

GENETICS, GENOMICS AND FERTILITY

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INTRODUCTION

Early studies investigating the genetic material of life began with Gregor Mendel, who published his research results in 1866. Through the use of peas in crossbreeding experiments, Mendel reported the inheritance of characteristics or traits occurred via units (which would later be described as genes; Mendel, 1866).

In 1944, scientists at the Rockefeller Institute in New York (Avery, MacLeod, and MacCarty, 1944) reported that the genetic material was "DNA" or deoxyribonucleic acid. Watson and Crick, in 1953, described the structure of DNA as a double helix. Twenty-two years later, in 1975, Sanger and Coulson developed a method of DNA sequencing, that is, determining the sequence of the building blocks of DNA, referred to as nucleotides. A nucleotide consists of three components: a sugar molecule (deoxyribose in DNA), a phosphate group and a nitrogen-containing base. The nitrogenous bases include cytosine (C), guanine (G), adenine (A), and thymine (T). The double helix, similar to a spiral staircase, allows DNA the ability to store and transmit information. The bases link across the two strands of the double helix: cytosine (C) pairs with guanine (G), and adenine (A) pairs with thymine (T).

The genetic code describes the relationship between the bases (A, C, G, and T) along a single strand. The alphabet (A, C, G, and T) forms a codon --- a group of three letters --- that code for specific amino acids, e.g., "AGC" is the codon for the amino acid serine. A gene is a sequence of nucleotides along a DNA strand that spells out codons (in three-letter sequences) that direct the sequence of amino acids necessary to form a protein. Therefore, each gene combines the four bases in various order to spell out three-letter codons that specify which amino acid is needed at every step in making a protein which determines the form and function of the organism.

GENOME, GENETICS, GENOMICS, AND SNPs

What is the difference between genome, genetics, and genomics? Genome refers to the haploid genetic material of an organism. Genetics is focused on heredity and variation of organisms. In simpler terms, genetics focuses on the characteristics or traits that are passed from one generation to another. However, genomics is more focused on the molecular aspects of genetics: DNA sequencing, genetic mapping, and analysis of the complete genome of an organism, including organizing the results in databases.

When gametes are produced (sperm in the male and oocytes in the female) each gamete may not carry the exact same DNA sequence, i.e., a polymorphism (poly=many, morph=form) may occur which involves one of two or more variants of a particular DNA sequence. The most common

polymorphism involves variation at a single nucleotide, or base pair. This variation is called a single nucleotide polymorphism, or SNP (pronounced “snip”), and may serve as a marker for a variety of genes. Scientists are currently studying how SNPs in the genome are associated with disease, production traits, and fertility in livestock.

THE BIG PICTURE: GENOMICS, ANIMALS, AND HUMANS

The National Center for Biotechnology Information, National Library of Medicine contains an up-to-date list of genome information (www.ncbi.nlm.nih.gov/genome). The list is exhaustive, and includes the cat (*Felis catus*), dog (*Canis lupus familiaris*), horse (*Equus caballus*), and cattle (*Bos taurus* and *Bos indicus*).

Genomic analyses have been used to discover new information pertaining directly to the animal and (or) in relation to similar diseases in humans. For example, the domestic dog exhibits great diversity in body size. Sutter et al. (2007), following a genome-wide association analysis (GWAA), reported the discovery of a single IGF1 (insulin-like growth factor-1) SNP common to all small breeds, but nearly absent from giant breeds, thereby providing evidence the same causal sequence variant is a major contributor to body size. Further research provides evidence that approximately 50% of the variance in body size of dog breeds can be explained by seven markers, including GHR (growth hormone receptor), IGF1, and IGF1R (insulin-like growth factor-1 receptor) (Rimbault et al. 2013).

A GWAA also revealed a gene mutation (superoxide dismutase-1 or SOD1) in canine degenerative myelopathy that resembles amyotrophic lateral sclerosis in humans (Awano et al., 2009), while the gene ADAMTS20 has been identified as a risk variant for cleft lip and palate in both dogs and humans (Wolf et al., 2015). Thus, genomics can be used to identify SNPs associated with important or desirable traits in animals and humans.

DAIRY CATTLE GENOMICS AND FERTILITY

In 2009, after six years of research by 300 scientists from 25 countries, the genome of cattle was decoded (Elsik et al., 2009). The bovine genome has approximately 22,000 genes, of which 80% are the same as human genes.

The focus of this manuscript, and the research funded by USDA National Institute of Food and Agriculture (2013-68004-20365) discussed herein, is dairy cattle genomics and fertility. As described by Spencer et al., (2014) there is sufficient genetic variability within major cattle breeds for fertility traits that are complex and polygenic (poly=many, genic=genes). Therefore, genomic selection strategies will require many different markers developed from analysis of carefully phenotyped animal populations (Spencer et al., 2014). Genomic technologies are currently available to identify genes to improve dairy cattle fertility without negatively affecting milk yield. Ultimately, the goal of research into genomics and fertility is to increase dairy cow fertility, thereby increasing the sustainability of dairy enterprises.

Ample evidence exists that fertility of lactating dairy cows has declined (Lucy, 2001; Washburn et al., 2002). Lucy (2001) reported conception rates decreased from 55% in the 1950s (Casida, 1961) to 45% for cows that received artificial insemination (AI) following spontaneous estrus by the 1990's (Dransfield et al., 1998). Presynchronization during the voluntary waiting period, coupled with improvements to Ovsynch, have resulted in conception rates of 45% following timed AI (Brusveen et al., 2008), while use of double Ovsynch has resulted in conception rates >50% in

primiparous, but not multiparous cows (Souza et al., 2008; Herlihy et al., 2012). Moeller et al. (2010), however, using data from 85 herds, 231,288 cows and 649,495 matings, reported that the upper 20th percentile of dairy herds achieved first service conception rate of greater than 38% for timed AI and AI upon detected estrus, providing evidence that many herds struggle with reproduction.

As cow fertility declined in recent years, average milk yield per lactation increased (Lucy, 2001; Washburn et al, 2002). A negative genetic correlation between milk yield and fertility exists in dairy cattle (VanRaden et al., 2004, Pritchard et al., 2013). The heritability of fertility traits is low (1-10%) (Sun et al., 2010) in comparison with milk yield which is considered to be moderately heritable (20-40%) (Hayes et al., 2010, Kemper and Goddard, 2012). Veerkamp and Beerda (2007) suggest selection on milk yield without concomitant selection for fertility is a major cause of the decline in cow fertility in spite of low heritabilities for reproductive traits. Lucy (2007) argued that poor fertility of dairy cattle involves many factors including anovulation, inadequate expression of estrus, irregular estrous cycles, and pregnancy loss.

To enhance the sustainability of dairy businesses, new management tools are needed to increase fertility of dairy cattle. Genomic selection has been successfully used by AI studs to screen potential sires and significantly decrease the generation interval of sires (Sattler, 2013; Schefers and Weigel, 2012). Buoyed by the success of genomic selection on the male side, coupled with continuing fertility challenges on the female side, researchers are investigating genomics and the potential to increase the fertility of lactating dairy cattle.

RESEARCH UPDATE: LOCI ASSOCIATED WITH FERTILITY IN HOLSTEIN HEIFERS AND COWS

The objective of the first portion of our study, as described by Moraes et al. (2015), was to identify genomic loci (particular positions or locations) associated with fertility in nulliparous Holstein heifers. Breeding and health records of Holstein heifers (n= 2,333) were analyzed from a commercial heifer raising facility in Southwestern Idaho. Of these, 1,114 heifers were classified as highly fertile (conceived on first AI service) and 209 were identified as subfertile (did not conceive until after the fourth AI service or were culled due to failure to conceive). Blood samples were obtained from the fertility-classified heifers, and DNA was extracted from 497 high fertile and 209 subfertile heifers. The DNA was genotyped with the Illumina Bovine HD Genotyping BeadChip (Neogen, Lincoln, NE). After quality control, 581,918 SNPs, 468 highly fertile, and 188 subfertile heifers remained for analysis. Subsequently a GWAA was conducted and heritability estimate determined for identified SNPs.

Moraes et al. (2015) reported the GWAA identified two quantitative trait loci (QTL) with strong association with fertility, while 72 loci were identified with a moderate association with fertility. The heritability estimate for fertility in Holstein heifers was 0.56 (Moraes et al., 2015). Keuter et al. (2016) investigated breeding and health records of Holstein heifers (n=926) from the same facility as Moraes et al. (2015). Heifers received artificial insemination (AI) at observation of estrus and were subsequently classified into two groups: highly fertile (n=497; conceived on first AI service) and subfertile (n=429; did not conceive until after the fourth AI service or were culled due to failure to conceive). DNA was extracted from blood samples and genotyped as previously described by Moraes et al. (2015). After quality control, 590,904 SNPs, 466 highly fertile and 368 subfertile heifers remained for analysis. A GWAA was conducted and identified 153 SNPs representing 147 QTLs that were moderately associated and 34 SNPs representing 26 QTLs that were strongly associated with heifer fertility. Taken together, the results of Moraes et al. (2015) and Keuter et al.

(2016) provide evidence that selection for fertility in dairy heifers is feasible and has the potential to improve fertility as the trait is moderately heritable and QTLs with large effects have been identified.

In a companion investigation, with the objective to identify genomic loci associated with fertility in primiparous Holstein lactating cows, samples have been collected for analysis in late 2016. Briefly, records will be used to classify primiparous Holstein lactating cows as highly fertile, subfertile, or infertile. Blood samples from highly fertile (n=500; pregnant as a result of first AI), subfertile (n=500; pregnant after fourth AI), and infertile (n=500; not pregnant after six or seven AI attempts and removed from herd) classified primiparous Holstein cows have been collected and samples from 2,000 unclassified primiparous Holstein cows will be collected for a validation study. Genomic DNA will be isolated and stored for genetic analyses. The cows used for fertility classification must have a normal reproductive tract, uncomplicated pregnancy, and no records of diseases (mastitis, retained placenta, metritis or uterine infection, milk fever, displaced abomasum, clinical lameness) preceding or after AI. Separate research, by another group of collaborating scientists, investigating genomics, animal health, and reproduction is also currently underway (Santos et al., 2015).

RESEARCH UPDATE: SNPs IN GENES ASSOCIATED WITH DAUGHTER PREGANANCY RATE

Daughter pregnancy rate (DPR) involves use of days open (DO), which is computed from breeding dates for current cows and from calving interval for historical cows, which are subsequently transformed into a pregnancy rate. Bulls generally range between +3.0 and -3.0 in DPR. An increase of 1% in DPR corresponds to a decrease of 4 DO (VanRaden et al., 2004); therefore, daughters of the highest and lowest DPR sires differ by 24 DO per lactation. As DPR is correlated to fertility traits such as days to first service, conception rate, and pregnancy rate, dairy producers can expect daughters of higher DPR bulls to have improved fertility across management systems.

The heritability of DPR has been estimated at 4% (VanRaden et al., 2004); therefore, genetic selection for fertility has been hampered. Cochran et al. (2013) argued that identification of SNPs for specific genes involved in reproduction might improve reliability of genomic estimates for a low-heritability trait such as DPR. Briefly, semen from over 500 Holstein bulls of high (≥ 1.7) or low (≤ -2) DPR was genotyped for 434 candidate SNPs. (The candidate approach focuses on specified genes of interest and phenotypes). As stated by Cochran et al. (2013), the goal of the investigation was to identify SNPs in candidate genes affecting reproduction, to ultimately explain genetic variation in DPR. An additional goal included the evaluation of SNPs for their relationship to other traits, such as milk, fat, and protein yield.

Cochran et al. (2013) reported a total of 40 SNPs associated with DPR. Additionally, 22 SNPs were associated with heifer conception rate, 33 with cow conception rate, 36 with productive life, 34 with net merit, 23 with milk yield, 19 with fat yield, 13 with fat percent, 19 with protein yield, 22 with protein percent, and 13 with somatic cell score (Cochran et al., 2013). Perhaps most exciting were the results that there were 29 SNPs associated with DPR that were not negatively associated with production traits.

Nevertheless, genetic markers in one study are often not predictive in other studies (Siontis et al., 2010; Ioannidis et al., 2011). Consequently, Ortega et al. (2016), in a follow-up investigation to Cochran et al. (2013), stated the objective was to evaluate SNPs in candidate genes previously associated with genetic merit for female fertility in Holstein bulls (Cochran et al. 2013) in a separate population of Holstein cows. Briefly, 69 SNPs in genes previously related to fertility and production

traits for their relationship to DPR were evaluated in a separate population of Holstein cows grouped according to their predicted transmitting ability [< -1 ($n=1,287$) and > 1.5 ($n= 1,036$)] for DPR. Ortega et al. (2016) reported 29 SNPs associated with DPR, and of the SNPs reported to be associated with DPR by Cochran et al. (2013), 19 were significantly associated with DPR in the Ortega et al. (2016) study. For 15 of the 19 genes, Ortega et al. (2016) reported the beneficial allele was the same as that found by Cochran et al. (2013), providing evidence that many of the candidate gene SNPs found by Cochran et al. (2013) are likely to represent true causal variants.

Failure of many of the SNPs found by Cochran et al. (2013) to have a significant effect on DPR in the Ortega et al. (2016) investigation may be related to a variety of factors, including false positives, and the reality that reliabilities of the cow population used in the Ortega et al. (2016) study were lower than the reliabilities for the bull population used by Cochran et al. (2013).

An unfortunate characteristic of some GWAA and candidate gene studies is that associations between genotype and phenotype are not repeatable (Ioannidis et al. 2011). Ortega et al. (2016) provides evidence that many SNPs previously related to fertility traits in Holstein bulls (Cochran et al. 2013) had similar relationships in a separate population of cows. Furthermore, Ortega et al. (2016) assert that the inclusion of these genes in genetic evaluations can improve reliabilities of genomic estimates for fertility. These results, when taken together with the conclusions of Cochran et al. (2013) that numerous SNPs associated with DPR were not negatively associated with production traits, support the premise that it should be possible to select for DPR without compromising production.

RESEARCH UPDATE: SNPs IN GENES ASSOCIATED WITH DAYS OPEN

Previously, a candidate gene approach identified 51 SNPs associated with genetic merit for reproductive traits and 26 SNPs associated with genetic merit for production in dairy bulls. Ortega et al. (2015) evaluated the association of these 77 SNPs with days open for primiparous Holstein cows. Cows were grouped based on DPR: ≤ -1 ($n = 1,220$) and ≥ 1.5 ($n = 1,053$) and were housed on 11 farms in Florida and California. To evaluate phenotypes, records were retrieved from on-farm computers and combined with records from the national genetic evaluation system. Days open were lower for cows in the high DPR group as compared with the low DPR group (97.8 ± 2.6 d vs 163.0 ± 2.9 d). There were 6 SNPs with significant additive effects on days open. For example, days open for cows with 0, 1, or 2 copies of the minor allele for COQ9 (coenzyme Q9 which is involved in protein coding) averaged 139.4 ± 3.5 , 134.3 ± 2.8 , and 123.6 ± 3.5 d, respectively. (A minor allele is considered the least common member of a pair of genes occupying a specific spot on a chromosome that controls the same trait). Days open for cows with 0, 1, or 2 copies of the minor allele for FST (Follistatin: protein coding) averaged 124.9 ± 3.3 , 134.8 ± 2.6 and 135.8 ± 4.4 d, respectively. Ortega et al. (2015) concluded: 1) SNPs in specific candidate genes are associated with phenotypic differences in days open for primiparous Holstein cows; and 2) SNPs related to genetic and phenotypic estimates of fertility are likely to be informative markers for selection.

CONCLUSIONS

Genome-wide association assays are powerful tools being used by scientists to gain greater insight into the genetic make-up and potential of animals. Genomic testing and selection is underway on many dairies today. Producers are sampling animals while still young and employing a variety of management strategies to optimize use of their cattle. In fact, nearly 250,000 females were genotyped in 2014, with greater than 100,000 sampled before 6 months of age (Cole, 2015,

unpublished). Promising research results provide evidence that: 1) there may be ample opportunity to make significant gains in Holstein heifer fertility using genomic selection; and 2) there are a large number of SNPs associated with DPR that are not negatively associated with production traits, perhaps allowing for selection for DPR without compromising production. Lastly, identification of genomic loci associated with fertility in primiparous Holstein lactating cows is underway, with results expected in late 2016-2017.

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