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Importance of Epigenetics in Animal Breeding: Genomic Imprinting

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Epigenetik i Husdjursavel: Genomisk Prägling

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Abstract

The aim of this study was to give an overview of the evidence for genomic imprinting in livestock and other mammals as well as outline the potential use of gene imprinting in livestock breeding. Epigenetics is the mitotical and meiotical partial hereditary variation in genomic activity without any alterations of the DNA sequence. An example of epigenetic regulation is genomic imprinting where one allele's expression differs depending on which parent it was inherited from. These parent-of-origin effects are currently overlooked in livestock production. Many economically important traits in livestock are so called quantitative traits, some of which have shown indication of being partially controlled by imprinted genes. A variety of imprinted genes have been found in livestock that affect traits such as milk yield, growth and carcass traits, fat and meat deposition and fetal development. The implementation of breeding programs taking imprinting into account, will require changes in the current standard breeding programs. More focus on the maternal contribution will be needed. It will also provide different breeding values for males and females, dominance deviations and additive genetic variances.

Sammanfattning

Syftet med denna studie var att sammanfatta tidigare studier inom området genomisk prägling (på engelska imprinting) hos lantbrukets husdjur och andra däggdjur samt diskutera potentiella användningsområden inom husdjursaveln. Epigenetik är den mitotiska och meiotiska partiella nedärvbara variationen i genomisk aktivitet som inte påverkas av DNA sekvensen. Ett exempel på epigenetiska förändringar är genomisk prägling där en allels uttryck skiljer sig beroende på vilken förälder den ärvdes ifrån. Dessa effekter beaktas inte i dagens avelsarbete trots att många ekonomiskt viktiga produktionsegenskaper till viss del kontrolleras av genomisk prägling. Flera präglade gener har hittats som associeras med egenskaper såsom mjölkavkastning, tillväxt och fosterutveckling. Implementeringen av avelsprogram som beaktar genomisk prägling kräver mer fokus på moderdjurets del i nedärvningen och kommer även att innebära skilda avelsvärden på honor och hanar, dominansavvikelser och additiva genetiska varianser.

Introduction

What is epigenetics?

Epigenetics is the partial hereditary variation in genomic activity without any alterations of the DNA sequence (Goldberg et al. 2007). Inheritance refers to the memory of such activity; transferred between cellular generations through mitosis, and between organismal generations through meiosis (Esteller, 2011). It is a link between genotype and phenotype that controls the expression of a locus. An example of epigenetic regulation is cellular differentiation; where different cells all share a common genotype but differ in phenotype (Goldberg et al. 2007). Other examples of epigenetic processes are the essential developmental mechanisms of gametogenesis, aging, embryo genome activation, X chromosome inactivation and genomic imprinting (Attig et al. 2010). Epigenetics introduces a level of genetic regulation independent to the DNA sequence, the epigenome (Reviewed by Jirtle and Weidman, 2007). Abnormality in the epigenome is related to developmental disorders and late-onset adult diseases such as mental and metabolic disorders (Attig et al. 2010).

Currently epigenetics is partly overlooked in livestock production. A great deal of research has been done on for example quantitative and molecular genetics, in contrast very little have been done in the field of epigenetics. However, in the last three decades there has been a rise in number of articles related to epigenetics, with main focus on humans, mice and plants (Attig et al. 2010). The current genetic improvement schemes in livestock assume that the expression of desirable traits is independent of parental origin (de Vries et al., 1994). These traits show a complex inheritance which is the result of multiple combined genetic and environmental factors. Until now there has been little evidence against the Mendelian inheritance and expression of genes affecting these traits (de Vries, 1994). Animal breeding theory assumes that most traits are affected by an infinite number of genes that each only contribute very little to the variance of the trait. However, through recent advancements in molecular and statistic tools, new research has shown that individual gene effects, or more precisely, effects of chromosomal regions have been detected in quantitative traits (de Koning, 2001). Some of these so called quantitative trait loci (QTL) show parent-of-origin specific effects. This is indicative of genomic imprinting, a special case of epigenetics (Cooney et al., 2002)

Genomic imprinting is the mechanism where one allele's expression differs depending on which parent it was inherited from (Monk, 1995). This implies that imprinted genes are dissimilarly altered in the egg or sperm, or perhaps seen as different in the early zygote (Monk, 1995). Imprinting is established during development of germ cells into sperm or egg (Reik and Walter, 2001). An imprinted gene functions as a haploid which makes it more vulnerable to negative mutational effects (Reviewed by Jirtle and Weidman, 2007). Consequently, a single mutation can change the epigenome. The epigenome can also change through stimulation by the environment. Deletion or mutations in imprinted genes are often connected to diseases, such as cancer, obesity, asthma, diabetes as well as several developmental and behavioral disorders (Reviewed by Jirtle and Weidman, 2007).

The aim of this study is to give an overview of the evidence for genomic imprinting in livestock and other mammals as well as outline the potential use of gene imprinting in livestock breeding.

Detection of imprinted genes

The existence of imprinting became evident more than 20 years ago, during experiments with mammalian embryos possessing only maternal or paternal chromosomes (Reviewed by Jirtle and Weidman, 2007). Their phenotypes differed greatly; those with maternal chromosomes developed normally, however their placental tissues grew poorly and the embryos died during mid-pregnancy (Reviewed by Jirtle and Weidman, 2007). Defects in maternal behaviors such as pup retrieval, nest building and placentophagy were also detected during functional loss of the paternal allele (Wilkins and Haigz, 2003). The embryos containing only paternal chromosomes on the other hand, exhibited severe growth retardation but with normal placental development (Reviewed by Jirtle and Weidman, 2007). Conclusively this meant that normal mammalian development, regardless of the actual DNA sequence, depended on different gene expression from the maternal and paternal copies (Randy et al. 2007).

By studying the genomic sequence; location, identification and prediction of an imprinted gene's preferred parental expression have been documented. Luedi et al. (2005) collected information such as statistics on repetitive elements, transcription factor binding sites and CpG islands from DNA sequences at each locus. Based on this the genes were predicted to be

imprinted or nonimprinted and subsequently the parental alleles' expression preferences were predicted for all imprinted candidates. Nezer et al. (2002) compared in their study sequence analysis on pig, human and mouse to outline organization and possible regulatory elements of three pig genes associated with QTL effects. Determining the amount of imprinted genes can be difficult due to two factors; it may be problematic to know whether or not a gene is imprinted because it is development and tissue-specific, and the definition of a gene must be defined (Spencer, 2009). Firstly, the determining of a parent-of-origin gene expression is limited to specific tissues and development stages. If the gene is studied during the wrong developmental stage, in the wrong tissue or sometimes even in the wrong individual; this could lead to the determination that a gene is not imprinted when in fact it might be imprinted. In addition, complete inactivation of an imprinted gene may not occur, even when tissues are considered to show imprinting of that gene. Some studies have showed a significant degree of biallelic expression which may falsely label the gene as not imprinted. (Spencer, 2009) Furthermore, genetic variation between alleles is essential to define biallelic expression (Knott et al., 1998).

Secondly, since a DNA sequence can be transcribed in dissimilar ways, so-called alternative splicing, to produce different functional proteins the sequence could be defined as more than one gene and not all transcripts create proteins either (Spencer, 2009).

Overview of known imprinted genes

According to the website Geneimprint (2011) there are a total number of 161 detected imprinted genes in mouse, human, pig, sheep, rat, dog and cattle and several predicted (see table 1). As seen in table 1, a larger portion of the imprinted genes overall are paternally imprinted (~50.3% see chart 1) and many of the imprinted genes are currently only defined in human and/or mouse. All of the known imprinted genes have also been found in either mouse or human, however only 29 are imprinted in both species (Morison et al., 2005). Most have first been found in these species and subsequently studied in others. Some scientists have argued that up to 600 mouse genes may be imprinted (Luedi et al., 2005). As seen in chart 1, the total number of confirmed imprinted genes in livestock is 19 and most are found in pigs. In chart 1, other refers to isoform dependent and unknown. Thus, it is quite likely that additional imprinted genes will be defined in livestock genomes in the future.

Table 1. Imprinted genes and predicted imprinted genes

		Maternal	Paternal	Isoform Dependent	Other
Imprinted Genes	Homo sapiens	23	37	4	1
	Mus musculus	38	32	4	0
	Sus scrofa	3	7	0	1
	Rattus norvegicus	1	1	0	1
	Ovis aries	1	3	1	1
	Bos taurus	0	1	0	0
	Canis lupus familiaris	1	0	0	0
Predicted	Homo sapiens	66	45	0	0
	Mus musculus	2	0	0	0
	Sus scrofa	0	0	0	0
	Rattus norvegicus	0	0	0	0
	Ovis aries	0	0	0	0
	Bos taurus	0	0	0	0
	Canis lupus familiaris	0	0	0	0

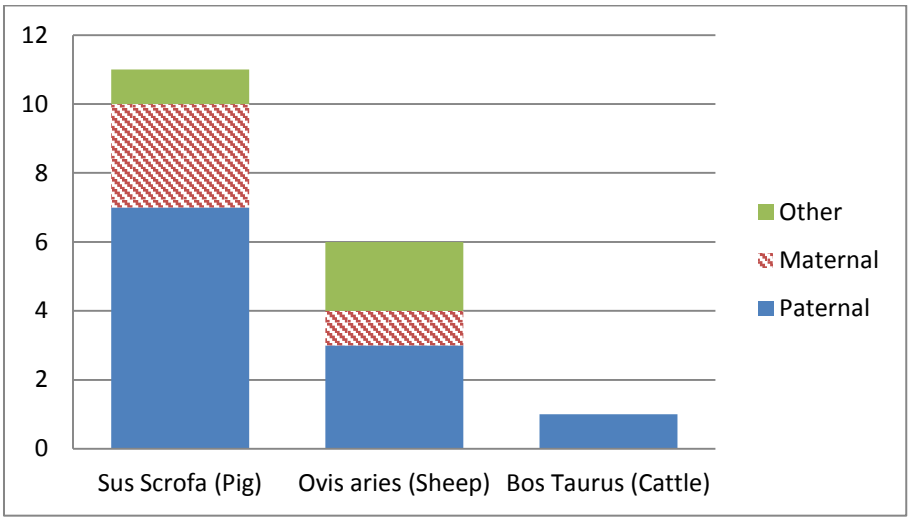

Chart1: detected imprinted genes in livestock.

Imprinting mechanism, regulation and function

Even though there are a few functional relationships between some proteins such as fetal development and growth, there have been no common features recognized when comparing protein sequences encoded by imprinted genes (Reik and Walter, 2001). However, two general features of the DNA sequence environment have been detected. An unusual abundance in CpG islands have been found; about 80% of imprinted mouse genes have these islands in comparison to the average 47%. Also, direct repeats are common within or near to these islands. These repeats are thought to be involved in maintaining differential methylation (Reik and Walter, 2001). However, Doi et al. (2009) found that during epigenetic reprogramming of human fibroblasts, extensive alterations in DNA methylation occurs on the CpG island shores' tissue specific differentially methylated regions (T-DMR) and not directly on the CpG islands. The study also proved the importance of CpG island shores and T-DMRs during development as well as somatic cell reprogramming (Doi et al., 2009).

Allele-specific expression can be viewed as a multistep developmental process that requires several factors to reach success (Pfeifer, 2000). The factors include marking, maintaining the mark, recognizing it as well as erasing and resetting it (Pfeifer, 2000).

The first factor, marking of the chromosome to its parental origin, occurs probably during gametogenesis or before the maternal and paternal chromosomes fuse in the zygote (Pfeifer, 2000). The two recognized epigenetic mechanisms for marking are DNA methylation and chromatin packaging of DNA through post-translational histone modifications (Jammes et al., 2011). The different processes of post-translational histone modifications include acetylation and deacetylation, methylation, histone phosphorylation and sumoylation. The acetylation and deacetylation processes are temporary and enzyme controlled (Jammes et al., 2011). In all histones, histone acetyltransferases (HATs) can acetylate a variety of lysine residues in the N-terminal tail of histones. This process is linked to the opening of the chromatin and subsequently allowing gene transcription. Methylation is a process which can both silence and activate transcription. It can also occur at both lysine and arganine residues; where the process is mediated by histone methylteransferases (HMTases) (Jammes et al., 2011). Histone phosphorylation is also a post-translational histone modification in which the process occurs

on serine residues and is associated with transcriptional activation. Finally, lysine residues can be sumoylated, a process involving the covalent attachment of small ubiquitin-related modifier proteins (SUMO proteins) to lysine residues (Jammes et al., 2011). The attachment or detachment of SUMO proteins to other proteins modify the proteins' functions. SUMO proteins are involved in several cellular processes such as apoptosis and stress response in cells.

The second factor, the maintenance and stability of the mark, is vital during cell division and differentiation (Pfeifer, 2000). The imprinting must, after fertilization, survive the reprogramming in the pre-implantation embryo which includes DNA methylation, alterations in histone modifications and protamine-histone exchange (Folami et al. 2008). The mark may in this case may be identical or a second derivative to its original form (Pfeifer, 2000).

The third factor involves the recognition of the parent-of-origin mark by the transcriptional machinery, resulting in a mono-allelic expression (Pfeifer, 2000). When the imprints have been presented in the germlines, sustained in the premature embryo and developed during differentiation they need to be read (Reik and Walter, 2001). This involves the alteration of methylation or chromatin imprints to differential gene expression, mainly at a transcriptional and perhaps even post-transcriptional level. This is a complex mechanism due to the fact that imprinted genes are usually clustered which involves interactions between neighboring genes and their control sequences. Around 80% of currently known imprinted genes are linked together in clusters with other imprinted genes (Reik and Walter, 2001).

The last factor is more specific to germ cells; in this step the mark must be erased and reset (Pfeifer, 2000), in somatic cells the imprints are modified and maintained during development (Reik and Walter, 2001). In the germline the resetting of imprints in mature gametes is important so that they will reflect the sex of that germline (Reik and Walter, 2001). Evidence shows that there is probably two stages for this resetting process in most imprinted genes and the first one is erasure followed by establishment. Preliminary evidence shows that methylation imprints are present and functional before erasure. In mice, by embryonic day 12-13 there is marked and genome wide demethylation in germ cells in both sexes (Reik and Walter, 2001). Current research indicates that all methylation imprints will be erased at this stage and imprints inherited from a specific parent with the same sex as the embryo are erased and changed. After erasure, experiments reveal that imprints have been severely altered and differential replication of DNA has been erased in both germlines (Reik and Walter, 2001). In female germlines the DNA has been erased during demathylation and in male germlines it occurs after birth. After erasure the process of establishment begins in both germlines at late fetal stages and continues after post-partum (Reik and Walter, 2001).

If any of these steps fail the effect will be a loss of imprinting mutation (LOI) (Pfeifer, 2000). Abnormal imprinting is supposedly a contributing factor in several human diseases and LOI occurs frequently in cancer cases (Lee et al., 1999). Other diseases which seem to be linked to genomic imprinting are Beckwith–Wiedemann syndrome (BWS), a disorder of prenatal overgrowth and Wilm's tumor which is a susceptibility to embryonal malignancies, in particular kidney tumors (Lee et al., 1999).

Current studies on livestock

Most economically important traits in livestock are so called quantitative traits. These traits can be expressed in calculable units or be measured on a continuous scale; for example

number of lambs born and weaned respectively kg of live weight are quantitative traits. The traits are often affected by many genes and often by non-genetic influence, referred to as environment. The term environment includes elements such as feed- and management-standard of the animal, geographic and climate influences as well as other factors such as disease exposure (Simm, 2010).

Quantitative trait loci (QTL) are regions that contain the genes for quantitative traits (Simm, 2010) and imprinting effects on QTL can be estimated when the parental origin of the alleles are detectable. There is a strong need to incorporate parent-of-origin effects in QTL models (Spencer, 2009). Mice with partial uni-parental disomy (Beechy, 1999), imprinted diseases with chromosomal anomalies in humans (Nicholls et al., 1998) and identifying methylation patterns (Peters et al., 1999) have in the past been valid markers for detection of imprinted areas in the genome. Genome scans can be used to detect imprinted QTL (Knott et al., 1998) but are often performed on experimental crosses with different inbred lines in plants and animals (Haley et al., 1994). In most livestock species inbreeding and line breeding is avoided (Simm, 2010); therefore crosses are carried out between livestock breeds that are divergent for the phenotype of interest, making detection of QTL underlying the phenotypic differences possible (Haley et al., 1994). However, such designs are less powerful in comparison to inbred populations since the markers are not entirely informative (Haley et al., 1994). The outbred lines will however often allow tracing the parental origins of the alleles from the F_2 generation to the F_1 which is essential to testing for genomic imprinting (Knott et al., 1998). Subsequently this excludes crosses between completely inbred lines since F1 parents will be heterozygous for the same alleles (Knott et al., 1998), unless reciprocal backcrosses are made (Clapcott et al., 2000).

Current pig production breeding programs focus on sire lines when directing production and meat quality traits such as: daily gain, muscle depth (MD), backfat thickness (BF), and intramuscular fat content (IMF) (de Koning, 2001). Maternal lines are only adressed when fertility and mothering abilities are in question. Growth and carcass trait selection is restricted due to the negative genetic correlations between fertility and production traits. Thus, the genetic potential of traits such as backfat thickness and daily gain is compromised by the maternally inherited alleles (de Koning, 2001).

Imprinting introduces different female and male breeding values, additive genetic variances and dominance deviations (Spencer, 2002). For example, a correlation between breeding values and dominance deviations will occur which restricts the traditional dividing of the two components of additive and dominance variances (Spencer, 2002). Other effects of imprinting will be phenotypic changes where silencing the maternal gene expression in the offspring will induce a resemblance to the parent who's copy is not suppressed. Correlations with half siblings will also be smaller if the shared parent suppresses its transmitted genes (Spencer, 2002).

IGF2 and H19

The most extensively studied imprinted mammalian gene is *IGF2*, insulin-like growth factor 2 gene, which encodes IGF-II; a fetal mitogenic protein structurally related to insulin (O'Dell and Day, 1998; Reik and Walter, 2001). According to Spencer (2008), *IGF2* is imprinted in all examined eutherian and marsupial species; domestic mouse, human, rat, sheep, cow, pig, opossum, tamar wallaby and two species of deer mouse.

In most embryonic tissues, *IGF2* is favorably expressed from the allele of paternal origin and the maternal copy is inactive. Earlier studies of parthogenic sheep indicated that *IGF2* was paternally expressed in the species (Feil et al., 1998). However, newer studies suggest that the gene is maternally expressed (Killian et al., 2001; Thurston et al., 2008).

IGF2 forms a gene cluster with *H19*; a maternally expressed reciprocally long non-coding RNA gene whose function remains unclear but is highly expressed in fetal and embryonic tissue (Bartolomei, 1991; Rachmilewitz et al., 1992; Giannoukakis et al., 1993; Feil et al., 1998; McLaren and Montgomery, 1999; Dindot et al., 2004a; Dindot et al., 2004b; Zhang et al., 2004; Li et al., 2008). Studies have shown that a loss of *H19* in mice is not lethal however a change in phenotype occurs where the mice express overgrowth (Leighton et al., 1995).In contrast overexpression of *H19* leads to a dominant and highly lethal mutation (Brunkow and Tilghman, 1991). The phenotypic change which occurs during loss of *H19* suggests that the function of *H19* RNA expression regulates the expression of IGF2 (Leighton et al., 1995) due to overexpression of IGF2 is generally expressed during the absence of *H19* and can lead to abnormal accelerated growth in fetuses (Leighton et al., 1995). *IGF2*and *H19* are also associated with the insulin gene *INS*; which encodes the insulin hormone peptide (Benett et al., 1995; Akers, 2006). These three genes seem to have a close interaction and are extensively studied due to the association of several diseases (Benett et al., 1995). Overexpression of *IGF2* or interference of imprinting patterns between *IGF2* and *H19* is associated with mainly growth disorders and tumours in humans (Reviewed by Nezer et al., 2002). In the 5' region of *H19*, several repeats have been found which harbor epigenetic marks essential to imprinting of both *H19* and *IGF2* (Trembley et al., 1995; Thorvaldsen et al., 1998). It acts like an insulator (Kaffer et al., 2000) and seems to be modulated by the methylation status of this region (Holmgren et al., 2001; Reed et al., 2001). The activity of the insulator is dependent on CTCF, an enhancer-blocking vertebrate protein whose function marks the *IGF2*/*H19* expression region in a parent-of-origin dependent manner (Ohlson et al., 2001). The 3' region of *H19* contains numerous enhancer elements that affect expression of *IGF2* and *H19* (Webber et al., 1998) and could have a tissue-specific action (Nezer et al., 2002).

IGF2 and H19 in livestock

There is an increasing interest in the role of *IGF2* in livestock (Berkowicz et al., 2011). *IGF2* is presumed to play a role in the variation of complex production traits such as muscle mass and fat deposition in pigs as well as meat and milk production in beef and dairy cattle (Jeon et al., 1999; Nezer et al., 1999; Nezer et al., 2002; Van Laere et al., 2003). As an example, Casas et al. (2003) found in their QTL mapping study that a bovine chromosome mapped by *IGF2* contained a QTL which influenced muscle mass. Newer studies have identified an *IGF2* sequence associated to meat traits and body weight in beef cattle (Flisikowski et al., 2007; Goodall and Schmutz, 2007; Sherman et al., 2008; Bagnicka et al., 2010). Studies have reported conflicting data on *IGF2* gene sequences that supposedly result both in increase of average daily weight gain (Schmutz and Goodall, 2005) as well as lighter birth weight (Sherman et al., 2008)

The paternal expression of porcine *IGF2* has been proven (Nezer et al., 1999). In porcine *IGF2*, DNA sequence variation has been directly linked to growth and carcass traits and one regulatory region is directly responsible for a QTL influence of muscle mass and fat deposition in pigs (Van Laere et al., 2003). In this study Van Laere et al. (2003) found evidence of a single nucleotide substitution A>G, suspected to be a causative quantitative trait nucleotide (QTN), in a region associated with *IGF2* and *INS*. Markljung (2009) found

association between the protein Zbed6 and *IGF2* wherestudy showed that Zbed6 binds the QTN region in a specific *IGF2* CpG island region. Zbed6 is expressed in several tissues such as skeletal muscle and heart. Markljung (2009) also found that silencing of Zbed6 during cellular differentiation of myoblasts in mice resulted in an increase in *IGF2* mRNA expression. This increase did however not occur until day 6, when cellular differentiation would occur. Before differentiation no effect on *IGF2* expression was found which indicates Zbed6 only affects IGF2 during cellular differentiation.

Newer studies on pigs have found association with meat production, body size, carcass traits, fertility and survival traits (Vykoukalova et al., 2006; Stinckens et al., 2007; Heuven et al., 2009; Oczkowicz et al., 2009; Hou et al., 2010; Stinckens et al., 2010). Also, effects on black coat colour in pigs have been reported (Hirooka et al., 2001). When this allele is paternally inherited individuals display enhanced muscle growth and reduction in body fat due to elevated expression of padumnal *IGF2* mRNA (Van Laere et al., 2003 and Stinckens et al., 2007). The word padumnal refers to paternally expressed genes opposed to madumnal which refers to maternally expressed genes (Sandor and Georges, 2008).

In ovine the *H19* gene has been found to be very similar in structure to the human and mouse *H19*, although slightly closer structurally to the human H19 (Young et al., 2003). *IGF2-H19* have been found to be imprinted in sheep and studies of ovine parthenogenotes indicated *IGF2* repression on the maternal allele (Young et al., 2003) and *H19* transcription mainly from the maternal allele.

Since *IGF2* plays an important part in encoding fetal mitogen it is not surprising that correlations between this gene and certain carcass and growth traits have been identified (DeChiara et al., 1991; Giannoukakis et al., 1993). However, although functional genetic experiments have found a link between a spontaneous mutation for muscle mass and fat deposition in pigs, there has been no such link established to the *IGF2* gene in cattle (Berkowicz et al., 2011).

Effects of IGF2 on milk performance

The mammary gland morphogenesis expands through a long period of an animal's life. Beginning during embryonic development and proceeding postnatally through puberty, pregnancy, lactation and involution; it corresponds to periods of cell proliferation, differentiation and apoptosis due to gene expression patterns (Sjaastad et al., 2003). Epigenetics is suspected to play a role in the development of the mammary gland (Rijnkels et al., 2010) and studies have shown that *IGF2* may be involved this process (Berkowicz et al., 2010). Prosser et al's (1994) study indicated that local IGF-II infusion increased milk production in goats. In mice locally secreted IGF-II facilitates a prolactin effect on the mammary glands. Prolactin is a hormone which induces synthesis of milk proteins such as α-lactalbumin and casein, causing milk secretion (Sjaastad et al., 2003).

A correlation between milk protein gene expression and DNA methylation in mammary gland and other tissues has also been detected (Jammes et al., 2011). Berkowicz et al. (2011) found a significant correlation between *IGF2* and milk yield and milk protein yield. The gene was negatively associated with milk protein percentage (Berkowicz et al., 2010). Other studies have shown an indication of *IGF2* correlating with a QTL for milk production traits (Berkowicz et al., 2010) and one study demonstrated an association between *IGF2* and estimated breeding values for milk yield, milk fat yield and milk protein yield in Holstein-Friesian bulls (Flisikowski et al., 2007).

Another possible candidate of QTL for milk production is the bovine insulin gene (INS) which encodes the insulin hormone peptide. Insulin protein affects mammalian gland development and lactation in dairy cattle (Akers, 2006). A certain region on the human INS gene is suspected to be involved with insulin-dependent diabetes mellitus (Benett et al., 1995). This particular region also seems to influence *IGF2* expression of human placenta *in vivo* (Paquette et al., 1998).

Other QTL associated with imprinting in livestock

There have been few studies conducted on imprinting in avian species, most are conflicting (Koski et al., 2000; Nolan et al., 2001; O'Neill et al.,2000). In poultry, imprinted QTLs have been found for traits such as egg weight, age at first egg, feed intake, egg quality and body weight; all economically important traits (Tuiskula-Haavisto, 2004). Traits in poultry that have shown reciprocal effects are thought to originate from sex-linked genes, maternal effects (Fairfull, 1990) or parent-of-origin specific expression (Tuiskula-Haavisto, 2004). In the study by Tuiskula-Haavisto (2004), several QTLs affecting age at first egg, egg weight, number of eggs, feed intake, body weight and egg quality showed a significant association to imprinting. In other studies, imprinting effects have also been found for immune traits (Siwek et al., 2003) and egg laying traits (Tuiskula-Haavisto et al., 2004). Rowe et al. (2009) found suggestive evidence was for maternally expressed QTL for weight and conformation score in chickens. The studied chromosomal region has been associated with many fat and carcass traits (Ikeobi et al., 2002; Kerje et al., 2003; Abasht et al., 2006; Sewalem et al., 2002; Ikeobi et al., 2004) as well as egg production (McElroy et al., 2006 and Tuiskula-Haavisto et al., 2004) and skeletal traits (Sharman et al 2007).

Imprinting in sheep is also not extensively studied, although evidence of imprinting has been found. Parthenogenetic development studies in sheep fetuses have found effects of growth reduction and subsequently death (Feil et al., 1998; Loi et al., 1998). Several genes imprinted in human and mouse have been found repressed on one of the parental alleles in sheep (Feil et al., 1998; McLaren and Montgomery, 1999; Charlier et al., 2001). It has also been evident that in embryo manipulation and *in vitro* culture during the preimplantation stage might influence growth and phenotypic expression of the fetus (Doherty et al., 2000; Khosla et al., 2001; Young et al., 2001), and these abnormal phenotypes are referred to as Large Offspring Syndrome (LOS) in cattle (Young et al., 1998). Somatic cell cloning in cattle and sheep has found similar effects (reviewed by Wilmut et al., 2002) and are assumed to be caused by epigenetic deregulation of genes. These epigenetic abnormalities are thought to affect imprinted genes in particular (Nagy et al., 1993; Dean et al., 1998; Moore and Reik, 1996; Young et al., 1998; Feil, 2001; Young and Fairburn, 2000; Khosla et al., 2001).

X-linked QTL

Genes located on the X chromosome are subjected to X-inactivation, another type of mono-allelic expression. In females one copy of each X chromosome is silenced and in contrast to imprinting this copy is supposedly randomly selected. Subsequently, this will regulate the number of X chromosomes working in the cell. X-linked inheritance is thus the pattern of inheritance caused by silencing or expression of X-chromosomes. Several diseases in humans are the result of X-linked inheritance, including haemophilia. Due to the random selection most X-linked diseases are not phenotypically expressed in females, however several are expressed in males.

In pigs a QTL for backfat thickness has been reported on the X-chromosome (Rohrer and Keele, 1998). Harlizius et al. (2000) also found an area on the porcine X-chromosome to harbor loci that significantly influence backfat thickness and intramuscular fat content in both genders; supporting the theory of key genes for obesity and carcass composition in pigs demonstrating non-Mendelian inheritance and expression. X-linked QTL affecting adiposity and weight of individual fat depots in male mice have also been detected (York et al., 1997) as well as X-linked QTL affecting obesity strictly in males (Taylor et al., 1999).

In commercial pig breeding different purebred lines are used to produce high quality pork (de Koning, 2011). Breeding programs are used to enhance these lines and the breeding practices include selection of traits such as reproduction, meat quality and growth. This is achieved through purebred breeding for additive genetic progress and later crossbreeding for a heterosis effect.

X-linked QTL and genomic imprinting could have a large impact on the common practice of crossbreeding in commercial pig production (de Koning, 2001). De Koning (2001) suggests that the use of imprinted genes and X-linked QTL can allow slaughter pigs to be tailored to four different markets using the same purebred lines. In his thesis, four different pig types were proposed: the pork pig, the bacon pig, the Parma pig and the Japan pig; most differing in traits such as fatness, growth rate and muscle depth. To achieve this, several imprinted genes were proposed and one X-linked QTL, all of which affected economically important traits in commercial pig production. The discussed scenario is said to be only one example of many possible QTL breeding schemes (de Koning, 2001). Further information on the possible implementations of X-linked and imprinted QTL is presented by Koning (2001) in the discussion chapter of “Identification of (non-) mendelian factors affecting pork production”.

Discussion

According to the website Geneimprint (2011), 18 imprinted genes have so far been confirmed in sheep, cow and pig. Most have been studied in pig, several in sheep and only one has been found in cattle. This confirms the earlier statement of imprinting not being highly considered as a factor in commercial livestock breeding. This in spite of the fact that recent studies show that genomic imprinting plays a vital role in the development of certain commercial traits as well as fetal development (Attig et al. 2010; Reviewed by Jirtle and Weidman, 2007; Jeon et al., 1999; Nezer et al., 1999; Nezer et al., 2002; Van Laere et al., 2003). Economically important traits such as milk yield and milk quality, backfat thickness, body weight and growth seem to be associated with imprinted and X-linked QTL. As demonstrated in this review *IGF2* and *H19* play a vital role in several valuable traits such as muscle mass, fat deposition, meat and milk production (Jeon et al., 1999; Nezer et al., 1999; Nezer et al., 2002; Van Laere et al., 2003) and has been studied extensively. Considering this, imprinting could become an important factor to be noted in future breeding schemes. Scientists have looked at using other statistic tools such as bioinformatics and Next generation sequencing with promising results. Now that we have these genomic tools and we know more about the animals’ genomic sequences one could argue that implementation of these methods in livestock breeding would be advisable. Future study on the effects of imprinting on chicken and sheep could also be economically beneficial if more imprinted genes are found and possibly utilized in breeding schemes.

The implementation of breeding programs which take imprinting into account will require more focus on the maternal contribution (de Koning, 2001). Today a large focus is placed on

the sire lines regarding production and meat quality traits, the maternal lines are only considered for fertility traits and maternal skills. However, since there is a negative correlation between certain production traits and fertility the genetic potential of many commercial traits are compromised by the maternally inherited alleles (de Koning, 2001). The consideration of these imprinting effects, accompanied by the subsequent introduction of different male and female breeding values, dominance deviations and additive genetic variances (Spencer, 2002) could have a large impact on current breeding schemes. In de Koning's (2001) thesis, he discusses the possible impact of X-linked and imprinted QTL on commercial pig production. According to de Koning (2001) this may enable slaughter pigs from the same purebred lines to be tailored to four different markets, which is only one of several possible scenarios. Using imprinting effects could enable differentiation between F1 sows and their offspring in traits such as fertility, mothering skills, backfat and meat percentage. In theory one could produce F1 sows with enough body fat to maintain large litters and yet produce offspring with high meat percentage and low backfat content.

Conclusion

Genomic imprinting could become a very useful tool to increase production of commercial traits in livestock. More research on how imprinted and X-linked QTL could be utilized in livestock production is needed.

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