoropatellar, the tarsocrural, and metatarsophalangeal joints were scored. In total, 24 locations were scored (locations defined in Van Grevenhof *et al.*, in press). An experienced veterinarian judged all joints. The joint surfaces of a location were macroscopically scored on a categorical scale from A-E, as used in a previous study on the macroscopic classification of OC in horses (Van Weeren and Barneveld, 1999). Score A indicated no abnormalities, score B flattening of the joint contour, score C slightly irregular cartilage, score D severely irregular cartilage, and score E indicated a 'classic' lesion with fragmentation, or the presence of an osteochondrotic cyst (See van Grevenhof *et al.*, in press for photographic illustrations).

Data transformation

In order to use the OC scores in a statistical analysis, we transformed the categorical observations into quantitative traits. For the transformation of categorical scores into quantitative values, we assumed a continuous normally distributed liability underlying the categorical scores (Van Grevenhof *et al.*, in press). A liability model is a common way to analyze polygenic traits showing discrete phenotypic categories (Falconer, 1965). In this model, liability values within a certain range correspond to a particular phenotypic category of an OC score, the range being determined by the incidence of that category. Each categorical score was transformed into the mean liability value for that score (Falconer, 1965). This procedure resulted in five quantitative traits, one for each of the five joints. An OC score for the entire animal was calculated by summing the values of the five joints. For details of the data transformation see in van Grevenhof *et al.* (in press).

Statistical analysis

The OC scores were transformed for two purposes. To investigate the relationship between OC and growth or BW, OC scores were divided into three

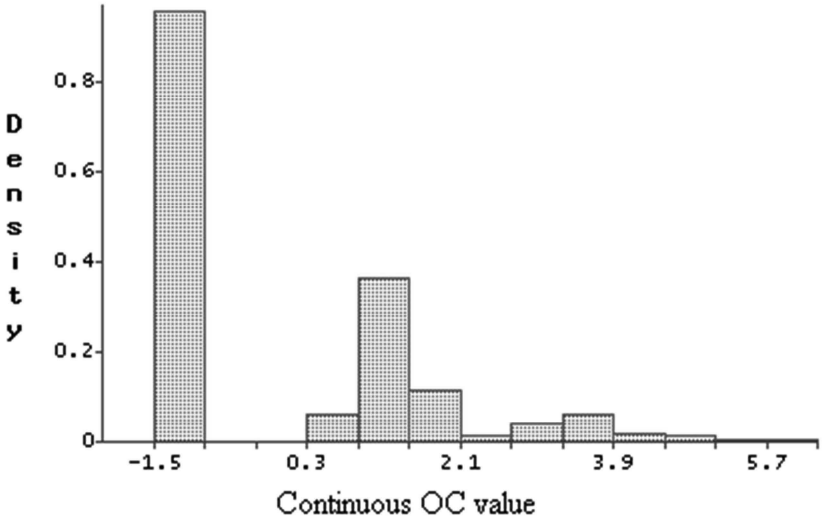

Figure 5.1 Density of the frequency distribution of all pigs, visualizing the peak of pigs without osteochondrosis (OC−) and the two groups with OC (OC+ and OC++).

groups (Figure 5.1) based on OC severity (distribution of the continuous OC values), and to investigate the relationship between OC and growth or BW within specific joints, OC scores were divided into five categories based on OC occurrence per joint.

Curves

To investigate the relationship between OC and growth or between OC and BW over time expressed in growth curves, pigs were grouped in three groups based on their OC severity. The distribution of OC in the total population shows that OC divides into three subgroups (Figure 5.1); 'OC−' indicates no OC, 'OC+' indicates mild OC in one location (scores B and C) and 'OC++' indicates OC in multiple locations or severe OC at a single location (scores D and E). Using three groups, visualising the results in growth curves will show the relation of pigs without OC, minor or severe OC with growth or BW. Therefore, a univariate linear mixed model was used to estimate the effect of OC group on BW and GR, and curves were created by plotting the LSMeans.

$$Y_{ijklmnop} = \mu + \text{IS treatment}_i + \text{Batch}_j + \text{Housing}_k * \text{Feeding}_l + \text{Sex}_m + \text{OC}_n + \textit{Mother}_o + e_{ijklmnop}$$

where $Y_{ijklmnop}$ is BW or mean growth per day (increase in weight between two time points, divided by duration of the interval), from intermittent suckling(IS) treatment i, in batch j, with housing system k, feeding strategy l, sex m, OC group n and mother o of pig p. The symbol μ stands for the overall mean; IS treatment i is the fixed class effect of IS treatment (i= 1, 2, 3, 4 or 5); batch j is the fixed class effect of the batch (j= 1 or 2); housing k is the fixed class effect of housing system (k =conventional, deep litter); feeding l is the fixed class effect of feeding strategy (l =*ad libitum*, restricted); sex m is the fixed class effect of sex (m = boar, gilt); OC n is the fixed class effect of the defined OC group (n=OC−, OC+, OC++) Mother o is the random effect of mother (o= 1, 2, …, 41); $e_{ijklmno}$ is the random residual of pig p.

Joint-level

To investigate the relationship between OC and growth or between OC and BW for a specific joint, an analysis was performed at joint level. The population was divided into 5 categories, where; 'OC–' indicates no OC (n=190), 'E+' indicates OC in the elbow joint but not in any other joint (n=15), 'FP+' indicates OC in the femoropatellar joint but not in any other joint (n=21), 'TC+' indicates OC in the tarsocrural joint but not in any other joint (n=84), and 'OC++' indicates OC in more than one joint (OC in elbow and femoropatellar joints (n=4) or OC in femoropatellar and tarsocrural joints (n=14)) or in the metacarpal- and metatarsophalangeal joints (n=5). Using this distribution, it can be distinguished whether and how growth curves differ between pigs suffering from OC within specific joints. For these analyses, the same univariate linear mixed model was used as described above for BW and GR.

Statistical analyses were performed on univariate models using the PROC GLM statement of SAS 9.1.3 (©2002-2003 by SAS Institute Inc.) (SAS, 2005).

5.3 Results

In compliance with animal experimentation regulations, 17 of 344 pigs had to be euthanized prematurely for welfare reasons. Some of these pigs suffered from lameness (the cause of which was not investigated), or were severely injured by pen mates. The majority of these problems appeared shortly after mixing of the animals at the start of the experiment, and seemed to be related

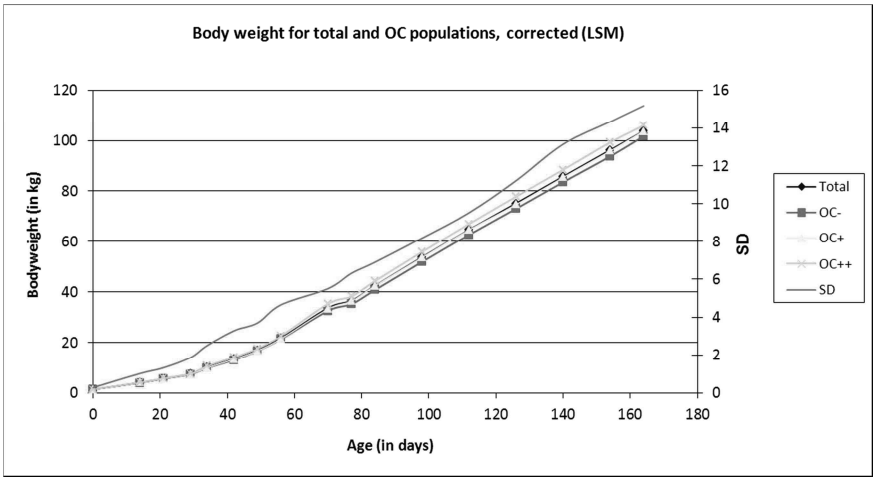

Figure 5.2a The increase in BW for the mean population (total), OC− group (no OC), OC+ group (mild OC) and OC++ group (severe OC) in pigs, corrected for the treatments and gender. The SD is expressed on the second Y-axes.

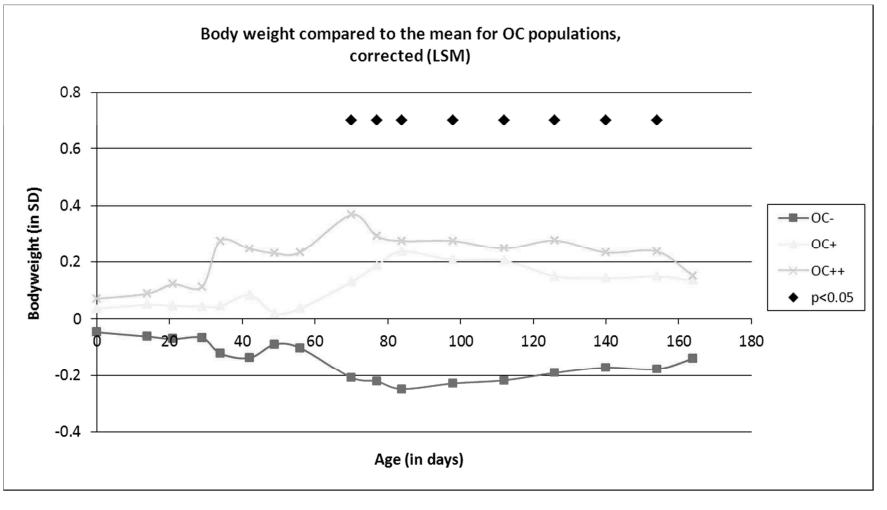

Figure 5.2b The difference between the BWs for the OC− group (no OC), OC+ group (mild OC) and OC++ group (severe OC) in pigs, corrected for the treatments and gender, compared to the mean (set at 0) expressed in standard deviations (Y-axis), over the entire fattening period (X-axis). The significance level of P<0.05 is indicated by a ♦ above each time point.

to behavioral interactions, not to OC. Later euthanasia were due to tail biting or disease, not OC. The euthanized pigs were nearly equally distributed over the treatments.

The mean slaughter weight was 102 kg. Differences in mean slaughter weights were found between batches, housing and feeding systems. *Ad libitum* fed pigs, either housed conventionally (110 kg) or in deep litter (110 kg) were significantly ($P<0.05$) heavier than restrictedly fed pigs, either conventionally (98 kg) or deep-litter housed (93 kg) (Van Grevenhof *et al.*, in press). Pigs severely affected by OC (OC++) were significantly ($P<0.05$) heavier (106 kg) at slaughter than OC+ pigs (103 kg) and OC− pigs (102 kg).
Fifty-nine percent of the animals showed no signs of OC (score A at all locations). Animals with score B through E had either one location (33%), two locations (8%) or three locations (1%) affected. In total, 12% of all animals showed severe OC (OC++; score D or E).

Curves
BW and OC. Until day 70, there was no significant ($P>0.10$) difference in BW between the three OC groups (Figure 5.2). To visualize the difference between the OC groups, the BWs of the OC groups were compared to the mean BWs of all pigs, and expressed in standard deviation units (Figure 5.2b). After day 30, OC++ pigs have the highest BW until the slaughter age, whereas pigs without OC (OC−) have the lowest BW up to slaughter. The largest significant ($P=0.002$) difference in BW compared to the mean was 0.57 SD at day 70. The largest absolute difference was more than 5.5 kg BW at day 154, between OC− and OC++ (Figure 5.2). In total, 8 of the 17 univariate model results showed a significant relation ($P<0.01$) between OC and BW.

GR and OC
The growth curve based on the univariate analyses of each period showed similar patterns for all three OC groups (Figure 5.3). Around day 70, after regrouping and consequently dominance fighting, a reduction in growth is visible for all groups (Figure 5.3a). Only between day 28 and day 35, and between day 56 and day 84, the groups did significantly ($P<0.05$) differ from each other (Figure 5.3b). In order to visualize the differences between each of the OC groups, the curves were expressed in standard deviation units as deviations from the mean growth curve of all pigs (Figure 5.3b).

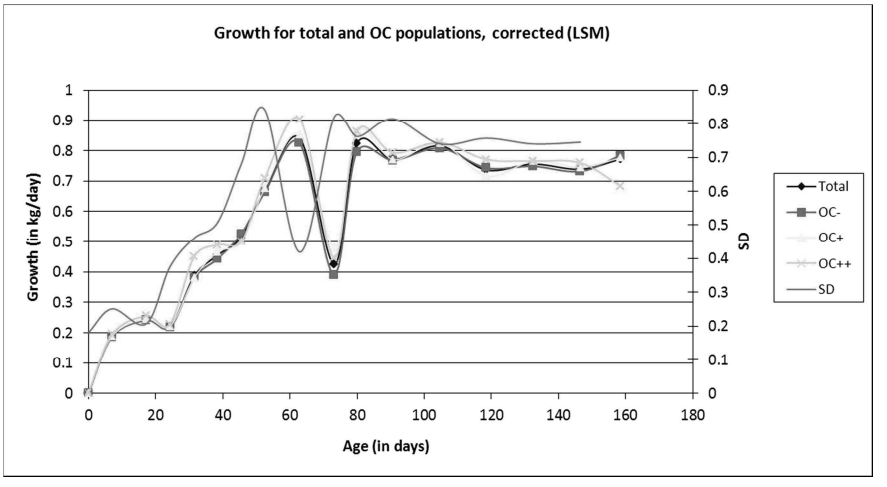

Figure 5.3a The increase in growth for the mean population (total), OC− group (no OC), OC+ group (mild OC) and OC++ group (severe OC) in pigs, corrected for the treatments and gender. The SD is expressed on the second Y-axes.

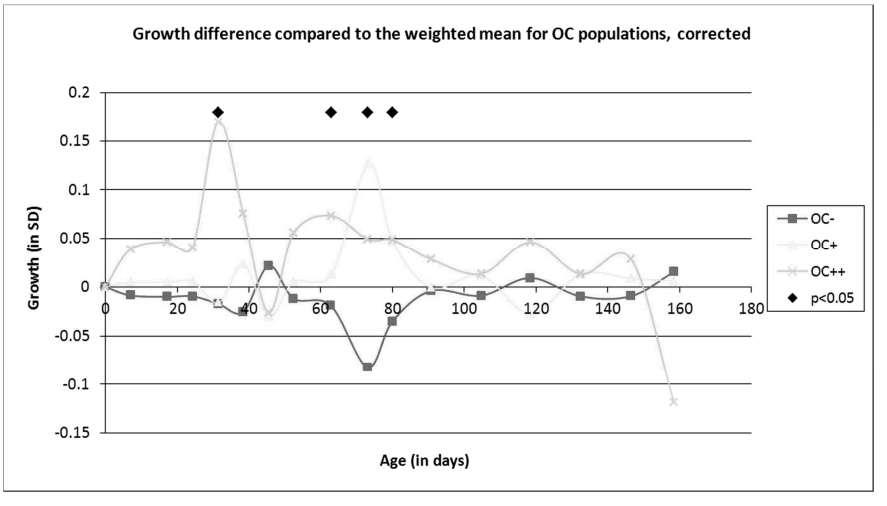

Figure 5.3b The differences in growth for the OC− group (no OC), OC+ group (mild OC) and OC++ group (severe OC) in pigs, corrected for the treatments and gender, compared to the mean (set at 0) expressed in standard deviations (Y-axis), over the entire fattening period (X-axis). The significance level of P<0.05 is indicated by a ♦ above each time point.

Pigs from the OC++ group showed a significant increased GR in the period from 28 days. Pigs from OC+ groups show increased GR after day 56 until day 84. The largest significant ($P<0.05$) difference in GR compared to the mean was 0.19 SD in the period from day 28 till day 35.

Joint-level

Until day 42, there was no significant ($P>0.05$) difference in BW between OC in the different joints (Figure 5.4). After day 42, pigs with OC in the elbow joint (E+) and pigs with OC in more than one joint or in the metacarpal- or metatarsalphalangeal joints (OC++) had the highest BW until slaughter at 164 days; whereas pigs without OC (OC−), with OC in the femoropatellar joint (FP+) or OC in the tarsocrural joint (TC+) seem to have BWs close to the mean of the population. To visualise the difference of OC in the different joints, in Figure 5.4b the BW of the pigs with OC in different joints is compared to the mean BW of all pigs, expressed in standard deviation units.

5.4 Discussion

BW and GR

Pigs with severe OC had higher BWs. These pigs might show a reduced physical activity due to painful joints, a reduction in activity might enhance higher BWs. Bone growth, strength and metabolism are all enhanced by exercise or physical activity (Jørgensen, 2003). During growth and with increasing body mass, the articular cartilage changes from a primarily growth-oriented structure to a structure that permits both growth activity and resistance to biomechanical stress. A genetically determined lower resistance to biomechanical loading could cause overloading in the developing joints. The increased GR, as evidenced in the curves of pigs with OC compared to pigs without OC, could result in overloading of the skeleton and hence in the development of lesions in the joint (Jørgensen, 2003). Other studies have found no, or only weak, relationships between growth parameters and osteochondrosis (Uhlhorn *et al.*, 1995; Woodard *et al.*, 1987). This lack of correlation might be caused by the lack of measurable effect of growth on osteochondrosis during a specific age of development, or caused by the fact that OC is influenced by many factors and therefore no large effects will be found due to a single factor. Conversely, Busch and Wachmann (2010) found a 20% increase in risk of OC for each additional 100 gram of average daily gain in the weaning period or in the finishing period.

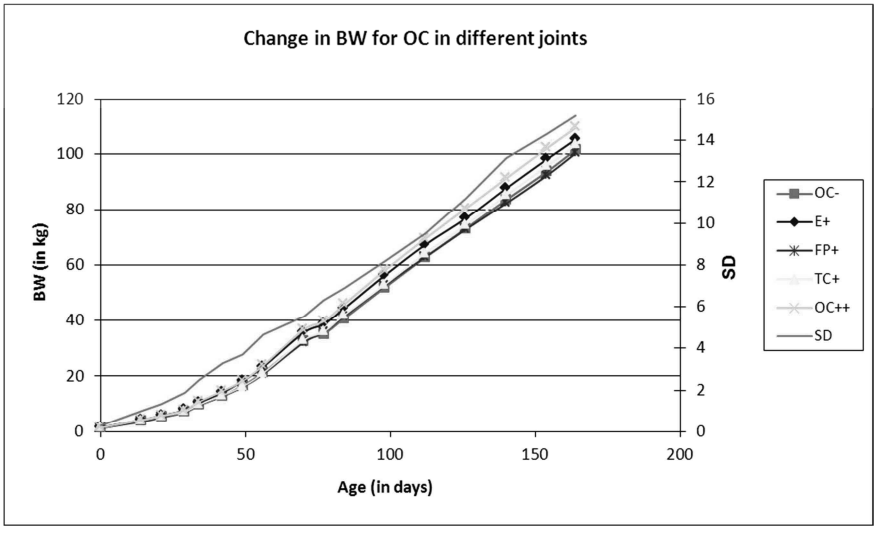

Figure 5.4a The increase in BW for the group without osteochondrosis (OC−), group with OC in the elbow joint (E+), group with OC in the femoropatellar joint (FP+), group with OC in the tarsocrural joint (TC+), or group with OC in more than one joint or in the metatarsal/ metacarpal joints (OC++) in pigs, corrected for the treatments and gender. The SD is expressed on the second Y-axes.

Window of susceptibility

Differences in weight gain were observed between the OC groups. These differences occurred during the period between weaning at 56 days until 84 days of age, which may be an indication that the so-called window of susceptibility, when the maturing cartilage is most vulnerable for developing osteochondrotic lesions, occurs within this period (Van Weeren and Barneveld, 1999). Although, no evidence could confirm the presence of OC in these pigs at earlier time points, as OC was only measured at slaughter age. Between day 28 and day 35 high growth was seen in pigs who eventually showed severe OC at slaughter age. After 90 days of age, no major differences in weight and gain were observed between pigs with and without OC. During lactation piglets cannot show their full growth due to limited amounts of milk. After weaning when fed *ad libitum*, pigs can show their potential and compensate the growth. Within this model, influences of IS treatment, housing and feeding system are corrected for. Periods of fast increasing or decreasing growth are likely indications of unbalanced skeletal growth. Research in horses, in which virtually all diarthrodial joints in the body were examined (van Weeren and Barneveld

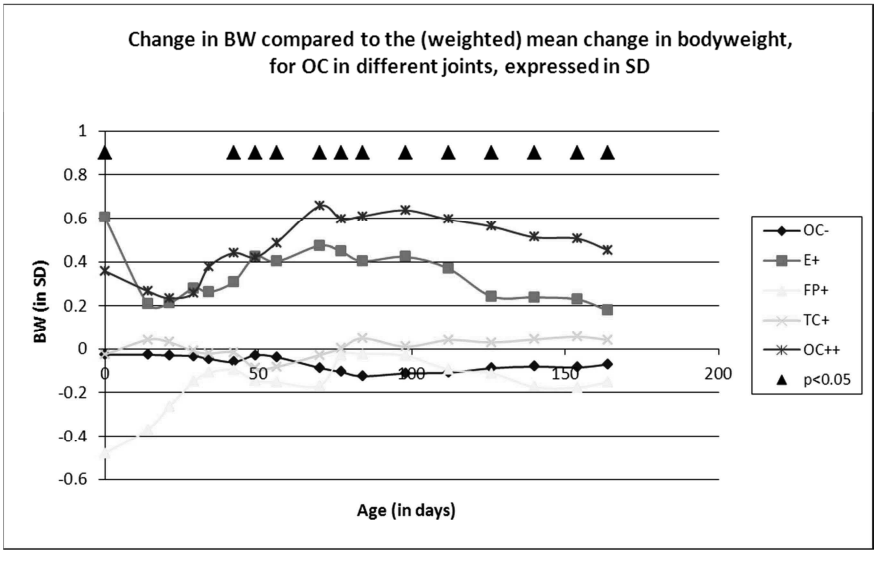

Figure 5.4b The difference between the BWs for the group without osteochondrosis (OC−), group with OC in the elbow joint (E+), group with OC in the femoropatellar joint (FP+), group with OC in the tarsocrural joint (TC+) or group with OC in more than one joint or in the metatarsal/ metacarpal joints (OC++) in pigs, corrected for the treatments and gender, compared to the mean (set at 0) expressed in standard deviations (Y-axis), over the entire fattening period (X-axis). The significance level of P<0.05 is indicated by a ▲ above each time point.

1999), has shown that OC is a highly dynamic and very common process in that species. In this process, osteochondrotic lesions cannot only develop, but may regress spontaneously as well during the window of susceptibility. In the horse, there was some evidence that growth rate may be one of the important intrinsic factors that determines the occurrence of OC. In foals, a positive relationship between OC status in the stifle joint and weight gain in the third and fifth month was found, which coincides with the timeframe of 'the window of susceptibility' defined for foals (van Weeren *et al.* 1999).

Biphasic growth curve

Biphasic growth curves were also fitted to this data to compare with the results from the BW and growth curves. These curves (Figure 5.5) differed from the curves of the univariate analyses (Figure 5.2a), which may be a consequence of the reduction in growth after regrouping. These growth reductions are difficult to fit using biphasic growth curves.

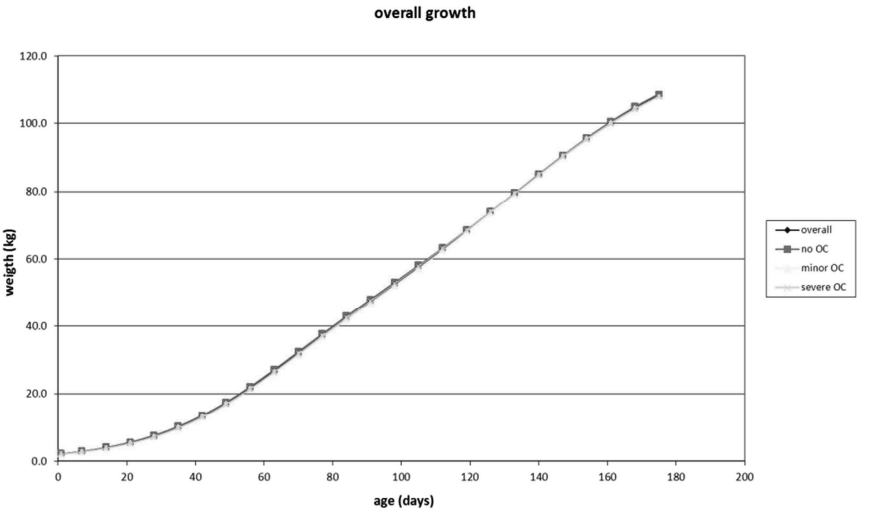

Figure 5.5 The biphasic growth curves for three defined osteochondrosis (OC) groups in pigs, no OC, minor OC and severe OC, including the overall mean.

Biphasic growth models have the strength to combine data points within one animal and thereby account for the interrelations of the body weight measurements within the animal. However, since these data points are connected into growth curves using six estimated parameters, an outlier caused by a sudden reduction in growth could possibly have a large effect on the total shape of the biphasic growth curve. In our study, the results of both methods are diverging and the combined univariate analyses were closest to the shape of growth curves drawn from raw uncorrected data. However, the fact that advanced biphasic growth models cannot correctly estimate the shape of the growth curves, using several BW measurements per pig reflects the instability of fitting biphasic growth curves to practical data. Therefore, a univariate model was a better fit to this data.

Joint-level

The high BW of pigs with OC in the elbow joint or with OC in two joints might be related to overloading of the skeleton. Most of the pigs of group 'OC++' were found to have OC in two joints (78%), either in both the femoropatellar and the tarsocrural joints, or in both the elbow and femoropatellar joints. Early skeletal lesion development could cause an increased weight gain by a reduced willingness to move or decreased general activity. In 67% of the cases, pigs with

OC in the elbow joint were housed in a conventional system. Conventionally housed pigs were 1.5 times less active compared to deep litter housed pigs. More pigs that were conventionally housed were lying passively compared to pigs that were deep litter housed (46% vs 24%), less pigs were sitting actively (2.7% vs 5.0%) and less pigs were walking actively (3.7% vs 6.8) rather than standing. Feeding strategy did not affect OC in the elbow joint, as pigs were equally divided over the feeding strategies. Almost all (93%) pigs with OC in the elbow joint were males, while there was no significant (P>0.10) effect of sex on slaughter weight. Birth weight has a significant effect (P=0.02) on OC at joint level. Both E+ and OC++ pigs showed high birth weights, 1.57 and 1.50 respectively. Higher birth weights will be an advantage during growth and in establishing the ranking within the litter or pen.
The low BW of the pigs with OC in the femoropatellar joint alone might be the result of low birth weight and consequently a lower individual feed intake due to ranking of these pigs within a litter or pen. These pigs might have had a lower intake of nutritional components, which possibly causes a metabolic imbalance during the process of ossification. Pigs with OC in the FP joint had low birth weight (1.26) indeed, compared to all other groups of OC at joint level ('OC–'= 1.39; 'E+'= 1.57; 'TC+'= 1.39; 'OC++'= 1.50). There was no effect of feeding strategy on OC in the FP joint, as these pigs were equally divided over both feeding treatments. More pigs with OC in the FP joint (67%) were housed in deep litter system, which leads on average to higher activity.

The results at joint level indicate that looking at OC in general might blur the informative figure of pigs with OC compared to pigs without OC, as pigs with OC in the elbow joint show an opposite relation with growth compared to pigs with OC in the femoropatellar joint. These two diverging results will conceal the relation between growth and OC. In combination, one effect would thus obscure the other, giving the false impression of no effect at all. However, from these analyses it cannot be determined whether the relationship between OC and growth is causal or dependent.

5.5 Conclusions

After 28 days of age, piglets with severe OC at slaughter started to grow faster, after 70 days of age pigs with OC became significantly heavier than pigs without OC, although the severity of lesions were not determined until the pigs were

slaughtered. The relationship between OC and GR showed no differences before 56 days of age between the three defined OC groups based on the severity of OC. Between 56 days and 84 days of age, pigs with minor and severe OC show increased growth, which may indicate the position in time of a 'window of susceptibility' for OC in pigs.

Pigs with OC in the elbow joints or with OC in two joints had higher BWs, whereas pigs with OC in the femoropatellar joints had lower BWs compared to the mean BW. The results at joint level indicate that looking at OC in general might blur the informative figure of pigs with OC compared to pigs without OC, as pigs with OC in the elbow joint show an opposite relation with growth compared to pigs with OC in the femoropatellar joint. The results of this study suggest that OC might be related to selection of pigs with high GRs during a specified time period.

Acknowledgement

The authors thank the Dutch Product Board of Animal Feed for financial support in this project.

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6

Potential of genomic selection for traits with a limited number of phenotypic records

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Concept article (2011)

Abstract

In the last 10 years, genomic selection has developed enormously. Simulations and results of real data suggest that breeding values can be predicted with high accuracy using genetic markers alone. To reach high accuracies, however, large reference populations are needed. In many livestock populations or even species, these cannot be realized when traits are difficult or expensive to record, or when population size is small. The value of genomic selection becomes questionable then. In this study, we compare traditional breeding schemes based on own performance or progeny information to genomic selection schemes, when the number of phenotypic records is limiting. For this goal, deterministic simulations were performed using selection index theory. Results showed that genomic selection schemes suffer more from the Bulmer effect than traditional breeding schemes, so that comparison of traditional versus genomic breeding schemes should focus on Bulmer-equilibrium response to selection. To maximize the accuracy of genomic EBVs when the number of phenotypic records is limiting, the phenotyped individuals, rather than progeny tested individuals, should be genotyped. When the generation interval cannot be decreased when implementing GS, large reference populations are required to obtain equal response as with own performance selection or progeny testing. The accuracy of genomic EBVs, however, increases non-linearly with the size of the reference population, showing a diminishing-return relationship. As a consequence, when a GS scheme has a small decrease in generation interval, relatively small reference population sizes are needed to obtain equal response as with own performance selection or progeny testing. When the trait of interest cannot be recorded on the selection candidate, GS schemes are very attractive even when the number of phenotypic records is limited, because traditional breeding would have to rely on progeny testing schemes with long generation intervals.

Key words: breeding program, genomic selection, selection response, reference population, Bulmer effect

6.1 Introduction

Genomic selection is a variant of marker-assisted selection in which genetic markers covering the whole genome are used so that all quantitative trait loci (QTL) are in linkage disequilibrium with at least one marker (Goddard and Hayes, 2007). Although more validation is required, simulation results and practical data in dairy cattle suggest that breeding values can be predicted with high accuracy using genetic markers alone (Schrooten *et al.*, 2005; De Roos *et al.*, 2008; VanRaden *et al.*, 2009). Since the introduction of the idea by Meuwissen *et al.* (2001) ten years ago, there have been a number of developments and the first practical applications occured in cattle breeding (Schrooten *et al.*, 2005; De Roos *et al.*, 2008; VanRaden *et al.*, 2009). In the past, the major limitation for the implementation of genomic selection has been the large number of markers required and the cost of genotyping these markers. Recently, both these limitations have been overcome in most livestock species, following the sequencing of the genomes and the subsequent availability of high density SNP-chips (Goddard and Hayes, 2007). It is now feasible to meet the requirements for the implementation of genomic selection in breeding programs. In fact, after deriving a prediction equation from a reference population that uses markers and phenotypes as input and predicts breeding values as outputs, there is no need at all to record phenotypes of the candidates for the selection. Thus genomic selection can potentially cut costs for producing and testing potential breeding animals considerably. Moreover, genomic selection will have a large impact on breeding programs for many livestock species as it will shorten generation intervals, which is of special importance in long-lived species such as dairy cattle and horses, or when trait values become available late in life or on progeny only. This is important, as genotyping can be applied to new-born animals or even embryos (Bredbacka, 2001), and because of the largely reduced need for progeny testing.

A limitation of genomic selection, however, is that large reference populations are needed to obtain high accuracies of estimated breeding values. When the size of the reference population increases, the accuracy of estimated breeding values can reach high values, approaching 0.8 to 1.0 (Daetwyler *et al.*, 2008; Meuwissen *et al.*, 2001; Zhao *et al.*, 2007). Reference population sizes used in simulations sometimes even exceeded 100,000 animals (Goddard and Hayes, 2009), whereas reference populations in practise are in some cases limited to less than 1,000 animals (Calus, 2010). In many livestock populations, or even in

some species, it is hardly feasible to create larger reference populations for many traits, especially when phenotypes are difficult or expensive to record, such as methane emission in cattle or disease resistance. If large reference populations cannot be obtained, genomic selection will reach relatively low accuracies, and may yield no, or a relatively small, additional response compared to traditional selection based on phenotypic records. This applies particularly to traits that are determined by large numbers of genes, which appears to be common in livestock (Hayes *et al.*, 2006). The more genes involved, the smaller the effect of individual genes, and the larger the reference population needs to be to reach a certain accuracy. For those reasons, it is important to investigate when genomic selection offers advantages over traditional selection in case the size of the reference population is limited.

In this study, we compare genomic selection (GS) to phenotypic selection on own performance (OP) and to progeny testing (PT), to predict the response to selection with a limited number of phenotypic records. First, we investigate the optimal construction of the reference population when the number of phenotypic records is limited. In dairy cattle, construction of a reference population has started with genotyping progeny tested bulls, merely because accurate EBVs based on progeny testing were available for these bulls. When the number of phenotypic records is limiting, however, it may be suboptimal to use progeny tested bulls for construction of the reference population. Second, we investigate the reduction of accuracy and response to selection due to the effect of selection on the genetic variance, the so-called Bulmer effect (Bulmer, 1971). Results will show that response to genomic selection suffers more from the Bulmer-effect than response to traditional selection. It is, therefore, essential that traditional and genomic selection schemes are compared at the Bulmer-equilibrium response, rather than at first generation response. Finally, we determine the minimal size of the reference population at which GS becomes advantageous over traditional selection, and investigate the dependency of this break-even point on heritability and generation interval.

6.2 Material and methods

Bulmer effect

The comparison of the Bulmer effect between traditional and genomic selection schemes will be investigated both theoretically using mathematical derivations, and numerically using deterministic simulations. The theoretical derivations and

results will be presented in the results below. The deterministic simulations are based on selection index theory, using the SelAction software. SelAction predicts the response to selection and accuracy of selection for breeding programs. The software accounts for reduction in variance due to selection (Bulmer, 1971) and for the use of pedigree information, as with selection on BLUP-EBVs. Features of SelAction and the theoretical background are described in Rutten *et al.* (2002).

Genomic selection schemes can be simulated in SelAction by including an additional trait representing the marker information (Schrooten *et al.*, 2005; Dekkers *et al.*, 2007). The marker information was modelled as a trait with a heritability of 0.99, which was genetically correlated to the trait of interest. The genetic correlation between the marker information and the trait of interest was equal to the accuracy of genomic EBVs, $r_{g\hat{g}}$, which depends on the reference population. The $r_{g\hat{g}}$ represents the accuracy of genomic EBVs in an unselected population, and is calculated using Equation 1 given below (Daetwyler *et al.*, 2008). Because it is assumed that the marker information is fully heritable, it has no residual variance and the environmental correlation between the marker information and the trait of interest can be set to zero in SelAction, $r_E = 0$. Further details of this approach are given in Dekkers *et al.* (2007).

Because the accuracy of genomic EBVs established in the reference population, $r_{g\hat{g}}$, refers to the accuracy in a random sample of the population, there is a distinction between $r_{g\hat{g}}$ and the Bulmer equilibrium accuracy of a breeding scheme based on genomic selection, denoted r_{IH}. The Bulmer effect reduces the proportion of genetic variance explained by the markers, so that r_{IH} will be smaller than $r_{g\hat{g}}$ in an on-going breeding scheme. The results of the deterministic simulations is, therefore, used to calculate the equilibrium accuracy and response that are reached after a few generations of selection for all selection schemes.

Optimal construction of the reference population

Accuracies of genomic EBVs depend on the size of the reference population (n_P), the number of potential loci affecting the trait (n_G), and the reliability of

the observed record in the reference population (r^2). In a random sample of the population, the accuracy of a genomic EBV, $r_{g\hat{g}}$, being the correlation between the estimated genomic breeding value and the true breeding value, can be calculated using

$$r_{g\hat{g}} = \sqrt{\frac{\lambda \cdot r^2}{\lambda \cdot r^2 + 1}}, \qquad \text{(Equation 1a)}$$

where $\lambda = n_P / n_G$, n_P is the number of individuals in the reference population with both phenotypic records and genotypes (Daetwyler *et al.*, 2008). The potential number of loci affecting the trait depends on the historical effective size of the population (N_E) and on the size of the genome, *L* (Hayes *et al.*, 2009),

$$n_G = 2N_E L. \qquad \text{(Equation 1b)}$$

When using own performance records in the reference population, *i.e.* when genotyping and phenotyping the same individuals, the reliability of records in the reference population is equal to the heritability of the trait,

$$r^2 = h^2. \qquad \text{(Equation 1c)}$$

When the reference population is based on progeny-tested individuals, *i.e.*, when genotyping parents while phenotyping their offspring, the reliability in Equation 1a equals the reliability of EBVs obtained with progeny testing,

$$r^2 = \frac{1/4 \cdot N \cdot h^2}{(1 + 1/4 \cdot (N-1) \cdot h^2)}, \qquad \text{(Equation 1d)}$$

where *N* is the number of progeny on which the EBV is based. To investigate the optimal construction of the reference population, $r_{g\hat{g}}$ -values were compared

for different numbers of progeny per sire and reference population sizes, for a fixed heritability of 0.3.

Response of traditional versus GS breeding schemes

For the comparison of GS with selection based on phenotypic information, deterministic simulations were performed with SelAction, using the approach described above in the section on the Bulmer effect. Alternative breeding schemes were compared based on the Bulmer-equilibrium response to selection. Several selection schemes were evaluated, to illustrate the general characteristics of phenotypic selection versus genomic selection. For GS, the reference population size (n_P), and heritability (h^2) were varied, to investigate the effect of these parameters on response to selection. All other parameters were kept constant across scenarios. In these scenarios, selection was for a single trait and in males only, to distinguish the effects of varying input parameters from effects of having two sexes. To mimic absence of selection in females, the selected proportion in females was set to 0.99 in SelAction. The number of selection candidates and the total number of animals selected were kept fixed.

Three scenarios were simulated:

1. Phenotypic selection using pedigree and own performance information (OP).
2. Phenotypic selection using pedigree and progeny information (PT).
3. Genomic selection using pedigree and marker information on selection candidate (GS).

To investigate the added value of genomic information on top of phenotypic information, the genomic selection scheme (Alt. 3) was applied both with and without own performance information on the candidates for selection.

The following assumptions were made in all scenarios:

- The population had discrete generations and a fixed number of sires and dams per generation;
- There was an active population of 1,000 dams per generation;
- 20 sires were used per generation;
- Each dam produced 2 male and 2 female offspring per generation;

- In the case of progeny testing, 10 progeny per sire were available in the progeny test.
- The historical effective population size was assumed to be 100 (required for Equation 1b).
- There was one-stage selection, with a selection proportion of 0.02 in sires and 0.99 in dams.

Results will be presented in two ways. First, we compare responses to selection on own performance, progeny or genomic information, where GS schemes either include or exclude own performance information. Second, we identify the break-even size of the reference population at which GS without own performance information yields the same response to selection as a traditional breeding scheme. In this approach, we model the break-even size of the reference population as a function of the reduction in generation interval that can be achieved when implementing GS.

6.3 Results and discussion

Bulmer effect

Results of the deterministic simulations revealed that GS schemes suffer more from the Bulmer effect than schemes based on phenotypic information. This occurs because GS targets a proportion of the genetic variation with full accuracy, whereas traditional selection targets the full genetic variation with limited accuracy. As a consequence, the genetic variance utilized by GS, *i.e.* the variance in the marker effects, is strongly reduced by selection, which in turn reduces the accuracy. Compare, for example, mass selection with an initial heritability of 25% to GS with an initial accuracy of 0.5, for a trait with unity phenotypic variance. In an unselected population, both schemes have the same accuracy of 0.5. With selected proportions of 5%, however, results of the deterministic simulations showed that the equilibrium accuracy and additive genetic variance were 0.47 and 0.21 for the mass selection scheme, but 0.39 and 0.22 for the GS scheme. Consequently, the equilibrium response was 14% lower than the first generation response for the mass selection scheme, but 27% lower for the GS scheme. Hence, at equilibrium, the mass selection scheme yielded 118% of the response of the GS scheme. In fact, an accuracy of 0.59 prior to selection would have been required for the GS scheme to be equivalent to the mass selection scheme at equilibrium. This example illustrates that

comparison at equilibrium parameters is essential when comparing GS schemes to traditional breeding schemes.

Results of the deterministic simulations also revealed a second difference between GS and traditional breeding schemes. With traditional selection, the reduction of response due to the Bulmer effect is greater at higher accuracy. With mass selection and a selected proportion of 5%, for example, response is reduced by only 7% when h^2 = 0.10, but by 21% when h^2 = 0.50. With GS, in contrast, the reduction in response due to the Bulmer effect did not depend on the accuracy of selection. With a selected proportion of 5%, the Bulmer effect always reduced response by 27% in GS schemes, irrespective of the accuracy. Again, this occurred because the genetic variance used by GS is known with full accuracy.

The above results followed numerically from the deterministic simulations, but can also be explained theoretically. The full genetic variance can be partitioned into the variance of the estimated marker effects and the prediction error variance (PEV); $\sigma_A^2 = \sigma_M^2 + PEV$. The variance in the estimated marker effects in the next generation equals

$$\sigma_{M_{t+1}}^2 = \tfrac{1}{2}\sigma_{M_t}^2 (1-k) + \tfrac{1}{2}\sigma_{M_0}^2 ,$$

where the first term in the right-hand side is the between-family variance, which is reduced by selection of the parents on the estimated marker effects. Because the markers can be observed with certainty, the reduction in between-family marker-variance does not depend on accuracy of the EBV. The *k* is the proportional reduction in variance due to truncation selection of parents, and is determined entirely by the intensity of selection, $k = i(i - x)$, where *i* is the intensity of selection and *x* the standardized truncation point (Tallis, 1961). (To keep this example as simple as possible, equal selection intensity is assumed for both sexes). The $\tfrac{1}{2}\sigma_{M_0}^2$ is the Mendelian-sampling variance, which is half the variance of the estimated marker effects in an unselected population (Bulmer, 1971).

The equilibrium follows from substituting $\sigma^2_{M_{t+1}} = \sigma^2_{M_t}$ and solving for $\sigma^2_{M_t}$, which yields

$$\sigma^2_{M_{eq.}} = \frac{\sigma^2_{M_0}}{1+k} \; . \qquad \text{(Equation 2a)}$$

For example, for $\sigma^2_P = 1$, $h^2_0 = 0.3$, an initial accuracy of GS of $r_{g\hat{g}}$ = 0.8, and a selected proportion of 5% so that *k* = 0.86, the equilibrium variance of estimated marker effects equals $(0.8^2 \times 0.3 \times 1)/1.86$ = 0.103. Since response (R) to GS equals $i\sigma_M$, which is proportional to σ_M rather than σ^2_M, the relative reduction of response to GS due to the Bulmer effect equals

$$\frac{R_0 - R_{eq}}{R_0} = 1 - \sqrt{\frac{1}{1+k}} \; , \qquad \text{(Equation 2b)}$$

which is independent of the accuracy of selection. For example, for a selected proportion of 5%, the Bulmer effect reduces response to GS by $1 - \sqrt{1/1.86}$ = 27%. The reduction in response with traditional selection is always smaller, except when accuracy of selection approaches 100% in both sexes, in which case the reduction will be the same. Hence, when comparing GS in bulls with traditional progeny testing of bulls in dairy cattle, the reduction of response due to the Bulmer effect will be similar for both schemes because the accuracy of progeny EBVs in dairy cattle is close to 100%. Note that the result becomes different when not only bull sires, but also bull dams, are selected based on genomic information. The above calculations of the Bulmer effect will be approximations when marker effects are updated each generation. Nevertheless, the pattern is expected to remain the same, because the size of the update will usually be much smaller than the already existing reference population.

The ranking of traditional breeding schemes based on response to selection is little affected by the Bulmer effect, so that comparison at equilibrium is not essential (Dekkers, 1992). However, because GS schemes suffer more from the Bulmer effect than traditional breeding schemes, the relative response to GS is

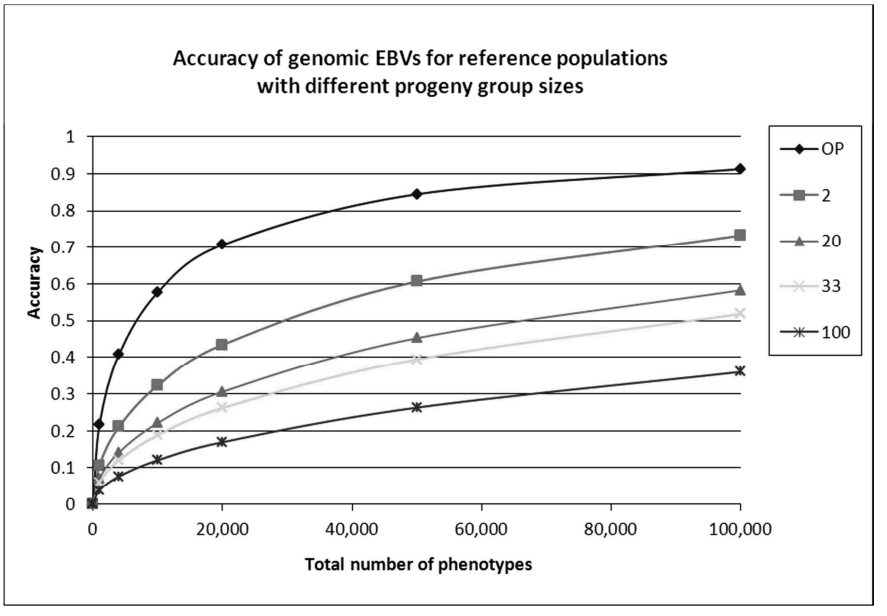

Figure 6.1a Accuracy of genomic EBV ($r_{g\hat{g}}$) when the reference population is varying in size and consists of varying group sizes of progeny information (EBV). OP = own performance. When the progeny group size is for example 20, the number of genotyped individuals in the reference population (n_P)equals the total number of phenotypes divided by the size of the progeny group. The $r_{g\hat{g}}$ is calculated from Equations 1a-d, for N_E = 100 and h^2 = 0.3.

overpredicted when ignoring the Bulmer effect, particularly when compared to traditional schemes of low accuracy. Thus, a comparison at Bulmer-equilibrium response to selection is essential when comparing traditional and GS breeding schemes. In the results below, therefore, breeding schemes will be compared based on the equilibrium response that is reached after a few generations of selection.

Optimal construction of the reference population

When the total number of phenotypic records is kept constant, a reference population with OP information on the genotyped individuals yields a greater accuracy of genomic EBVs than a reference population with progeny information on the genotyped individuals (Figure 6.1a). With progeny information, the number of sires on which the reference population is built will

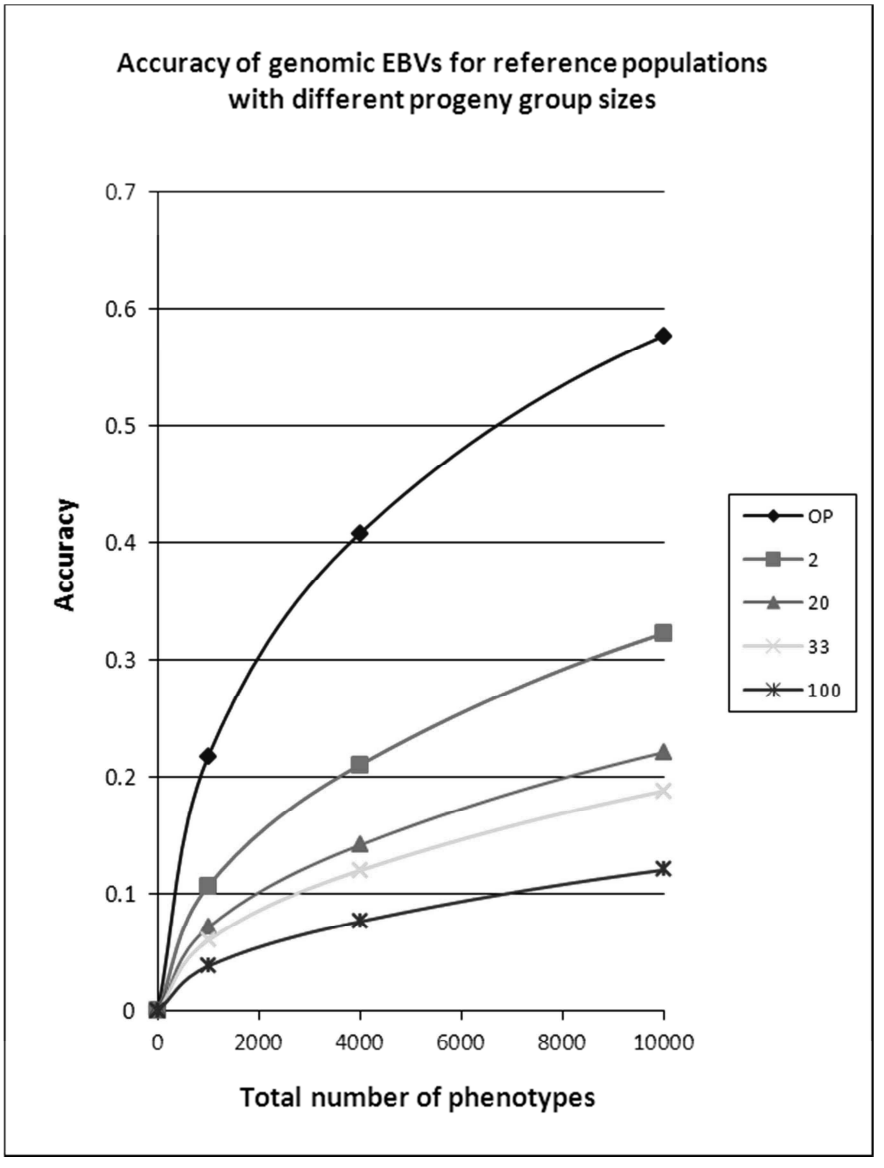

Figure 6.1b Detail of Figure 6.1a, focussing on a small number of total phenotypic records.

obviously decrease when increasing the number of progeny per sire, which consequently reduces the accuracies particularly when the number of phenotypic records is small (Figure 6.1b). For example, with 4,000 phenotypic

records and 20 progeny per sire, the reference population will consist of n_P = 200 genotyped sires with EBVs based on 20 progeny, whereas with 2 progeny per sire, the reference population will consist of n_P = 2,000 genotyped sires with EBVs based on two progeny. Even though increasing the size of the progeny groups will increase the accuracy of the sire EBVs, the number of sires upon which the GS reference population is built has a much larger impact on the accuracy of genomic EBVs. Thus, when the number of phenotypic records is limiting, it is optimal to genotype the individuals that produce the phenotype, not their parents. Reference populations used to obtain the results presented below, therefore, assume that the same individuals are both phenotyped and genotyped, so that n_P refers not only to the number of phenotypic records but also to the number of genotyped individuals in the reference population.

Figures 1a and b show that the increase of accuracy with the number of phenotypic records is strongly non-linear, showing a diminishing-return relationship. The non-linearity is greatest when using own performance information. As a consequence, reducing the total number of phenotypic records reduces accuracy less than proportional. With OP information, for example, reducing the number of phenotypes from 10,000 to 5,000, which is a 50% reduction, reduces accuracy by only 24% (Figure 6.1b).

Response of traditional versus GS breeding schemes

Figure 6.2a compares response to selection per generation between traditional breeding schemes and genomic selection schemes in which the selection candidates do not have own performance information. The results show that large reference population sizes are required for GS to outperform traditional breeding schemes. Even with reference population sizes of 10,000 individuals, GS did not generate a larger response per generation.

Figure 6.2b compares traditional breeding schemes to genomic selection schemes in which the selection candidates also have own performance information. Hence, in the GS schemes in Figure 6.2b, genomic information is available on top of own performance information. Results show that, when phenotypes of the selection candidates are available, genomic information is of little additional value over phenotypic information, unless the reference population is large. Figures 2a and b show that the response pattern does not change with heritability, as the lines for different heritabilities are almost parallel. In conclusion, Figures 2a and b show that GS cannot compete with

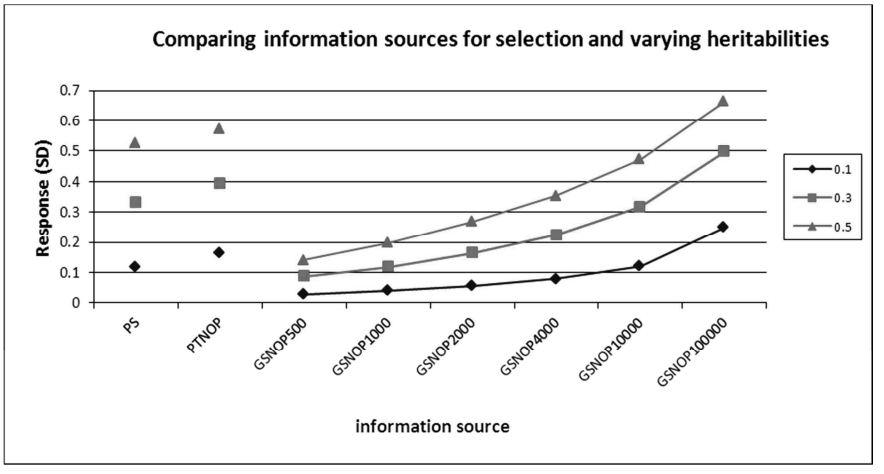

Figure 6.2a Bulmer-equilibrium response to selection per generation, for different information sources and reference population sizes. Response is expressed in phenotypic standard deviations. The GS schemes do not have own performance information on the selection candidate (NOP = No Own Performance). PS=phenotypic selection; PT=progeny testing; GS=genomic selection; number indicates the size of the reference population. Reference populations are based on own performance information. Note that the x-axis scale is non-linear.

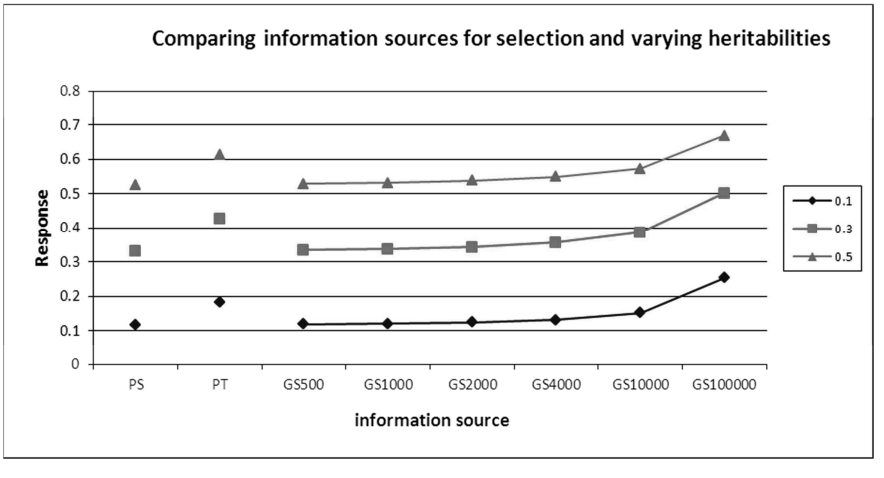

Figure 6.2b Bulmer-equilibrium response to selection per generation, for different information sources and reference population sizes. Response is expressed in phenotypic standard deviations. GS schemes also have own performance information on the selection candidate. PS=phenotypic selection; PT=progeny testing; GS=genomic selection; number indicates the size of the reference population. Reference populations are based on own performance information. Note that the x-axis scale is non-linear.

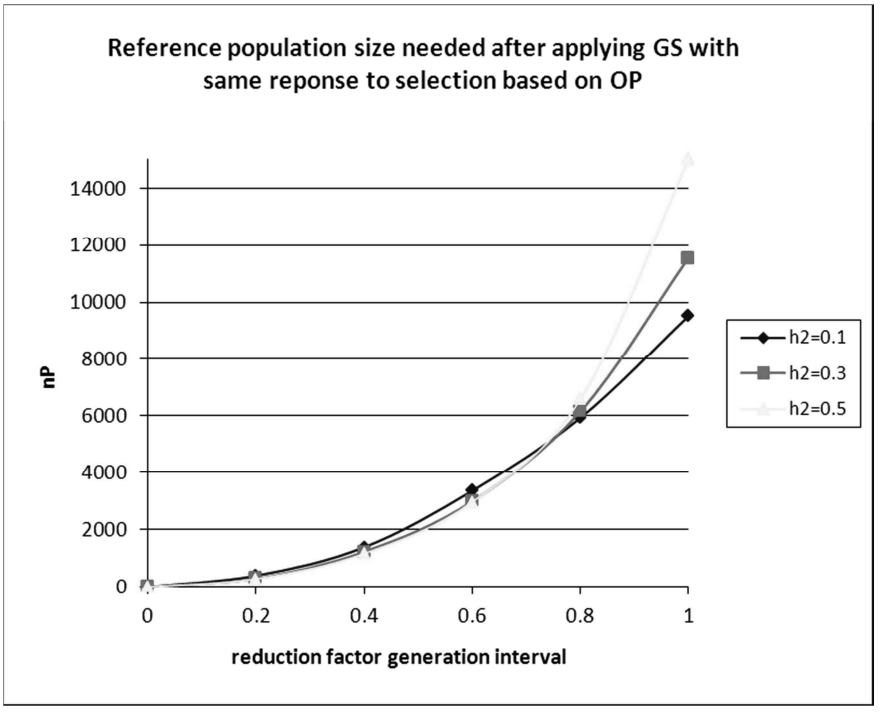

Figure 6.3a Reference population size needed for genomic selection to reach equal response per year as a traditional breeding scheme based on own performance. Reference populations are based on own performance information.

traditional selection when the number of phenotypic records is limited, unless the generation interval can be decreased by GS.

Figures 3a and b show the break-even size of the reference population that is needed to reach equal response as with traditional selection, as a function of the decrease in the generation interval that can be realized when implementing GS. When the generation interval cannot be decreased, large reference population sizes are required, particularly when heritability is high, which agrees with Figures 2a and b. When generation intervals can be decreased, however, the break-even size of the reference population decreases rapidly, particularly when heritability is high. The rapid decrease of the break-even size of the reference population with the decrease of generation interval originates from the non-linear relationship of accuracy with the number of phenotypes; a reduction in reference population size yields a less than proportional reduction in accuracy (Figure 6.1a,b). A reduction in generation interval, in contrast, yields a proportional increase in response. As a consequence, small reductions in

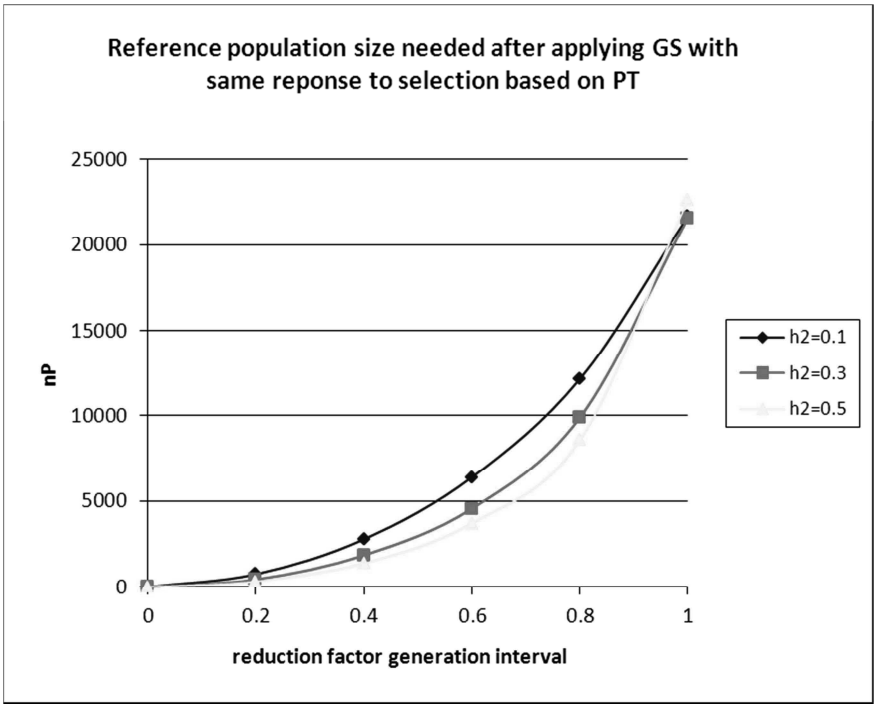

Figure 6.3b Reference population size needed for genomic selection to reach equal response per year as a traditional progeny-testing scheme having 10 progeny per sire. Reference populations are based on own performance information.

generation interval lead to relatively large reductions in the break-even size of the reference population.

Compared to an own performance breeding scheme, a reference population size of ~6,000 individuals is needed when the generation interval is reduced by 20%, whereas only ~2,000 individuals are needed when the generation interval is halved (Figure 6.3a). Compared to a progeny testing scheme, a reference population size of ~10,000 individuals is needed when the generation interval is reduced by 20%, whereas ~3,500 individuals are needed when the generation interval is halved (Figure 6.3b). Because traditional progeny testing schemes require rather large numbers of phenotypic records and long generation intervals, GS schemes will often be superior to progeny testing schemes when compared at an equal number of phenotypic records.

In this work, we based our calculations of the accuracy of GS on the expression presented in Daetwyler *et al.* (2008; Equation 1a), rather than applying stochastic simulations. An alternative prediction of the accuracy of genomic

breeding values was presented by Goddard (2008), as

$$r = \sqrt{\left[1 - \lambda/(2N\sqrt{a}) * \ln((1 + a + 2\sqrt{a})/(1 + a - 2\sqrt{a}))\right]},$$

where $a = 1 + 2\lambda / N$, and $\lambda = qk / h^2$, with $k = 1/\log(2N_E)$, where N_E is the effective population size. The difference between both predictions originates from a difference in the assumption on the accuracy of estimated QTL effects.

In contrast to Goddard (2008), Daetwyler *et al.* (2008) assumed this to be equal regardless of allele frequency. Hayes *et al.* (2009) compared the accuracies of genomic breeding value predicted by both methods, and found them to be very similar. The method of Daetwyler *et al.* (2008) yielded slightly lower accuracies of breeding values at low to moderate heritabilities. For traits with a limited number of phenotypes, heritabilities will mostly be in this low to moderate range (Hayes *et al.*, 2009). Hence, this may indicate that the accuracies presented here are slightly conservative.

6.4 Conclusions

GS schemes for traits that are expensive or difficult to record will be characterized by small reference populations and a relatively low response to selection per generation. Because GS schemes suffer more from the Bulmer effect than traditional breeding schemes, relative response to genomic selection is overpredicted when ignoring the Bulmer effect, particularly when compared to traditional schemes with low accuracy. A comparison of traditional versus GS breeding schemes should, therefore, focus on Bulmer-equilibrium response to selection. To maximize the accuracy of genomic EBVs when the number of phenotypic records is limiting, the same individuals should be genotyped and phenotyped, rather than genotyping progeny tested individuals. When the generation interval cannot be decreased with GS, large reference populations are required to obtain equal response as with own performance selection or progeny testing. The accuracy of genomic EBVs, however, increases non-linearly with the size of the reference population, showing a diminishing-return relationship. As a consequence, when GS schemes have a little decrease in generation intervals, relatively small reference population sizes are needed to obtain equal response as with own performance selection or progeny testing.

Thus, when the trait of interest cannot be recorded on the selection candidate, GS schemes are very attractive even when the number of phenotypic records is limited, because traditional breeding would have to rely on progeny testing schemes with long generation intervals.

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7

General discussion

7.1 Introduction

This final chapter, the general discussion, explores possibilities for the improvement of genetic selection to reduce the prevalence of OC in horse and pig populations. The first section deals with the impact of OC on locomotion and sports, in order to assess the relevance of OC for the ultimate breeding goal. The second section compares different breeding strategies designed for the Dutch warmblood horse population. Possibilities for genomic selection are evaluated and compared to selection strategies which have been in use until very recently (own performance of stallions), and which have recently been adopted (progeny testing). The third and last section of the general discussion focuses on selection to reduce the prevalence of OC in pigs. This is done in basically the same way, *i.e.* by identifying the relevance of OC in pigs and by consequently evaluating possibilities for the implementation of genomic selection.

7.2 The relevance of OC in horses

Relevance

This thesis focuses on the developmental disorder osteochondrosis (OC), but the practical relevance of OC is not so easy to assess and has therefore been questioned. An often heard comment from horse owners is: "I don't care if my horse has OC, as long as it jumps at international level" or "I'd rather have a very good sport horse with OC, than an average horse without OC". Non painful joint effusion is the most common sign of equine OC, not lameness (Jeffcott, 1997; Radiostits *et al.,* 2007). The incidence of OC is high, but varies per breed and per joint. Previous studies have reported prevalences of osteochondrotic lesions or fragments in the limb joints measured through radiographic screening between 7% and 64% (Grøndahl and Dolvik 1993; Hoppe 1984; Hoppe and Philipsson 1985; Jeffcott and Henson 1998; Philipsson *et al.* 1993; Pieramati *et al.* 2003; Ricard *et al.* 2002; Sandgren *et al.* 1993; Schober *et al.* 2003; Schougaard *et al.* 1990; Wittwer *et al.* 2006). In our analysis of an unselected population of Dutch warmblood yearlings, the prevalence was even 70% (Chapter 2), but the incidence of lameness due to OC is unknown.

Table 7.1 Estimated losses due to OC (Personal communication van Tartwijk *et al.*)

3.000 horses annually (25% of 12.000)		Million
5% loss in rearing	600 x 2.500	1,5
1.000 surgeries	1.000 x 1.000	1,0
economical loss	1.000 x 3.000	3,0
economical loss of rearing and training	1.000 x 2.000	2,0
Loss of important genetic material		2,5
Annual total loss		**10 Million**

Economical relevance

Knowing the OC status (e.g. for sale purposes) has an influence on the saleability and market value, even though lameness would not play a role for the sport performance of that specific horse. The estimated economical losses to the equine industry due to OC are high (Table 7.1), mainly caused by the loss of breeding potential and depreciation of market value of affected animals (Van Weeren, 2006). The presumed indicative value of radiographic findings in general, in the equine limbs for future performance capacity is reflected by their effect on the sales values of a horse as it poses a risk for the longevity of the horse, irrespective of its intended use (Van Hoogmoed *et al.*, 2003). Especially in export sales, radiographic evaluation plays an important role in decision making.

Practical relevance

In addition to sale value, OC also leads to other large expenses for the horse industry including surgery and losses during rearing and training (Table 7.1). The number of surgical interventions shows that horse owners perceive OC as relevant, as else this investment would not be worthwhile. Even with a reduced OC prevalence, preventative surgical removal of fragments may still need to be considered in some cases as arthroscopic intervention is safe, and is expected to prevent the development of problems at a later age. Besides the estimated economic losses and the practical consequences as described above, osteochondrosis can be considered relevant when the presence of the disorder influences sport performance. Sport performance has a high priority within the breeding goal of warmblood studbooks (Koenen *et al.*, 2004). To further assess the relevance of OC, the relations between OC and traits relevant for sport performance such as gait quality or longevity in sports are needed.

Aim

Therefore, in the first section of this chapter I consider the relation of osteochondrosis with sport and conformation, as an indirect measure of performance. In the following paragraphs, available literature will be described and results from data analyses will be provided on the relationship of radiographic findings, including OC, and certain conformational features.

To identify the OC status in horses, OC is generally scored using radiographic examination. Radiographic examination is an integral part of pre-purchase examinations and stallions performance tests, because radiographic findings are generally considered as useful predictors of future orthopaedic soundness. Although the true impact of abnormal radiographic findings on performance is limited (Couroucé-Malblanc *et al.* 2006, Grøndahl and Engeland 1995, Robert *et al.* 2006, Storgaard Jørgensen *et al.* 1997.

What is known of the correlation between direct measures of sport performance or lameness and OC?

Until now, selection to reduce the prevalence of osteochondrosis has not been very effective, despite the heritable character of OC. The ineffective breeding can unfortunately not be validated with results from other studies as no reliable field data are available (Koenen *et al.*, 2000). A reason for the ineffective selection might be the unknown relation between OC and sport performance traits, as negative relationships between these traits could cause slowing or even an entire lack of genetic progress, when selection is focused on sport performance. For most warmblood breeds, selection on sport performance is the most important breeding goal, next to maintenance of general health and character which are considered necessary for top sport performance (Koenen *et al.*, 2004).

Clinical importance of OC in relation to general locomotion disorders

The presence of osteochondrosis in joints has been considered to be a limiting factor for sport performance in horses (Hoppe, 1984). Locomotory problems have been often related to reduced performance (Rossdale *et al.*, 1985) and lameness has also been indicated as one of the causes (6% of total) of poor performance (Martin *et al.*, 2000). Although OC is assumed to affect locomotion capacity, the relationship is not straightforward. Riley *et al.* (1998) studied the clinical features, radiographic findings and treatments of draft horses with OC,

where most (13) of the 15 horses with OC in the femoropatellar (FP) joint were lame. However, this is certainly not the rule in other horse breeds. Radiographic findings of OC in the tarsocrural (TC) joint were related to the presence of synovial effusions, but no relation was found with lameness (Carlsten *et al.*, 1993). Some other studies found a weak relationship between lameness and OC (Sandgren *et al.*, 1993; Gaustad *et al.*, 1995).
Many studies focus on radiographic findings in general, which includes also other joint abnormalities, rather than to focus on OC only (Kane *et al.*, 2003; Storgaard Jørgensen *et al.*, 1997). In race horses, some data are available specifically on OC, but in riding horses the literature is extremely scarce.

Racing
Studies on the long-term effects of different radiolographic abnormalities have mostly been confined to racehorses, in which performance is easier and more objectively measurable than in riding horses (Stock and Distl, 2006a). In these studies the number of starts, the number of winnings or total earnings are mostly taken as measures of the lifetime performance of a horse. Many investigators did not find a negative effect of radiographic findings on the racing performance (Brehm and Staecker, 1999; Kane *et al.*, 2003; Storgaard Jørgensen *et al.*, 1997). Abnormal radiographic findings, including OC and other joint diseases, significantly decreased racing career longevity in French Standardbred trotters. Trotters that raced only one year had showed more frequent and more severe abnormal radiographic findings compared to horses that raced two years or more (Couroucé-Malblanc *et al.*, 2006). Also, Robert *et al.* (2006) found severe radiographic findings as well as multiple radiographic findings significantly compromised the future racing career of young French trotters.
However, the pressure put on the joints of trotters or Thoroughbreds differs considerably from the pressure put on the joints of riding horses. Gallop racing puts great demands on bones, joints and soft tissues of the front limbs. Trotting mainly stresses the hind limbs. Warmblood horses are used in various disciplines but loading on joints in itself is generally less heavy than in racing (Stock *et al.*, 2006a). One should take these differences into account when comparing results from this thesis with results reported in literature on racehorses or trotters.

Riding horses
The relation between performance of Warmblood riding horses and orthopaedic status at a young age has only been investigated in a few studies.

These studies did not reveal clear evidence for a negative effect of a less than optimal overall orthopaedic status on performance or longevity (Holmstrøm and Philipsson, 1993; Willms *et al.*, 1996; Wallin *et al.*, 2001; Wallin *et al.*, 2003). Stock and Distl (2006a) investigated this relationship in detail, and reported that genetic correlations between radiographic findings in the limbs and the sport performance of Hanoverian Warmblood horses were in many cases close to zero. This is the only study on genetic correlation between individual bone or joint diseases and sport performance in dressage and show jumping competitions.

The correlation of OC with indirect measures of performance or soundness

Given the paucity of data on the direct relationship of OC with performance measures and/or clinical data, one is forced to return to more indirect measures of performance to assess the practical influence of OC. The most important indirect measure is conformation.

Conformation

Linear scoring of conformation is correlated to performance (Koenen *et al.*, 1995; Ducro *et al.*, 2007). For example, linear scoring of free jumping is highly correlated genetically (r_a=0.87) with jumping performance (Ducro *et al.*, 2007), and linear scoring of croup length is correlated negatively (r_a=-0.49) with dressage performance (Koenen *et al.*, 1995).

The genetic correlation found between certain conformation traits and radiographic findings can be explained by effects on the distribution of biomechanical loads within joints. An unfavourable conformation will affect the load distribution and also the gait pattern of a horse. During sport, joints may be heavily challenged and the load distribution in the joints changes continuously. Differences in loading can only be adequately met when the tissue is biomechanically adapted to withstand these changing loading conditions (Brommer *et al.,* 2005). Juvenile joints consist of dynamic tissues that are capable of continuous remodelling, which makes them capable to respond adequately to the changes in biomechanical loading (Van de Lest *et al.,* 2002). This is in contrast to mature joints, where remodelling activity has decreased to a low level and consequently the capacity for functional adaptation is limited. Constitutional irregularity of limb loading might, therefore, directly relate to bony remodelling in joints and the occurrence of lesions of cartilage and subchondral bone.

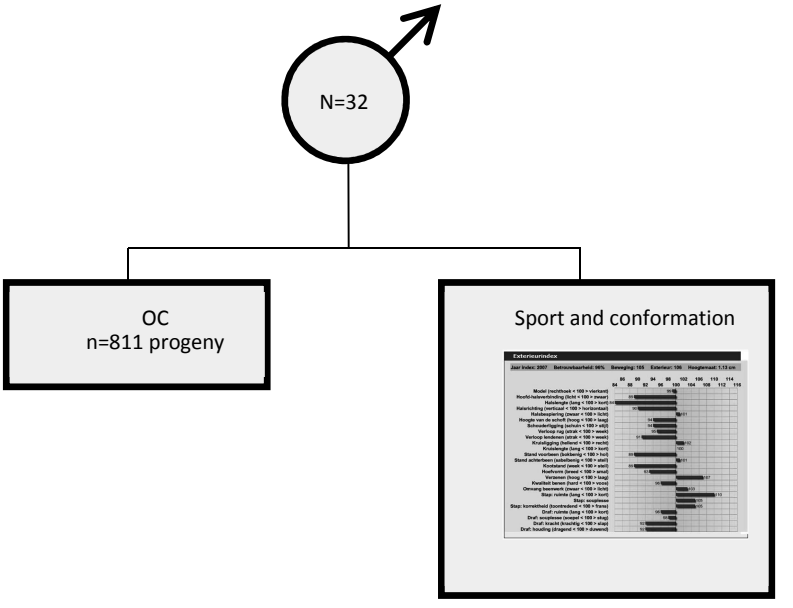

Figure 7.1 Data of two separate progeny groups used to estimate the correlations between OC and sport performance or conformation.

Use of data from this thesis to assess the practical relevance of OC

As indicated above, limited information is available in literature concerning the relevance of OC. In an attempt to generate additional information from the data presented in this thesis, I used available data on estimated breeding values (EBVs) of OC and EBVs of both sport and conformation traits from the same stallions to estimate the correlation between OC and sport traits. EBVs for both traits have been collected in completely separate progeny groups of these stallions (Figure 7.1). Consequently, there can be no environmental correlation between both traits which helps to estimate the correlation. Results of this study are correlations between OC, for both manifestations (fragments and flattened bone structures) and all joints separately, and sport performance or conformation.

Since correlations between EBVs do not fully reflect the true genetic correlation between two traits, formally the simple correlations should have been adjusted depending on their accuracy (Calo *et al.* 1973). This adjustment only affects the level of the correlation, not its significance.

Box 7.1 Available EBVs for performance and conformation

The descriptive traits are scored on a scale from 1 to 40, where 20 reflects the average of the population. The scale is defined such that a score below 20 is favourable, although extreme values are undesired. Subjective traits are scored on a scale from 40 to 100, of which the maximum reflects the ideal level for that trait (Ducro *et al.,* 2007). Using these scores, the KWPN calculates breeding values for movement and sports traits. For the estimation of the sports breeding values also sports performances and results from performance tests were included. The reliability of the estimated breeding values varies between 40% and 98%, and averages 78%. Results from sports competition are recorded by the national equestrian sports organization (KNHS) and the international sportorganization (FEI) as the highest classification ever achieved by a horse. For analysis, the classification scores are transformed to linear scores, using square-root transformation (Ducro *et al.,* 2007). Annually, the average of the total population is set at 100 points for every trait. The breeding values for the descriptive conformation traits and subjective conformation traits have a standard deviation of 4 points and the standard deviation for the sports traits is 20 points.

Osteochondrosis database

Radiographic data from 8 joints was used: the contralateral homologues of the FP, TC, metacarpalphalangeal (MCP) and metatarsalphalangeal (MTP) joints were used (Chapter 2). Osteochondrosis was scored on a categorical scale, which gives the opportunity to distinguish between flattened bone contours and fragments. Inclusion of the FP, TC, MCP and MTP joints gives the possibility to specify the possible relationship of OC at joint level, and conformation or sports performance traits.

Conformation and performance database

Estimated breeding values for conformation and performance traits were available for the 32 approved breeding stallions (Chapter 2). The EBVs were retrieved from the public database of KWPN. At studbook entry two different types of conformation traits are recorded on progeny of the stallions: descriptive and subjective traits. Table 7.2 shows the averages and standard

deviations of the conformation and performance breeding values for the 32 approved breeding stallions. More details on the traits can be found in Box 7.1.

Model description

To calculate the relationship of OC and sport performance, the EBVs of sport and conformation were correlated to the EBVs of OC at stallion level (n=32). For the estimation of breeding values for osteochondrosis, pedigree information on 5 generations was used, containing 7,799 horses. Genetic parameters were estimated univariately using ASReml software (Gilmour *et al.*, 2006). More details can be found in Box 7.2.

Pearson correlation coefficients were estimated between the EBVs for OC, the 38 breeding values for the conformation traits and the 2 breeding values for the sports traits, using PROC CORR of SAS 9.1® (SAS, 2004).

Results

There were no significant ($p<0.05$) correlations between EBVs for OC (either flattened bone contours or fragments) and dressage or jumping (Table 7.3). Significant correlations ($p<0.05$) were found between conformation traits and fragments in the FP joint, fragments in the TC joint and flattened bone contours in the MCP/MTP joints. All other correlations were either less significant ($p<0.10$) or not significant ($p>0.10$).

Fragments in the FP joint were found to be significantly ($p<0.05$) correlated with three locomotion or conformation traits (Table 7.3): movement ($r=-0.36$), head-neck connection ($r=0.36$) and length of stride at walk ($r=0.40$). More OC fragments was moderately related to worse movement. Traits such as a shortened stride length and weaker impulsion at trot had a tendency ($p<0.10$) towards a correlation with OC fragments in the FP joints (Table 7.3).

Fragments in the TC joint were significantly ($p<0.05$) correlated with two locomotion or conformation traits: movement ($r=-0.39$) and carriage at canter ($r=0.47$). The presence of more OC fragments moderately correlated to worse movement.

Fragments in the MCP/MTP joints were significantly ($p<0.05$) correlated with four locomotion or conformation traits: take-off jumping direction ($r=-0.43$), jumping technique of back ($r=-0.37$), scope ($r=-0.41$) and elasticity ($r=-0.41$).

Box 7.2 Calculation of EBVs for OC

The following linear animal model was used to calculate the EBVs for OC at stallion level (n=32):

$$Y_{ijklm} = \mu + sex_i + EVP_j + age_k + year_l + animal_m + e_{ijklm},$$

where Y_{ijklm} is the continuous OC value for ALL, FLAT, or FRAG of the animal or joint; μ is the mean; sex_i is the fixed class effect of sex (i = male or female); EVP_j is the fixed class effect of the equine veterinary practice responsible for taking radiographs (j = 1, 2, 3, …, 15); age_k is the fixed class effect of age in months (k = 9, 10, 11, …, 22); $year_l$ is the fixed class effect of the year of scoring (l = 2005 or 2006); $animal_m$ is the random additive genetic effect of the m^{th} animal (m = 1, 2, 3, …, 811); and e_{ijklm} is the residual (Chapter 3). All fixed effects in the genetic model had a significance level of $P \leq 0.10$. All radiographs were scored by one specialized veterinarian.

There was a tendency ($p<0.10$) for the presence of more flattened bone structures to moderately correlate with free jumping characteristics. Results cannot be compared to literature, as adequate results from other studies are limited as described above. Before drawing conclusions from these results, consequences of multiple testing will be considered.

Multiple testing

Many correlations have been calculated, which consequently leads to a certain amount of significant correlations by chance. With multiple hypothesis testing, problems occur when the sets of results are considered simultaneously, as in this analysis is the case. Calculating the False Discovery Rate (FDR) can quantify the expected proportion of false positives caused by this phenomenon. The FDR control is a statistical method used in multiple hypothesis testing to correct for multiple comparisons (Benjamini and Hochberg, 1995). In a list of rejected hypotheses, FDR controls the expected proportion of incorrectly rejected null hypotheses (type I errors).

Table 7.2 The descriptive and subjective traits of the studbook entry, and the averages and standard deviations (SD) of the conformation and performance estimated breeding values for the 32 approved breeding stallions (KWPN bewaarboek, 2006-2007).

	Descriptive traits	Score[1]	Score 40	Average EBV	SD EBV
	Trunk				
1.	Model	Rectangle	Square	95.0	7.5
	Front				
2.	Head-neck connection	Light	Heavy	96.2	5.8
3.	Length of neck	Long	Short	95.6	5.7
4.	Position of neck	Vertical	Horizontal	97.1	8.2
5.	Muscularity of neck	Heavy	Poor	97.3	4.3
6.	Height of withers	High	Low	98.1	4.9
7.	Position of shoulder	Sloping	Straight	98.1	6.0
	Body and rear				
8.	Line of back	Straight	Weak	98.5	7.8
9.	Line of loins	Straight	Weak	99.2	9.1
10.	Shape of croup	Sloping	Straight	98.8	5.2
11.	Length of croup	Long	Short	97.0	5.4
	Legs				
12.	Stance of forelegs	Over the knee	Back at the knee	99.4	7.0
13.	Stance of hind legs	Sickle-hocked	Straight	98.2	6.4
14.	Stance of pastern	Weak	Straight	101.6	6.9
15.	Shape of feet	Broad	Narrow	100.6	5.6
16.	Heels	High	Low	97.3	6.0
17.	Quality of legs	Lean	Blurred	96.6	5.5
18.	Substance of legs	Heavy	Light	100.1	4.4

	Movement				
19.	Walk: length of stride	Long	Short	96.5	7.0
20.	Walk: correctness	Toed in	Toed out	102.0	6.2
21.	Trot: length of stride	Long	Short	95.9	6.8
22.	Trot: elasticity	Elastic	Stiff	94.6	6.4
23.	Trot: impulsion	Powerful	Weak	95.4	6.5
24.	Trot: carriage	Carrying	On forehand	95.1	7.7
25.	Canter: length of stride	Long	Short	94.2	5.6
26.	Canter: impulsion	Powerful	Weak	94.6	7.2
27.	Canter: carriage	Carrying	On forehand	94.2	6.3
	Free-jumping				
28.	Take-off: direction	Upwards	Forwards	96.9	9.0
29.	Take-off: speed	Fast	Slow	97.3	8.3
30.	Technique: foreleg	Bend	Stretched	97.6	9.2
31.	Technique: back	Rounded	Hollow	97.0	8.8
32.	Technique: haunches	Open	Tight	97.3	10.9
33.	Scope	Much	Little	97.4	9.8
34.	Elasticity	Elastic	Stiff	96.2	10.1
35.	Care	Careful	Reckless	97.5	8.5
	Subjective traits	**Score 40**	**Score 100**	**Average**	**SD**
1.	Conformation	Bad	Good	106.5	4.3
2.	Movement	Bad	Good	105.8	5.4
3.	Free-jumping	Bad	Good	103.1	9.3
	Sports traits	**Score 0[1]**	**Score 200[1]**	**Average**	**SD**
1.	Jumping	Bad	Good	112.4	38.8
2.	Dressage	Bad	Good	119.9	28.8

[1] Original marks, before square-root transformation.

For this particular situation, in which 40 conformation traits were correlated with 6 OC traits at joint level (Table 7.3), there were in total 240 observations predicted to be different or not from the null hypotheses. Using a significance level of 0.05, 12 false positives would be expected, in case the null hypothesis is true. In fact, only 9 correlations showed to be significantly different from the null hypotheses, which thus might merely be false positive correlations. With a significance level of 0.10, 24 false positives would be expected. In fact, 24 correlations were significantly ($p<0.10$) different from the null hypotheses, all of which could therefore be false positives. In fact, overall there were less positive correlations than expected by chance. Therefore, the results were not adjusted depending on their accuracy, as these results could not be interpreted because of a multiple testing problem.

Future research

OC

Results from both literature and this study do not provide enough evidence to prove any, let alone a strong, relationship of OC with sport performance in horses. Ideally, the radiographic status of a horse should be available at young age, before sales or culling introduce preselection in the data, but after the moment that the osteochondrotic status of a joint has stabilized (Dik *et al.*, 1999; Van Weeren *et al.*, 1999). This is at approximately 12 months.

Sport

Competition data provide some basis for statistical analyses in sport horses, but suboptimal recording of data is common and represents a major limitation of riding horse data analysis (Stock and Distl, 2006a). Besides, waiting for sport performance records is time consuming, especially in dressage horses and show jumpers that perform at relatively old age, and therefore (too) costly in terms of genetic response and economic values. In the current situation, KWPN stallions can be exported abroad for breeding purposes, phenotypes of progeny will then not be available within the Netherlands, while the semen could be available for breeding. To be able to use phenotypic data generated in other countries, the EBVs for sport traits estimated in other countries can be deregressed in order to obtain a phenotypic record corrected for fixed effects. This phenotypic record can then be used in a mixed-model analysis in the Netherlands. Deregression is a common procedure in international genetic evaluation of dairy cattle bulls (Goddard, 1985). Differences in accuracy among stallions can be accounted for by weighting the records.

Table 7.3 Correlations between breeding values for osteochondrosis at joint level and breeding values for conformation- and sports traits[1].

Animal/Joint	Animal		FP		TC		MCP/MTP	
Manifestation of OC	**FLAT**	**FRAG**	**FLAT**	**FRAG**	**FLAT**	**FRAG**	**FLAT**	**FRAG**
Sports traits:								
Dressage	.09	-.23	.10	-.22	.27	-.23	-.19	.01
Jumping	.09	.14	-.00	.28	-.20	.10	.35*	-.08
Subjective traits:								
Conformation	-.10	-.32[2]	-.13	-.25	-.02	-. 27	-.04	-.08
Movement	-.18	-.37[3]	-.11	-.36[3]	.00	- 39[3]	-.25	.06
Free-Jumping	.10	.08	-.00	.25	-.16	.03	.33[2]	-.10
Descriptive traits:								
Trunk model	-.19	.12	-.07	.15	-.03	.13	-.22	-.11
Head-neck connection	.26	.35[2]	.16	.36[3]	.12	.17	.17	.17
Length of neck	.18	.20	.06	.14	.07	.06	.18	.23
Position of neck	.18	.36[2]	-.10	.29	.04	.28	.36[2]	.10
Muscularity of neck	.07	.19	-.22	.16	.17	.20	.16	-.06
Height of withers	.01	.18	-.03	.19	-.02	.10	.06	.08
Position of shoulder	.10	.17	-.02	.21	-.10	.00	.28	.16
Line of back	-.08	.06	-.02	.14	-.07	.05	-.05	-.13
Line of loins	-.13	-.01	-.05	.14	-.24	-.03	.04	-.11
Shape of croup	.26	.11	.30	.12	.02	.09	.14	-.01
Length of croup	.09	.09	.22	.26	-.06	.15	.02	-.26
Stance of forelegs	.04	.07	.04	-.08	.13	.05	-.11	.14
Stance of hindlegs	-.20	-.08	-.15	-.06	-.33[2]	.06	.11	.23
Stance of pastern	-.11	-.04	-.28	-.13	.16	.00	-.08	-.03
Shape of feet	.06	.11	-.05	-.22	.22	.09	-.07	.32[2]
Heels	.06	.01	.12	.22	-.05	-.09	.04	-.02
Quality of legs	.14	.23	.09	.16	.23	.33[2]	-.05	-.17
Substance of legs	-.11	-.24	-.10	-.23	-.29	-.30	.16	.16
Walk: length of stride	.20	.11	.19	.40[3]	.18	-.10	.02	.04
Walk: correctness	-.00	-.32[2]	.15	-.22	.13	-.23	-.27	-.12
Trot: length of stride	.11	.25	.05	.31[2]	-.15	.16	.29	.00
Trot: elasticity	.08	.27	.10	.29	-.11	.23	.15	-.03

Trot: impulsion	.06	.33[2]	-.05	.34[2]	-.11	.30	.26	-.09
Trot: carriage	.06	.34[2]	-.05	.26	-.11	.34[2]	.26	-.04
Canter: length of stride	-.04	.11	-.07	.07	.20	-.04	-.16	.26
Canter: impulsion	-.15	.18	-.15	.03	.28	.26	-.35[2]	-.06
Canter: carriage	-.07	.32[2]	-.11	-.01	.29	.47[3]	-.26	-.04
Take off: direction	-.13	-.13	-.03	-.35[2]	.26	.09	-.43[3]	-.10
Take off: speed	-.21	-.03	-.12	-.18	.15	-.01	-.36[2]	.10
Technique: foreleg	.02	-.05	.09	-.16	.14	.14	-.18	-.16
Technique: back	-.15	-.26	.02	-.31[2]	.10	-.11	-.37[3]	-.11
Technique: haunches	-.09	-.12	.07	-.22	.21	-.02	-.41[2]	-.02
Scope	-.06	-.12	.05	-.23	.27	-.08	-.41[3]	.09
Elasticity	-.11	-.04	-.03	-.20	.27	.12	-.41[3]	-.07
Care	-.09	-.10	.04	-.23	.24	.00	-.41[2]	.01

[1] ALL = overall value, including both flattened bone contours and fragments; FLAT = represents only flattened bone contours; FRAG = represents only fragments; FP = FemoroPatellar; TC = TarsoCrural; MCP/MTP = MetaCarpoPhalangeal/ MetaTarso-Phalangeal; [2] P-value < 0.10; [3] P-value < 0.05.

Preselection

Recording OC at an early age, but sport performance at the later age of training or even competition seems practical, but may introduce substantial preselection, as horses with (severe) OC influencing sport performance or even training possibilities will have been culled before that moment. One should realize that the economic losses, the need for surgery and the loss of training potential create preselection in the sports data, which may bias the estimated relationship between OC and sport traits. It can, therefore, be expected that the estimated genetic correlation between the level of sport performance and OC is biased. Ideally, phenotypes on sport performance longevity should be available for the offspring, as thus far there is more evidence for a correlation between osteochondrosis and sport performance longevity than for any correlation between OC and the level of sport performance (Couroucé-Malblanc *et al.*, 2006). This measure should ideally also correct for horses which do not reach the competition level, which may be a result of (severe) OC.

Breeding programs

For future research, a practical opportunity for data collection is measuring OC in randomly selected yearlings of all newly approved stallions, as implemented by KWPN since 2009 for progeny testing. The genetic correlation between OC and sport traits can be estimated directly using multitrait mixed model equations implemented with ReML. A reduction of the prevalence of OC is desirable and can best be achieved by selection, considering the heritable character of OC. In the following section, breeding schemes aiming to reduce the prevalence of OC will be compared. The comparison includes phenotypic selection based on own performance, progeny testing schemes, and genomic selection schemes.

In genomic selection, breeding values are estimated using genetic markers alone. Therefore, a reference population is needed in which both phenotypes and genotypes are known, to estimate the effects of genetic markers. Hence, from the reference population, a prediction equation can be generated that combines all the marker genotypes with their effects to predict the breeding value of an animal from its markers. Those EBVs can then be used to select the parents for the next generation.

For OC, the phenotypes collected in the progeny testing program of the KWPN can be used for building a reference population. The reference population will grow over time with annually on average 20 new stallions approved, of which 20 yearling progeny of each stallion will be judged on OC by the studbook. By genotyping these yearling, OC records of on average 400 yearlings will become available each year to build the reference population. In the following section of the general discussion, the potential of genomic selection applied to a horse population will be evaluated.

7.3 Genomic selection against OC

Breeding against osteochondrosis in horses

In 1994, the KWPN evaluated the effect of selection to reduce the prevalence of OC over the last 10 years (KWPN, 1994). The general conclusion was that stallions with OC had 19% of their offspring affected with OC, against 13% for stallions without OC. These results show that selection on OC status is effective. However, phenotypic selection of stallions becomes ineffective in reducing the prevalence once all stallions are free from OC. At that moment, phenotypic selection needs to be replaced by selection on estimated breeding values based

on information from relatives if the prevalence of OC is to be reduced any further. Therefore, a study based on phenotypic selection using progeny testing was set up to increase the accuracy, as described in Chapter 2, with the aim to improve the response to selection in the near future.

In Chapter 6, conclusions are drawn for selection based on a trait with a low number of phenotypes, *i.e.* a small reference population. Osteochondrosis is a typical example of such a trait in which phenotyping of individuals is technically challenging (in a random sample), expensive and time-consuming. Data can be collected from progeny, but the entire process of breeding, selecting, raising, preparing and presenting young colts takes a long time and is expensive.

Alternatives

Quantitative genetic approaches, such as selection using estimated breeding values for stallions based on progeny information, can also be effective, considering the heritabilities found (Chapter 3) and the heritable factors that contribute to OC (Van Weeren 2006).

The first results using high-throughput molecular genetic screening of DNA indicate that the genetic background of OC probably is extremely complicated, with different locations regulated by different genes (Wittwer *et al.* 2007). Consequently, selection based on known genes is not possible yet. However, this is not necessary, as molecular information can also be used in genomic selection which does not require the identification of genes. Genomic selection has recently been introduced in dairy cattle and poultry. In this part of the discussion I will explore whether genomic selection can possible be used to reduce the prevalence of OC effectively in an equine population.

The population sample generated for progeny testing can be used for building a reference population, which enables genomic selection (Figure 7.2). As shown in Chapter 6, simulations can be performed to predict genetic gain of genomic selection and to compare this with results of traditional phenotypic selection on own performance or performance of progeny.

Aim

Three selection strategies, phenotypic selection on own performance (the standard until recently), progeny testing (recently introduced), and genomic selection will be compared with regard to the effectiveness in optimizing the long-term response to selection against OC.

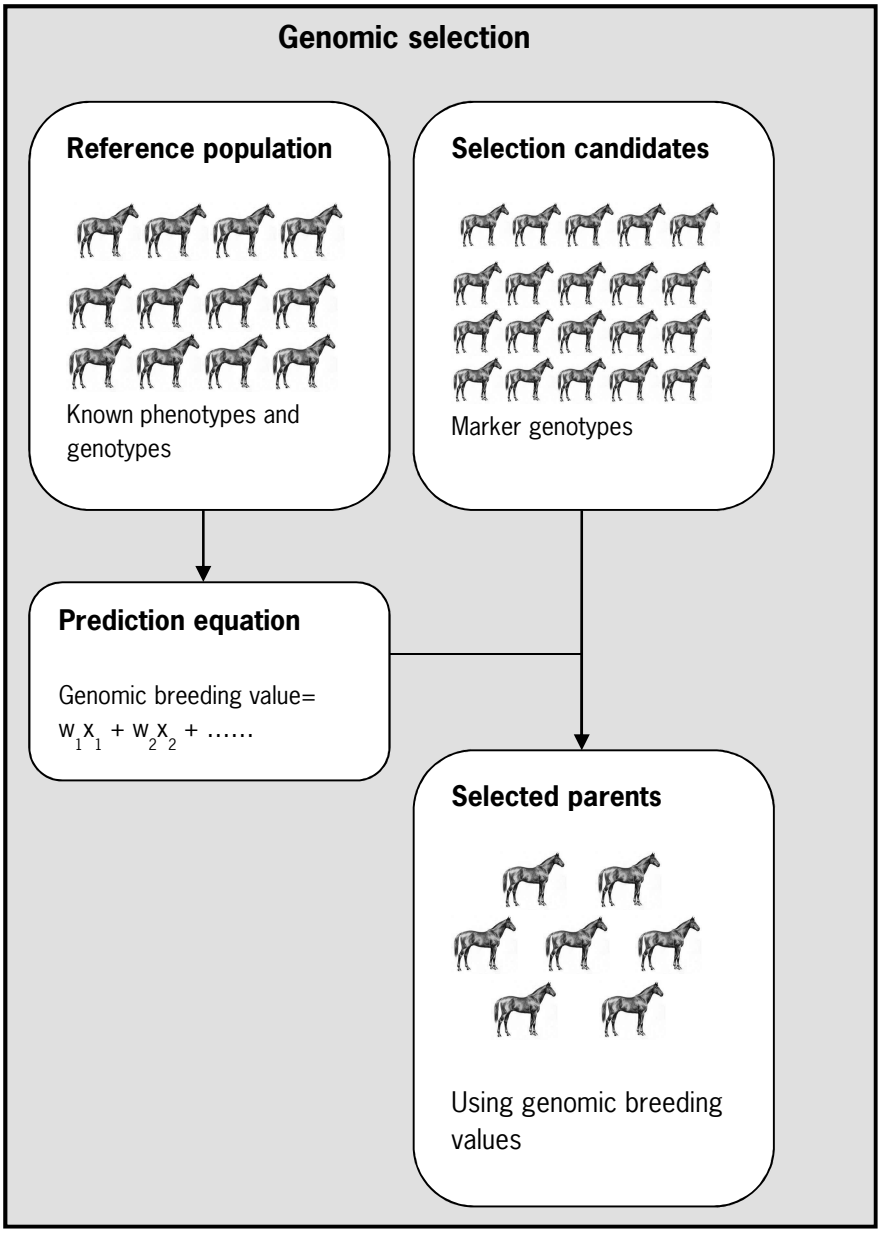

Figure 7.2 Schematic overview of data collection for genomic selection in a reference population. A prediction equation can be generated that combines all marker genotypes with their effects (w) to predict the breeding value of each animal. This value can then be used to select the best parents (after Goddard, 2009).

Scenarios

For the comparison of GS with phenotypic selection based on own performance and with progeny testing in the KWPN situation, deterministic simulations were performed using SelAction (Rutten *et al.*, 2002). Selection schemes were evaluated to illustrate the response of phenotypic selection and genomic selection. In these scenarios, selection was on one trait only. The number of selection candidates and the total number of animals selected were fixed.

Three different basic scenarios were simulated:

1. Phenotypic selection using pedigree and own performance (OP).
2. Phenotypic selection using own performance, pedigree and progeny information (PT) in a two stage selection.
3. Genomic selection using pedigree and marker information (GS).

Breeding programs

To model the breeding programs, the following assumptions were made in the basic scenarios:

- The population had discrete generations and a fixed number of stallions and mares;
- There was an active population of 12,000 dams per generation;
- 300 sires were used per generation;
- Each dam produced 1 male and 1 female offspring per generation;
- From the offspring, 20 progeny per sire were available in the progeny test.
- The effective historical population size used was 100.
- The heritability of OC was 0.23 (Chapter 2)
- In scenario 1, which involves one-stage selection using OP, there is a selection proportion of 0.45 or 0.3 in sires and 0.99 or 0.75 in dams (Table 7.4).
- In scenario 2, which involves two-stage selection using PT, there is a selection proportion of 0.3 based on own performance in the first stage and 0.75 or 0.5 in the second stage in sires and 0.99 in dams based on progeny testing (Table 7.4).
- In scenario 3, which involves one-stage selection using GS, there is a selection proportion of 0.3, 0.1 or 0.025 in sires (Table 7.4).
- GS will be applied for selection without own performance information.

Table 7.4 Simulation input of scenarios tested.

	P_{male}		P_{female}					
Scenario[1]	1st stage	2nd stage	1st stage	2nd stage	OP	PT	GS	R^2
OP_m45	0.45		1		x			0.098
OP_m30	0.30		1		x			0.128
OP_f75	0.30		0.75		x			0.173
PT75	0.30	0.75	1	1	x	x		0.178
PT50	0.30	0.50	1	1	x	x		0.228
GS	0.025		1				x	0.255
GS	0.10		1				x	0.185
GS	0.30		1				x	0.124

[1] Genomic selection based on a reference population of 10,000 individuals with own performance information. OP=own performance with the selected proportion in males (m) or in females (f); PT=progeny testing with the selected proportion; [2] R= selection response (in phenotypic SD), P_{male}= selected proportion in males, P_{female}=selected proportion in females.

In the simulations, no other than the phenotypic trait OC was simulated, which consequently leads to a selected proportion equal to the prevalence of horses without OC. Considering both the hock and stifle joints, the prevalence of horses without OC is 0.45 in the Dutch KWPN population. If the hock, stifle and fetlock joints are included, the prevalence of horses without OC reduces to 0.3. Using progeny testing, we assumed that the selected proportion would be 0.75, as we expect mare owners not to use the worst 25% of stallions. As an alternative, progeny testing with a selected proportion of 0.50 is compared, but it is not likely that breeders will disregard half of the stallion population because of the EBV for OC. In the case of genomic selection, the selected proportion is not determined by the prevalence and can therefore be stricter. A selected proportion of 0.3 is used for a direct comparison with phenotypic selection on own performance. Using GS, 0.1 is used as a stricter selected proportion. Although in theory much higher proportions can be realized. To illustrate selection solely on OC in which 20 stallions can be genomically selected from the 800 stallions phenotyped for OC in the selection based on own performance (Figure 1.4), a selected proportion of 0.025 could theoretically be reached. Although, stallions will be selected based on several traits (e.g. sport) else than OC, but this illustrates the possibilities of stricter genomic selection. The environmental correlation has little or no effect and is kept constant (r_e=0) over the scenarios. A break-even point is calculated at which GS is able to yield equal

response to selection compared to phenotypic selection on own performance or progeny testing. Results will be discussed in break-even point expressed in terms of response and consequently the necessary size of the reference population (Chapter 6; Criteria for comparing breeding schemes).

Comparing scenarios

Own performance

Decreasing the selected proportion from 0.45 to 0.3 by taking more joints into account will lead to a 31% increase (Table 7.5) in the response to selection on the liability scale (Chapter 2). When a mean prevalence of 30% at animal level (Chapter 2) is considered, this corresponds to an increase in prevalence of horses without OC from 33.5 to 34.6, When the expected selection intensity in females selected on own performance changes from 0.99 to 0.75, the response increases by 35% (Table 7.5).

Progeny testing

Using progeny testing will result in a higher predicted response than when using own performance. The response of two-stage selection using progeny testing (PT75) is 80% higher than the response of one-stage selection using own performance (OP_m45) (Table 7.5). If mare owners are willing to use only the 50% best stallions with respect to OC, the response to selection using progeny testing is 78% better than the response to selection using own performance. However, progeny testing is expensive, time-consuming (earliest age of selection in stallions is 5 years of age instead of 3 when selecting on own performance) and requires collection of information from yearlings. Progeny testing can soon be applied and this analysis shows that it can be effective to reduce the prevalence of OC. It is important to keep in mind that we used single trait selection.

Genomic selection

Using genomic selection with a reference population of *i.e.* 10,000 horses, the predicted response to selection with a selected proportion of 0.3 is comparable with the response gained using phenotypic selection on own performance with a selected proportion of 0.3 (Table 7.5). Decreasing the selected proportion of genomic selection to 0.1, the response increases with 49%, and decreasing the selected proportion of genomic selection to 0.025, the response doubles compared to genomic selection with a selected proportion of 0.3.

Table 7.5 Reference population size (ref pop size) needed to reach equal response to selection using genomic selection with a selected proportion of 0.1 compared to the other scenarios, assuming no reduction in generation interval.

GS selected proportion	0.025		0.1	
GS compared to scenario[1]	$r_{g\hat{g}}^2$	ref pop size	$r_{g\hat{g}}^2$	ref pop size
OP_m45	0.21	1,200	0.28	2,225
OP_m30	0.27	2,140	0.36	4,010
OP_f75	0.37	4,150	0.49	8,250
PT75	0.38	4,400	0.51	9,175
PT50	0.49	8,250	0.65	19,100

[1] OP=own performance with the selected proportion in males (m) or in females (f); PT=progeny testing with the selected proportion. [2] $r_{g\hat{g}}$ is the accuracy of genomic selection (Daetwyler *et al.*, 2008).

The size of the reference population should preferably be as small as possible. Genomic selection with a selected proportion of 0.1 is the selection scenario which is considered to be a realistic alternative to progeny testing (PT75; Table 7.4). Besides, genotyping will be used in the future for selection on many traits in the breeding goal. Therefore, the actual cost for building a reference population against OC will consist of phenotyping costs. One of the advantages is that selected proportions can be strict when using genomic selection, irrespective of the disease prevalence.

Reference population size

It is useful to estimate the minimal sizes of the reference populations that are needed when using genomic selection to reach an equal response to the other scenarios (Table 7.5). Relatively large reference population sizes are required when a selected proportion of 0.3 is used. In contrast, to reach equal response to the selection on own performance based on OC in two joints (OP_m45) when applying genomic selection with a selected proportion of 0.1, a reference population of not more than 2,225 horses will be necessary (Table 7.5). To reach a response equal to progeny testing on all considered joints (PT75), a reference population of 9,175 horses will be needed. As a comparison, genomic selection with a selected proportion of 0.025, will need a reference population size of 4,400 individuals to reach equal response to selection on progeny testing with a selected proportion of 75% (Table 7.5). GS can also be applied to the mare population, which will increase the response and possibly lower the generation interval.

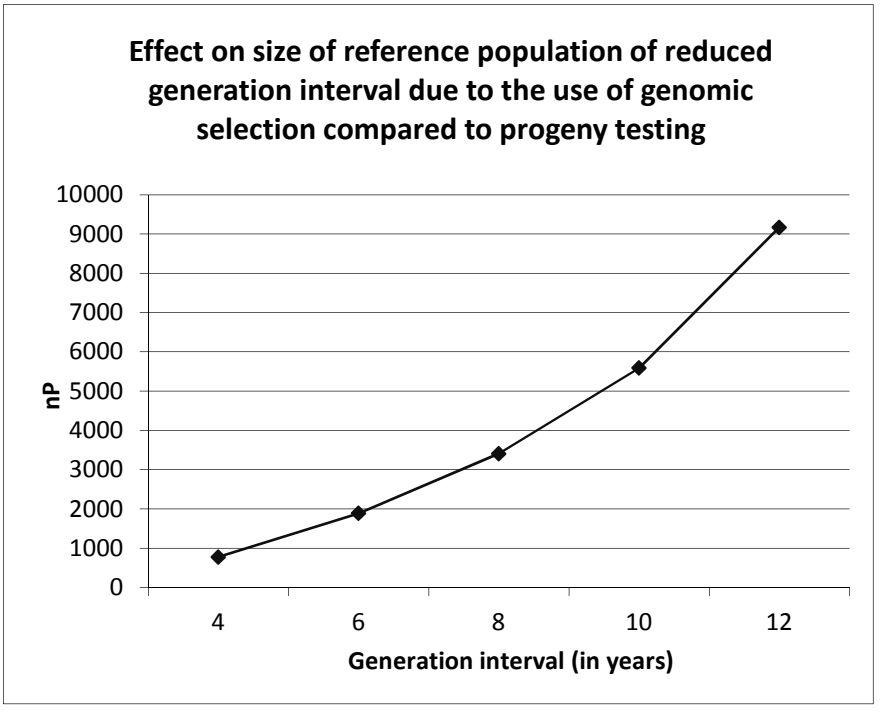

Figure 7.3 The size of the reference population needed after applying genomic selection with a selected proportion of 0.1 to reach the same response as with progeny testing with a selected proportion of 0.75 (in the 2nd stage), in case of generation interval increase.

Generation interval

When using genomic selection the generation interval can be reduced by (at least) 2 years, because genotypes can be collected at very young age and no time is needed for phenotyping of progeny. Using genomic selection in young (potential) breeding stallions, the generation interval can be reduced from 12 to 10 years. As a consequence, the size of the reference population needed can be lowered by 40% (from 9,175 to 5,590) to reach the same response with genomic selection using a selected proportion of 0.1 compared to the use of progeny testing with a selected proportion of 0.75 in the second stage (PT75) (Figure 7.3).

Conclusion

It can be concluded that genomic selection is a realistic option in the KWPN horse population for selection against OC. Even though dealing with a difficult trait such as OC, the size of the reference population that is needed to reach equal response to selection using genomic selection compared to phenotypic selection on own performance is not more than 2,225, which is certainly feasible. When genomic selection is compared with phenotypic selection on progeny testing, as will be applied in the near future, this figure is larger (n_p=9,175), but when taking into account the reduction of the generation interval with two years, the number falls to 5,590, which must be considered feasible as well. When systematic phenotyping progeny from newly approved stallions is used to build a reference population, annually approximately 400 individuals can be genotyped and phenotyped and the reference population can be built in 14 year. When all available phenotypic information is used and also data from other horses such as phenotyped mares, the time required can be reduced. Therefore, it can be concluded that progeny testing will lead to the highest accuracy, until the required reference population (5,590) has been built for genomic selection, when henceforth genomic selection will lead to the highest gain.

Discussion

Measuring and scoring of OC in horses, as planned in the KWPN stallion selection procedure, involves the use of progeny groups. Considering GS in this situation, some of the estimated accuracy of the genomic breeding values may in this case be the result of SNPs capturing the effect of relationship (Habier *et al.*, 2007), whereas the deterministic predictions assume that the accuracy of the breeding values is a result of the SNPs capturing the effect of QTL (Hayes *et al.*, 2009b). Therefore, it is possible that the accuracy of direct measurements from the horse population is overestimated, leading to possible differences between observed accuracies and predictions.

The Bulmer effect (Bulmer, 1971) as described in Chapter 6 has a larger influence on genomic selection than on phenotypic selection. In the results, the equilibrium response and accuracies are presented. Therefore this issue has been taken into account. It should be realized that, when dealing with selection results not corrected for the Bulmer effect, the equilibrium response can still be lower with genomic selection, even when this selection method has a larger predicted accuracy compared to for example progeny testing.

A great disadvantage of progeny testing is that some of the progeny produced before selection of sires on progeny test results will show undesirable EBVs for OC and thereby maintain a certain amount of OC in the population. Using genomic selection, the selection moment can be applied earlier and stallions can be impeded from producing progeny when their genomic EBVs for OC are too low.
In genomic selection, the reference population built from phenotyped progeny will consist of both females and males. Therefore, selection only starts after building the reference population, as the reference population cannot be used to select individuals as parents for the next generation. These progeny are then considered as horses without preselection. As genomic selection can only be applied after the reference population has been established, progeny testing could be used to maximize response to selection in the meantime.

Future breeding
Most studies on the possibilities for genomic selection have been performed on Holstein bulls or using simulation data based on cattle populations (Meuwissen *et al.*, 2001; Muir, 2007; Calus *et al.*, 2008; VanRaden, 2009; De Roos *et al.*, 2009). It is known that the accuracy increases with the size of the reference population (Goddard, 2008). Genomic selection has been introduced in several countries, which increased their reference population by genotyping more bulls (Wiggans *et al.*, 2010) or international sharing of genotypes. Reference populations up to 16,000 Holstein bulls are used and reach accuracies of over 0.8, which is higher than accuracies reached in other (cattle) breeds because of the use of smaller reference populations in those breeds (Wiggans *et al.*, 2010).
In horses, especially in case of OC, these high accuracies does not have to be reached as selection against OC using phenotypic selection cannot reach high accuracies either (Table 7.5). Therefore, genomic selection will already be advantageous over phenotypic selection with relatively low accuracies, compared to cattle, and consequently smaller total reference populations are needed.

Selection in mares yields additional response, but this type of selection cannot be imposed in horse breeding, as mare owners decide independent from the studbook whether they deem the mare suitable for breeding purposes or not. Therefore, gain in mares can only be accomplished on a voluntary basis by individual breeders, who are willing to phenotype (or genotype) their breeding

mares. Nowadays, mare owners are encouraged by the studbook to phenotype mares.

Building a reference population for selection against OC

The accuracy of genomic breeding values for individuals may differ from the average accuracy for a population and depends on its genetic relationship to the reference population (Habier *et al.*, 2007; Hayes *et al.*, 2009b). Evidently, individuals that have many close family members in the reference population have higher accuracies than individuals without family in the reference population. This is because family members share large chromosome segments that are not broken down by recombination (De Roos, 2011).

The relationship of an individual with the reference population is not taken into account in Daetwyler's formula (2008) for the accuracy, but it can be used within families to estimate the accuracy (De Roos, 2011). Therefore, using large offspring groups of stallions in the reference population of OC would increase the accuracy (De Roos, 2011).

The effect of the degree of relatedness to the reference population should be taken into account when designing a reference population, both in terms of overestimation and higher accuracy. In the future, stallions will be genotyped for several traits in the breeding goal and osteochondrosis can be added to these genotypes. OC is not the main goal for obtaining genotypes, which can be used for several traits if the relevant phenotypes are available. Therefore, the costs for building a reference population for selection against OC will basically consist of the costs for phenotyping. Ideally, the reference population is built from progeny of the newly approved stallions. If approximately 20 offspring per stallion are phenotyped, as performed nowadays for selection based on progeny testing, the reference population will express an average of the OC status of the total population. This will not be the case when (potential) breeding stallions will be used for building a reference population, as they are a preselected sample from the stallion population. When using progeny groups of 20 individuals for building a reference population, each individual will have 19 half sibs in this reference population. This reference population can be built over a few years until the desired accuracy has been reached, while in the meantime OC can be reduced using progeny testing. The total reference population will then exist of progeny groups of approved stallions who will, consequently, also sire the next generation of the potential newly approved sires on which genomic selection can be applied. The reference population will

most likely contain family (sibs) of the genotyped individuals, which will increase the accuracy of the genomic selection of stallions.

7.3 Osteochondrosis in pig breeding

This section of the general discussion has three objectives: assessment of the relevance of OC in pigs, answering the question how the prevalence of OC in pigs can be reduced by improvement of the environment or genetics, and, finally, opportunities to breed for reduced prevalence of OC in pigs.

Relevance of OC in pigs

Genetic selection has increased production levels of many species of livestock considerably. However, apart from this positive effect, single trait selection for high production efficiency might lead to an increased risk for behavioral, physiological and immunological problems (Rauw *et al.*, 1998). There are over 100 references describing unfavorable relationships of metabolic, reproduction and health traits with production efficiency in broilers, pigs and dairy cattle.

Initially, genetic improvement in the pig has focused on production (growth, carcass, and meat quality) and reproduction characteristics (litter size and piglet survival). In the past decade, due to the increased emphasis on animal welfare, the emphasis on functional traits, such as longevity, piglet survival and quality of feet and legs, has increased (Jørgensen and Andersen, 2000). The major economic impact of functional traits of sows originates from effects of productive life. A longer productive life decreases replacement costs in pig production substantially. A number of longevity studies have recently been performed in pigs (e.g., Yazdi *et al.*, 2000; Serenius and Stalder, 2004; Tarrés *et al.*, 2005; Engblom *et al.*, 2009).

The improvement in production traits and the increase in the number of piglets born per sow have led to an increase in the incidence of leg weakness in the past 20 years (Busch and Wachmann, 2010; Rauw *et al.*, 1998). OC is seen as an important risk factor for leg weakness. Leg weakness is a serious problem and a common reason for culling in sows (Dial and Koketsu, 1996; Friendship *et al.*, 1996; Engblom *et al.*, 2008; Yazdi *et al.*, 2000). Culling rates between weaning and slaughter of growing pigs are in general very low (less than 2% in The Netherlands) and culling due to OC is not very common. Nevertheless, OC leads to reduced production and welfare in slaughter pigs (Yazdi *et al.*, 2000). However, OC is considered to be a more of a problem in sows than in fattening

pigs. Sows have a longer productive life, and OC might reduce longevity. Consequently, OC has a larger economic and welfare impact in sows than in fattening pigs (Jørgensen and Sørensen, 1998). Additionally, selection for reduced of OC in sows will also indirectly contribute to a reduction of the prevalence of OC in fattening pigs. A good understanding of the biological processes underlying OC and leg problems in general is needed for the implementation of effective breeding and management strategies to improve longevity of breeding sows and welfare of pigs. As OC is determined by genetic as well as environmental factors such as housing, feeding level and growth rate, improvement of these factors is expected to result in a reduction of the prevalence of OC, and hence in an increase of the longevity of sows.

Environmental factors

Housing conditions are considered to have an effect on leg weakness in general (Nakano *et al.*, 1987). Pigs housed on straw bedding showed less leg weakness problems compared to pigs housed on conventional types of flooring (Jørgensen, 2003; Scott *et al.*, 2007; Scott *et al.*, 2006). There are only a few studies reporting relations between OC and housing conditions (Jørgensen, 2003), but this field is insufficiently explored thus far and needs further attention. The type of floor, number of pen-mates, and surface per animal are all factors that affect the development of OC (Chapter 4).

A higher growth rate, either caused by genetic selection or feeding level, is considered to affect the development of OC (Chapter 5; Busch, 2006; Carlston *et al.*, 1988; Kadarmideen *et al.*, 2004; Nakano *et al.*, 1987; Ytrehus *et al.*, 2007). A negative genetic correlation between OC and growth rate suggests a negative side-effect of the many years of selection for high growth rates (Busch, 2006; Jørgensen and Andersen, 2000). Some studies reported that *ad libitum* fed pigs are more affected with OC than restricted fed pigs (Goedegebuure *et al.*,1980), but other studies failed to support this claim (Grøndalen, 1974; Jørgensen, 1995). However, the latter studies did not use strongly contrasting feeding regimes, which might explain the lack of effects on OC. There is a need to establish unequivocally the effect of feeding level and growth rate on OC status of specific joints, rather than looking only at generalized OC within the entire pig (Chapter 5).

Housing and feeding

In Chapter 4, the relationship between prevalence of OC and feeding and housing conditions was investigated.

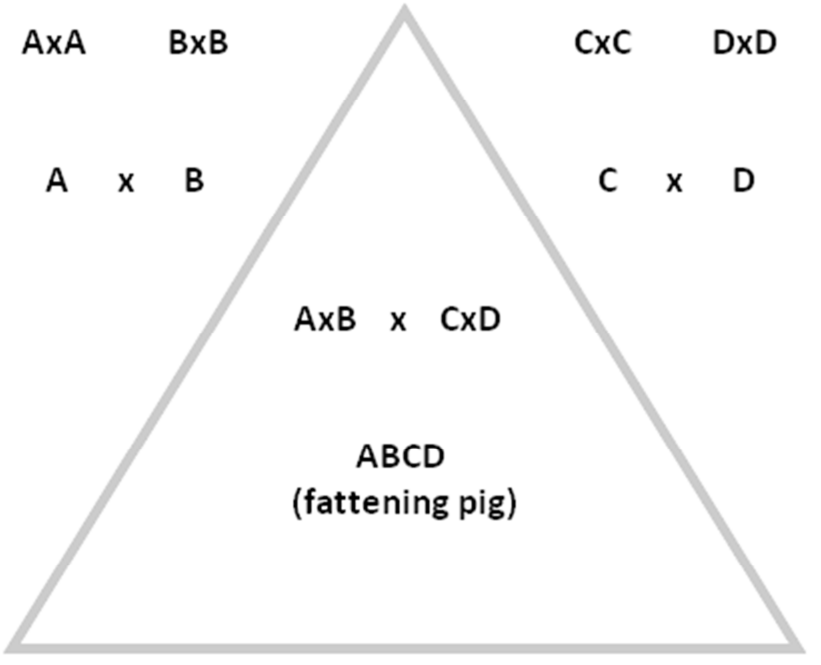

Figure 7.4 General breeding scheme of pig breeding, using pure boar (A and B) and sow (C and D) lines to produce crossbred fattening pigs, the 'pyramid' of pig breeding.

The results showed that OC had the highest prevalence (57.5%) in pigs that received a treatment of conventional housing combined with *ad libitum* feeding, both for mild and severe OC (Chapter 4). OC was least prevalent (33.7%) in pigs that received the treatment deep litter housing with restricted feeding (Chapter 4). In general, *ad libitum* feeding showed the highest prevalence of OC, and restricted feeding showed least severe OC (Chapter 4). These results suggest that, by changing only the housing system into deep litter, OC prevalence in *ad libitum* fed pigs can be reduced by 16.8% compared to conventional housing. By improving both the housing system and feeding strategy, OC can be reduced by 23.8% compared to conventional housing and *ad libitum* feeding. However, this latter management system entails a decrease in growth rate or an increase in slaughter age (Chapter 4), which is undesirable from a farmers' point of view. The reduction of OC due to improvement of the housing system only, which does not have any consequences for bodyweight at slaughter, may therefore be the most realistic alternative.

Pig breeding

Additional to changes in the environment, OC can also be reduced by genetic selection. Pig breeding involves selection in several pure lines, which are subsequently crossed to produce crossbred sows and fattening pigs (Figure 7.4). Purebred sow lines (C and D) are selected mainly for large litters with high survival rates, whereas the purebred boar lines (A and B) are selected for fast growth, high weights at slaughter and high carcass quality. Formation of AxCD or BxCD crosses result in fattening pig populations.

Selection against OC might improve sow lines and will thereby also result in a reduction of the OC prevalence in the fattening pigs. This genetic improvement will result in improved welfare and longevity in the whole pyramid of pig breeding.

As the effort to reduce OC is focused on improving purebred sow lines and not the fattening pig population, selection will have to be performed directly in the sow lines. If OC would be a problem in the fattening pig population, selection in boar lines would be faster and more adequate, as selection intensity in these lines is generally higher.

Genetic selection versus environmental improvements

The genetic improvement resulting from a breeding scheme can be increased by using genomic selection, as explained in Chapter 6. In the following section, the necessary characteristics of genomic selection to reach a reduction in OC of either 16.8% or 23.8%, which are the gains feasible by improving the environment (see above), is estimated. It should be realized that the prevalence of OC can be reduced even further by combining improvement of both the environment and the genetics.

For the comparison of genomic selection with the gain reached by changing housing system and feeding level, deterministic simulations were performed using SelAction (Rutten *et al.*, 2002). In the genomic selection scenarios, selection was on OC only. The number of selection candidates and the total number of pigs selected were fixed. To model the breeding programs, assumptions made in the basic scenarios are presented in Box 1.

When using genomic selection, the selected proportion is not determined by the prevalence and can therefore be smaller compared to normal selected proportions used for breeding goal traits. The results (Table 7.6) are discussed

Box 7.3 Assumptions in the deterministic simulations of the breeding programs

The assumptions made were:

- The population had discrete generations and a fixed number of boars and sows
- There was an active population of 5,000 sows per generation (Bergsma *et al.*, 2008)
  40 boars were used per generation (Bergsma *et al.*, 2008)
- Each sow produced 6 male and 6 female piglets per generation; the generation interval was set to 15 months (Bergsma *et al.*, 2008)
- The historical effective population size was 100
- The heritability of OC was set to 0.21 (Averaged from Yazdi *et al.*, 2000; Stern *et al.*, 1995; Jørgensen and Nielsen, 2005; Luther *et al.*, 2007; Storskrubb *et al.*, 2010 and Kadarmideen *et al.*, 2004)
- There was one-stage selection, with a selection proportion of 0.041 in boars and 0.395 in sows (Bergsma *et al.*, 2008)
- Genomic selection was applied for selection without the availability of own performance information of OC
- Only OC was considered, therefore, the economic weight was set to 1
- The phenotypic variance was set to 1.

in terms of time (in generations and in years) needed to reach a response equal to the gain reached by improvement of the environmental factors. The response needed is calculated using prevalences expressed on the liability scale.

Simulation results

When using small reference populations of 1,000 or 2,000, as unavoidable when selecting on a trait such OC which is expensive and time-consuming to phenotype, an response of 16.8 or 23.8% can be reached within an time span of four to eight generations (Table 7.6). If the reference populations go up to 10,000, this can even be reached within 2.1 to 2.9 generations. The response gained by breeding is cumulative over generations, whereas progress due to environmental improvements can only be realized once.

Table 7.6 Time expressed in generations and years to reach equal a response in prevalence of OC of 16.8% and 23.8%, with different reference population sizes.

		Time	
Desired gain (in %)	Reference population size	Generations	Years
16.8	1,000	5.7	7.1
	2,000	4.1	5.1
	5,000	2.7	3.4
	10,000	2.1	2.6
23.8	1,000	8.1	10.1
	2,000	5.9	7.3
	5,000	3.9	4.8
	10,000	2.9	3.7

In a practical sense, it is important to realize that selection against OC will have to be performed at the level of pure sow lines, whereas the environmental improvements need to be implemented at the lower part of the pig pyramid, which involves a larger number of farms. This needs to be taken into account in a cost-benefit analysis of alternatives.

One should realize that these figures correspond to selection on OC only. Selection on OC only is not a realistic option for pig breeding, as major breeding goal traits such as production and reproduction also need to be not considered in selection. Nevertheless, Table 7.6 illustrates that there are realistic opportunities to select for reduced prevalence of OC.

Genomic selection will most likely be implemented in pig breeding for other traits in the near future. Therefore, genotypes of individuals will already be collected for other traits in the breeding goal. To use genomic selection to reduce the prevalence of OC, reliable phenotypes on the individuals are necessary in the reference population. It seems justified to seriously consider whether the investment of collecting those phenotypes in the reference population is worthwhile. In the longer term, selection potentially leads to a larger reduction in the prevalence of OC than by improvement of the environment. Opportunities need to be explored to add OC to the traits already recorded on individuals in the reference population.

Future research

For many years, pig improvement schemes have aimed exclusively at improving growth rate, meat quality and litter size. More recently, breeding goals have been broadened by including reproduction of sows, survival of piglets and longevity of sows. Measuring OC would offer new opportunities for improving longevity of sows and leg quality of growing pigs. To realize this, reliable phenotypic information and information on the genetic variation in OC status and the relationship between OC and longevity and other traits of pigs are needed. A number of studies have found genetic variation in OC within breeds (Goedegebuure *et al.* 1980, Uhlhorn *et al.* 1995) and a number of countries have had OC in their breeding goals for decades already (Ytrehus *et al.*, 2007; Yazdi *et al.*, 2000; Grondalen *et al.*, 1974a). However, there is currently no information on the genetic relations of joint-specific OC with longevity, and on the consequence of selection against OC for growth rate. This information is essential if a breeding program that aims to exploit information on OC to improve longevity of sows is to be designed.

Diagnosis of OC

For the evaluation of both environmental influences and genetic factors, a reliable phenotypic diagnosis is a must. The golden standard for the diagnosis of OC in pigs is histology. In the pig, detailed histological analyses have permitted the classification of OC in various meaningful grades (Ytrehus *et al.*, 2004). Histology is commonly performed on dead animals, which cannot be candidates for selection. Nevertheless, these animals can still provide phenotypes and genotypes for the construction of a reference population to be used for genomic selection. One of the advantages of genomic selection is that phenotyped animals do not necessary have to be available as selection candidates themselves.

Relationship between OC and longevity

Data will be needed from breeding sows to estimate the genetic relationship between OC and longevity. These databases can be collected on a small number of farms with breeding and production sows that feature an electronic management system for the registration of information on birth date, breed, reproductive performance and longevity of all sows on the farm. Longevity can be defined as the number of days between first farrowing and culling of the sow. Osteochondrotic lesions can then be scored macroscopically on all sows that are culled from these farms, which can be carried out at the

slaughterhouse. Further, OC data can be recorded on sows that died or needed to be euthanized at the farm because of serious health problems. In this set-up, data can be collected on >1,000 sows within a year. Additional farm characteristics need be collected on feeding, housing and possible other risk factors to determine genetic and environmental correlations of traits that can be used as input for subsequent simulations to analyze breeding possibilities.

Consequences of breeding programs

It is possible that there are unfavorable genetic correlations between OC, locomotion, growth-related parameters and longevity. Knowledge of these genetic relationships is essential for the development of breeding programs. The genetic parameters can be used in deterministic simulation (e.g. in the SelAction program), to predict the genetic response from alternative breeding schemes. Alternative breeding schemes may differ in the emphasis placed on different traits in the breeding goal, and in the information collected on traits.

Genomic selection

As a first step for the identification of genes that play a role in the development of OC, a whole genome association (WGA) study can be applied to the 1,000 sows. Pedigree information on sows is usually not known, but to avoid a pedigree problem all culled sows can be genotyped for a small number of SNP markers, a technique that has been developed recently for pedigree reconstruction. The SNP information can then be used to estimate genetic relatedness between sows. As a next step, the genetic relatedness can be used for the estimation of the heritability of OC. This procedure has recently applied in a population of fish without pedigree (Blonk *et al.*, 2009). High-low sampling and genome-wide association studies can be used to detect genes that contribute to genetic variation, making two groups of sows, sows without signs of OC (low) and sows with serious OC (high), that are to be genotyped and used in the genome wide association study.

When strong candidate genes for OC are found, they can be sequenced in the two groups of animals to identify potential candidate SNPs affecting OC, or to identify additional SNPs that show a stronger association with OC. These SNPs can then be combined in a genotyping assay to evaluate their usefulness to predict the occurrence of OC in different pig populations or breeds, and to reduce the incidence of OC by genomic selection. Christensen *et al.* (2010) identified QTLs associated with six osteochondrosis traits in pigs, which

explained a large part of the genetic variation. The study comprised a large number (n=7,172) of animals, in contrast with previous studies (Lee *et al.*, 2003; Andersson-Eklund *et al.*, 2000). Information on genes contributing to OC will help to increase our understanding in the underlying mechanisms and might help to predict correlated responses to selection. Furthermore, knowledge on identified QTL can help to improve the efficiency of genomic selection.

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Summary
Samenvatting

Summary

Osteochondrosis (OC) is a disturbance in the process of endochondral ossification during skeletal growth. The most common clinical sign of OC is a non-painful joint distension; less common clinical signs are varying degrees of lameness. The disease is common in a number of livestock species such as cattle, pigs, horses, dogs, chicken and even in humans. In this thesis, the focus is on horses and pigs. OC occurs in many horse populations. In pigs, OC is the main cause of leg weakness, which is the second reason for culling sows. OC is, therefore, important for both economical and welfare reasons. OC is multifactorial in origin, and both environmental and genetic influences play a role. The presence of genetic effects imply a potential for breeding against OC. In general, breeding aims at improvement of livestock by selection of the best individuals as parents of the next generation. The response to selection depends on the number of recorded phenotypes and on the accuracy of those phenotypes, such as the OC-status of an animal. Even when genomic selection can be applied in the near future, these two features of a breeding scheme remain of great importance. This thesis focuses on phenotypic and genetic parameters of OC, and on and the possibilities of selection against osteochondrosis.

The objectives of this thesis were to assess the prevalence and genetic parameters of various manifestations of OC, and to facilitate optimization of breeding against osteochondrosis in horses and pigs. In chapter 1, OC is defined and introduced. Chapter 2 and 3 of this thesis focus on prevalences and genetic parameters of OC in Dutch warmblood horses. Chapter 4 and 5 present prevalences of OC in pigs, and the effects of housing system, feeding level and growth rate on the prevalence of OC. In chapter 6 the general potential of genomic selection for improvement of traits on which a limited number of phenotypic records is available is evaluated. In the General Discussion, three topics relating to selection against OC are discussed: the relevance of OC in horse breeding goals, the design of the current breeding schemes to select against OC in the Dutch horse population, and the relevance of and possibilities for selection against OC in pigs.

In chapter 2, the prevalence of the various manifestations of OC as detected radiographically were assessed, and possible relationships between their occurrence within the same joint and between different joints was evaluated. In total, 70% of all horses showed joint abnormalities in a least one of the 28 locations judged. Joint-specific prevalences were between 31% and 39% and the correlation between flattened bone contours and fragments was low. Results shows that a detailed phenotypic description of OC, as used in this study, is required to identify the full genetic component of OC.

In chapter 3, genetic parameters were estimated for the various manifestations of OC. The overall heritability of OC was 0.23, while the heritability was 0.08 for flattened bone contours and 0.22 for fragments. The contralateral joint homologues had very similar heritabilities. OC in the hock and stifle joints was highly genetically correlated, whereas the correlations between the fetlock and the other joints were moderate. These correlations imply that selection on the stifle and hock joint, as performed nowadays, should not create a considerable correlated response for OC in the fetlocks.

In chapter 4, the effects of housing system and feeding strategy on the prevalence and severity of OC are investigated. The housing system was either a conventional concrete partial slatted floor, or a deep litter floor with extra space allowance. The feeding strategy was either *ad libitum* or restricted to 80% of *ad libitum*. The overall prevalence of OC in the experimental population was 41%, and 12% of the individuals had severe lesions. The stifle joint was most affected, showing a prevalence of 30%. OC scores between the different joints were not correlated. Boars were more affected than gilts in the elbow joint. Conventionally housed pigs had more OC than deep litter housed pigs, and *ad libitum* fed pigs had more OC than restrictedly fed pigs. Prevalence was highest for the conventional floor with *ad libitum* feeding (58%), and lowest for the deep litter floor with restricted feeding (34%). These results demonstrate that the prevalence of OC can be reduced by applying deep litter floors with extra space allowance and/or restricted feeding of fattening pigs.

In chapter 5, the differences in growth patterns between pigs with OC in different joints were investigated. Therefore, repeated measurements on body weight of pigs were taken over time, and their OC-status was recorded after slaughter. Pigs were divided into three groups based on their OC-status. After 28 days of age, piglets with severe OC at slaughter started to grow faster.

Although the severity of lesions were not determined until the pigs were slaughtered, the results showed that pigs with OC were significantly heavier than pigs without OC after 70 days of age. Pigs with OC in the elbow joints or with OC in at least two joints had higher bodyweights, whereas pigs with OC in the stifle joints only had lower bodyweights compared to the mean. Those results indicate that defining OC per joint may be required to identify its effect on growth rate, as OC in the elbow joint show had an opposite effect on growth compared OC in the femoropatellar joint. The results of this study suggest that OC might be related to selection of pigs with high growth rates during a specified time period.

In chapter 6, the potential of genomic selection for improvement of traits with a limited number of phenotypic records was evaluated. Selection responses were compared for breeding schemes using phenotypic selection based on own performance or progeny testing and genomic selection. In many livestock populations or even species, large reference populations cannot be realized for many traits. The value of genomic selection becomes then questionable. The results of this study show that, when genomic information has an equal accuracy as phenotypic information before selection, genomic selection will have a lower equilibrium response to selection because it has a larger Bulmer effect. It was illustrated that comparison at Bulmer-equilibrium parameters is essential when comparing genomic selection schemes to traditional breeding schemes. When the number of phenotypic records is limiting, a reference population using own performance information will give larger response to selection than a reference population using EBVs of the sires of the phenotyped individuals. Especially for low heritable traits, genomic selection showed to be of additional value over traditional selection, even though it was less good in absolute terms than with high heritable traits.

In the General Discussion, three topics relating to selection against OC are discussed: the relevance of OC in horse breeding goals, the design of the current breeding schemes to select against OC in the Dutch horse population, and the relevance of and possibilities for selection against OC in pigs. Results from the first part and from the literature were too weak to draw conclusions about the relevance of OC for horse breeding. Adequate and more focused research is needed to determine whether there are direct relationships of OC with performance of sport horses. The second part of the general discussion showed that, despite the inability of this and other studies to prove a genetic

correlation between OC and sport performance, OC has both economical and practical relevance for the current horse industry. Therefore, selection against OC is desirable and different selection strategies were compared. As a consequence of selection, a decrease in the prevalence of OC will reduce the need for surgery, and the devaluation and loss of genetic potential, and therefore decrease the economic loss. Results showed that a disadvantage of the current progeny testing scheme is that the progeny produced before selection takes place may will show undesirable EBVs and thereby maintain a certain amount of OC in the population. With genomic selection, the selection moment can be applied earlier to avoid stallions producing progeny when their EBVs are still unknown. Simulations showed potential for genomic selection, even with small reference populations, compared to the current breeding program in the Dutch warmblood horse population (KWPN). The third part of the general discussion showed that OC and thereby leg weakness is a serious problem and is a common culling reason in sows. OC is considered as a more serious problem in sows than in fattening pigs, because sows have a longer productive life. A decrease in OC prevalence will, therefore, lead to a larger economic and welfare impacts then apparent from results on fattening pigs. In this part, simulations indicate possibilities for using genomic selection. Future research should be performed to design a reference population of adequate size and construction. Finally, directions for future research are proposed.

Samenvatting

Osteochondrose (OC) is een aandoening aan de gewrichten die optreedt tijdens de groei. Bij normale groei verbeent het kraakbeen dat bij jonge dieren aanwezig is en wordt bot. Bij OC is dit proces verstoord, waardoor er schade aan het kraakbeen en bot ontstaat in de gewrichten. Het meest voorkomende symptoom van OC is een niet-pijnlijke gewrichtsovervulling, maar kreupelheid komt ook voor. De aandoening komt bij diverse diersoorten voor, zoals koeien, varkens, paarden, honden en kippen, en ook bij mensen. Dit proefschrift gaat over OC bij paarden en varkens. OC komt in veel paardenrassen voor. Omdat OC een belemmering vormt in de paardensport is het terugdringen van OC een belangrijk doel in de paardenfokkerij. Bij varkens is OC de belangrijkste oorzaak van beenwerkproblemen, wat de tweede reden van uitval bij zeugen is. Om die reden is het terugdringen van OC belangrijk voor zowel de economie als het dierenwelzijn.

OC wordt door veel factoren beïnvloed. Naast omgevingsfactoren zoals voeding en huisvesting, speelt erfelijke aanleg een belangrijke rol. Dit betekent dat fokkerij gebruikt kan worden om OC terug te dringen. Fokkerij heeft als doel de beste ouders te selecteren om een volgende generatie te fokken die beter is dan de huidige. De selectie van deze ouders is afhankelijk van het registreren van uiterlijke kenmerken (fenotypen) en van de nauwkeurigheid waarmee deze kenmerken worden gemeten. De ouders kunnen geselecteerd worden op basis van hun eigen kenmerken, of op basis van kenmerken gemeten aan hun nakomelingen; de nakomelingen zijn namelijk een afspiegeling zijn van de erfelijke aanleg van hun ouders. Deze twee vormen van selectie noemen we fenotypische selectie. Tenslotte is het sinds een aantal jaren ook mogelijk om dieren te selecteren direct op basis van hun genen. Dit noemen we genomische selectie. Ook bij genomische selectie blijft het regelmatig meten van fenotypen van groot belang. Deze fenotypen zijn namelijk nodig om de effecten van genen te berekenen, wat nodig is om genomische selectie mogelijk te maken.

Dit proefschrift gaat over het vóórkomen van OC bij varkens en paarden (de zgn. prevalentie), de erfelijkheid van OC, en de mogelijkheden om OC terug te dringen met behulp van fokkerij. In hoofdstuk 1 wordt OC nader uitgelegd en beschreven. In hoofdstuk 2 en 3 worden de prevalentie en erfelijkheid van OC bij Nederlandse warmbloed paarden (KWPN) onderzocht. In hoofdstuk 5 en 6 worden prevalenties en effecten van huisvesting, voeding en groei onderzocht

bij varkens. In het 6e hoofdstuk worden de mogelijkheden van genomische selectie onderzocht voor kenmerken die moeilijk te meten zijn, of waarvan het meten duur is. OC is een voorbeeld van een dergelijk kenmerk, omdat het bij paarden met röntgenfoto's gemeten moet worden. Bij dat soort kenmerken is het aantal dieren met fenotypen beperkt, waardoor fokkerij moeilijker is. In hoofdstuk 7, de algemene discussie, komen drie onderwerpen aan bod: de relevantie van OC voor het fokdoel bij paarden, een vergelijking van fokprogramma's om tegen OC te selecteren bij Nederlandse warmbloed paarden, en een evaluatie van de relevantie en selectiemogelijkheden tegen OC bij varkens.

In hoofdstuk 2 wordt de prevalentie van de diverse vormen van OC bij paarden onderzocht zoals deze door middel van röntgenfotografie kan worden gediagnosticeerd. Daarnaast wordt de relatie tussen het vóórkomen van deze vormen in hetzelfde gewricht en tussen verschillende gewrichten onderzocht. In totaal bleek 70% van alle paarden gewrichtsafwijkingen te hebben in tenminste een van de 28 onderzochte locaties. Gewrichtsspecifieke prevalenties varieerden van 31 tot 39%. Er was een zwakke relatie tussen milde vormen van OC, waarbij een kleine afvlakking van het botoppervlak zichtbaar is, en ernstiger vormen van OC, waarbij losse botfragmenten aanwezig zijn. Zo'n zwakke relatie betekent dat het niet van zelfsprekend is dat beide vormen tegelijk voorkomen in hetzelfde dier. Deze resultaten maken duidelijk dat een gedetailleerde beschrijving van OC noodzakelijk is voor nauwkeurige metingen en genetische studies.

In hoofdstuk 3 is de erfelijkheid berekend voor de diverse vormen van OC bij paarden. Op dierniveau, waarbij alle gescoorde locaties van een dier zijn opgeteld, is de erfelijkheid van OC geschat op 23%. De erfelijkheid voor afvlakkingen werd geschat op 8%, en voor fragmenten op 22%. De erfelijkheid voor de gewrichten aan het linker en het rechter been waren vergelijkbaar. Het spronggewricht en het kniegewricht zijn genetisch het sterkst aan elkaar gerelateerd, en minder sterk gerelateerd aan de kogelgewrichten. Dit betekent dat selectie tegen OC in de knie en spronggewrichten niet automatisch een verbetering te weeg zal brengen in de kogelgewrichten. Het is daarom belangrijk dat in de fokkerij rekening wordt gehouden met OC in zowel de knie, sprong als kogelgewrichten.

In hoofdstuk 4 zijn de effecten van twee huisvestingssystemen en twee voerstrategieën op OC onderzocht. De huisvesting bestond ofwel uit een conventioneel systeem met deels een roostervloer en deels een betonnen bodem, ofwel uit een zaagselbed met meer ruimte in het hok. De dieren werden onbeperkt gevoerd, of beperkt tot 80% van de vrijwillige voeropname. De prevalentie van OC in dit experiment was 41%, en 12% van de dieren had ernstige OC. De kniegewrichten waren het meest aangetast waarbij 30% van de dieren OC had. In het ellebooggewricht hadden beren meer OC dan zeugen. OC bleek het meest voor te komen (58%) in conventionele huisvesting met een onbeperkt voer, en het minst (34%) op de zaagselvloer met beperkt voer. Deze resultaten laten zien dat de prevalentie van OC verminderd kan worden door het gebruik van zaagselvloeren en beperkte voeding.

In hoofdstuk 5 zijn de verschillen onderzocht in groeipatronen tussen varkens met en zonder OC. Herhaalde metingen van lichaamsgewicht en OC scores op slachtleeftijd werden verzameld. De resultaten laten zien dat biggen met een ernstige vorm van OC vanaf een leeftijd van 28 dagen sneller gingen groeien, en na 70 dagen zwaarder werden dan varkens zonder OC, ondanks dat de OC pas na slachten werd gemeten. Deze resultaten zouden kunnen duiden op een gevoelige periode in de groei met betrekking tot de ontwikkeling van OC. Varkens met OC in het ellebooggewricht of met OC in twee of meer gewrichten hadden een hoger lichaamsgewicht, terwijl varkens met OC in het kniegewricht juist een lager lichaamsgewicht hadden. Dit betekent dat de relatie tussen OC en groei per gewricht bekeken moet worden, omdat de resultaten in elleboog en kniegewricht elkaar op dierniveau zouden kunnen maskeren.

In hoofdstuk 6 is onderzocht of genomische selectie een alternatief zou kunnen zijn voor traditionele selectiemethoden die gebaseerd zijn op metingen aan het dier zelf of aan nakomelingen. Deze vergelijking is toegespitst op kenmerken die moeilijk of kostbaar zijn om te meten, waardoor het totaal aantal metingen beperkt is. In dat geval is het onduidelijk of genomische selectie waardevol is. Selectiemethoden worden beoordeeld op basis van de genetische vooruitgang die behaald kan worden. Deze vooruitgang is berekend met behulp van computersimulaties. Voor genomische selectie is een referentiepopulatie nodig. Een referentiepopulatie bestaat uit een groep dieren waaraan zowel fenotypen als genotypen zijn gemeten, en wordt gebruikt om de effecten van genen te berekenen. De resultaten laten zien dat, wanneer het aantal te meten dieren beperkend is, de grootste vooruitgang behaald kan worden als er metingen aan

het dier zelf worden gebruikt voor het bouwen van een referentiepopulatie, niet aan de nakomelingen. Vooral bij laag erfelijke kenmerken heeft genomische selectie meerwaarde.

In de algemene discussie worden drie onderwerpen worden besproken; de relevantie van OC in paarden, de mogelijkheden voor fokkerij tegen OC in het paard, en tenslotte de relevantie en selectiemogelijkheden tegen OC in het varken.
Voor de relevantie van OC in de paardenfokkerij is weinig direct bewijs te vinden in de wetenschappelijke literatuur. De aanvullende analyses die zijn uitgevoerd om de relatie tussen OC en sportkenmerken te onderzoeken, laten zien dat deze relatie niet betrouwbaar in kaart gebracht kan worden met de gegevens die op dit moment beschikbaar zijn. Zowel uit dit onderzoek als uit de beschikbare literatuur blijkt dat er betere gegevens noodzakelijk zijn. Ondanks dat er geen direct bewijs is voor een relatie tussen OC en sportprestaties is OC duidelijk relevant voor de paardensector, zowel economisch als praktisch. Omdat selectie tegen OC wenselijk is, worden er verschillende methoden vergeleken om tegen OC te selecteren in paarden. Door selectie tegen OC zal de prevalentie afnemen. Dit zal leiden tot een verminderde waardedaling van paarden door OC, minder operaties als gevolg van OC, en de beschikbaarheid van meer divers genetisch materiaal voor de fokkerij. Als selectie tegen OC wordt gebaseerd op metingen aan nakomelingen is de betrouwbaarheid hoger dan bij selectie op basis van metingen aan het dier zelf. Een nadeel van deze selectiemethode is dat er altijd een bepaalde hoeveelheid nakomelingen met OC in de populatie gehandhaafd zal blijven. Uit de resultaten bleek dat genomische selectie een veelbelovend alternatief is voor de fokkerij van Nederlandse warmbloed paarden (KWPN). Het derde en laatste onderwerp laat zien dat OC een serieus probleem is in zowel mestvarkens als zeugen, omdat het een aantasting is van het dierenwelzijn maar ook een grote economische schadepost doordat veel zeugen moeten worden afgevoerd vanwege pootproblemen. Simulaties laten ook hier zien dat OC door middel van genomische selectie teruggedrongen kan worden.

P

Publications
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Dankwoord

Peer-reviewed publications

E.M. van Grevenhof, S. Ott, W. Hazeleger, P.R. van Weeren, P. Bijma and B. Kemp. 2011. The effects of housing system and feeding level on the joint-specific prevalence of osteochondrosis in fattening pigs. Livestock Sc. 135: 63-71.

E. M. van Grevenhof, A. Schurink, B. J. Ducro, P. R. van Weeren, J. M. F. M. van Tartwijk, P. Bijma and J. A. M. van Arendonk. 2009. Genetic parameters of various manifestations of osteochondrosis and their correlations between and within joints in Dutch warmblood horses. Journal of Animal Science. 87: 1906-1912.

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K. Frankena, P.W. White, J. O'Keeffe, E. Costello, S.W. Martin, **I. van Grevenhof**, S.J. More. 2007. Quantification of the relative efficiency of factory surveillance in the disclosure of tuberculosis lesions in attested Irish cattle. Vet. Rec. 161: 679-684.

E.M. van Grevenhof, B. Ducro, H.C.M. Heuven and P. Bijma. 2007. Identification of environmental factors affecting the prevalence of insect bite hypersensitivity in Shetland and Friesian horses in the Netherlands. Equine Vet. J. 39 (1): 69-73.

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Financed project proposals

Project proposal 'Identification of genetic and environmental risk factors determining the prevalence and the impact on welfare of osteochondrosis (OC) in pigs', financed by (Technologie-Stichting) STW. 2009.

Project proposal 'Development of intervention strategies for insect bite hypersensitivity (IBH) in horses', financed by (Technologie-Stichting) STW. 2007.

Conference proceedings

E.M. van Grevenhof, W. Hazeleger, P. Bijma and B. Kemp. 2010. The effects of housing system, feeding level and genetic line on osteochondrosis in pigs. 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany, August 1-6, 2010.

E.M. van Grevenhof, A. Schurink, B.J. Ducro, J.M.F.M. van Tartwijk, P. Bijma and J.A.M. van Arendonk. 2008. Genetic parameters of various manifestations of osteochondrosis in Dutch Warmblood horses (KWPN). EAAP - 59th Annual Meeting, Vilnius, Lituania, August 24-27, pg 280.

A. Schurink, B.J. Ducro and **E.M. van Grevenhof**. 2008. Heritability and repeatability of insect bite hypersensitivity in Dutch Shetland mares. EAAP - 58th Annual Meeting, Vilnius, Lituania, August 24-27, pg 281.

E.M. van Grevenhof, B. Ducro, P. Bijma and J.M.F.M. van Tartwijk. 2007. Correlations of osteochondrosis between joints and body measurements in Dutch Warmblood horses (KWPN). EAAP - 58th Annual Meeting, Dublin, Ireland, August 26-29, 2007.

H.C.M. Heuven, B.T.T.M. van Rens, **E.M. van Grevenhof** and H. Bovenhuis. 2007. Weight gain of F2-gilts depends on its paternally inherited IGF2-allele. EAAP - 58th Annual Meeting, Dublin, Ireland, August 26-29, 2007.

E.M. van Grevenhof, B. Ducro, H.C.M. Heuven and P. Bijma. 2006. Environmental and genetic factors affecting prevalence of insect bite hypersensitivity in Shetland and Friesian horses in the Netherlands. 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte-MG, Brazil, August 13-18, 2006.

Training and Supervision Plan

The basic package

WIAS Introduction course	2007
Course on philosophy of science and ethics	2009

Scientific exposure

Congresses	
World congress on genetics, Belo Horizonte, Brasilia	2006
EAAP, Dublin, Ireland	2007
EAAP, Vilnius, Lithuania	2008
World congress on genetics, Leipzig, Germany	2010

Seminars and workshops

Fokkerij&genetica connectiondays, Vugt, Netherlands	2006
Fokkerij&genetica connectiondays, Vugt, Netherlands	2008
Fokkerij&genetica connectiondays, Vugt, Netherlands	2010
Conservation genetics of animal populations, Wageningen	2006
WIAS science day	2007
WIAS science day	2009
WIAS science day	2010
Workshop Samen meer paardenkracht, Kootwijkerbroek	2007
Workshop OC, Wageningen, Netherlands	2007
Symposium Equine performance, Wageningen, Netherlands	2008
Symposium Karkater van een sportpaard, Leeuwarden, Netherlands	2008
Symposium Contactgroep gedragsgenetica, Utrecht, Netherlands	2008
Symposium Equine research, Hoogmierde, Netherlands	2008
Workshop OC, Stockholm, Sweden	2008
Workshop OC, Utrecht, Netherlands	2008
Interstallion Seminar, Uppsala, Sweden	2010

In-depth studies

Biological basis for management and selection tools	2005
QTL detection and fine mapping in complex pedigrees	2005

Science meets society	2007
Linear models in animal breeding	2007
WIAS Advanced statistics course	2007
Epigenesis and epigenetics	2008
Quantitative genetics with a focus on selection theory	2010
Quantitative Discussion Group	2005-2010

Professional skills support courses	
Techniques for scientific writing	2007
Supervising MSc thesis work	2008
College geven	2008
ASReml	2009
1-op-1 gespreksvaardigheden	2009

Research skills training	
Preparing own PhD research proposal, OC in horses	2006
Research proposal, IBH in horses	2007
External training period, Foulum, Denmark	2008
Research proposal, OC in pigs	2009

Didactic skills training	
Lecturing	
Guest lectures, genetic improvement of livestock	2005/2007
Guest lectures, equine locomotion and genetics, VHL	2006-2008
Supervising practicals and theses	
Animal breeding and genetics practical	2007
Genetic improvement of livestock practical	2007
Inleiding dierwetenschappen tutorship	2006-2007
Supervising 7 MSc theses	2006-2009
Supervising 4 BSc theses	2006-2007

Education and training total	**67 ECTS**

About the author

Elizabeth Maria van Grevenhof was born on the 1st of January 1980 in Beverwijk, the Netherlands. She was raised in Heemskerk and in 1998 she obtained her highschool diploma at the Augustinus College. The same year, Ilse started her bachelor study at the Van Hall-Larenstein, University of Applied Science, in Deventer. She did her bachelor thesis at the department of epidemiology of the University of Liverpool. In 2002, Ilse obtained her bachelor degree and started for her master degree in Animal Science at Wageningen University. For her MSc-degree, she specialised in animal breeding and genetics and in quantitative veterinary epidemiology, for which she carried out two theses. The first thesis was carried out using data of broilers collected by Nutreco, the Netherlands and indicators predicting the susceptibility for ascites were analysed. The second thesis was carried out in collaboration with the University College Dublin, Ireland, where she quantified the efficiency of factory surveillance in the exposure of tuberculous lesions in attested Irish cattle. Ilse graduated in 2004. In 2005, she started as a research assistant at the Animal Breeding and Genomics Centre of Wageningen University, where she started her PhD-research in 2006. The result of that project is this thesis. During this thesis, she collaborated with the University of Utrecht in writing a research proposal, which resulted in funding for a project on osteochondrosis in pigs. Currently, Ilse is working on that project as a post-doc researcher at the Animal Breeding and Genomics Centre.

Over de auteur

Elizabeth Maria van Grevenhof is geboren op 1 januari 1980 in Beverwijk en groeide op in Heemskerk. In 1998 behaalde ze haar HAVO-diploma aan het Augustinus College. Datzelfde jaar begon ze aan haar studie diergezondheid aan de HAS Van Hall-Larenstein te Deventer. Ze heeft haar afstudeeropdracht vervuld bij de Universiteit van Liverpool, en in 2002 haar diploma ontvangen. In 2002 begon ze een Master opleiding in Dierwetenschappen aan Wageningen Universiteit. Voor het behalen van haar MSc-graad is ze in twee specialisaties afgestudeerd, fokkerij en genetica en kwantitatieve veterinaire epidemiologie. Voor haar eerste specialisatie heeft ze gebruik gemaakt van vleeskuiken data van Nutreco om indicatoren te identificeren voor de gevoeligheid voor ascites. Voor de tweede specialisatie heeft ze samengewerkt met University College Dublin in Ierland om de efficiëntie te bepalen van het ontdekken van tuberculose in slachthuizen bij koeien. In 2004 behaalde Ilse haar MSc-diploma, waarna ze in 2005 als onderzoeksassistent begon bij de leerstoelgroep Fokkerij en Genetica van Wageningen Universiteit. Aansluitend is ze in 2006 begonnen aan haar promotieonderzoek, waar dit proefschrift het resultaat van is. Gedurende haar promotieonderzoek heeft ze samengewerkt met de Universiteit van Utrecht voor het schrijven van een projectvoorstel op het onderwerp: osteochondrose bij varkens. Momenteel is Ilse op dat project werkzaam als post-doc onderzoeker bij Fokkerij en Genetica.

Dankwoord

Het is erg cliché, maar daarom niet minder waar: onderzoek doe je niet alleen! Om die reden wil ik graag van deze ruimte gebruik maken om in dit misschien wel belangrijkste hoofdstuk iedereen te bedanken die op welke manier dan ook direct of indirect een bijdrage heeft geleverd aan de voltooiing van dit proefschrift.

In het bijzonder wil ik natuurlijk Piter bedanken, voor je altijd en eeuwige tijd en behulpzaamheid. In de afgelopen 6 jaar hebben we veel samen gedaan op de fiets, en op het werk. Je hebt altijd je best gedaan de begeleiding zo goed mogelijk te doen en je volledig te geven als we aan het eind van een fietsritje nog 'even' die berg opknallen. Dat siert je!
Op een iets grotere afstand van 50 km verderop richting het midden van ons land heb jij, René, een enorme interesse gehad en altijd een goede bijdrage geleverd aan de artikelen. De passie waarmee je zelfs naar andermans stukken (en 700 varkenspootjes) kunt en wilt kijken is erg bijzonder en aanstekelijk! Ondanks je nieuwe Prof. titel en bijkomende overvolle agenda heb je altijd weer de tijd gevonden om met grote zorgvuldigheid naar de stukken te kijken. Ik vond het dan ook een zeer plezierige en effectieve samenwerking, bedankt!
Johan, ik wil je ontzettend graag bedanken! Voor alles! Voor de mogelijkheid om hier te werken, voor de mogelijkheid dit project succesvol tot een eind te brengen, voor je waardevolle kritische blikken op mijn ideeën en stukken, de vrijheid binnen dit project, maar zeker ook voor de vele gezellige fietsuren op de racefiets en op de mountainbike! Toch wel leuk hè, door het bos?

En dan al die mensen van Fokkerij en Genetica die me steeds weer verder hielpen, met discussies, ideeën of samenwerking, maar bovenal met de gezellige, vermakelijke, humoristische en 'nog-veel-meer...' koffiepauzes, enorm bedankt! In het speciaal wil ik nog graag een paar mensen bedanken. Bart, heerlijk dat we tussen de koffiepauze door nog even over paardenmeisjes eh onderzoek konden praten, jij met SAS altijd weer leuke ideeën had en mmm dat lammetje was heerlijk! Henk, bedankt voor je wijsheden en wanneer gaan we weer fietsen? Henri, je bent nog steeds een jonge hond, een tikje ongecontroleerd maar altijd zo enthousiast, leuk! Han, bedankt voor je openheid en interesse in mijn onderzoek, discussies met jou zijn altijd leuk! Ane, many thanks for the years together, especially for everything we could share so well, and thanks for sitting next to me again during this day! I miss the

running together, watching fantasy movies en dinners… Gus, haha, ik moet al lachen als ik aan je denk. Bedankt voor 'always being such an Aussie gentlemen', je woord van de week en je humor.

Maar ook buiten Fokkerij en Genetica zijn er veel mensen die een grote rol hebben gespeeld in mijn onderzoek die ik graag persoonlijk wil bedanken. Wouter, bedankt voor je vrolijkheid, enthousiasme, creativiteit en wilskracht. Wat hebben we een leuk project gedraaid, met een nog mooier resultaat! Op naar nog 4 jaar veel plezier en mooie resultaten! Bas, bedankt dat je mee hebt gedacht, erin durfde te geloven en de mogelijkheden hebt gecreëerd! Al waren het heel veel varkenspootjes! KWPN, met name Johan, Hans en Daniëlle, bedankt voor de mooie data, de samenwerking en het meedenken voor de praktische vragen en toepassing. Mogens and Ole, it was great to collaborate, thanks for all your time, knowledge, help, good fun and great enthusiasm!

Dan tenslotte, mijn vrienden en familie, hoe kan ik jullie bedanken? Frans, Franca en Floor, heerlijk dat jullie met zoveel liefde hebben kunnen helpen aan een fijne afronding van mijn proefschrift, enorm bedankt! Bedankt Fleur, Arjen en Ellen, Annemarie en Dick, omdat het altijd zo gezellig is samen! Ellen, lief dat je met mij daar vooraan wilt zitten! Marieke, je weet wel waarom! Bedankt voor al je onvoorwaardelijke, lieve, steun en toeverlaat, alles van jou en onze vriendschap is zo bijzonder. Daar zijn geen woorden voor! Robbert, bedankt voor alle leuke, kritische blikken op foto's, we gaan er nog meer leuks van maken! Acda en de Munnik, wat kunnen woorden toch mooi zijn. Christa, gewoon omdat je mijn zusje bent, en van mij een hele trotse tante hebt gemaakt. Paps en mams, bedankt voor alle mogelijkheden, steeds maar weer. Ik weet dat jullie trots op me zijn, maar oh wat ben ik ook trots op jullie!

En boven alles, Cyriel, bedankt voor alles wat we samen zijn en hebben, iedere dag! Bedankt voor jouw pracht bijdrage in 'onze' genetica! Marit, wat ben je een ontzettend mooi wezentje! Iedere dag ben ik weer dankbaar, trots en blij als ik je zie, bedankt voor al je vrolijkheid, je mooie lach! Wonder boven wonder in mijn buik, ik hoop dat jij het ook zo gezellig gaat vinden bij ons!

Colophon

Data used in this thesis was financed by the Royal warmblood studbook of the Netherlands, KWPN.

The printing of this thesis was funded by the Animal Breeding and Genomics Centre, Wageningen University, Netherlands

The cover of this thesis is designed by X-pressions.nl

This thesis was printed by GVO drukkers & vormgevers B.V. | Ponsen & Looijen, Ede, Netherlands