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# Genomic heritability and genome-wide association analysis of anti-Müllerian hormone in Holstein dairy heifers

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## ABSTRACT

Anti-Müllerian hormone (AMH) is an ovarian growth factor that plays an important role in regulation of ovarian follicle growth. The objectives of this study were to estimate the genomic heritability of AMH and identify genomic regions associated with AMH production in a genome-wide association (GWA) analysis. Concentrations of AMH were determined in 2,905 dairy Holstein heifers genotyped using the Zoetis medium density panel (Zoetis Inclusions, Kalamazoo, MI) with 54,519 single nucleotide polymorphism (SNP) markers remaining after standard genotype quality control edits. A linear mixed model was used to model the random effects of sampling day and genomics on the logarithm of AMH. The genomic heritability (± standard error of the mean) of AMH was estimated to be 0.36 ± 0.03. Our GWA analysis inferred significant associations between AMH and 11 SNP markers on chromosome 11 and 1 SNP marker on chromosome 20. Annotated genes with significant associations were identified using the Ensembl genome database (version 88) of the cow genome (version UMD 3.1; https://www.ensembl.org/biomart). Gene set enrichment analysis revealed that 2 gene ontology (GO) terms were significantly enriched in the list of candidate genes: G-protein coupled receptor signaling pathway (GO:0007186) and the detection of chemical stimulus involved in sensory perception (GO:0050907). The estimated high heritability and previously established associations between AMH and ovarian follicular reserve, fertility, longevity, and superovulatory response in cattle implies that AMH could be used as a biomarker for genetic improvement of reproductive potential.

**Key words:** genome-wide association, heritability, reproduction

## INTRODUCTION

The rate of genetic improvement for reproductive performance in dairy cattle is relatively slow (García-Ruiz et al., 2016), partly because heritability estimates of conventional reproductive traits of dairy cattle are low, typically ranging from 0.02 to 0.17 (Berry et al., 2014). Reliable biomarkers highly correlated with reproductive performance, expressed early in life, and moderately to highly heritable have not yet been discovered. Such a discovery would be useful in identifying cattle with superior reproductive potential and designing breeding programs for faster genetic gain in reproductive efficiency to enhance profitability of the dairy industry.

Anti-Müllerian hormone (**AMH**) has been proposed as an important biomarker of reproductive potential of cattle. It is a growth factor produced by granulosa cells of ovarian follicles and Sertoli cells of testes, and it was first discovered to play an important role in sex differentiation during embryo development (Baarends et al., 1994; Allard et al., 2000). In adults, however, AMH is known to regulate ovarian follicle growth (Tiftik et al., 2016) as it prevents initial recruitment and the premature depletion of the follicular population in the ovary (Durlinger et al., 1999, 2002). Anti-Müllerian hormone may also inhibit FSH-induced follicular growth because it reduces the sensitivity of follicles to FSH treatments (Durlinger et al., 2001). Consequently, AMH may play a role in determining which follicles undergo selection and grow, and which follicles undergo atresia.

Our research group has shown that circulating AMH concentration is positively correlated with ovary size, number of antral follicles growing during follicular waves, and size of the ovarian reserve (total number of morphologically healthy oocytes in ovaries; Ireland et al., 2008). Anti-Müllerian hormone has been determined to predict ovarian progesterone (Ireland et al., 2009), androgen production (Mossa et al., 2010), herd longevity (Jimenez-Krassel et al., 2015), maintenance of pregnancy, and pregnancy rate in dairy cows bred on estrus (Ribeiro et al., 2014). It is also positively cor-

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related with response to superovulation (Ireland et al., 2007) and embryo production in dairy cattle (Monniaux et al., 2010; Guerreiro et al., 2014). Although highly variable among individuals, serum AMH concentration is highly repeatable within animals and not affected by the stage of estrous cycle (Ireland et al., 2008, 2011). Thus, AMH concentration is a reliable phenotypic marker, not only for size of the ovarian reserve, ovarian function, and response to superovulation, but also for fertility and herd longevity. Taken together, these findings imply that AMH may be useful for identification and subsequent genetic selection of cattle with superior reproductive potential, provided that serum AMH is at least moderately heritable.

The first objective of the present study was to estimate the genomic heritability of AMH. Our second objective was to use genome-wide association (**GWA**) analyses to help identify genomic regions associated with AMH using a medium-density SNP genotype panel.

## MATERIALS AND METHODS

All experiments involving cattle were approved by the Institutional Animal Care and Use Committee at Michigan State University. Holstein heifers (n = 3,252, 11–15 mo old, located at Green Meadow Farms Inc., Elsie, MI) were each subjected to 2 intramuscular injections of $PGF_{2\alpha}$ spaced 11 d apart to synchronize estrous cycles. Heifers were synchronized in groups of 95 to 124 heifers once or twice a month for a total of 29 groups or sampling dates. At 96 h after the last $PGF_{2\alpha}$ injection, a single tail-vein blood sample was taken from each heifer to measure serum AMH concentration. Blood samples were taken beginning on April 14, 2014 (sampling date 1) and ending on December 4, 2015 (sampling date 29). Follicle hair samples were also collected at these sampling dates for genotypic analyses. Freemartins (n = 144) were not included in the statistical analyses.

### AMH Assay

The commercially available ELISA kit for bovine AMH (MiniTube of America, Verona, WI) was used to measure serum AMH concentrations in duplicate 20-μL serum samples in cattle per kit instructions. The 2-site AMH assay has been validated (Ireland et al., 2008) for use in cattle and does not cross-react with other members of the transforming growth factor β (**TGFβ**) superfamily including TGFβ, bone morphogenic factor-4, inhibin, or activin (Kevenaar et al., 2006). The interassay coefficients of variation for 105 assays for low, intermediate, and high quality controls were 17, 16, and 20%, respectively. In addition, to evaluate potential AMH degradation during storage, serum AMH concentrations for 5 heifers in the study after ~1 mo storage at −80°C were determined to be 75, 55, 1,117, 436, and 208 pg/mL. When samples from these same individuals were assayed ~12 mo after storage at −80°C, AMH concentrations were 59, 48, 1,227, 435, and 191 pg/mL, respectively. These results implied that AMH is relatively stable during long-term storage.

### Genotyping and Pedigree Information

A total of 2,939 Holstein heifers were genotyped for SNP markers using a Zoetis proprietary medium-density SNP panel (Zoetis Genetics, Kalamazoo, MI). Four-generation pedigree information of these animals was also retrieved to construct an additive relationship matrix. The genotypes were imputed to the standard USDA 60,671 bovine SNP set (Wiggans et al., 2016) by the USDA Animal Genomics and Improvement Laboratory (Beltsville, MD). Animal genotypes and SNP markers were retained for analysis only if they fulfilled the following criteria: missing values <20%, minor allele frequency (MAF) >0.05, and pairwise linkage disequilibrium (**LD**) value of $r^2 < 0.95$. The final data set used for statistical analysis after data editing included phenotypes and genotypes for $m = 54{,}519$ SNP markers on each of 2,905 cows and their 4-generation pedigree information.

### Statistical Analysis

A linear mixed model was used to model the random effects of sampling day and genomics on the logarithm of AMH to estimate the genetic, sampling day, and residual variance components. The linear model used for analysis is provided in Equation [1]:

$$y_{ij} = \mu + d_i + a_j + e_{ij}, \qquad [1]$$

where $y_{ij}$ is the AMH phenotype taken on day $i$ on heifer $j$; $\mu$ is the overall mean; $d_i \sim NIID\left(\mathbf{0}, \sigma_d^2\right)$ is the random effect of the date of sample collection, $i = 1, 2, \ldots, 29$, with $NIID$ = normally, identically, and independently distributed; and $e_{ij} \sim NIID\left(\mathbf{0}, \sigma_e^2\right)$ is the residual term for the record on animal $j$ taken on date $i$. Furthermore, $a_j$ is the genomic merit of animal $j$, $j = 1, 2, \ldots, 2{,}905$, with $\mathbf{a} = \left\{a_j\right\} \sim N\left(0,\ \mathbf{A}\sigma_a^2\right)$. Here, $\sigma_d^2$ refers to variance of the effects of date of sample collection, $\sigma_a^2$ is the additive genetic variance, and $\sigma_e^2$ is the residual variance.For pedigree-based heritability estimation, **A** was the matrix of additive genetic relation-