Genetic and clinical studies of teat traits in the pig

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Abstract

A major goal of pig breeding is to produce sows with good longevity, which can raise many uniform litters with healthy, fast-growing piglets. The fitness of the sow, including the presence of sufficient number of functional teats, is one key to the success of the initial rearing period of the piglets. A frequently occurring type of non-functional teat, believed to not deliver sufficient amounts of milk from which piglets cannot suckle easy which is thereby less valuable for the suckling piglets, is the inverted teat. The aim of this thesis was to provide new genetic and clinical knowledge about pig teat traits, to create implements for accurate registrations and optimum focus on these traits in the breeding work. The long term aim is to provide knowledge that contributes to an increased piglet survival and a higher daily piglet growth.

The present study shows that many of the inverted teats will protrude between the age of 5 month and early gestation. However, some teats remain inverted and neither inverted teats nor small teats will supply enough milk to the suckling piglets and they are therefore undesired. At three weeks of age the number of functional and non-functional teats is the same between male and female pigs. However this changes at live weight of 100 kg and the number of functional and non-functional teats differs between gender. The difference noted may be caused by differences in the recording because of, e.g. divergent development during puberty. Total teat number, number of functional teats and inverted teats are heritable traits and the number of functional teats can therefore be improved by selection. There is no significant genetic correlation between litter size and teat number. This motivates that the number of functional teats should be included in any genetic evaluation which includes litter size. Present study has identified tentative chromosome regions for the total number of teats, the number of inverted and number of functional teats. Further research is needed to fine-map and verify the candidate genes. This thesis has assisted in answering important questions regarding teat traits in the pig. The complexity of the interaction between the nursing sow and its piglets was further emphasized, but there are still questions that need to be focused and new questions have been raised.

Keywords: sow, litter size, inverted teats, functional teats, mammary, SNP, genome-wide association

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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Chalkias, H., Ekman, E., Lundeheim, N., Rydhmer, L. and Jacobson, M. (2013) Inverted teats (*Mammillae invertitae*) in gilts – effect on piglet survival and growth rate *(Manuscript*).

II Chalkias H., Rydhmer L., Lundeheim N. (2013). Genetic analysis of functional and non-functional teats in a population of Yorkshire pigs. *Livestock Science* 152, 127-134.

III N. Lundeheim, H. Chalkias & L. Rydhmer , Acta Agriculturae Scandinavica, Section A – Animal Science (2013): Genetic analysis of teat number and litter traits in pigs, Acta Agriculturae Scandinavica, Section A – Animal Science, DOI: 10.1080/09064702.2013.841749

IV Chalkias, H., Jonas, E., Andersson, L.S., Jacobson, M., de Koning D.-J., Lundeheim, N. and Lindgren, G. (2013) A genome-wide association study for inverted teats in sows reveals novel candidate genes (*Manuscript*).

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Related Work by the Author

Chalkias, H., Jonas, E., Andersson, L.S., Jacobson, M., de Koning D.-J., Lundeheim, N. and Lindgren, G. (2013) A genome-wide association study for stayability traits reveals highly associated novel chromosome regions and markers (*Manuscript*)

Abbreviations

D100	age (days) to 100 kg live weight
DNA	deoxyribonucleic acid
DWG	daily weight gain
EDTA	ethylenediaminetetraacetic acid
FT	number of functional teats
FT100	number of functional teats at 100 kg
FT3	number of functional teats at 3 weeks of age
Fteat	number of functional teats
FTT	fidelity to teat
GC	genomic control
GWA	genome-wide association
IBD	identical by descent
IgG	immunoglobulin G
IT	number of inverted teats
LW1	litter weight at 3 weeks, 1st parity
LW2	litter weight at 3 weeks, 2nd parity
MAF	minor allele frequency
NFT100	number of non-functional teats at 100 kg
NFT3	number of non-functional teats at 3 weeks of age
NFteat	number of non-functional teats
pp	post-partum
PT	protruded teat, a teat that at 100 kg was inverted in the gilt, but before farrowing was protruded
PTHLH	parathyroid hormone-like hormone
QTL	quantitative trait locus
RLN	relaxin gene
Row	the row of teats, upper or lower, which the piglets were suckling

S100	sidefat thickness (mm) at 100 kg live weight
SB1	number of stillborn piglets, 1st parity
SB2	number of stillborn piglets, 2nd parity
Side	the side, left or right, which the sow was laying on during nursing
SNP	single nucleotide polymorphism
TB1	number of total born piglets in 1st parity
TB2	number of total born piglets in 2nd parity
TOT100	total number of teats at 100 kg
TOT3	total number of teats at 3 weeks of age
TP	teat pair
TT	total number of teats
Tteat	total number of teats

Introduction

Since their domestication, pigs have been selected for a number of traits including litter size. This process has progressed slowly throughout domestication but has become more rapid during the last decades when new animal breeding techniques such as us of breeding values were applied. As a consequence, litter size in domestic pigs has increased significantly in comparison to that of the wild boar ancestor. The number of functional teats in sows is critical for piglet survival, since each piglet needs a teat. Consequently, with growing litter sizes the piglet mortality has also increased (Vasdal *et al.*, 2011). This is an economical and animal welfare issue, as well as a work environment problem for the herd staff. The two main causes of pre-weaning mortality are crushing or starvation, mostly during the first two to three days of life (Devillers *et al.*, 2011; Moustsen *et al.*, 2013). The very low energy reserve of newborn piglets rapidly depletes and they are therefore sensitive to chill, hypoglycaemia and weakness (Le Dividich & Noblet, 1981; Le Dividich & Noblet, 1983; Herpin *et al.*, 2002). The increased litter size also leads to decreased birth weight and heterogeneous weight within litter (Roehe, 1999; Wolf *et al.*, 2008; Canario *et al.*, 2010) as well as heterogenic maturity at birth (Herpin *et al.*, 1993). Further, the selection for lower fat deposition of the slaughter pigs has decreased the body reserves of the sows (Bergsma *et al.*, 2009). Also, the complex nursing behaviour argues the importance of individual teats for the piglets. Additionally, newborn pigs are born with low immunity and are totally dependent on an intake of protective antibodies via the colostrum, the first milk produced in the sow udder (Dividich *et al.*, 2005). The reason for this is that the uterus of the sow differs from many other species because there is no transfer of maternal antibodies across the placenta. Thus it is vital for the survival of young pigs to get access to an adequate amount of colostrum. It can therefore be concluded that healthy udder with well-functioning mammary glands and teats of the sow is the basis for a successful initial nourishment of the piglets.

Background

The sow plays a central role for the piglet production, as piglets need to be fed approximately every hour. To make this feasible each piglet needs a teat of its own. Successful breeding for larger litter sizes has led to an increased focus on number and quality of the teats.

Pig breeding

Modern systematic pig breeding started approximately 100 years ago. At that time, the traits as well as the breeding goal did differ greatly from those of today's pig breeding programmes. Typically fat was appreciated due to physically demanding work during the first part of the 20th century, whereas today, lean meat is targeted. Before the era of computers and prior to the development of technologies such as artificial insemination, selection was based on quite simple performance and pedigree information. Today, with the aid of computational power, genetic evaluation is based on sophisticated software that makes use of information on a huge number of animals, and integrates their relationship in a matrix to link information between related animals. This method is called BLUP (best linear unbiased prediction) (Henderson, 1975). This method made it possible to more effectively select for traits with low heritability, such as litter size. The litter size in wild pigs is usually 4-6 piglets (Gethöffer *et al.*, 2007) and in the conventional dam-line breeds of today, the total number of piglets born is on average 14 piglets, with an increase of about 0.2-0.3 increase per year (Thorup, 2012). Since each piglet needs its own teat, the breeding for increased litter size has made the number of functional teats in the sow critical in the modern pig production. The large litter sizes, in pigs are unusual for mammals of this body size. Generally, the reproductive strategy of large mammals is to produce one or two offspring each gestation whereas small mammals produce a large number of offspring.

However in the dog, the small breeds often have smaller litters than large breeds (Borge *et al.*, 2011).

The structure of pig breeding resembles typically in a pyramid. The nucleus herds, with the smallest number of breeders, are at the top of the pyramid. In the nucleus herds, the selection within each breed takes place and the genetic improvement is generated. The multiplier herds build the level below. These herds purchase purebred sows from the nucleus herds, and inseminate them with semen of the other dam line. Crossbred gilts, born in the multiplier herds are sold to the bottom layer of the pyramid, the commercial herds. In these herds, these crossbred sows are inseminated with a terminal sire breed producing the 3-breed cross for slaughter.

Genetic evaluation

In Sweden, a national pig breeding program was developed during the 1920s, subsidised by the government (Hansson & Lundeheim, 2013). Testing stations were established, a nation-wide litter recording system was introduced, and a litter recording scheme registry started. Since late 1920s with the litter recording scheme, the total number of teats has been recorded. In the 1960s, the on-farm performance testing, using the ultrasonic technique was introduced (Hansson & Lundeheim, 2013). Along with the on farm performance testing the number of non-functional teats has been recorded. During the 1980s the government support was cut down, leading to a polarisation of the breeding companies: the farmers' cooperative slaughter organisation and the private slaughter plants' organisation. Also in Sweden pig breeding is organised in a pyramid. Typically Landrace or Yorkshire sows are used as purebred females in the multiplier herds. The terminal sire breeds are commonly Hampshire or Duroc in Sweden.

Genetic evaluation - recorded traits

Pig breeding has had the main focus on production and reproduction traits (the economically valuable) such as growth rate, feed efficiency, lean meat percentage and litter size. Since the late 1980s leg strength and longevity have been included in the breeding goals, with the intension to improve animal health and welfare. Traits under selection are recorded in nucleus herds, multiplier herds and test-stations, and the breeding organisation stores the registrations from all pigs in the nucleus herds, registered both by the herd staff and technicians from the breeding organisation. Today, in Sweden, the best boars are sent to a test station at two to three months of age, and raised in a standardized environment. This allows the accurate recording of feed efficiency and additional evaluation of carcass quality of those boars slaughtered and not used as future breeding boars. The registration performed by herdsmen in the nucleus herd includes litter size, piglets born alive, litter weight, teat quality and teat number at three weeks of age. Technicians visit all nucleus herds when the pigs in each batch are about 5 months old, the whole process is performance testing. At performance testing the body weight, sidefat thickness, clove health and teat numbers including teat status are registered while the pigs are locked up in the scale. The technician observes and palpates the teats on both male and female pigs to determine which teats are considered non-functional. This recording is physically demanding for the technicians; the light is often poor, there is a risk for injuries during the palpation if the animal moves in the scale, and the technician must bend knees and back. Yet this procedure can cover as many as 200 animals in one day of testing. When the pig approaches the scale and when it leaves the scale, scoring of the body conformation, such as legs and movements, is performed.

The genes behind traits in the genetic evaluation

The molecular genetics techniques have developed greatly over the past decade and can be used to find the underlying genes or mutation for a trait or a disorder (Hirschhorn & Daly, 2005; McCarthy *et al.*, 2008). The techniques also provide complement to the traditional genetic evaluation to improve accuracy and speed up the genetic improvement. With next generation sequencing techniques the identification of Single Nucleotide Polymorphisms (SNPs) and development of commercial SNPChips (Ramos *et al.*, 2009) has facilitated mapping of genetic traits. At present, the focus is on the study of association of phenotypes with genes which increases our understanding of gene function. The domestic animals are in many ways optimal for genomic studies (Andersson, 2009). Since domestication, production animals have been selected based on spontaneous mutations (for example coat colour). This has made the domestic animals more phenotypically diverse than their wild ancestors. Furthermore, large pedigrees can be easily accessed in production animals, their environments are rather standardised and tissue sample are easy to collect, either on the live animal or at slaughter. Also, within breeds of farm animals the linkage disequilibrium (LD) is higher than between breeds, which is advantageous when fine-mapping genes.

Monogenic and polygenic traits

Monogenic traits depend on a single allele and the polygenic traits depend on multiple alleles and environment (quantitative traits). The genetic regulation of quantitative traits is complex and identifying association with the genes that underlie genetic variation or the molecular mechanism behind the phenotypic expression is still a huge challenge.

Mapping strategies

Both the selection of candidate genes and different genome mapping strategies have been used in domestic animals for the identification of genes that underlies a trait. Candidate gene studies are based on an assumed gene that might be involved in the trait development and require some previous knowledge about the trait (such as the physiological background), to be able to predict whether the gene plays a role in the trait. The method is relatively cheap and fast, but due to the assumptions made, it often fails. Alternatively genome mapping strategies such as linkage mapping can be used. Although linkage mapping has been frequently used in the past and has been successful for mapping genes that underlie monogenic traits, it has not performed so well for traits influenced by mutations in multiple genes throughout the genome. Linkage mapping requires fewer markers than association studies, but the confidence intervals of the regions identified are larger and additional fine-mapping studies are often required. To be useful, markers tested for association must either be located on the causal gene or be highly associated markers, in linkage disequilibrium, with the causal allele. In the pig production malignant hyperthermia (MH) is correlated to pale soft exudative (PSE) meat and causing major economic losses. The identification of the causative mutation in ryranodine receptor 1 (*RYR1*) is one example of the successful identification of a QTL, and the mutations which is today used as a marker in commercial breeding programs (Fujii *et al.*, 1991). As shown on this successful example, development of a diagnostic test can provide the basis for elimination of such detrimental mutations in livestock breeding programs.

Genome-wide association studies

Genome-wide association is a powerful method that allows the identification of major genes for complex traits (McCarthy *et al.*, 2008). It is also very successful for monogenic trait mapping. Genome-wide association studies are based on thousands of genetic markers distributed all over the wholegenome. It requires more markers than classical linkage mapping but due to the development of medium and high-throughput genotype platforms, this method is today commonly used. The method is based on genotyping of many individuals (usually hundreds in domestic animals), using tens of thousands of SNPs across the whole genome, and performing association tests between the genotypes and the phenotypic measurements. The method can be used both for monogenic traits and for polygenic traits and the approach allows both classical case-control studies (affected or not) as well as studies of quantitative traits (for example height). The genome-wide association methods could allow the identification of genes and mutations underlying variation in pig production and reproduction traits.

Because no assumptions are made about the genomic location of the causal variants it is an unbiased method, but several issues need to be accounted for. The sample sizes needed depend on the trait as well as the genome and population structure of the species. The trait of interest need to be well defined and accurate phenotyping is crucial. One risk when performing a genome-wide association study is the risk of false-positives. Correction of the p-values can be made by the Bonferroni-method, which accounts for the multiple testing. Another cause for false-positives could be population stratification that might be due to sub-populations within the sample population. By comparing the observed p-values with the expected p-values this can be detected.

Milk production in sows

With increased litter size, increased piglet growth and at the same time leaner sows, requirements of the sow are extreme and the allocation of resources is crucial. The sow lactation performance is important since production and composition of colostrum and milk is essential for the piglets' ability to survive and grow during lactation (Cabrera *et al.*, 2010).

Nursing behaviour

The milk ejection reflex is highly conserved across all mammals and is essential for successful lactation. The way the sow interacts with her piglets influences the success of the nursing period. The piglets are motile at birth and within minutes after birth they are moving towards the udder. The piglet often has nose to nose contact with the sow immediately after birth. The milk let-down occur at frequent intervals and milk is available more or less all the time. Initially the piglets suckle from several teats along the udder. Only hours after farrowing the first teat disputes can be seen. About 12 hours after farrowing (Fraser, 1980), the milk let down occur at regular intervals (30- 70 minutes). The sow milk let-down and oxytocin release is connected with an increased grunting rate by the sow. In contrast to the dairy cow, the mammary gland of sows does not contain a milk cistern and the milk is only stored in the alveoli and milk ducts (Hartmann & Holmes, 1989). Therefore it is difficult to measure the milk yield. Suckling (milk let-down) lasts 10-20 seconds and occurs approximately every 60 min. There is a competition among piglets for a functional teat or, in large litters, a competition for any teat (Andersen *et al.*, 2011). Piglets generally return to the same teat (teat fidelity) and defend their teat (De Passillé *et al.*, 1988). Fights occur, resulting in facial lesions on piglets when other piglets try to conquer the occupied teats (De Passille & Rushen, 1989). When the piglets return to the same teat between sucklings's the teat order is established (Skok & Škorjanc, 2013).

Mammary gland and milk production

There are more than 4,000 species of mammals with similarities in the structure and function of their mammary glands, but also with unique milk components, variations in the number of mammary glands, and suckling strategies (Oftedal, 2002). The milk is the main source for growth of the piglets the first weeks of life. For the piglet, it takes 4 L of milk to grow 1 kg live weight (Noblet & Etienne, 1989). In the second week of lactation, the milk becomes a limiting factor for the piglet growth (Zimmerman *et al.*, 2012) and in large litters they need complementary feeding to keep the high growth rate (Auldist *et al.*, 1998; Auldist *et al.*, 2000).

Colostrum and milk composition varies between teats, sows and breeds (Zou *et al.*, 1992). It is also influenced by diet (Farmer & Quesnel, 2009) and body conditions. The lactose content, which provides energy to the piglets, is about 50% lower in colostrum from the Meishan compared to Yorkshire sows at farrowing (Zou *et al.*, 1992). On the contrary, Meishan sows have a higher fat content in the milk compared to Yorkshire sows throughout the lactation period. Fat is an important energy source for the growing piglet. There has not been the same focus on milk production in sows as in dairy cows, but milk production gets more relevant as sows in current farming systems are high producing. Measuring the appoximate milk yield in sows can be easily performed, by either weighing the piglets before and after the suckling or the use of isotope dilution (Theil *et al.*, 2002). The most used method in genetic evaluation is to measure the weight gain of the piglets and then recalculate the milk yield (Devillers *et al.*, 2007).

The blood vessel system of the mammary gland of the sow, consists of the arterial, venous and lymphatic circulation and is located on each side of the ventral midline. In contrast to ruminants, where only one artery supplies both sides of the udder, the mammary glands on each side of the sow receive blood through several arteries. Different arteries supply the anterior mammary gland pairs 1 to 5, and the posterior mammary glands. Venous blood from the mammary glands uses two distinct pathways(Renaudeau *et al.*, 2002). The anterior glands drain cranially and the posterior glands drain into the external vein. There is a venous anastomosis between the right and left mammary gland of each pair of glands (Trottier *et al.*, 1995; Renaudeau *et al.*, 2002). Nutrients from the blood are synthesized by the epithelial cells in the alveoli into milk components and transported into the alveolar lumen where milk is produced. Removal from the alveoli and milk ducts, require induction of the milk ejection reflex. Stimulation by the piglets will induce this milk-ejection in order to obtain a release of oxytocin that will induce milk ejection (Fraser, 1980).

Colostrum

Colostrum is rich in energy and also contains immunoglobulins for the new-born piglets and is an important factor for piglet survival during the first days of life (Noblet *et al.*, 1997; Rooke & Bland, 2002; Quesnel, 2011). Due to six layers of cells (three cell layers in the sows and three cell layers in the foetus) between maternal and foetal circulation, no immunoglobulins will be transferred across the sow placenta (Rooke & Bland, 2002). The piglets are therefore born without a fully activated immune system (Rooke *et al.*, 2003). The passive transfer of immunoglobulins via colostrum is essential and must take place within 24 hours after birth, before the intestinal closure occurs. Le Dividich *et al.* (2005) showed that the content of colostrum immunoglobulin G (IgG) decreased by a third three hours after the farrowing started. A piglet that misses the colostrum will be more sensitive to infections and also to starvation.

Teats

The function of the teat is quite simple but it is essential for piglet survival. There has been discrepant use of the terms teat and nipple in the literature. The teat has a single canal, as found in cows and sheep, and that the nipple has multiple canals, like in humans and dogs. Throughout this thesis the word teat is used, as most other studies on the pig. The mostly two milk canals in the pig teat (and the horse teat) have separate cavity systems. The number of teats in pigs ranges from 8-18, but varies between breeds. The Yorkshire sow has on average 14 mammary glands and two ductal systems per gland. Some of the teats are believed to be less functional than others. Examples on this are teats that are inverted, small, blind, extra, flat or telescopic. It is thought that these types of teat cannot be suckled by piglets, or that the related mammary glands produce a reduced amount of milk. Small teats are often found in a posterior location. Small teats in-between two normal teats are often called "extra teats" or "blind teats". It is assumed that these extra teats cannot produce sufficient milk because they are associated with small mammary glands, because they cannot be gripped by piglets, or do not have milk canals. Flat teats are teats that are injured early in the life of the pig, and remain as a flat scar. Telescopic teats are not yet described in the literature and their influence on nursing is not documented. This type of teat is not protruded completely in a gilt of 100 kg live weight; it looks as though it is trying to "come out of its shell". The teat is regarded as non-functional if it cannot be extruded when gripped during clinical examination (pers. comm. Avelspoolen, Nilsson, 2011; Nordic Genetics, Jönsson, 2011).

Inverted teats

Inverted teats exist in many mammals such as mouse, dog, pig and human. In an inverted teat the top of the teat, or even the entire teat, is inverted to form a crater. In some cases the tip of the teat can protrude when stimulated, but in others the inversion remains, regardless of stimuli (Nordby, 1934). The occurrence of inverted teats is not uncommon in humans, (with a reported frequency of 3% by Park *et al.* (1999)). Different methods to correct flat and inverted nipples in humans have resulted in varying degrees of success and also complications have been reported. The protruding of the teat is linked to pain in humans and as a consequence delayed lactating period (Dewey *et al.*, 2003; Chakrabarti & Basu, 2011). Inverted teats limit the rearing capacity of the sow and also increase its vulnerability to mastitis (Jonas *et al.*, 2008). These teats are often found near to the umbilicus (Beilage *et al.*, 1996). During the embryonic development, the mammary glands can be seen as a thickening of epidermis, called the mammary ridge, at day 28 (Patten, 1948). Failure of the foetal mammary pit to evert before birth will result in an inverted teat. The mechanism behind this failure is unknown, but may be related to the connective tissue proliferation (Günther, 1984). No correlations between inverted teats and inflammation or trauma during early growth have been reported (Beilage *et al.*, 1996).

Hormones and factors affecting teats

Many inherited disorders are traits with polygenic background, as is the case with inverted teats in pigs. The trait is difficult to identify in the routine breeding work and identification of genetic factors that influence inverted teats is therefore important. Sherwood (2004) showed that the hormone relaxin (*RLN*) promotes growth and development of the mammary apparatus in pig and rat. This hormone inhibits collagen synthesis and the major effects of *RLN* on mammary growth and development target the nipple in the rat, whereas they target the glandular parenchyma in the pig. *RLN*-dependent growth of the nipple in rats is required for normal lactational performance. *RLN* is required for the development of the mammary nipples in rodents. *RLN* knockout-mice show impaired mammary development and exhibit abnormalities in the collagen remodelling. The phenotype is similar to inverted teats and the teat cannot be suckled by the offspring (Kamat *et al.*, 2004). *PTHrP* stimulate mammary bud outgrowth and nipple formation. *PTHrP* is secreted from mammary epithelial cells of the mammary bud. Other factors trigger epithelial outgrowth and inhibits hair follicle formation within the nipple sheath (Foley *et al.*, 2001; Robinson, 2007; Watson & Khaled, 2008). In mouse mutants Pth1r-/- failure of nipple development occurs and in transgenic mice over-expression of *PTHLH* leads to mismatch of the mammary glands (Mikkola & Millar, 2006). Significant Quantitative Trait loci (QTL) for number of teats have been identified on several pig chromosomes. The chromosome that have QTL for both number of teats and inverted teats are SSC3, SSC5 and SSC11 (Rohrer, 2000; Bidanel *et al.*, 2008; Jonas *et al.*, 2008). The number of inverted teats can be related to the total number of teats and the QTL can be expected to be partly the same (Jonas *et al.*, 2008). Genes that have some physiological relevance for inverted teat and are within the QTL region may be possible candidate genes (Ron & Weller, 2007). Genomic research advances our understanding of factors that influence health and disease and advances have been made within molecular genetics in the identification of chromosomal regions or genes that affect a trait.

Aims of the thesis

The purpose of this thesis was to provide knowledge about pig teat traits, and to create tools for accurate registration and optimum use of these traits in the selection. The long term aim is to provide knowledge that contributes to an increased piglet survival and a higher piglet growth rate.

The specific aims are to:

I evaluate the function and impact of inverted teats during nursing

II-III estimate genetic and phenotypic variation for the teat traits with effect of gender and reproductive traits

II-III investigate the genetic correlations between teat traits and performance and reproductive traits

IV identify genetic markers associated with the total number, the number of functional and inverted teats in pigs

(number in roman style in the margin correspond to papers 1-4)

Present studies

Inverted teats (Mammillae invertitae) in gilts – effect on piglet survival and growth rate (Paper I)

In the modern pig industry, the increasing number of piglets born per litter and the increasing piglet mortality during the suckling period augments the importance of the number of functional teats in the sow. One example of non-functional teats, teats that are believed to be less functional and thereby less valuable for the suckling piglets, are the inverted teats. Today (2012) the pre-weaning mortality of liveborn piglets in Sweden is about 18% (PigWin, 2013). The aim of this study was to evaluate the function and importance of inverted teats during nursing, and to analyse structural and functional differences between the mammary glands of inverted teats versus normal teats. Furthermore we wanted to evaluate the growth rate of piglets that have suckled previously inverted teats that have protruded before farrowing.

Material and methods

Of the 16 gilts selected for this study only nine were finally included in the study. The remaining sows had health problems and were euthanized before mating or returned to oestrus. Gilts excluded from the study had all a high number of inverted teats. The gilts were purebred Yorkshire gilts, except one that was a cross between Swedish Yorkshire and Norwegian Landrace. In connection with performance testing (at 100 kg live weight), the number of inverted teats was recorded in the gilts, see Table 1. Additionally, three of the gilts had one small teat each (approximately half the size of the normal teat) and one had an additional extra teat. Ten weeks before expected farrowing, the teats were counted, palpated and subjected to a thorough inspection. After parturition, the piglets were counted, marked individually and all excessive, functional posterior teats were blocked with adhesive bandage, to ensure that the number of available teats equalled the number of piglets. For each piglet, the suckled teat was registered as “inverted”, “protruded” (previously inverted), “small” or “normal” and the teat pair (TP) was registered and numbered from 1-8, starting from the front. The fidelity to teat was registered, excluding the first 48 hours *p.p.* The piglet weight was recorded daily during the first week of life and thereafter once a week until weaning at four weeks of age. All piglets that died during the study were subjected to necropsy. The gilts were euthanized immediately after weaning and the mammary gland for each teat was dissected and weighed. A total number of 26 teats were examined histologically, 2 inverted, 10 protruded, 3 small and 10 normal teats, from a total of six sows. These samples were embedded in paraffin, sectioned and stained with haematoxylin and eosin. In order to visualize collagen and smooth muscle Masson´s trichrome stain was used and Weigert´s elastin stain was used to visualize elastic fibres. The weight and growth rate was analysed using repeated observation mixed-model analysis of variance. The mammary gland weight was analysed using a generalised linear model (GLM) with sow, teat type (normal/protruded) and sex as fixed factors. The correlation of mammary gland weight and weight gain was estimated with Pearsson’s correlation. Chi2-test was used to identify any differences in fidelity.

Results

From the nine farrowing gilts, 94 piglets were included in the study. These piglets suckled normal teats (59), suckled previously inverted teats (32) and suckled small teats (3). All inverted teats had protruded at farrowing, except for two gilts where one teat in each of the gilts remained inverted. Most of the inverted teats (94%) had protruded already during early gestation.

Four sows developed fever (0, 3, 7, and 13 p.p., respectively), and were treated with antibiotics. There was significant lower weight (p=0.02) and the daily weight gain (p=0.006) in piglets of treated sows (0.332 kg; 36 gram/day) that influenced the piglets suckling protruded teats as well as piglets suckling normal teats. One of the farrowing gilts had 18 liveborn piglets and in this litter, both the average birth weight and the average weaning weight was low (normal teats=6.3 kg; inverted teats; 5.6kg; small teat=3.2 kg). Several of the piglets were weak and four were euthanized at birth, due to weakness and the fact that the teats were fewer than the number of piglets. Further, this was one of the sick sows and the sow did not expose the entire udder during the first days of lactation.

The two piglets that suckled the inverted teats were euthanized 4 and 8 days after birth, respectively, due to loss of body weight.

Table 1. Teat and litter information of the nine studied sows. Y=Yorkshire sow, LY= crossbred sow (Landrace^{x}Yorkshire)

Sow	Teats at 100 kg	Teats at farrowing	No. live born piglets	No. blocked teats	No. piglets weaned	Video
1 Y	10 normal 4 inverted	10 normal 4 protruded	13	2	12	no
2 Y	10 normal 4 inverted	10 normal 3 protruded 1 inverted	13	5	9	no
3 LY	6 normal 8 inverted	6 normal 7 protruded 1 inverted	13	5	9	no
4 Y	10 normal 4 inverted	10 normal 4 inverted	10	5	9	yes
5 Y	10 normal 5 inverted	10 normal 5 inverted	12	3	11	yes
6 Y	8 normal 6 inverted	8 normal 6 protruded	11	4	10	yes
7 Y	11 normal 2 inverted 1 small	11 normal 2 protruded 1 small	15	2	12	yes
8 Y	13 normal 1 inverted 1 small	13 normal 1 protruded 1 small	16	4	11	yes
9Y	13 normal 1 inverted 1 small	13 normal 1 protruded 1 small	18	3	12	yes

Within the litters with two or more protruded teats, the difference in weight between piglets suckling normal and protruded teats varied (Figure 1). In seven out of the nine sows, the weaning weight of the piglets suckling previously inverted teats was numerically lower as compared to the piglets suckling normal teats, although the difference was not significant. A significant difference in growth rate of 27 g/day ($p=0.04$) was seen between the two groups during week one but not during the following weeks (2-4). Piglets nursing small teats i.e. considerably smaller teats, had lower weaning weight (4.8 kg) and the corresponding mammary tissue also had lower weight.

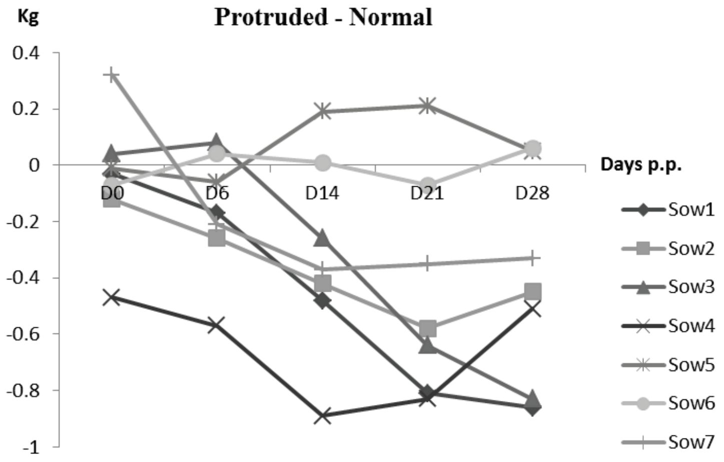


Figure 1: Difference (kg) within litter between the weight of piglets suckling protruded teats and piglets suckling normal teats, for litter 1-7 having two or more protruded teats.

The average weight of the mammary glands did not differ significantly between the two groups. The amount of mammary gland tissue at necropsy was positively correlated to the average daily weight gain.

The two inverted teats were shorter than the normal teats and the protruded teats, and broader than the small teats. The histological examination revealed no obvious morphological differences in arrangement of the connective tissue or muscles fascicles between the groups. Neither did the presence of vessels in the subcutaneous tissue differed between the groups. The elastin content in the connective tissue were similar in normal teats, protruded teats, inverted teats and in the small teats.

Genetic analysis of teat number, production and reproduction traits in pigs (Papers II and III)

The successful breeding for increased litter size has made the number of functional teats on the sow, needed for piglet growth, an important topic. Some of the teats found on young gilts at the time of selection (~100–150 kg live weight) are considered to be less functional. Examples of these are inverted, small or extra teats. A phenotypic threshold selection, based on the number of normal teats, mostly at least 14 functional teats has been applied in many breeding organisations. As a result of the threshold selection, animals with very good genetic capacity for production and reproduction traits might be culled because of the low phenotypic number of functional teats. Genetic analyses of teat traits are needed to facilitate a more accurate process of selection for increased number of functional teats.

The aims of these studies were;

to analyse genetic and phenotypic variation in teat traits; to evaluate the effect of gender, birth litter size, birth parity number, birth season and herd on teat traits and to investigate the genetic relationship between teat traits and performance traits and reproduction traits.

Material and methods

These studies are based on routinely collected data from nucleus herds with purebred Yorkshire sows, within the pig breeding company Nordic Genetics in Sweden. Teat number and teat quality are recorded twice for these pigs (boars and gilts), at approximately three weeks of age (when they are ID-marked) by the herdsmen and five months of age (at performance testing) by technichians. The number of functional teats at three weeks of age (FT3), number of non-functional teats at three weeks of age (NFT3), numbers of functional teats at 100 kg (FT100), and number of non-functional teats at 100 kg (NFT100) are recorded separately at both occasions. Small teats, extra teats and inverted teats are classified as non-functional. Litter size is recorded at birth. In nucleus herds litter weight at three weeks of age is recorded both for purebred and crossbred litters. Almost all purebred piglets are given an unique identification (ID) number at three weeks of age, and on average 80% of these pigs are performance tested.

SAS software version 9.1 (SAS Inst. Inc., Cary, NC) was used to edit the data and to run phenotypic analyses. The genetic analyses were performed using the DMU-package (Madsen & Jensen, 2007). After editing data (excluding herds with less than 1000 performance tested pigs during the period studied and obviously erroneous data), 83,804 pigs from 15 nucleus herds (Paper II) and 56,884 pigs from 14 nucleus herds (Paper III) were included in the analyses. In paper III reproductive data on 10,000 1st-parity litters and 6,500 2nd-parity litters was included for analysis. In paper II the teat traits were analysed by gender and included registration both at three weeks of age and at 100 kg live weight. In paper III only teat records from performance testing were included and not separated by gender. Difference in the number of functional teats and the number of non-functional teats between the registration at three weeks of age and that at 100 kg live weight was calculated (Paper II). The age and sidefat thickness at performance testing were pre-adjusted to 100 kg live weight and included in the analyses as Age (days) to 100 kg live weight (D100) and Sidefat thickness (mm) at 100 kg live weight (S100) (Paper II).

Table 2. Descriptive statistics for the data, after editing (Paper II).

Trait	N[a]	Mean	SD	Min	Max
Total number of piglets born in the litter	9,310	12.9	3.56	1	26
Parity number	9,310	1.9	1.29	1	10
Number of functional teats at three weeks (FT3)	83,804	14.4	0.92	6	21
Number of non-functional teats at three weeks (NFT3)	83,804	0.1	0.33	0	8
Total number of teats at three weeks (TOT3)	83,804	14.5	0.94	10	22
Number of functional teats at 100 kg live weight (FT100)	70,068	14.2	1.22	1	19
Number of non-functional teats at 100 kg live weight (NFT100)	70,068	0.3	0.87	0	14
Total number of teats at 100 kg live weight (TOT100)	70,068	14.5	0.94	10	20
Age (days) at performance test	70,761	159.1	13.96	95	278
Weight at performance test, kg	70,761	100.5	12.97	62	152

[a] Number of litters (first two variables)/number of pigs

Results

In the phenotypic analyses (paper II) there were significant differences between males and females for all analysed teat traits. Males showed a higher number of functional teats and females three times more non-functional teats at 100 kg live weight. Approx. every 5th gilt at performance testing had non-functional teats. The proportion of animals with at least one NFT3 was 6% for males and 8% for females. At 100 kg live weight, the corresponding proportions were 8% and 18%. For the animal that had non-functional teats the most common was to have one or two (range 0-14). In 69% of all litters, all piglets were free of NFT3. At 100 kg live weight, the proportion of litters completely free from pigs with non-functional teats had decreased to 47%. Among the parental pigs with teat information that was included in the data set, 8% of the sows and 6% of the boars had at least one non-functional teat recorded at their own performance testing.

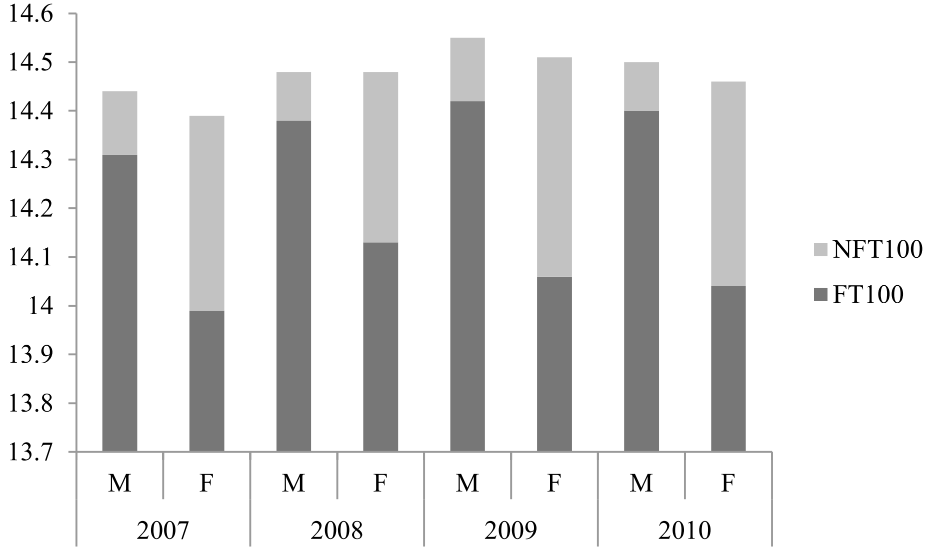


Fig. 2. Least squares (LS) means for number of functional teats (FT) and non-functional teats (NFT) at 100 kg live weight by birth year in males (M) and females (F). NFT100 + FT100 = TOT100

The average TOT3 was the same as that found for TOT100 for both males and females. In total, 98% of both the males and the females had exactly the same total number of teats at both occasions. There was a significant effect of birth herd on all the teat traits analysed (both gender). For all herds, the number of non-functional teats was higher at 100 kg live weight than at three weeks of age. Birth month had a significant influence on TOT100, with the highest number in August.

The estimated heritability of total number of teats and that of number of functional teats was moderate (paper II and III), both at three weeks of age and at 100 kg live weight (paper II). The heritability for the number of non-functional teats was low (Paper III) and for the males it was low both at three weeks of age and at 100 kg live weight (Paper II). Surprisingly, at 100 kg live weight, the heritability of non-functional teats was moderate for females (0.29).

Table 3. Estimated heritability (with SE as subscript) including both gender, within gender, and the genetic correlation between genders, within trait.

Trait	Both gender	Male	Female	Genetic correlation[a]
FT3		$0.41_{(0.01)}$	$0.39_{(0.01)}$	$0.99_{(0.01)}$
FT100	0.31	$0.42_{(0.02)}$	$0.36_{(0.02)}$	**0.84**$_{(0.02)}$
NFT3		$0.02_{(0.01)}$	$0.02_{(0.01)}$	$1.00_{(0.06)}$
NFT100	0.09	$0.03_{(0.01)}$	$0.29_{(0.02)}$	**0.72**$_{(0.06)}$
TOT3		$0.40_{(0.01)}$	$0.38_{(0.02)}$	$0.98_{(0.01)}$
TOT100	0.39	$0.42_{(0.02)}$	$0.38_{(0.01)}$	$0.98_{(0.01)}$

[a] Correlation in bold are significantly different from unity.

Traits: FT3 = Number of functional teats at three weeks of age; NFT3 = Number of non-functional teats at three weeks of age; TOT3 = Total number of teats at three weeks of age; FT100 = Number of functional teats at 100 kg live weight; NFT100 = Number of non-functional teats at 100 kg live weight; TOT100 = Total number of teats at100 kg live weight.

Number of functional teats and number of non-functional teats were both positively correlated with the total number of teats (Paper II and III) and the genetic correlation, between the total number of teats at three weeks of age and the total number of teats at 100 kg live weight was high for both genders (Paper II). In paper III the genetic correlation between FT100 and NFT100 was negative, but within gender (paper II) the genetic correlation between FT100 and NFT100 was low and not significant in males and showed a strong negative correlation for females (–0.69). For number of functional teats as well as non-functional teats the genetic correlation between genders was high (0.99–1.0) at three weeks of age, but somewhat lower (0.72–0.84) at 100 kg live weight (paper II).

Table 4. Genetic correlations (with SE as subscript), within gender, between different teat recordings.

	Gender	FT100	NFT100	TOT3	TOT100
FT3	M	$1.00_{(0.00)}$	$0.31_{(0.09)}$	$1.00_{(0.00)}$	$1.00_{(0.00)}$
FT3	F	$0.63_{(0.02)}$	$0.14_{(0.04)}$	$1.00_{(0.00)}$	$1.00_{(0.00)}$
FT100	M		$0.18^{a}_{(0.10)}$	$0.99_{(0.00)}$	$0.99_{(0.00)}$
FT100	F		$-0.69_{(0.02)}$	$0.63_{(0.02)}$	$0.64_{(0.03)}$
NFT3	M		$0.69_{(0.08)}$	$0.62_{(0.07)}$	$0.58_{(0.08)}$
NFT3	F		$0.26_{(0.10)}$	$0.46_{(0.09)}$	$0.57_{(0.08)}$
NFT100	M			$0.32_{(0.09)}$	$0.29_{(0.09)}$
NFT100	F			$0.14_{(0.04)}$	$0.12_{(0.04)}$
TOT3	M				$1.00_{(0.00)}$
TOT3	F				$1.00_{(0.00)}$
TB1	M+F	$-0,06^{a}_{(0.0.8)}$	$0.20_{(0.10)}$		$0.05_{(0.11)}$
TB2	M+F	$-0.02^{a}_{(0.08)}$	$0.05^{a}_{(0.11)}$		$0.05_{(0.11)}$
LW1	M+F	$0.02^{a}_{(0.08)}$	$-0.06^{a}_{(0.08)}$		$0.05_{(0.11)}$
LW2	M+F	$0.13^{a}_{(0.08)}$	$-0.04^{a}_{(0.10)}$		$0.05_{(0.11)}$
SB1	M+F	$-0.02^{a}_{(0.09)}$	$0.12^{a}_{(0.12)}$		$0.05_{(0.11)}$
SB2	M+F	$-0.16^{a}_{(0.11)}$	$0.34_{(0.14)}$		$0.05_{(0.11)}$

[a] Correlation not significantly different from zero.

Traits: FT100 = Number of functional teats at 100 kg live weight; NFT100 = Number of non-functional teats at 100 kg live weight; TOT100 = Total number of teats at 100 kg live weight; TB1= Total number of born piglets first parity; TB2 = Total number of born piglets in second parity; LW1 = Number of liveborn piglets in first parity; LW2 = Number of liveborn piglets in second parity; SB1= Number of stillborn piglets in first parity; SB2 = Number of stillborn piglets in second parity.

Significant genetic correlation between NFT100 and total number of piglets born in first parity was found, as well as between NFT100 and number of stillborn piglets in second parity. The only significant genetic correlation found between the teat traits and performance traits (paper II) was for male pigs; the genetic correlation between the number of non-functional teats at three weeks of age and the age at 100 kg was –0.2, indicating that slowly growing pigs have a lower number of non-functional teats.

A genome-wide association study for inverted teats in sows reveals novel candidate genes (Paper IV)

The combination of well monitored phenotypes and available genomic tools including the draft genome sequence and genotyping platforms make the pig attractive for gene mapping studies and genome-wide association (GWA) studies. One important criterion of the maternal ability of the sow is the number of functional teats. Each piglet needs its own functional teat for supplement of colostrum and survival during the first weeks of the nursing period. The inverted teat is a common type of non-functional teat with genetic background. These teats are difficult to identify on the gilt before her 1st lactation period, and therefore phenotypic selection remains difficult. The aim of this study was therefor to identify chromosome regions associated with the total number of teats, number of functional teats and number of inverted teats.

Material and methods

The pigs in this study, 230 Swedish Yorkshire pigs from the breeding company Nordic genetics (www.nordicgenetics.com), were selected based on the number of inverted teats and kinship. At performance testing, technicians from Nordic Genetics routinely score animals for the total number of teats, the number of functional teats, the number of non-functional teats, as well as other productions traits such as weight and side fat thickness. Pedigree information and detailed data on teat traits are stored in Nordic Genetics databases. Our approach was, based on the relatedness in the Swedish Yorkshire population, to collect full sib pairs of the same gender including one pig with inverted (case) and one pig without inverted teat (control) out of the same litter. When inverted teats at performance testing were present we visited the current herds and collected blood samples. Blood samples were collected in EDTA tubes from the jugular vein of each pig and stored in -20^{o}C. The technicians from the Nordic Genetics in south of Sweden did also collect samples from these herds. We managed to collect 401 blood samples in 7 different herds during the period 2008-2012. Based on the criteria above, 100 full sib pairs and 15 pairs from different litters were chosen for further analysis.

DNA was extracted from blood samples of all pigs using the QIAGEN QIAsymphonySP Midi kit (www.qiagen.com). The DNA concentration was measured using the Nano Drop 8000 spectrophotometer (Thermo Scientific) and ranged between 49-444 ng/ul (on average 232 ng/ul), and 260/280 ratio > 1.50 (1.79-1.87). The samples were genotyped using the Illumina

PorcineSNP60K BeadChip (Illumina, San Diego, USA) containing 61,565 SNPs.

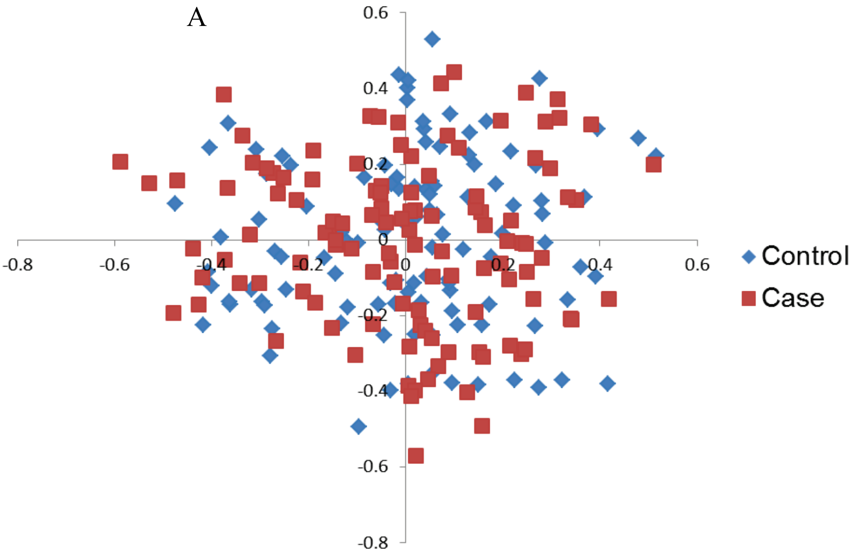
Poorly genotyped and precarious data was excluded. This quality control excluded markers with low call-rates (<0.95) and minor allele frequencies (<0.03), resulting in a total of 46,652 SNPs that were included in the association analysis using the GenABEL software. Possible subpopulations were estimated using identity-by-state (IBS) distance clustering. We expected to see a subpopulation, since twelve of the samples had Finnish ancestors. To view for population stratification in the sample population, quantile-quantile plot (QQ-plot) was used. Under the null hypothesis of no association at any SNP, the points would be expected to follow the slope lines. Deviations from the slope lines correspond to loci that deviate from the null hypotheses. Inflated line with inflation factor above 1, indicate population stratification or difficulties to define relatedness. With population stratification the risk is false-positive results, showing the population structure instead of an association with the trait and genes. Deflated line, indicate not consistent phenotyping of the samples or difficulties to define relatedness.

Phenotypic records used in the analyses were total number of teats (TT), number of functional teats (FT) and number of inverted teats (IT). To avoid false-positive result and without the parents genotypes in the sib-pair approach (Boehnke & Langefeld 1998), the GRAMMAR-gamma (Amin et al. 2007; Aulschenko et al. 2007a; Chen et al. 2007) implemented in the version 1.7-4 of GenABEL software (Aulschenko et al. 2007b), through R ver. 2.15.1 (http://CRAN.R-project.org), was suitable for the genome-wide association analysis. The GRAMMAR-gamma approach first accounts the residuals with linear mixed model and in a second step treating the residuals as phenotypes and test the markers effect (Svischeva et al. 2012). For the teat traits we included sex, birth herd and birth month as fixed effects in our model, based on previous study (Chalkias et al. 2013). Corrections for multiple testing and significant levels were set with Bonferroni significant level.

Results

IBS distance clustering showed that the pigs with Finnish ancestors could be classified in the Swedish population (Figure 3), suggesting no significant genetic difference existed between these two groups. The analysis for inverted teats did not reveal any significant association using Bonferroni correction for multiple testing. Both for number of inverted teats and number of functional teats, the QQ-plots were deflated with an inflation factor (λ) of 0.65 and 0.74 respectively. However we identified the same top markers for IT and FT and also markers in the same region on chromosome 2 and 18 (Figure 4-5). The empirical p-value of these markers did not exceed the significant threshold. The marker ASGA0093674 is in the SPRY4 gene. SPRY4 is an inhibitor of the mitogen-activated protein kinase (MAPK) and suppresses the insulin receptor. ALGA0098367 and DRGA0017032 are located 13 kb from each other and about 60 kb from CPVL gene. The protein encoded by this gene is a carboxypeptidase, but the exact function of this protein, however, has not been determined. A number of candidate genes for IT including PTHLH have previously been discussed (Tetzlaff et al. 2009; Martínez-Giner et al. 2011), however we could not identify a significant association on chromosome 5. We identified top markers for TT on chromosome 6, 8, 10, 13, and 14 (Table), but the empirical p-value of the top markers were not significant. One of the most significantly associated marker where identified on chromosome 14.

The analysis for inverted teats and functional teats had same top markers in the suggestive genes and for total number of teats we also had markers within the same region. Our study could identify new suggestive candidate regions for the teat traits investigated, suggesting that some regions might harbour favourable (total number of teats and functional teats) and unfavourable (inverted teats) alleles, but need to be further investigated. The present results indicate that the number of functional and non-functional teats is a complex trait and may be influenced by many genes with small effects.



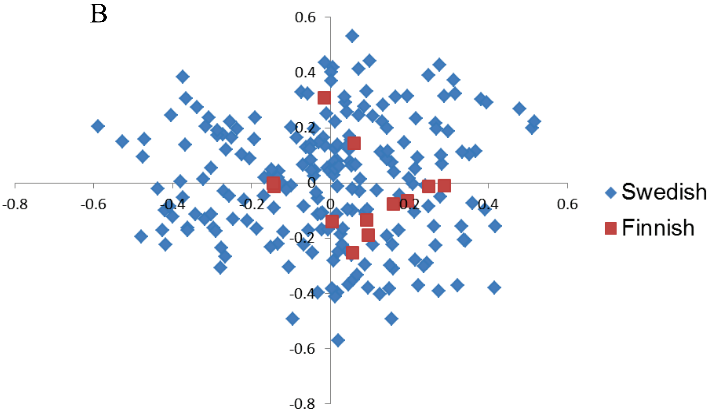


Figure 3. Multi Dimensional Scale (MDS) plot of the entire sample set of animals MDS-plot of the sample population. (A) showing the pigs with inverted teats (case) versus pigs without inverted teats (control) indicating cases in blue and controls in red (B) showing the pigs with Finnish ancestors (red) versus the Swedish pigs (blue) belonging to sample population

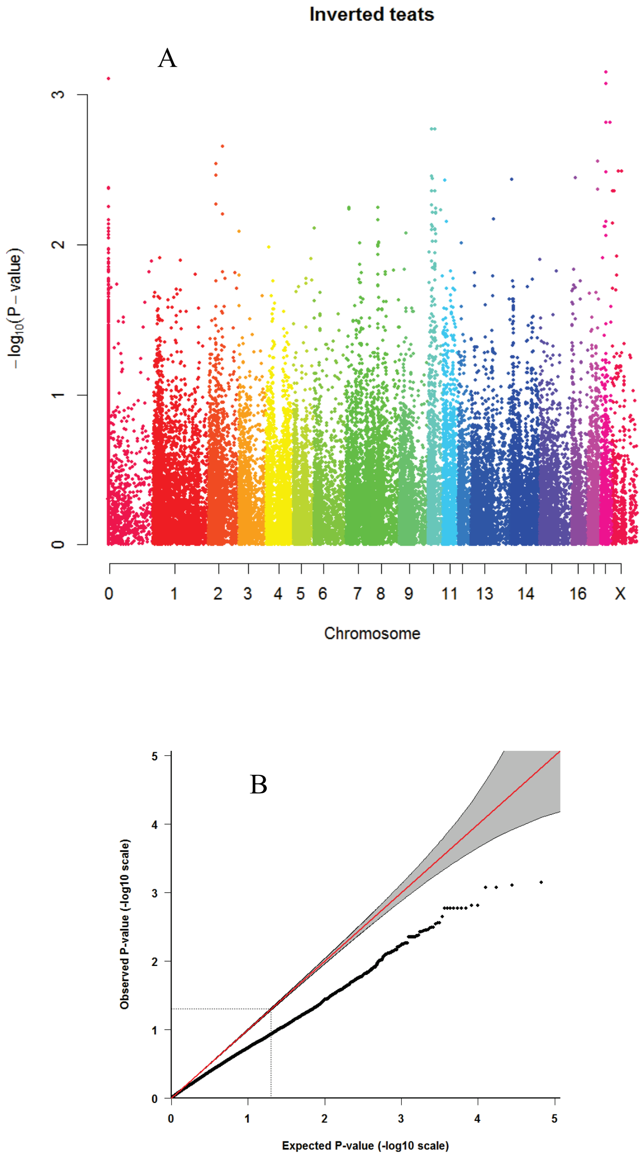


Figure 4. The association with the number of inverted teats and QQ-plot for the number of inverted teats. (A) Manhattan-plot showing the negative logarithm of the p-value from the genome-wide association analysis for the number of inverted teats. Different colours represent chromosomes from 1 to X; results from unmapped markers not yet mapped to the pig reference genome are shown on the left side. (B) QQ-plot from the Grammar-Gamma analysis, inflation factor =0.65

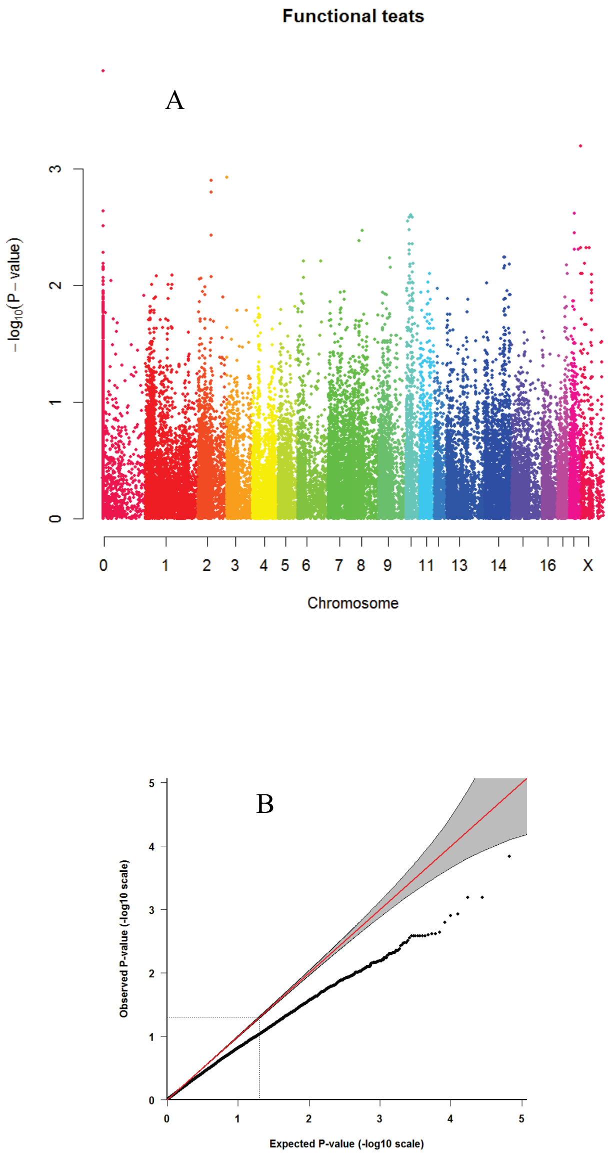


Figure 5: The association with the number of functional teats and QQ-plot for the number of functional teats. (A) Manhattan-plot showing the negative logarithm of the p-value from the genome-wide association analysis for the number of functional teats. Different colours represent chromosomes from 1 to X; results from unmapped markers not yet mapped to the pig reference genome are shown on the left side. (B) QQ-plot from the Grammar-Gamma analysis, inflation factor =0.74

General discussion

A major goal of the pig breeding is to produce healthy, fast-growing piglets and sows with good longevity, which can raise many uniform litters. The fitness of the sow, including the presence of sufficient number of functional mammary glands and functional teats, is one key to the success in the initial rearing period of the piglets. This study clearly shows the importance of an adequate number of well-developed and well-functioning teats for the growth and development of the piglets.

Teat development

Both genes and environment influences the quantity and quality of the teats in the pig. In the sow uterus, the future number of teats in the piglet is influenced by the sex ratio of the litter (Drickamer *et al.* (1999).

A high growth rate in gilts leads to early puberty, while low fat deposition delays puberty. The stage of development of the teats at 100 kg live weight could thus be influenced both by age and by fat deposition. The genetic correlation for males between the number of non-functional teats at three weeks of age and the age at 100 kg was negative and significant; the correlation for females was also negative but not significant. This may imply that decreased growth rate, i.e. in pigs that are older at 100 kg live weight, results in fewer non-functional teats. This indicates that the fast growing gilts may be too young when they start their reproductive life.

The number of teats in the sow has a large impact on her production results. Skjervold (1963) found a significant correlation between teat number in the sow and the number of piglets in the litter at three weeks of age. A positive genetic correlation between these traits should mean that selection for a larger litter size would lead to an increase in the number of teats. In this study no significant genetic correlation between the litter size and the number of teats was found, indicating that selection for larger litters will not result in more teats. This agrees with a previous study by Zhang *et al.* (2000).

The nursing sow

Wold (2009) showed that the major part of the non-functional teats in Norwegian Landrace was composed of inverted teats. The gilts in the present study had various numbers of inverted teats at 100 kg live weight, but it was not possible to predict whether or not the teats would protrude before farrowing. This might explain the different opinions reported among the pig farmers concerning the importance of the inverted teat (pers. communication 2008, Swedish Yorkshire nucleus herds).

Following performance testing, animals that do not have enough functional teats are usually culled. This has led to a lack of information on the development of the inverted teat after performance testing. According to the literature, the inverted teat can sometimes protrude at farrowing (Nordby, 1934) and one theory is that the increased mammary gland volume and pressure will force the inverted teat to protrude. In our study, to our surprise, most of the inverted teats protruded already in early gestation. This is in agreement with Thorup (2012) reporting that out of 25 inverted teats recorded at mating, 10 that were rechecked at weaning of the first litter were functional.

The present study concludes that teats that are still inverted at farrowing are of no value to the suckling piglets. Therefore, it is necessary to emphasise the importance of this trait in the genetic evaluation and that nucleus herds exclude animals with inverted teats to avoid the risk of accumulation in the population. In the breeding program for Norwegian Landrace (Norsvin, 2013), the total teat number and the number of inverted teats have been included in the genetic evaluation since 2001 and 2010, respectively. This has led to a clear reduction in number of inverted teats and an increase in the total number of teats (pers. commun. Dan Olsen, Norsvin).

The milk production of the sow is important as emphasized throughout this thesis. Milk production is at a maximum in the second week p.p. and thereafter it will become a limiting factor for piglet growth, especially in large litters (Auldist *et al.*, 1998), although the milk yield may be increased by frequent nursing (Auldist *et al.*, 2000). The small teats clearly had less corresponding mammary gland tissue and the piglet growth rate was also lower, but the possible effect of an increased nursing activity may be doubted in these cases.

A high number of non-functional teats were genetically associated with more stillborn piglets and this might indicate that genes causing non-functional teats also have a negative impact on the piglets' ability to survive birth. This has major implications in commercial production and needs to be investigated further.

The small but significant correlation between mammary gland weight and the position of the teat pair is in agreement with Skok *et al.* (2007) and Wu *et al.* (2010). According to Orihuela and Solano (1995) these differences may also depend on the piglet birth weight, since heavier piglets may massage the udder more vigorously, resulting in a greater blood flow, thereby increasing the oxytocin release (Fraser, 1984; King *et al.*, 1997). Both Kim *et al.* (2000) and Nielsen *et al.* (2001) presented a positive correlation between mammary gland weight and daily weight gain of the piglets. This is in agreement with our results in the present study.

Piglet production

To increase profitability in piglet production, a selection has been made for larger litters. The larger litter size leads to lower birth weight and heterogeneous weight within litter (Roehe, 1999; Wolf *et al.*, 2008; Canario *et al.*, 2010) as well as heterogenic maturity at birth (Herpin *et al.*, 1993). One of the farrowing gilts in the clinical study had 18 liveborn piglets and both the average birth weight and the weaning weight was low. Several of the piglets were weak and four were euthanized at birth, due to weakness and the fact that the number of teats was less than the number of piglets. This was also one of the sows that were treated and this sow was not able to expose the entire udder during the first days of lactation, thus further decreasing the piglet milk yield.

The increased number of piglets born has led to the use of cross-fostering to supply a teat to each piglet. However, it cannot solve the problem if there are more piglets than functional teats in the batch. Therefore the number of functional teats is still important. Producers claim that litter size today is large enough, but the homogeneity of the litter should be increased to enable the sows to raise their litter without much human interference. In other multiparous species such as the dog, this problem is unknown since the selection has not been as intensive for larger litters. In addition, the fact that dogs are companion animals makes artificial nursing easier. Further, puppies do not require a nipple of their own and they do not establish a nipple order (Arteaga *et al.*, 2013).

Many of the selected gilts in our clinical study had health problems, returned to oestrus or died before insemination. In the Swedish piglet production, around 20% of the gilts are culled before first lactation and most of these, about 50%, are culled due to reproductive disorders (pers. communication 2013, Engblom L.). This warrants further investigation and correlation between non-functional teats and reproduction disorders or health disorders should be estimated.

Poor udder health is a large cause of the sow removal in Swedish commercial herds (Engblom *et al.*, 2007), thus emphasizing the importance of the sow's lactating performance. The decreased piglet weight and growth rate in the piglets from the treated sows highlights the importance of a good health status in the sow especially during the lactation, since the high milk production in the sow, similarly to the dairy cow, is very demanding. Overall, this study emphasizes the complexity of the interaction between the nursing sow and piglets and the importance of a good health status in the sow especially during lactation.

Gender differences

For all teat traits analysed there were significant differences between males and females. The recorded numbers of non-functional teats were still as low at 100 kg as at 3 weeks of age in males, but almost three times higher in the females. Further studies on the development of non-functional teats between 3 weeks of age and 100 kg live weight is needed.

Differences between sexes might be caused by biological factors or by subjective differences in the recording. The development of mammary cells during puberty in the sow is controlled by a complex signalling network. This may have an impact on teat development in females that results in the difference noted at 100 kg. Also, more emphasis may be put on the gilts because they are the potential mothers of the next generation. In addition, there is an obvious risk that some non-functional teats may be missed at the recording, especially in males. For example, extra teats (narrow teat spacing) around the umbilicus can be missed if it is not possible to make observations from both sides of the penis. In contrast to this study Beilage (1996) reported a higher prevalence of inverted teats of males at slaughter. In our study there were differences between males and females in the number of functional teats: males showed a higher number of functional teats at 100 kg live weight. This agrees with the study by (Zhang *et al.*, 2000), which was based on pigs from Chinese × European breeds.

For both the number of functional and the number of non-functional teats at three weeks of age, the genetic correlation between the genders was high. At 100 kg live weight the genetic correlation was lower, which indicates that these traits at 100 kg live weight are not really the same in males and females.

For the total number of teats, the genetic correlation between three weeks of age and at 100 kg live weight was 1.00 for both genders, which indicates that these parameters measure the same trait.

Heritabilities

The heritability of the number of inverted teats in the Swedish Yorkshire breed has not been estimated, due to the fact that the trait is not recorded. However Long *et al.* (2010) estimated the heritability in the Norwegian Landrace to 0.3 in gilts and slightly lower in boars.

The low heritability for the number of non-functional (not only inverted) teats recorded at three weeks of age, argues that this trait should not be included in the genetic evaluation.

The heritability for non-functional teats at 100 kg live weight was moderate for female but still low in males and concludes that records on females could be included in the genetic evaluation.

Including the number of functional teats with a heritability around 0.3 in the genetic evaluation, could easily result in a substantial genetic progress also for this trait.

In present study, both males and females had a moderate heritability for the total number of teats at three weeks of age as well as at 100 kg live weight. The heritability for the total number of teats in previous studies varies from low (Enfield and Rempel, 1961; Smith et al., 1986; Ligonesche et al., 1995) to moderate (Von Willham and Whatley, 1963; Pumfrey et al., 1980; Toro et al., 1986; McKay and Rahnefeld, 1990; Rydhmer, 2000; Hirooka et al., 2001; Fernández et al., 2004).

The genetic associations among the litter traits show that the number of stillborn piglets in the litter increase with increased number of total born piglets, and that there is a negative association between litter size and litter weight.

Genome-wide association

The molecular techniques provide complement to the traditional genetic evaluation to improve accuracy and speed up the genetic improvement. The present study found chromosome regions associated with inverted teats on chromosome 2, 10 and 18; with the functional teats on chromosome, 2, 3, 10, 18 and X; and with the total number of teats on chromosome 6, 8 10, 13 and 14. QTL have been previously reported on chromosome 2 both for inverted teats (Jonas *et al.*, 2008) and for extremely small teats (Sato *et al.*, 2006). Another QTL for inverted teats on chromosome 18 have been reported, although with a confidence interval exceeding almost the whole chromosome (Jonas *et al.*, 2008). Surprisingly, the associated markers for inverted teats were located within the same region and even the same markers were associated. Interestingly, for functional teats, the fibroblast growth factor 1 (*FGF1*) is a suggestive candidate gene. In the mouse, *FGF1* is assumed to be involved in promoting mammary ductal development during sexual maturity. For the inverted teat, the aquaporin 3 is a current candidate gene. Aquaporin 3 is a glycerol transporter in the mammalian skin (Hara-Chikuma & Verkman, 2008).

Most of the associated markers for total number of teats were identified on the chromosomes 6, 8, 10, 13, and 14. The associated markers were not in the same region as the markers for inverted teats and functional teats. Interestingly, no previously identified QTL had been reported on chromosome 14, where we identified one of the most significantly associated marker, but QTL's on chromosomes 6, 8, 10 and 13 among most other chromosomes are also reported in the PigQTL database (Hu *et al.*, 2013).

Our study could identify new suggestive candidate regions for the number of functional teats and the number of inverted teats, proposing that some regions might harbour favourable alleles. One of the hypotheses for the lack of identification of strong associations is that animals from the Yorkshire population have been highly selected for maternal traits, including number of teats. It is possible, that some of the unfavourable alleles have a minor allele frequency below the threshold used for the quality control. Furthermore, animals used for this study are from a single breed highly selected for maternal traits, including number of teats. The observed phenotypes did verify this, as no animal with an extremely high or low number of teats was found.

One of the hypotheses was that the Finnish offspring would differentiate from the rest of the population in the MDS-plot, but the results displayed that they fitted well with the Swedish Yorkshire population. During the 1980's there were some exchanges of animals between the countries. The deflated QQ-plots may indicate non-consistent phenotyping and the technicians may have an effect, which needs to be further investigated. Almost all studies on Genome-wide association methods discuss the problem with population stratification with inflated QQ-plots, but there is very little information regarding this problem in the literature.

The present results suggest that the total number of teats, the number of functional and number of inverted teats are complex traits and may be influenced by many genes with small effects, or simply that any major gene was not tagged by the SNP chip.

Implementation in the genetic evaluation

This study concludes that there is no genetic correlation between the litter size and the three teat traits. Therefore selection for litter size requires that at least one teat trait also is included in the breeding goal. With no selection on teat traits (or just a phenotypic threshold selection of gilts and young boars for replacement) there is a risk that the number of functional teats will become a limiting factor for piglet growth. This might be of increasing importance, when the genetic improvement for litter size is substantial.

At present selection for pig teats in Sweden is performed by both threshold selection and by inclusion in the breeding evaluation. Breeding gilts are required to have at least 14 functional teats. During the last 20-year period (1992-2012), the mean total number of teats increased from 14.4 to 14.6 in the Swedish Yorkshire pig. This mere increase of 0.2 functional teats demonstrates that this selection method is not an efficient way to improve the number of functional teats. In addition, almost every fifth breeding gilt has at least one non-functional teat. The threshold selection is not addressing this issue and it will therefore not solve the problem.

In this study the number of functional teats and total number of teats have a high positive genetic correlation and thus it is enough to include one of the traits in the genetic evaluation. Total number of teats is the easiest teat trait to record objectively, because it requires merely that all teats are counted; there is no need to consider the possible teat function during nursing, but there is one bias that may occur; the rudimental teats. It is important that these rudiments are equally recorded. The genetic correlation between number of non-functional teats and total number of teats is also positive and significant but not as strong as between functional teats and total number of teats.

In this study there were also significant differences between herds for the teat traits. This is interesting since the genetic material is quite similar, since the same AI-boars are used. The technicians often visit different herds but some herds have more than one technician visiting the herds at regular intervals. This should be further investigated to see the effect of technicians and heritabilities at herd levels. Also, this observed difference between herds might indicate strong influence of environmental factors.

An accurate identification of non-functional teats early in life would be of great value, because it would permit early selection, reduce the cost for the recording as well as reduce the workload of the technicians. Identification of the genes that influence the development of non-functional teats should allow for a more accurate selection against this trait. Identification of genes that influence the number of functional teats or total number of teats should allow faster progress on that or those traits. However, at this stage we have not identified the genes and further research is required before marker-based selection could be implemented.

For recording, the total number of teats registration at three weeks of age is to prefer. This number is in most cases the same as at 100 kg live weight but recorded earlier, easier and on more animals, making the later registration unnecessary. The genetic correlation between the number of functional teats and total number of teats is high and therefore only one of them is necessary to include in the breeding evaluation. Instead additional attention should be on reducing non-functional teats. Non-functional teats on the gilts at 100 kg can be included already today, but the alternative recording of the inverted teats should be discussed. Also the recording of occurrence or the actual number of non-functional teats needs to be discussed. At 100 kg live weight the knowledge whether the inverted teat will protrude or not is lacking, but this study indicate that even if they do they are not of the same value as the functional teats. Selection against inverted teats have been implemented in Norway with good results.

Conclusions

This thesis has assisted in answering important questions about teat traits in the pig. There are still questions that need to be emphasised and new questions have been raised which emphasises the complexity of the interaction between the nursing sow and its piglets. The conclusions made based on the results from the studies (given in roman numerals in the margin) are:

I The teat quality at 100 kg live weight is not a valid predictor of the function of the teats at farrowing, since many of the inverted teats at 100 kg will protrude during early gestation

I If the inverted teat is still inverted at farrowing it will not supply enough milk to enable a suckling piglet to survive. Small teats are less valuable for the suckling piglets and the corresponding mammary gland have less weight.

II-III At three weeks of age the presence and heritability of the number of functional and non-functional teats is the same for males and females

II-III At 100 kg live weight, the number of functional and the non-functional teats differ between gender as well as the heritability of the number of non-functional teats

II-III The number of functional teats and inverted teats are heritable traits and the number of functional teats can be improved by selection. Similarly, the presence of number of non-functional teats can be decreased by selection

II-III The total teat number is accurately recorded in the young pig

II-III There is no genetic correlation between the litter size and the total number of teats and further selection on increased litter size should include the number of functional teats in the genetic evaluation.

IV The three teat traits total number of teats, number of functional teats and number of inverted teats are likely to be traits with a complex inheritance

IV We have identified chromosome regions and candidate genes for the total number of teats, the number of functional teats and the number of inverted teats

Future prospect

- We were able to show that the inverted teats have a developing phase between the age of 5-6 month (performance testing) and early gestation, as shown in paper I. Two important questions still remain to be answered; how does the non-functional teats develop from performance testing until farrowing, i.e. how many of the inverted teats will protrude and can we predict this? This is a topic that needs priority, but the high cost for such a study is a limiting factor. The pig production industry would however benefit from such knowledge.

- Further behaviour studies on nursing in the presence of non-functional teats would be interesting to investigate. Our study lack 24-hours video recording or comprehensive registrations of behavioural parameters. Has the non-functional teat a negative effect on the nursing behaviour?

- Four sows developed fever during nursing period, but none developed mastitis. Is the risk for mastitis really higher with inverted teats?

- After the gilts were selected for the study, they were kept for insemination. A large part of the selected gilts, returned to oestrus, had health problem or died. This leads to the question; are non-functional teats and reproductive disorders correlated?

- How does the milk composition vary within and between dam line breeds?

- The genetic correlations among the teat traits show that both functional and non-functional teats increase with total teat number.

This means that selection for a higher total number of teats will increase the number of functional as well as non-functional teats. This raises the question whether a non-functional teat disturbs the function and use of the surrounding functional teats. How much do the non-functional teats disturb the suckling behaviour of the piglets suckling the functional teats?

- We have estimated the heritability for number of non-functional teats in the population of Yorkshire registered by Nordic Genetics. The registration of teats at three weeks of age is performed by the herdsmen, i.e. by a different individual for each herd, and at 100 kg live weight different technicians perform the recording in different herds. It would be interesting to estimate the heritability of the teat traits at herd level and the genetic correlations among herds for these traits.

- The chromosome regions for the most associated markers for IT, FT and TT should be further analysed. Even though the empirical p-value of these markers did not exceed the significant threshold, we consider the result as interesting. As mentioned before, the Bonferroni method is conservative. The candidate regions could be analysed in more detail and with incorporation of haplotype patterns.

Genetiska och kliniska studier av spenar hos grisen

Avhandlingen handlar om onormala spenars utveckling, funktion och genetiska bakgrund. Syftet är att främja avel för fungerande spenar och därmed högre smågrisöverlevnad och bättre smågristillväxt.

Grisens livmoder skiljer sig från många andra djurslag genom att de livsnödvändiga antikropparna inte kan passera den så kallade placentabarriären. Den nyfödda grisen föds därför med låg immunitet och är helt beroende av att få i sig skyddande antikroppar från råmjölken som är den första mjölken som produceras i suggans juver. Eftersom spädgrisar föds med låg immunitet och även med små energireserver, är det i bokstavlig mening livsnödvändigt för den unga grisens överlevnad att den snabbt får i sig råmjölken. Suggans mjölknedsläpp som sker under korta intervall (ca 20 sekunder, en gång i timmen), gör det viktigt att varje smågris har tillgång till var sin fungerande spene. Om antalet funktionsdugliga spenar hos suggan är färre än antalet födda smågrisar, riskerar vissa smågrisar att bli utan råmjölk och mjölk. Genom avel har kullstorleken ökat och det ställer högre krav på att antalet fungerande spenar. En av de vanligaste typerna av icke-fungerande spenar är s.k. inverterad spene, där spenspetsen är indragen, vilket försvårar eller helt omöjliggör diandet av denna spene. I genomsnitt har vår svenska moder-ras yorkshire drygt 14 fungerande spenar, men därtill har mer än var sjätte sogris minst en inverterad spene.

Syftet med artikel I var att se om tillväxten hos smågrisarna skiljer sig mellan de som diat normala spenar respektive diat inverterade spenar eller inverterade spenar som ”ploppat ut” under dräktigheten. Vi kunde visa att om en spene fortfarande är inverterad vid grisningen klarar inte den nyfödda grisen att dia denna spene och överlever därmed inte. Det är anmärkningsvärt att flertalet inverterade spenar “ploppade ut” före grisning, redan två månader före grisning. Gyltor med inverterade spenar vid ekolodning bör ändå inte användas för avelsändamål. Tillväxthastighet och avvänjningsvikt för de grisar som diat ”utploppade spenar” var numeriskt lägre än för de grisar som diat normala spenar.

Svenska avelsorganisationen Nordic Genetics registrerar spenantal och spenkvalitet på renrasiga grisar vid 2 olika tillfällen: då djuren ID-märks vid ca 3 veckors ålder och vid ekolodning. I stort sett alla djur ID-märks och ca 80 % av de ID-märkta djuren ekolodas. Registreringen vid 3 veckor utförs av uppfödaren. Normala och onormala spenar räknas. Inverterade, små spenar och ”extraspenar” betraktas som onormala. Vid 3 veckor är det svårt att skilja inverterade spenar från normala. Registreringen vid ekolodning utförs av avelstekniker från Nordic Genetics. Bedömningen sker då djuret befinner sig i vågen, och teknikern för in handen mellan vågens spjälor, tittar på spenarna samt känner och drar i spenar som bedöms vara onormala. Denna bedömning upplevs som jobbig. Syftet med artikel II och III var att beräkna arvbarheten för antalet spenar, normala spenar och onormala spenar. Även det genetiska sambandet mellan spen-, produktions- och reproduktionsegenskaper analyserades. Resultaten visade att spenkvaliteten kan förbättras via avelsurval. Antal spenar och normala spenar har en moderat arvbarhet och onormala spenar har en låg arvbarhet vid 3 veckor, för gyltor vid ekolodning var den dock högre. Vi kunde inte hitta något genetiskt samband mellan spenantal och kullstorlek.

Avel för fler spenar kan genomföras genom att räkna antal spenar vid tre veckor och ta med egenskapen i avelsvärderingen. Avel för färre inverterade spenar kräver en lättare och säkrare metod. I artikel IV var vårt mål att identifiera kromosomregioner och gener som inverkar på uppkomsten av inverterade spenar för att på ett säkert sätt kunna välja ut djur med förutsättningar att fungera som goda mödrar och därigenom förbättra smågrisarnas överlevad. För att hitta kromosomregioner i genomet som skiljer sig mellan grisar med inverterade spenar och grisar utan inverterade spenar, genomförde vi en så kallad Genome Wide Association (GWA) analys av grisens genom. Analysen innebär att man med hjälp av markörer (drygt 60000 hos gris) över hela genomet letar efter områden i genomet som kopplar till den egenskap man undersöker. Vi hittade markörer associerade till inverterade spenar och även fungerande spenar. För att kunna identifiera den exakta genen eller mutationen som påverkar en egenskap måste varje position i det associerade området analyseras vidare, s.k. finmappning. Inverterade spenar kan förväntas bero på flera gener som samverkar och även miljön spelar in.

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