

## Genomic Selection and Reproductive Efficiency in Dairy Cattle

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### TAKE HOME MESSAGES

- New management tools are necessary to increase fertility of dairy cattle.
- Genomic loci associated with fertility have been identified in nulliparous Holstein heifers.
- Research is underway to determine genomic loci associated with fertility in primiparous Holstein cows.
- Single nucleotide polymorphisms favorably associated with daughter pregnancy rate have been discovered and are not negatively associated with production traits.
- Single nucleotide polymorphisms in specific candidate genes are associated with phenotypic differences in days open for primiparous Holstein cows.
- Early research results provide hope that genomic selection for fertility will be a reality on farms in the future.

### INTRODUCTION

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), whereas use of Double Ovsynch has resulted in conception rates >50% in primiparous, but not in 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 >38% for timed AI and AI upon detected estrus, providing evidence that many herds still struggle with reproduction.

As cow fertility declined during 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). Heritability of fertility traits is low (1 to 10%) (Sun et al., 2010) in comparison with milk yield which is considered to be moderately heritable (20 to 40%; Hayes et al., 2010, Kemper and Goddard, 2012). Veerkamp and Beerda (2007) suggested selection for milk yield without concomitant selection for fertility is a major cause of the decline in cow fertility despite low heritabilities for reproductive traits. Lucy (2007) argued that the poor fertility of dairy cattle involves many factors including anovulation, inadequate expression of estrus, irregular estrous cycles, and pregnancy loss (Lucy, 2007).

To increase 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.

### **GENOME, GENETICS, GENOMICS, GENES, AND SNPs**

Before continuing the discussion of genomics and fertility, it is important to refresh our memory on a few basic terms (e.g., 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. Genomics, however, 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. A more thorough review of terminology may be accessed:

<http://www.extension.org/pages/73178/genetics-and-genomics:-an-introduction#.VgCHTpd4Vrs>.

In 2009, after 6 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 found in humans. A gene is a sequence along a DNA strand that directs the sequence of amino acids necessary to form a protein.

When gametes are produced (sperm in the male and oocytes in the female), each gamete may not carry the exact same DNA sequence. A polymorphism (poly= many, morph=form) may occur that 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.

As described by Spencer et al. (2014), sufficient genetic variability exists within major 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. The ultimate goal of research in genomics and fertility is to increase dairy cow fertility, thereby increasing the sustainability of dairy enterprises.

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

The objective of the first portion of the study, 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 at first AI service) and 209 were identified as subfertile (did not conceive until after the fourth AI service or were culled for 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) and, after quality control, 581,918 SNPs, 468 fertile, and 188 subfertile heifers remained for analysis. A subsequent genome wide association analysis (GWAA) and heritability estimate was conducted.

Moraes et al. (2015) reported the GWAA identified two quantitative trait loci (QTL) with strong association with fertility on chromosome 4 (BTA4) and chromosome 6 (BTA6), whereas 72 loci on chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, 21, 23, 25, 26, 27,

and 28 (BTA1, BTA2, BTA3, BTA4, BTA5, BTA6, BTA7, BTA8, BTA9, BTA10, BTA11, BTA12, BTA15, BTA18, BTA21, BTA23, BTA25, BTA26, BTA27, and BTA28) were identified with a moderate association with fertility. The heritability estimate for fertility in Holstein heifers was 0.56 (Moraes et al., 2015). The previous researchers concluded that ample opportunity exists to make significant gains in fertility in nulliparous Holstein heifers with genomic selection. Approximately 900 more samples from fertility classified heifers in this same study are scheduled to be analyzed in late 2015 and early 2016.

In a companion investigation, with the objective to identify genomic loci associated with fertility in primiparous Holstein lactating cows, samples were collected and will be analyzed in 2016. Briefly, records will be used to classify primiparous Holstein lactating cows as highly fertile, subfertile, or infertile. Cows in the highly fertile category are those that became pregnant after the first AI; subfertile cattle became pregnant only after the fourth AI; and infertile cows did not conceive after six or seven AI attempts and were culled. Blood samples from highly fertile (n = 500), subfertile (n = 500), and infertile (n = 500) 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 this 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.

**RESEARCH UPDATE:  
SNPs IN GENES ASSOCIATED WITH  
DAUGHTER PREGNANCY RATE**

Daughter pregnancy rate (DPR) involves use of days open, 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 days open (VanRaden et al., 2004); therefore, daughters of the highest and lowest DPR sires differ by 24 days open 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 more than 500 Holstein bulls of high ( $\geq 1.7$ ) or low ( $\leq -2$ ) 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. In addition, 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 percentage, 19 with protein yield, 22 with protein percentage, and 13 with somatic cell score (Cochran et al., 2013). Perhaps most exciting were the results that 29 SNPs associated with DPR were not negatively associated with production traits. Cochran et al. (2013) concluded: (1) it might be feasible to include these SNPs into genomic tests of reproduction and other traits; and (2) given the large number of SNPs associated with DPR that were not negatively associated with production traits, it should be possible to select for DPR without compromising production.

### **RESEARCH UPDATE: SNPs IN GENES ASSOCIATED WITH DAYS OPEN**

A previous 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 on the basis of DPR:  $\leq -1$  ( $n = 1,220$ ) and  $\geq 1.5$  ( $n = 1,053$ ) 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. Fewer ( $P < 0.001$ ) days open were detected for cows in the high DPR group compared with the low DPR group ( $98 \pm 3$  vs.  $163 \pm 3$  days). Six SNPs had 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  $139 \pm 3$ ,  $134 \pm 3$ , and  $124 \pm 3$  days, 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  $125 \pm 3$ ,  $135 \pm 3$ , and  $136 \pm 4$  days, 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**

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 there: (1) may be ample opportunity to make significant gains in Holstein heifer fertility using genomic

selection, and 2) 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 2016 to 2017.

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### **REFERENCES**

- Brusveen, D. J., A. P. Cunha, C. D. Silva, P. M. Cunha, R. A. Sterry, E.P.B. Silva, J. N. Guenther, and M. C. Wiltbank. 2008. Altering the time of the second gonadotropin-releasing hormone injection and artificial insemination (AI) during Ovsynch affects pregnancies per AI in lactating dairy cows. *J. Dairy Sci.* 91:1044–1052.
- Casida, L. E. 1961. Present status of the repeat-breeder cow problem. *J. Dairy Sci.* 44:2323–2329.
- Cochran, S. D., J. B. Cole, D. J. Null, and P. J. Hansen. 2013. Discovery of single nucleotide polymorphisms in candidate genes associated with fertility and production traits in Holstein cattle. *BMC Genet.* 14:49.
- Dransfield, M.B.G., R. L. Nebel, R. E. Pearson, and L. D. Warnick. 1998. Timing of insemination for dairy cows identified in estrus by radiotelemetric estrus detection system. *J. Dairy Sci.* 81:1874–1882.
- Elsik, C.G., R. L. Tellam, and K. C. Worley. 2009. The genome sequence of Taurine cattle: A window to ruminant biology and evolution. *Science* 324:522–528.
- Hayes, B. J., J. Pryce, A. J. Chamberlain, P. J. Bowman, and M. E. Goddard. 2010. Genetic architecture of complex traits and accuracy of genomic prediction: coat colour, milk-fat percentage, and type in Holstein cattle as contrasting model traits. *PLoS Genet.* 6:e1001139.

- Herlihy, M. M., J. O. Giordano, A. H. Souza, H. Ayres, R. M. Ferreira, A. Keskin, A. B. Nascimento, J. N. Guenther, J. M. Gaska, S. J. Kacuba, M. A. Crowe, S. T. Butler, and M. C. Wiltbank. 2012. Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. *J. Dairy Sci.* 95:7003-7014.
- Kemper, K. E., and M. E. Goddard. 2012. Understanding and predicting complex traits: knowledge from cattle. *Hum. Mol. Genet.* 21:R45-51.
- Lucy, M. C. 2001. Reproductive loss in high-producing dairy cattle: where will it end? *J. Dairy Sci.* 84:1277-1293.
- Lucy, M. C. 2007. Fertility in high-producing dairy cows: reasons for decline and corrective strategies for sustainable improvement. *Soc. Reprod. Fertil. Suppl.* 64:237-254.
- Moeller, L. M., N. A. Michael, J. C. Dalton, and G. C. Lamb. 2010. Assessment of reproductive outcomes in United States Holstein dairies by geographic region. In: *Proc. Amer. Assoc. Bov. Pract. Ann. Conf.*, Albuquerque, NM.
- Moraes, J., J. C. Dalton, T. E. Spencer, J. N. Kiser, G. W. Burns, A. Wojtowicz, M. Neupane, and H. L. Neibergs. 2015. Identification of loci associated with fertility in Holstein heifers. *J. Anim. Sci.* 93(E. suppl. 3):650.
- Ortega, M. S., A. C. Denicol, D. J. Null, J. B. Cole, and P. J. Hansen. 2015. Single nucleotide polymorphisms in specific candidate genes are associated with phenotypic differences in days open for first lactation in Holstein cows. *J. Dairy Sci.* 98(E. suppl. 2):648.
- Pritchard, T., M. Coffey, R. Mrode, and E. Wall. 2013. Genetic parameters for production, health, fertility and longevity traits in dairy cows. *Animal* 7:34-46.
- Sattler, C.G. 2013. Progeny testing and genomics: Where are we and where are we going? In: *Proc. Dairy Cattle Reprod. Council Annu. Conf.*, Indianapolis, IN, pp 98-103.
- Schefers, J. M., and K. A. Weigel. 2012. Genomic selection in dairy cattle: Integration of DNA testing into breeding programs. *Anim. Frontiers* 2:4-9.
- Souza, A. H., H. Ayres, R. M. Ferreira, and M. C. Wiltbank. 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. *Theriogenology* 70:208-215.
- Spencer, T. E., P. J. Hansen, J. B. Cole, J. Dalton, H. Neibergs. 2014. Genomic selection and reproductive efficiency in dairy cattle. In: *Proc. Dairy Cattle Reprod. Council Annu. Conf.*, Salt Lake City, UT, pp 16-31.
- Sun, C., P. Madsen, M. S. Lund, Y. Zhang, U. S. Nielsen, and G. Su. 2010. Improvement in genetic evaluation of female fertility in dairy cattle using multiple-trait models including milk production traits. *J. Anim. Sci.* 88:871-878.
- VanRaden, P. M., A. H. Sanders, M. E. Tooker, R. H. Miller, H. D. Norman, M. T. Kuhn, and G. R. Wiggans. 2004. Development of a national genetic evaluation for cow fertility. *J. Dairy Sci.* 87:2285-2292.
- Veerkamp, R. F. and B. Beerda. 2007. Genetics and genomics to improve fertility in high producing dairy cows. *Theriogenology* 68 (Suppl. 1):S266-S273.
- Washburn, S. P., W. J. Silvia, C. H. Brown, B. T. McDaniel, and A. J. McAllister. 2002. Trends in reproductive performance in Southeastern Holstein and Jersey DHI herds. *J. Dairy Sci.* 85:244-251.