GENOME WIDE ASSOCIATION STUDY AND GENOMIC HERITABILITY OF ANTI-MULLERIAN HORMONE IN DAIRY HOLSTEIN HEIFERS

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ABSTRACT

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The objectives of this study were to estimate the genomic heritability of Anti Mullerian hormone (AMH), identify candidate genes associated with AMH production, and establish phenotypic correlations between serum AMH concentrations and parameters of reproductive performance in Holstein heifers. AMH concentrations were determined in 2905 dairy Holstein heifers. Animals were genotyped using the Zoetis 70K SNP Panel. Genotypes were imputed to standard USDA 60,671 bovine SNP set with 54,519 SNP markers remaining after standard editing procedures. A linear mixed model was used to model the random effects of sampling day and genomics on the logarithm of AMH. Results showed that the genomic heritability (±SEM) of AMH was 0.36±0.03. We identified significant associations between AMH and 11 SNP markers on chromosome 11 and one marker on chromosome 20 based on a 5% false discovery rate. Some of the annotated genes in those regions have been previously identified as being important for AMH expression and ovarian function. There was no strong evidence of any association between conventional reproductive performance measures of dairy heifers and their serum AMH concentration. It seems that these associations should be studied in later parities as these heifers continue to mature. Nevertheless, the high heritability of AMH and a well-established association of AMH with super ovulatory response may make AMH a biomarker to genetically select cattle for larger gonads, more follicles and better response to superovulation.

This thesis is dedicated to my parents, their love and affection has always been a source of strength for me.

I dedicate this thesis to my wife, my brothers and my sisters whose continued support motivated me to accomplish everything I have achieved in my life.

I also dedicate this work to all my friends and teachers who believed in me and helped me sort out the challenges of graduate school life.

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CHAPTER ONE

Introduction

Dairy farming is a vital part of the global food system and it plays a key role in sustainability of rural areas in particular. Efficiency of dairy farming is an important consideration not only for global food security but also for profitability and hence, the sustainability of dairy farming. Milk production of individual cows depends on their ability to reproduce as the lactation cycle is initiated and renewed by pregnancy. Efficiency of reproduction is the primary determinant of dairy farm profitability because reproductive losses translate into a number of economic losses due to longer calving interval, shorter herd life, higher replacement cost and higher costs of veterinary treatment and drugs. The total loss due to reproductive failure equates to about 2% of the gross production value or 10% of an average dairy farmer's income (Dijkhuizen et al., 1984) . Therefore, farmers emphasize the optimization of reproductive performance of cattle.

Reproductive efficiency in dairy cattle has been decreasing, at least until 2005 (García-Ruiz et al., 2016). It was observed that modern dairy cows have lower first service conception rates (Butler, 1998; Lucy, 2001), longer intervals to first ovulation (Marion and Gier, 1967; de Vries and Veerkamp, 2000), higher incidences of anestrus and abnormal luteal phases (de Vries and Veerkamp, 2000), lower blood progesterone concentrations (Lucy et al., 1998), higher multiple ovulation and twinning rates (Fricke and Wiltbank, 1999), and greater embryonic losses (Lucy, 2001). The root causes of poor reproductive performance were a variety of physiological and management factors that had cumulative effects on reproductive efficiency. However, there is some evidence that a genetic antagonism exists between reproductive traits and milk production (Macmillan et al., 1996; Dematawewa and Berger, 1998; Hansen, 2000). The aggressive genetic selection of dairy cattle for increased milk production has been an important contributing factor to the decreased reproductive efficiency in modern cows. Realizing this situation, cattle breeding

schemes around the world have been broadened to include reproduction traits in dairy selection indices (Miglior et al., 2005). National genetic evaluation of cows for fertility was started in USA based on daughter pregnancy rates (DPR) (VanRaden et al., 2004). Consequently, an increase in estimated breeding values (EBV) of cows has been observed for DPR (García-Ruiz et al., 2016). However, the rate of gain in EBV of DPR has been slow due to its low heritability (0.04).

Animal breeding is defined as the selective breeding of animals for economically important traits. Traditional genetic selection of animals involves extensive phenotype recording of animals and their relatives to determine their EBV. Animals are selected as parents of next generation based on their EBV. This typically requires a long generation interval in dairy due to a long duration of pregnancy and higher age of puberty. There is also a high cost associated with progeny testing programs to calculate EBVs. Genomic prediction (Meuwissen et al., 2001) allows the determination of EBV of animals right after birth by using their genotype information. Genomic prediction assumes that genetic markers or specifically single nucleotide polymorphisms (SNP) are in linkage disequilibrium (LD) with underlying quantitative trait loci (QTL), which have an additive effect in expression of the trait. It focuses on estimating the cumulative effects of markers without testing for their significance. Therefore, we can predict the breeding values of animals without having to wait for them to mature and exhibit the phenotype. Rates of genetic gain have doubled by using genomic selection in cattle, in part because of a decrease in generation interval. However, the rate of genetic gain and the accuracy of genomic prediction also depends on the genomic heritability of the trait under selection. Heritability refers to the proportion of phenotypic variance due to genetic differences in the population. Unfortunately, the heritability of reproductive traits in dairy is very low (Berry et al., 2014).

Discovery of novel biomarker traits in genomic selection that have a high heritability and a relatively high genetic correlation with reproductive traits would increase the rate of genetic gain in reproductive potential.

While breeding programs use selection approaches based on genetic evaluations to identify phenotypically superior animals, assisted reproductive technologies (ART) such as artificial insemination, in vitro embryo production, and embryo transfer (Smidt and Niemann, 1999), are used to breed cows considered to be genetically superior. Genomic selection and ART are routinely being used by dairy farmers to improve cattle genetics and reproduction (Garcia et al., 2013). However, the success of the reproductive technologies depends on the individual characteristics of the animal. The high variability of animal responses to super-ovulatory treatments coupled with the high cost of treatments, are limiting factors for widespread use of these technologies. If some of this variation can be attributable to genetics, genomic selection may allow an efficient use of ART. Super ovulatory traits (fertilization and blastocyst rate, ovulation rate, total number of embryos per flush, the number of viable embryos per flush etc.) are difficult to measure, are highly invasive, and require expertise in ultrasound scanning, ovum pick up and in vitro fertilization procedure. Endocrine markers of super ovulatory response traits provide an opportunity to select animals more efficiently for ART.

Anti Mullerian hormone (AMH) is a potentially useful endocrine indicator of reproductive potential in dairy cattle. In recent studies, the circulating serum concentration of AMH has been found to be associated with antral follicle count (AFC) (Ireland et al., 2008; Rico et al., 2009; Batista et al., 2014). AFC in turn, is positively associated with size of ovary, total number of follicles in ovary, number of oocytes and embryos in response to super ovulation, and higher gonadotropin and progesterone concentration (Ireland et al., 2011). AMH has been identified as

an important marker of super ovulatory response (Monniaux et al., 2010; Souza et al., 2015),in-vitro embryo production (IVEP) in cattle (Guerreiro et al., 2014; Gamarra et al., 2015; Vernunft et al., 2015), and dairy herd longevity (Jimenez-Krassel et al., 2015). There is some evidence that AMH is correlated with fertility traits like maintenance of pregnancy between 30 and 65 days of pregnancy, and pregnancy rate in cows bred following natural estrus (Ribeiro et al., 2014). Furthermore, AMH is increasingly being viewed as an indicator of health. Mossa et al., (2013) showed that calves born to feed restricted mothers had lower AMH concentrations, larger aortic trunk size and high blood pressure as compared to calves born to control cows. Similarly, low AMH levels in men have been associated with cardiovascular disease in men (Dennis et al., 2013).

AMH is originally a growth factor, produced from the granulosa cells of pre-antral and small antral follicles in ovary and sertoli cells of testes in males. It is a 140-kDa dimeric glycoprotein hormone which belongs to the transforming growth factor-β (TGF-β) family (Cate et al., 1986). AMH gene in cow (ENSBTAG00000014955) is located on chromosome 7 and ranges from 22,696,978 bp to 22,699,843 bp on the forward strand. The AMH mRNA and protein exist in the granulosa cells of preantral and small antral follicles (Hirobe et al., 1992; Durlinger et al., 2001). In cattle, Rico (2011) showed that AMH is highly expressed in the cumulus cells and the outer layers of the granulosa cells close to the theca in healthy antral follicles while its expression is strongly diminished in atretic follicles, except in the cumulus cells of atretic follicles surrounding the oocyte. Studies involving circulating serum concentrations of AMH have been conducted by various researchers in dairy animals. These concentrations have been reported to be between 60 to 570 pg/ml in Bos taurus heifers and from 60 to 780 pg/ml in Bos indicus heifers (Batista et al., 2014; Guerreiro et al., 2014; Ribeiro et al., 2014). Another study has reported that plasma

concentration of AMH is higher in Bos taurus indicus (Zebu cattle) compared to Bos taurus taurus (European cattle) and found that relationship between AMH and reproductive parameters was found to be significantly greater in Zebu compared to European cattle (Stojsin-Carter et al., 2016). These results suggest that genetic background of cattle may impact their AMH levels.

AMH was first discovered to play an important role in sex differentiation in the fetal life (Munsterberg and Lovell-Badge, 1991; Lee et al., 2008). It inhibits formation of Mullerian but not Wolffian ducts during embryonic development. Wolffian ducts in turn give rise to parts of the male reproductive system like epididymis and vas deferens. AMH plays an important role in regulation of ovarian follicle growth and development in adults (Tiftik et al., 2016). AMH also prevents initial recruitment and the premature depletion of the follicular population in the ovary (Durlinger et al., 1999) and prevents activation of primordial follicles into the growing follicle pool (Durlinger et al., 2002). AMH may inhibit follicle stimulating hormone (FSH) induced follicular growth because AMH reduces sensitivity of follicles to FSH treatments Durlinger (2001). In the rat ovary, AMH was found to be expressed in pre antral and small antral follicles while absent in large antral follicles (Baarends et al., 1995). Some follicles inherently show lower AMH expression than others and these follicles may be more sensitive to FSH and more prone to be selected by the secondary estrous FSH peak (Visser and Themmen, 2005). As there is a certain FSH threshold level required for follicles to ovulate, different follicles might have a different threshold level due to their inherent differences in AMH expression. Therefore, AMH may play a role in determining which follicles undergo selection and grow and which follicles undergo atresia.

In the ovaries of domestic animals, AMH remains constant throughout the estrous cycle. A single blood sample taken on any day of the estrous cycle to measure serum AMH concentration is a

reliable phenotypic marker as it is independent of the stage of estrous cycle (Ireland et al., 2011). The concentration of AMH in 4 to 9 years old Holstein dairy cows did not change over a three-month period (Rico et al., 2009), and measurements of AMH taken before OPU protocols over a period of one year were also significantly correlated with each other (Rico et al., 2012). Several studies in humans also suggest that the concentration of AMH measured through the menstrual cycle does not show significant fluctuations (La Marca et al., 2004; Tsepelidis et al., 2007). Although concentrations may remain very constant within a female, female to female variability does exist. For example, the concentration of AMH varied from 25 to 228 pg/ml (Ireland et al., 2009) and 6 to 433 pg/ml (Ireland et al., 2011). As AMH appears to be greatly variable across individuals, and it has been established as an indicator of reproductive potential in animals, it may be useful for identification of cattle with superior reproductive potential.

Genomic prediction relies on the linkage disequilibrium between the SNP markers and the actual causative alleles or "Quantitative Trait Loci" (QTL) which control the expression of a phenotype. As different breeds of animals might have different LD between the markers and QTL, the marker effects estimated in any breed cannot be used to accurately predict the breeding values in another breed (Habier et al., 2007). One possible solution to this problem is to use the causative "Quantitative Trait Nucleotide" (QTN) in selection indexes (Weller and Ron, 2011) instead of relying on the linkage disequilibrium between markers and QTL. This can also facilitate the selection for negatively correlated traits. Selection indexes can incorporate information from markers that have positive QTN effects for one trait but, say, neutral effects for the negatively correlated trait. Furthermore, identification of QTN can also have biotechnological significance as well. Gene editing technologies like CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) can be used in to alter the genotype of an

individual to change its phenotypic performance (Cong; et al., 2013). Finally, by extracting functional information about the genes located near the QTL can possibly give rise to a handful of interesting hypotheses on the biological pathways that underlie economically important traits.

Genome wide association (GWA) studies are performed as a first step to help locate QTN. GWA studies are designed to detect the association between DNA markers and the traits of interest. Hundreds of GWA analyses have been performed in the last decade on a wide variety of traits in plants, animals and humans. Some of the putative QTLs identified can be validated by previously identified QTNs. For example Cole (2009) showed that the largest marker effects for fat percentage and protein yield were found on BTA14 flanking the diacylglycerol O-acyltransferase 1 (DGAT1) gene (Grisart et al., 2004) and BTA6 flanking the ATP-binding cassette, subfamily G, member 2 gene (Cohen-Zinder et al., 2005) respectively. Other studies have identified novel regions of the genome to search for candidate genes. It has led to new discoveries about genes and pathways involved in common diseases and complex traits (Visscher et al., 2012). For example, DENND1A was identified as a candidate gene for polycystic ovarian condition in human females (Welt et al., 2012). Later functional genomic studies revealed increased expression of DENND1A in theca cells (McAllister et al., 2015). It has been proposed as a diagnostic criterion for polycystic ovarian disease. Therefore, GWA studies can facilitate basic research in genetics and genomics by exploiting the functional meaning of the genes underlying economically important traits.

Therefore, the objectives of the study were to:

1. Estimate the genomic heritability of serum AMH concentration in dairy Holstein heifers
2. Perform a genome wide association analysis to identify the regions of genome significantly associated with serum AMH concentration

3. Study the correlations of serum AMH concentration with reproductive phenotypes in dairy Holstein heifers

LITERATURE CITED

LITERATURE CITED

Baarends, W.M., J.T.J. Uilenbroek, P. Kramer, J.W. Hoogerbrugge, Axel P. N. Themmen, C.M.V.L. Elisabeth, and J.A. Grootegoed. 1995. Anti-Mullerian Hormone and Anti-Mullerian Hormone Type II Receptor Messenger Ribonucleic Acid Expression in Rat Ovaries during Postnatal Development, the Estrous Cycle, and Gonadotropin-Induced Follicle Growth. Endocrinology 136.

Batista, E.O.S., G.G. Macedo, R. V. Sala, M.D.D. V Ortolan, M.F. Sá Filho, T.A. Del Valle, E.F. Jesus, R.N.V.R. Lopes, F.P. Rennó, and P.S. Baruselli. 2014. Plasma antimullerian hormone as a predictor of ovarian antral follicular population in bos indicus (nelore) and bos taurus (holstein) heifers. Reprod. Domest. Anim. 49:448–452. doi:10.1111/rda.12304.

Berry, D.P., E. Wall, and J.E. Pryce. 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8 Suppl 1:105–121. doi:10.1017/S1751731114000743.

Butler, W.R. 1998. Review: Effect of Protein Nutrition on Ovarian and Uterine Physiology in Dairy Cattle. J. Dairy Sci. 81:2533–2539. doi:10.3168/jds.S0022-0302(98)70146-8.

Cate, R.L., R.J. Mattaliano, C. Hession, R. Tizard, N.M. Farber, A. Cheung, E.G. Ninfa, A.Z. Frey, D.J. Gash, E.P. Chow, R.A. Fisher, J.M. Bertonis, G. Torres, B.P. Wallner, K.L. Ramachandran, R.C. Ragin, T.F. Manganaro, D.T. MacLaughlin, and P.K. Donahoe. 1986. Isolation of the bovine and human genes for müllerian inhibiting substance and expression of the human gene in animal cells. Cell 45:685–698. doi:10.1016/0092-8674(86)90783-X.

Cohen-Zinder, M., E. Seroussi, D.M. Larkin, J.J. Loor, A. Everts-Van Der Wind, J.H. Lee, J.K. Drackley, M.R. Band, A.G. Hernandez, M. Shani, H.A. Lewin, J.I. Weller, and M. Ron. 2005. Identification of a missense mutation in the bovine ABCG2 gene with a major effect on the QTL on chromosome 6 affecting milk yield and composition in Holstein cattle. Genome Res. 15:936–944. doi:10.1101/gr.3806705.

Cole, J.B., P.M. VanRaden, J.R. O'Connell, C.P. Van Tassell, T.S. Sonstegard, R.D. Schnabel, J.F. Taylor, and G.R. Wiggans. 2009. Distribution and location of genetic effects for dairy traits. J. Dairy Sci. 92:2931–2946. doi:10.3168/jds.2008-1762.

Cong;, L., F.A. Ran;, D. Cox;, S. Lin;, R. Barretto;, N. Habib;, P.D. Hsu;, X. Wu;, W. Jiang;, L.A. Marraffini;, and F. Zhang; 2013. Multiplex Genome Engineering Using CRISPR/Cas Systems. Science (80-.). 339:819–823. doi:10.1126/science.1231143.

Dematawewa, C.M.B., and P.J. Berger. 1998. Genetic and Phenotypic Parameters for 305-Day Yield, Fertility, and Survival in Holsteins. J. Dairy Sci. 81:2700–2709. doi:10.3168/jds.S0022-0302(98)75827-8.

Dijkhuizen, A.A., J. Stelwagen, and J.A. Renkema. 1984. Economic Aspects of Reproductive Failure in Dairy Cattle. I. Financial Loss At Farm Level. Elsevier Scmnce Publ. B V 385:251–263.

Durlinger, A.L.L., M.J.G. Gruijters, P. Kramer, B.A.S. Karels, H.A. Ingraham, M.W. Nachtigal, J.A.N.T.H.J. Uilenbroek, J.A. Grootegoed, A.P.N. Themmen, A.L.L.D. Reproduction, J.J.U. Th, and F. Medicine. 2002. Anti-Mullerian Hormone Inhibits Initiation of Primordial Follicle Growth in the Mouse Ovary. Endocrinology 143:1076–1084.

Durlinger, A.L.L., M.J.G. Gruijters, P. Kramer, B. Karels, T.R. Kumar, M.M. Matzuk, U.M. Rose, F.H. De Jong, J.T.J. Uilenbroek, J.A. Grootegoed, and A.P.N. Themmen. 2001. AMH attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology 142:4891–4899. doi:10.1210/en.142.11.4891.

Durlinger, A.L.L., P. Kramer, B. a S. Karels, F.H.D.E. Jong, J. a N.T.H.J. Uilenbroek, J.A. Grootegoed, A.P.N. Themmen, N. di Clemente, S. Ghaffari, R.B. Pepinsky, C. Pieau, N. Josso, R.L. Cate, and B. Vigier. 1999. Control of Primordial Follicle Recruitment by Anti-Mullerian Hormone in the Mouse Ovary. Endocrinology 140:5789–5796. doi:10.1210/en.140.12.5789.

Fricke, P.M., and M.C. Wiltbank. 1999. Effect of milk production on the incidence of double ovulation in dairy cows. Theriogenology 52:1133–1143. doi:10.1016/S0093-691X(99)00205-8.

Gamarra, G., C. Ponsart, S. Lacaze, B. Le Guienne, P. Humblot, M.C. Deloche, D. Monniaux, and A.A. Ponter. 2015. Dietary propylene glycol and in vitro embryo production after ovum pick-up in heifers with different anti-Müllerian hormone profiles. Reprod. Fertil. Dev. 27:1249–1261. doi:10.1071/RD14091.

García-Ruiz, A., J.B. Cole, P.M. VanRaden, G.R. Wiggans, F.J. Ruiz-López, and C.P. Van Tassell. 2016. Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection. Proc. Natl. Acad. Sci. 113:E3995–E4004. doi:10.1073/pnas.1519061113.

Garcia, J.F., R. V. Alonso, Y.T. Utsunomiya, and A.S. Carmo. 2013. Genomic selection and assisted reproduction technologies to foster cattle breeding. Anim. Reprod. 297–301.

Grisart, B., F. Farnir, L. Karim, N. Cambisano, J.-J. Kim, A. Kvasz, M. Mni, P. Simon, J.-M. Frère, W. Coppieters, and M. Georges. 2004. Genetic and functional confirmation of the causality of the DGAT1 K232A quantitative trait nucleotide in affecting milk yield and composition.. Proc. Natl. Acad. Sci. U. S. A. 101:2398–2403. doi:10.1073/pnas.0308518100.

Guerreiro, B.M., E.O.S. Batista, L.M. Vieira, M.F. Sá Filho, C.A. Rodrigues, A.C. Netto, C.R.A. Silveira, B.M. Bayeux, E.A.R. Dias, F.M. Monteiro, M. Accorsi, R.N.V.R. Lopes, and P.S. Baruselli. 2014. Plasma anti-Mullerian hormone: an endocrine marker for in vitro embryo production from Bos taurus and Bos indicus donors. Domest. Anim. Endocrinol. 49:96–104. doi:10.1016/j.domaniend.2014.07.002.

Habier, D., R.L. Fernando, and J.C.M. Dekkers. 2007. The impact of genetic relationship

information on genome-assisted breeding values. Genetics 177:2389–2397. doi:10.1534/genetics.107.081190.

Hansen, L.B. 2000. Consequences of Selection for Milk Yield from a Geneticist's Viewpoint. J. Dairy Sci. 83:1145–1150. doi:10.3168/jds.S0022-0302(00)74980-0.

Hirobe, S., W.W. He, M.M. Lee, and K. Donahoe. 1992. Mullerian Inhibiting Substance Messenger Ribonucleic Acid Expression in Granulosa and Sertoli Cells coincides with Their Mitotic Activity. Endocrinology 131:854–862.

Ireland, J.J., G.W. Smith, D. Scheetz, F. Jimenez-Krassel, J.K. Folger, J.L.H. Ireland, F. Mossa, P. Lonergan, and A.C.O. Evans. 2011. Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-Mllerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in . Reprod. Fertil. Dev. 23:1–14. doi:10.1071/RD10226.

Ireland, J.J., A.E. Zielak-Steciwko, F. Jimenez-Krassel, J. Folger, A. Bettegowda, D. Scheetz, S. Walsh, F. Mossa, P.G. Knight, G.W. Smith, P. Lonergan, and A.C.O. Evans. 2009. Variation in the Ovarian Reserve Is Linked to Alterations in Intrafollicular Estradiol Production and Ovarian Biomarkers of Follicular Differentiation and Oocyte Quality in Cattle. Biol. Reprod. 80:954–964. doi:10.1095/biolreprod.108.073791.

Ireland, J.L.H., D. Scheetz, F. Jimenez-Krassel, a P.N. Themmen, F. Ward, P. Lonergan, G.W. Smith, G.I. Perez, a C.O. Evans, and J.J. Ireland. 2008. Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle.. Biol. Reprod. 79:1219–1225. doi:10.1095/biolreprod.108.071670.

Jimenez-Krassel, F., D.M. Scheetz, L.M. Neuder, J.L.H. Ireland, J.R. Pursley, G.W. Smith, R.J. Tempelman, T. Ferris, W.E. Roudebush, F. Mossa, P. Lonergan, A.C.O. Evans, and J.J. Ireland. 2015. Concentration of anti-Müllerian hormone in dairy heifers is positively associated with productive herd life.. J. Dairy Sci. 98:3036–45. doi:10.3168/jds.2014-8130.

Lee, M.M., A. Noto, D. Schoenfeld, D.T. Maclaughlin, G. Mol-rr-, N.C. Grant, and L.E.E.E.T. Al. 2008. Substance in Humans : Normal 81:2–7.

Lucy, M.C. 2001. Reproductive Loss in High-Producing Dairy Cattle: Where Will It End?. J. Dairy Sci. 84:1277–1293. doi:10.3168/jds.S0022-0302(01)70158-0.

Lucy, M.C., W.J. Weber, L.H. Baumgard, B.S. Seguin, A.T. Koenigsfeld, L.B. Hansen, H. Chester-Jones, and B.A. Crooker. 1998. Reproductive endocrinology of lactating dairy cows selected for increased milk production. J. Anim. Sci. 76:246.

Macmillan, K., I. Lean, and C. Westwood. 1996. The effects of lactation on the fertility of dairy cows. Aust. Vet. J. 73:141–147. doi:10.1111/j.1751-0813.1996.tb10007.x.

Mossa, F., F. Carter, S.W. Walsh, D.A. Kenny, G.W. Smith, L.H. Janet, T.B. Hildebrandt, P.

Lonergan, J.J. Ireland, and A.C.O. Evans. 2013. Maternal Undernutrition in Cows Impairs Ovarian and Cardiovascular Systems in Their Offspring. Biol. Reprod. 88:1–9. doi:10.1095/biolreprod.112.107235.

La Marca, A., R. Orvieto, S. Giulini, V.M. Jasonni, A. Volpe, and V. De Leo. 2004. Mullerian-inhibiting substance in women with polycystic ovary syndrome: relationship with hormonal and metabolic characteristics.. Fertil. Steril. 82:970–2. doi:10.1016/j.fertnstert.2004.06.001.

Marion, G.B., and H.T. Gier. 1967. Factors Affecting Bovine Ovarian Activity After Parturition. J. Anim. Sci. 1621–1626.

McAllister, J.M., R.S. Legro, B.P. Modi, and J.F. Strauss. 2015. Functional genomics of PCOS: From GWAS to molecular mechanisms. Trends Endocrinol. Metab. 26:118–124. doi:10.1016/j.tem.2014.12.004.

Meuwissen, T.H.E., B.J. Hayes, and M.E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829. doi:11290733.

Miglior, F., B.L. Muir, and B.J. Van Doormaal. 2005. Selection indices in Holstein cattle of various countries.. J. Dairy Sci. 88:1255–1263. doi:10.3168/jds.S0022-0302(05)72792-2.

Monniaux, D., S. Barbey, C. Rico, S. Fabre, Y. Gallard, and H. Larroque. 2010. Anti-Mllerian hormone: A predictive marker of embryo production in cattle?. Reprod. Fertil. Dev. 22:1083–1091. doi:10.1071/RD09279.

Munsterberg, A., and R. Lovell-Badge. 1991. Expression of the mouse anti-mullerian hormone gene suggests a role in both male and female sexual differentiation.. Development 113:613–624.

Ribeiro, E.S., R.S. Bisinotto, F.S. Lima, L.F. Greco, A. Morrison, A. Kumar, W.W. Thatcher, and J.E.P. Santos. 2014. Plasma anti-Müllerian hormone in adult dairy cows and associations with fertility. J. Dairy Sci. 97:6888–6900. doi:http://dx.doi.org/10.3168/jds.2014-7908.

Rico, C., L. Drouilhet, P. Salvetti, R. Dalbìs-Tran, P. Jarrier, J.L. Touź, E. Pillet, C. Ponsart, S. Fabre, and D. Monniaux. 2012. Determination of anti-Mullerian hormone concentrations in blood as a tool to select Holstein donor cows for embryo production: From the laboratory to the farm. Reprod. Fertil. Dev. 24:932–944. doi:10.1071/RD11290.

Rico, C., S. Fabre, C. Medigue, N. d. Clemente, F. Clement, M. Bontoux, J.-L. Touze, M. Dupont, E. Briant, B. Remy, J.-F. Beckers, and D. Monniaux. 2009. Anti-Mullerian Hormone Is an Endocrine Marker of Ovarian Gonadotropin-Responsive Follicles and Can Help to Predict Superovulatory Responses in the Cow. Biol. Reprod. 80:50–59. doi:10.1095/biolreprod.108.072157.

Rico, C., C. Médigue, S. Fabre, P. Jarrier, M. Bontoux, F. Clément, and D. Monniaux. 2011.

Regulation of Anti-Müllerian Hormone Production in the Cow: A Multiscale Study at Endocrine, Ovarian, Follicular, and Granulosa Cell Levels1. Biol. Reprod. 84:560–571. doi:10.1095/biolreprod.110.088187.

Smidt, D., and H. Niemann. 1999. Biotechnology in genetics and reproduction. Livest. Prod. Sci. 59:207–221. doi:10.1016/S0301-6226(99)00028-7.

Souza, A.H., P.D. Carvalho, A.E. Rozner, L.M. Vieira, K.S. Hackbart, R.W. Bender, A.R. Dresch, J.P. Verstegen, R.D. Shaver, and M.C. Wiltbank. 2015. Relationship between circulating anti-Müllerian hormone (AMH) and superovulatory response of high-producing dairy cows. J. Dairy Sci. 98:169–178. doi:10.3168/jds.2014-8182.

Stojsin-Carter, A., K. Mahboubi, N.N. Costa, D.J. Gillis, T.F. Carter, M.S. Neal, M.S. Miranda, O.M. Ohashi, L.A. Favetta, and W.A. King. 2016. Systemic and local anti-Mullerian hormone reflects differences in the reproduction potential of Zebu and European type cattle. Anim. Reprod. Sci. 167:51–58. doi:10.1016/j.anireprosci.2016.02.003.

Tiftik, A.M., A. Kelimeler, and O. Kisti. 2016. Anti-Müllerian Hormone (AMH) in Adults with Polycystic Ovary Syndrome and Pregnants. Ann. Eurasian Med. 4:65–70. doi:10.4328/AEMED.92.

Tsepelidis, S., F. Devreker, I. Demeestere, A. Flahaut, C. Gervy, and Y. Englert. 2007. Stable serum levels of anti-Mullerian hormone during the Stable serum levels of anti-Mu menstrual cycle : a prospective study in normo-ovulatory women. Hum. Reprod. 22:1837–1840.

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. 87:2285–92. doi:10.3168/jds.S0022-0302(04)70049-1.

Vernunft, A., M. Schwerhoff, T. Viergutz, M. Diederich, and A. Kuwer. 2015. Anti-Muellerian hormone levels in plasma of Holstein-Friesian heifers as a predictive parameter for ovum pick-up and embryo production outcomes. J. Reprod. Dev. 61:1–6.

Visscher, P.M., M.A. Brown, M.I. McCarthy, and J. Yang. 2012. Five years of GWAS discovery. Am. J. Hum. Genet. 90:7–24. doi:10.1016/j.ajhg.2011.11.029.

Visser, J.A., and A.P.N. Themmen. 2005. Anti-Mullerian hormone and folliculogenesis. Mol. Cell. Endocrinol. 234:81–86. doi:10.1016/j.mce.2004.09.008.

de Vries, M.J., and R.F. Veerkamp. 2000. Energy Balance of Dairy Cattle in Relation to Milk Production Variables and Fertility. J. Dairy Sci. 83:62–69. doi:10.3168/jds.S0022-0302(00)74856-9.

Weller, J.I., and M. Ron. 2011. Invited review: Quantitative trait nucleotide determination in the era of genomic selection. J. Dairy Sci. 94:1082–1090. doi:10.3168/jds.2010-3793.

Welt, C.K., U. Styrkarsdottir, D.A. Ehrmann, G. Thorleifsson, G. Arason, J.A. Gudmundsson, C. Ober, R.L. Rosenfield, R. Saxena, U. Thorsteinsdottir, W.F. Crowley, and K. Stefansson. 2012. Variants in DENND1A are associated with polycystic ovary syndrome in women of European ancestry. J. Clin. Endocrinol. Metab. 97:1342–1347. doi:10.1210/jc.2011-3478.

CHAPTER TWO

Genome wide association analysis and genomic heritability of anti-Mullerian hormone in Holstein dairy heifers

ABSTRACT

Anti-Mullerian hormone (AMH) is a growth factor which has an important role in regulation of ovarian follicle growth. Recent studies have shown a positive association between AMH concentrations in serum with the number of follicles and oocytes in ovaries (ovarian reserve), response to superovulation and embryo production, and herd longevity in dairy and beef cattle. The objectives of this study were to estimate the genomic heritability of AMH and identify candidate genes associated with AMH production. AMH concentrations were determined in dairy Holstein heifers. Animals were genotyped using Zoetis 70K SNP Panel. Genotypes were imputed to standard USDA 60,671 bovine SNP set with 54,519 SNP markers remaining after standard editing procedures. A linear mixed model was used to model the random effects of sampling day and genomics on the logarithm of AMH. Results showed that the genomic heritability (±SEM) of AMH was 0.36±0.03. We identified significant associations between AMH and 11 SNP markers on chromosome 11 and one marker on chromosome 20 based on a 5% false discovery rate. Annotated genes in those regions were identified using the ENSEMBL genes database v. 88. of the cow genome (v. UMD 3.1). Some of these genes have been previously reported to be involved in maintenance of cell structure, cell signaling, cell viability, embryonic development of cardiac, gonadal and brain tissues, embryonic survival, oocyte competence, cancer suppression and immunity. The high heritability and a potentially positive association of AMH with some reproductive traits implies that AMH may be used as a biomarker to improve reproductive potential in dairy cattle.

INTRODUCTION

Reproductive efficiency is vitally important for the profitability of a dairy herd. Aggressive genetic selection of dairy cattle on milk production over the years led to a decrease in genetic merit for reproductive performance (Veerkamp et al., 2001). Reproductive performance was included in the breeding goals around the world only a decade ago. (Miglior et al., 2005). However, the rate of gain in genetic potential observed for reproduction in all these years is slow (García-Ruiz et al., 2016) as the heritability estimates of typical reproductive traits of dairy cattle are low (Berry et al., 2014). Moreover, as reproductive traits are necessarily expressed after puberty, selection decisions cannot be made early in the life of an animal thereby leading to a long generation interval. Reliable biomarkers that are highly correlated with reproductive performance, expressed early in life and are moderately to highly heritable have not been discovered. Such a discovery will be instrumental to identify cattle with superior reproductive potential and design breeding programs for faster genetic gain in reproductive efficiency and hence the profitability of the dairy industry.

Anti-Mullerian hormone (AMH) is considered to be a potentially important biomarker of reproductive potential of cattle. It is a growth factor, produced from the granulosa cells of ovary and sertoli cells of testes, and was first discovered to play an important role in sex differentiation in the fetal life (Lee et al., 2008). In adults, AMH regulates ovarian follicle growth (Tiftik et al., 2016). Recent studies have shown a positive association between AMH and dairy herd longevity (Jimenez-Krassel et al., 2015), maintenance of pregnancy, and pregnancy rate in cows bred on estrus (Ribeiro et al., 2014). It is also positively related with in vivo (Monniaux et al., 2010) and in vitro (Guerreiro et al., 2014) embryo production performance of dairy cattle. A single blood sample taken on any day of the estrous cycle to measure serum AMH concentration is a reliable

phenotypic marker as it is highly repeatable within an animal and not affected by the stage of estrous cycle. (Ireland et al., 2011). However, AMH appears to be highly variable across individuals. Therefore, it can be useful for identification and subsequently genetic selection of cattle with superior reproductive potential.

Genomic prediction allows one to predict the breeding values of animals using their genomic information (Meuwissen et al., 2001). It makes use of the single nucleotide polymorphism (SNP) which is a kind of genetic variation in which a mutation occurs at a single base pair in the deoxy ribo nucleic acid (DNA). Genomic prediction models typically assume that the SNP markers are roughly equally spaced and spread across the whole genome are in linkage disequilibrium (non-random association of markers, LD) with the underlying quantitative trait loci (QTL) that control the traits. However, the accuracy of genomic predictions depends on size of the reference population used to derive the prediction equation, the effective population size (which refers to the number of animals contributing as parents of the next generation), the number of SNP markers, the genetic architecture (distribution of the marker effects), and the heritability of the trait (Daetwyler et al., 2008; Goddard, 2009; Meuwissen, 2009). For lowly heritable traits, large number of animals with dense marker information are required for accurate genomic estimated breeding values (GEBV). Genome-Wide Association (GWA) analyses are performed to explore the genetic architecture of a trait i.e. the key genomic regions associated with a trait and the distribution of their effects by identifying the single nucleotide polymorphisms (SNP) significantly associated with the trait. Significant markers from a GWA study may not necessarily be the causal mutations (QTL) themselves, but can be in high LD with the QTL. GWA studies often serve as an important first step to identify the causative QTL. Such GWA studies can suggest candidate genes and formulate new hypothesis for further genetic research.

They may also indicate potential drug targets to improve phenotypic performance or cure a disease condition. Marker effects from a GWA study can also be included in genomic prediction models that put additional weight on the certain genomic regions to improve prediction accuracies. However, few GWA studies have been performed for traits associated with dairy cattle fertility (Berry et al., 2014).

This study aims to estimate the genomic heritability of AMH and conduct a genome wide association study using the 60k standard USDA SNP panel.

MATERIALS AND METHODS

All experiments involving cattle were approved by the IACUC at Michigan State University. Holstein heifers (*n* = 3252, 11-15 months old, located on Green Meadow Farms Inc, Elsie, MI) were subjected *once* to two 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 per month for a total number of 29 groups. 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 4/18/2014 and ending on 12/4/2015. Follicle hair samples were collected at these times for genotypic analyses. Freemartins (n=144) were not included in the statistical analyses.

AMH assay

The commercially available anti-Müllerian hormone ELISA serum sample test kit for bovine (MiniTube of America) was used to measure serum AMH concentrations in duplicate 20 ul serum samples in cattle per kit instructions. The two-site AMH assay was validated (Ireland et al., 2008) for use in cattle and does not cross react with other members of the transforming

growth factor beta (TGFβ) superfamily including TGFβ, bone morphogenic factor-4 (BMP4), inhibin or activin (Kevenaar et al., 2006). In addition, serum AMH concentrations for five heifers in the study were 75, 55, 1117, 436, 208 pg/ml. When samples from these same individuals were assayed ~12 mo after storage at -80°C, AMH concentrations were 59, 48, 1227, 435, 191 pg/ml, respectively. These results implied that AMH values are relatively stable during long-term storage.

Genotyping

A total of 2939 Holstein heifers were genotyped for single nucleotide polymorphisms (SNP) using the Zoetis 70K SNP Panel. The genotypes were imputed to standard USDA 60,671 bovine SNP set. SNPs were retained for analysis 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 genotype data set contained 54519 SNPs for 2905 cows.

Statistical analysis

A total of 2914 cows with both genotypes and phenotypes were used for GWA 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 and environmental variance components. The model was as follows:

$$y_{ij} = \mu + d_i + a_j + e_{ij} \quad (1.1)$$

Where y_{ij} is the vector of phenotype (AMH), μ is the overall mean, d_i is the random effect of the date of sample collection, i= 1,2,…,29, a_j is the genetic effect of animal j, j=1,2,…,2914, such that $\boldsymbol{a} = \{a_j\} \sim N(\mathbf{0}, \mathbf{G}\sigma_a^2)$ and $\mathbf{e}$ is the random error vector such that $\mathbf{e} = \{e_{ij}\} \sim N(\mathbf{0},$

$\mathbf{I}\sigma^2$). Here $\mathbf{G}=\mathbf{ZZ}`$ is the genomic relationship matrix between animals with **Z** being the standardized matrix of genotypes and obtained by standardizing the matrix of allelic dosages (**M**). (VanRaden, 2008)

$$\boldsymbol{Z} = \frac{M_{ij} - 2p_j}{\sqrt{m(2p_j(1-p_j)}} \tag{1.2}$$

Note that M_{ij}, element *ij* of **M**, indicates the number of copies of the reference allele on SNP marker *j* for animal *i* whereas p_j is the allelic frequency of the reference allele, and m is the number of markers.

The best linear unbiased predictions were calculated by maximizing the log likelihood of the model in E.q 1.1. The accuracy of estimated breeding values was calculated by using following method (Van Vleck, 1993) :

$$r = \frac{\sigma_a^2 - \boldsymbol{C_{ii}}}{\sigma_a^2} \tag{1.3}$$

Here r refers to the accuracy of prediction, $\boldsymbol{C_{ii}}$ refers to the ith diagonal element of $\boldsymbol{C^{aa}}$ which is the prediction error variance-covariance matrix or the portion of the inverse of the mixed model equations that corresponds to animal effects.

Breeding values were linearly transformed to estimate the marker effects as explained by (Gualdrón Duarte et al., 2014)

$$\hat{\boldsymbol{g}} = \boldsymbol{Z'G^{-1}}\hat{\boldsymbol{a}} \tag{1.4}$$

Here **Z** and **G** have been previously defined, $\hat{\boldsymbol{g}}$ is the estimate of marker effect, and $\hat{\boldsymbol{a}}$ is the best linear unbiased prediction (BLUP) of $\boldsymbol{a}$. The variances of genetic effects were estimated by the following equations as explained by (Gualdrón Duarte et al., 2014):

$$Var(\hat{\boldsymbol{g}}) = \boldsymbol{Z'G^{-1}Z}\sigma_a^2 - \boldsymbol{Z'G^{-1}C^{aa}G^{-1}Z} \quad (1.5)$$

We divided SNP effect estimates by their corresponding standard errors to obtain test statistics as follows:

$$SNP_j = \frac{\hat{g}_j}{\sqrt{Var(\hat{g}_j)}} \quad (1.6)$$

where the subscript *j* refers to marker *j*. Here *P* values were obtained using the z score test as follows:

$$P - value_j = 2(1 - \Phi\,(|SNP_j|)) \quad (1.7)$$

where Φ denotes the standard normal cumulative distribution function. The false discovery rate (FDR) (Storey and Tibshirani, 2003) of 5% was used as a significance criteria for multiple tests. A linkage disequilibrium (LD) heat map was constructed for significant markers.

Number of QTL per peak

To determine the number of QTL (quantitative trait loci) per peak we ran GWA analysis again by fixing the top most significant peak (Casiró et al., 2017). The model thus used was as follows

$$y_{ij} = \mu + \beta_{peak} + d_i + a_j + e_{ij} \quad (1.8)$$

Here all the terms are as described in Eq. 1.1 except β_{peak}which is the fixed effect of peak significant SNP.

Confidence interval of the QTL peak

We used cross validation approach to estimate the 99% confidence interval for the peak significant QTL proposed by Hayes (2013). In this method, we firstly conducted the GWA as

described above to get the position of peak significant QTL (*p=peak*). Then we divided the dataset into two parts to conduct GWA and get the position of the most significant SNP from both datasets and calculate the difference between them (Casiró et al., 2017). We repeated the process 300 times to get the standard error of half the difference between two peaks (*h*) as follows:

$$se(\bar{h}) = \sqrt{\frac{1}{4n}\sum_{i}^{n}(x_{1i} - x_{2i})^2} \quad (1.9)$$

Here $se(\bar{h})$is the standard error of half the distance between peak significant SNPs of the two split datasets, *n* is the number of data splits (in this case 300), x_{1i} and x_{2i} are the position of the peak significant SNP of the first and second data set respectively in the i^{th} data split. Assuming symmetric distribution of the peak significant SNP after *n* splits of data, we calculated the 99% confidence interval of the peak SNP as follows:

$$CI = p \pm z_{99.5}se(\bar{x}) \quad (1.10)$$

Proportion of genetic variance explained by the significant region

The proportion of genetic variance explained by the significant region was calculated by fitting a model that contains two genetic effects: the genetic effect of the 99% confidence interval of peak significant SNP and the genetic effect of the rest of SNPs.

$$y_{ij} = \mu + d_i + a_{peak,j} + a_{-peak,j} + e_{ij} \quad (1.11)$$

Here μ is the overall mean d_i is the effect of i^{th} date of blood collection, e_{ijk} is the residual,a_{peak} and $a_{-peak,j}$ correspond to genetic effects of the 99% confidence interval of peak significant

SNP marker and the genetic effects of the rest of the SNP markers respectively. The proportion of genetic variance can be calculated by:

$$\frac{Var(a_{peak,j})}{Var(a_{peak,j}) + Var(a_{-peak,j})} \quad (1.12)$$

Where $Var(a_{peak,j})$ is the estimated variance component associated with the significant genomic region and $Var(a_{-peak,j})$is the estimated variance component associated with the rest of genome. A likelihood ratio test was used to test the significance of identified region by comparing the likelihood of model in Eq 1.11 to a null model that does not contain a_{peak} as shown below:

$$y_{ijk} = \mu + d_i + a_{peak} + a_k + e_{ijk} \quad (1.13)$$

The variance components were estimated by restricted maximum likelihood method using the R package regress (https://cran.r-project.org/web/packages/regress/regress.pdf). GWA analysis was done using gwaR (https://github.com/steibelj/gwaR) package in R. All analysis was done using R version 3.2.0 (http://www.R-project.org/).

Identification of candidate genes

Firstly, a genomic region on chromosome 11 corresponding to 99% confidence interval of the peak significant SNP was identified as described above. Annotated genes within that region were identified from ENSEMBL genes database v. 88. of the cow genome (v. UMD 3.1) using "GenomicRanges" package in R (Lawrence et al., 2013).

RESULTS

Heritability

Serum concentrations of AMH in the dairy heifers had a phenotypic mean of 438.50 pg/ml and a standard deviation of 604.33 pg/ml. (Figure 1.1) The median AMH concentration was 333.3 pg/ml and it ranged from 2 pg/ml to 14350 pg/ml. The distribution of AMH concentration and log transformed AMH concentrations is also given below which shows an approximate normal distribution.

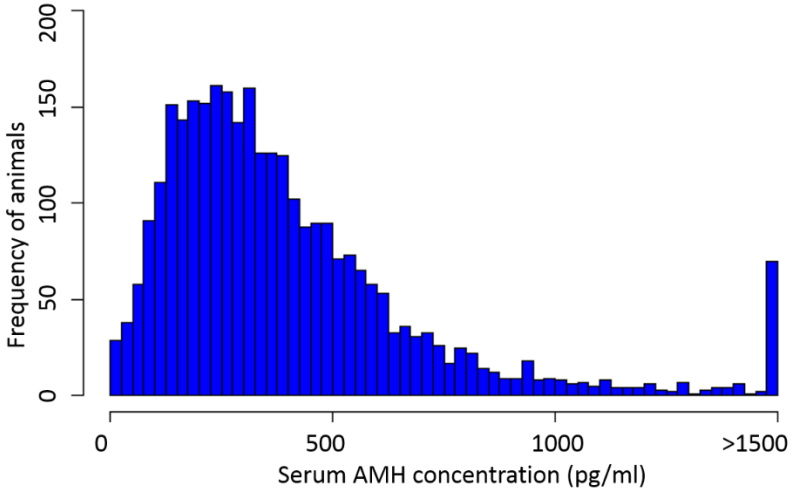

Figure 2.1: Distribution of serum concentration of AMH in dairy Holstein heifers

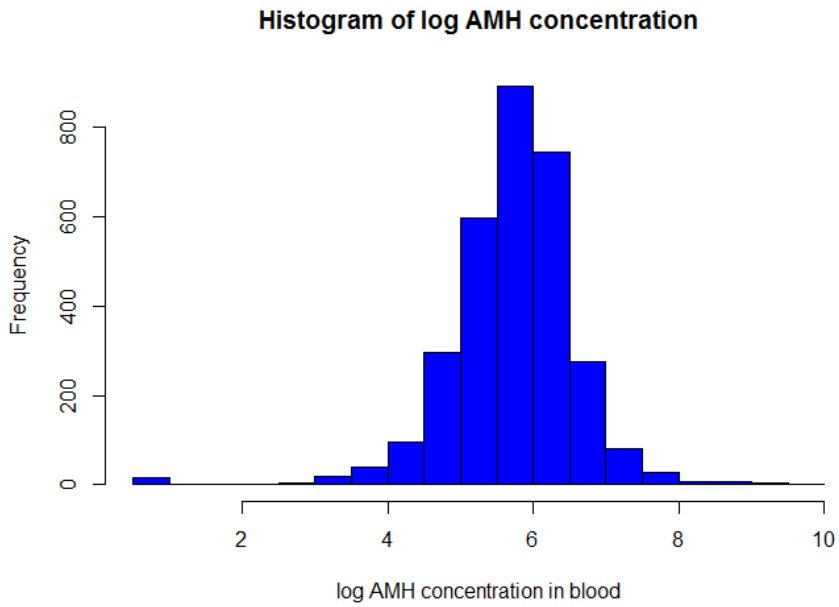

Figure 2.2: Distribution of log transformed serum concentration of AMH in dairy Holstein heifers

The variance associated with random day of blood sample collection was 0.015 ± 0.006. The estimated genetic variance associated with AMH was 0.28 ± 0.003 and the residual variance was 0.48 ± 0.020. Finally, the heritability of AMH was 0.36 with a standard error of 0.03.

We calculated the accuracy of the breeding values in our study by inverse of the set of linear model equations (the coefficient matrix). The average accuracy of prediction of breeding values in our study using 2905 animals and 54519 markers was 55.7%. The distribution of prediction accuracies is given below in figure 2.3.

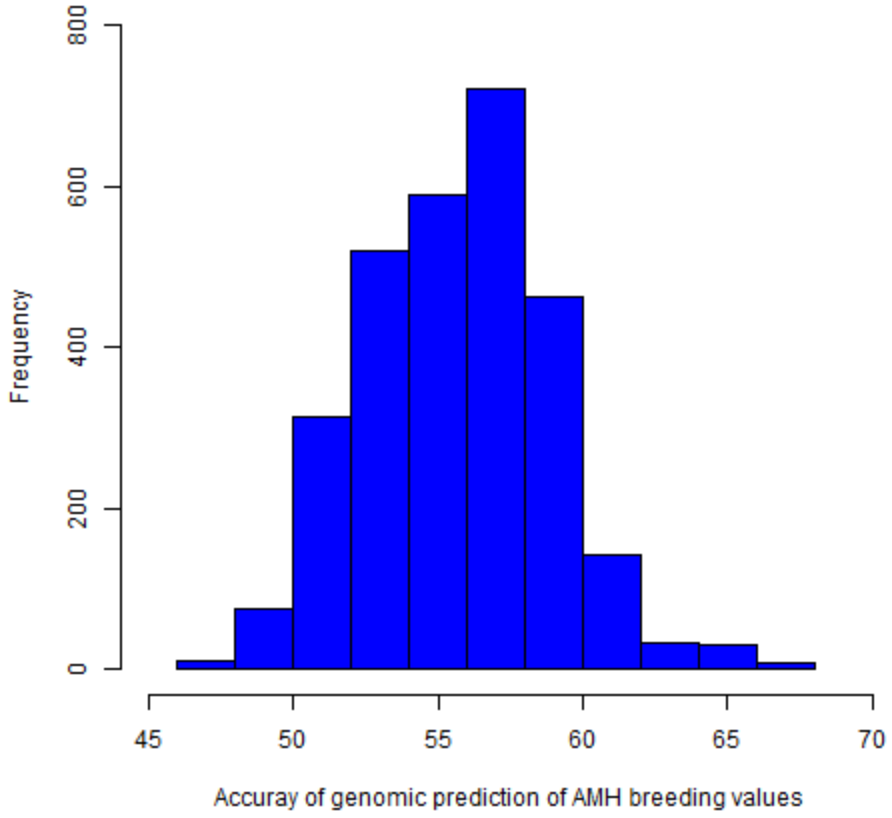

Figure 2.3: Distribution of accuracy of predicted genomic breeding values of AMH using 2905 animals and 54519 markers

GWA Study

The genome wide association analysis determined 11 significant SNPs on chromosome 11 and 1 SNP on chromosome 20 (Figure 2.4) based on a 5% false discovery rate (FDR). SNP marker "Hapmap41435-BTA-115556" on chromosome 11 (pos= 95026013 bp) turned out to be the peak significant SNP.

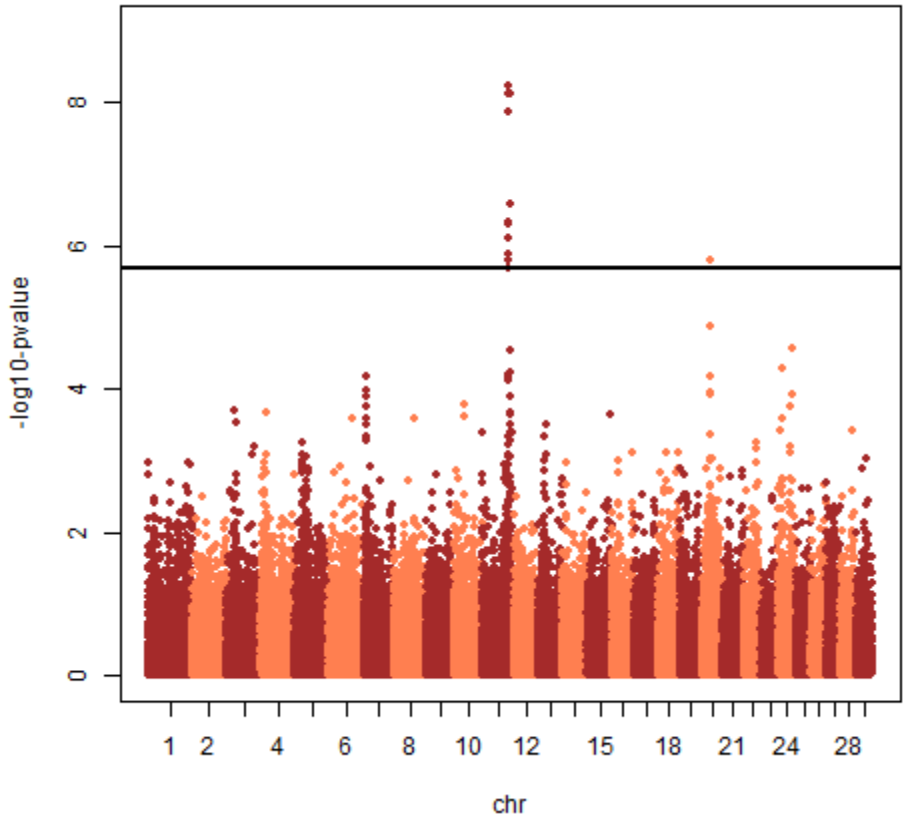

Figure 2.4: Manhattan plot of $-\log_{10}$ P values versus genomic location for logarithm of serum concentrations of AMH in dairy Holstein heifers

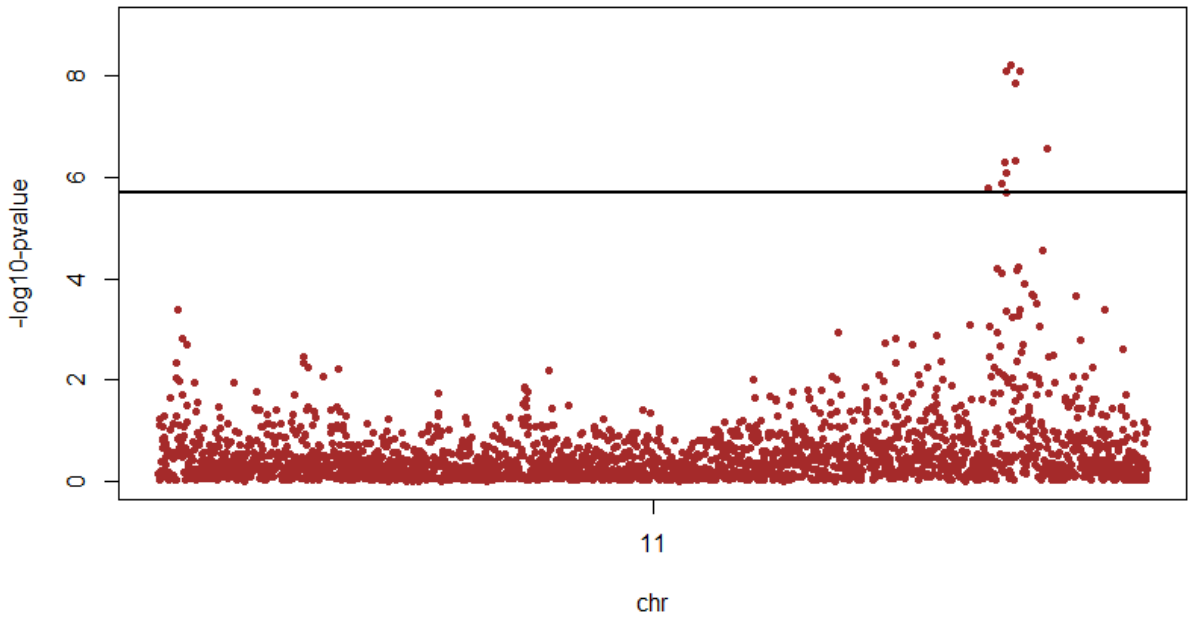

Figure 2.5: Manhattan plot of P values of markers on chromosome 11 for logarithm of serum concentrations of AMH in dairy Holstein heifers

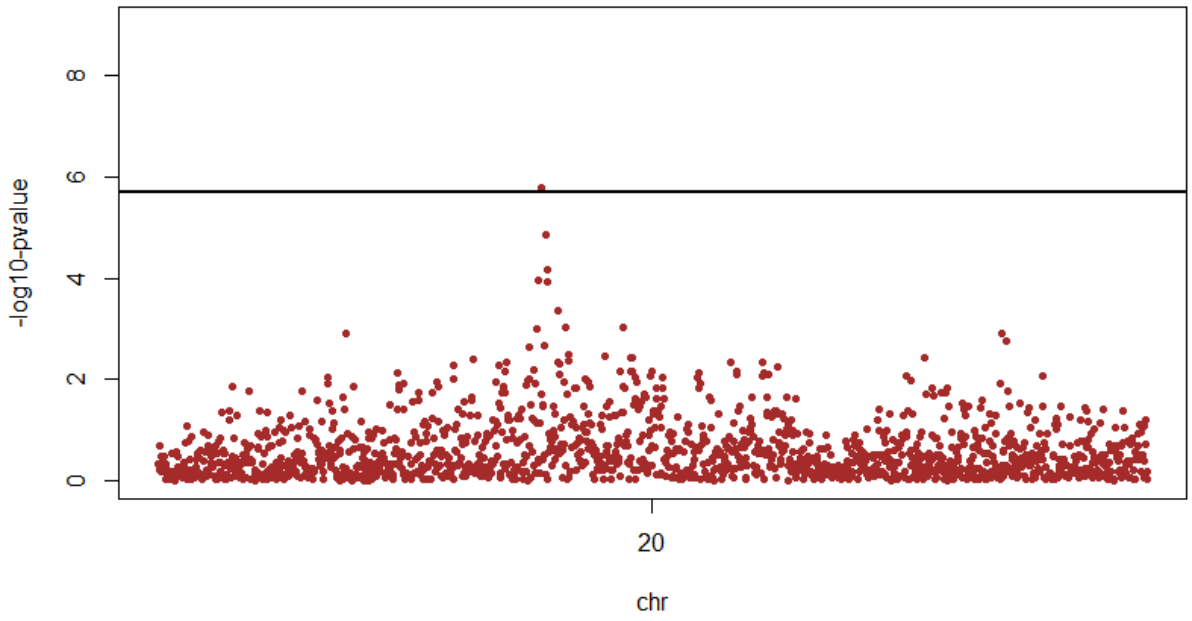

Figure 2.6: Manhattan plot of P values of markers on chromosome 20 for logarithm of serum concentrations of AMH in dairy Holstein heifers

Table 2.1: Significant associations from genome wide association analysis of logarithm of AMH in dairy Holstein heifers

SNP ID	Chr	Position (Mbp)	P-value	q-value	Effect	Minor Allele Frequency
BTA-17666-no-rs	11	92.05	1.59×10^{-6}	0.007	-	0.18
ARS-BFGL-NGS-118517	11	93.546	1.29×10^{-6}	0.007	+	0.47
Hapmap38572-BTA-115523	11	94.071	5.10×10^{-7}	0.003	-	0.22
BTA-115525-no-rs	11	94.101	7.77×10^{-9}	0.0001	-	0.20
BovineHD1100027436	11	94.202	2.00×10^{-6}	0.008	-	0.22
Hapmap46766-BTA-115526	11	94.446	7.92×10^{-7}	0.005	-	0.22
Hapmap41435-BTA-115556	11	95.026	5.94×10^{-9}	0.0001	-	0.18
ARS-BFGL-NGS-12334	11	95.516	4.65×10^{-7}	0.00386	+	0.37
Hapmap53866-rs29019867	11	95.568	1.40×10^{-8}	0.00018	-	0.14
Hapmap47514-BTA-115564	11	96.074	7.87×10^{-9}	0.00013	-	0.16
ARS-BFGL-NGS-114094	11	99.413	2.63×10^{-7}	0.00279	-	0.10
ARS-BFGL-NGS-110286	20	25.689	1.64×10^{-6}	0.00794	+	0.46

Number of QTLs

When the peak SNP (Hapmap41435-BTA-115556) was fixed in the model, none of the remaining SNP markers reached the level of statistical significance (5% FDR) (Figure 2.7) suggesting that all the significant SNP markers are in LD with a single QTL.

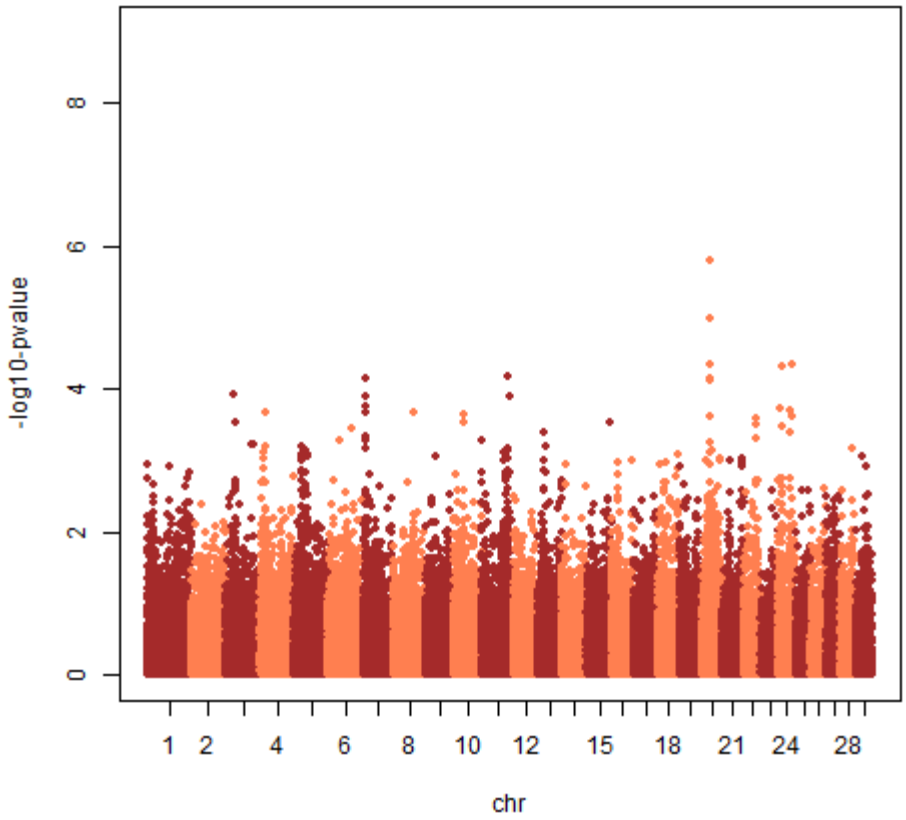

Figure 2.7: Manhattan plot after using the peak SNP as a fixed effect

We noticed that the only SNP on chr 20 (ARS-BFGL-NGS-110286) which was previously significant (q=0.007) and had a low correlation with the peak, was no longer significant (q=0.085) by fixing the peak SNP (Hapmap41435-BTA-115556). Although the *P*-value of that SNP did not increase ($p=1.56 \times 10^{-6}$-1.65×10^{-6}), it did not reach the level of significance because the new FDR was increased due to the loss of 11 highly significant SNPs on chr 11 having very low ($\sim 10^{-9}$) *P*-values. This implies that ARS-BFGL-NGS-110286 on chr 20 is moderately associated with AMH expression.

Confidence interval of the peak association on Chromosome 11

The 99% confidence interval of the peak significant SNP ranged from 92879023 bp to 97160473 bp on chromosome 11, a span of roughly 4.28MB. This region contained 68 SNPs and accounted for 7.9% of the genetic variance. When a likelihood ratio test was used to test whether this region accounted for a significant genetic variance, the test was highly significant ($p=1.7x10^{-7}$). This means that the 99% confidence interval of the peak significant SNP accounted for a significant portion of the genetic variance.

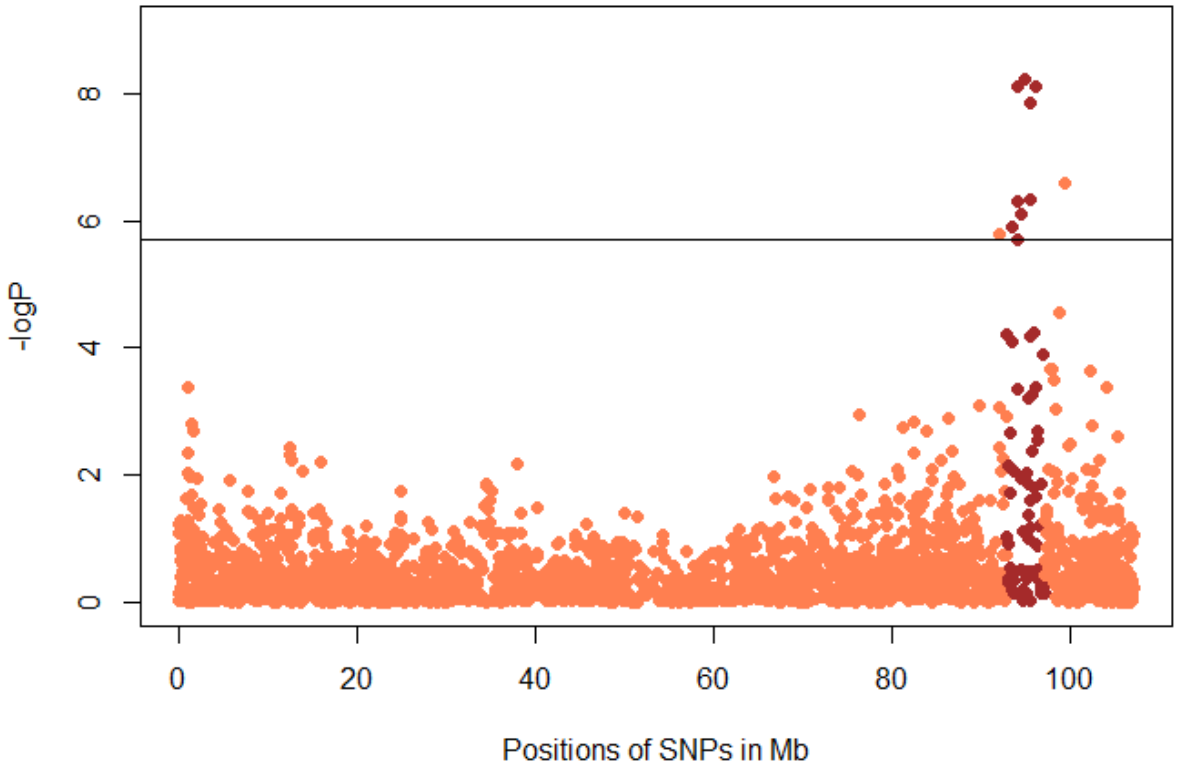

Figure 2.8: Manhattan plot of markers on chromosome 11. Markers with in the 99% confidence interval of the most significant SNP are shown in different color.

Confidence interval of the peak association on Chromosome 20

The 99% confidence interval of the peak significant marker on chromosome 20 (ARS-BFGL-NGS-110286) ranged from 25002762 bp and 26362752 bp, a span of roughly 1.35 MB. This region contained 25 SNPs and accounted for 5.25% of the genetic variance. When a likelihood ratio test was used to test whether this region accounted for a significant genetic variance, the test was significant (p=0.007). This means that the 99% confidence interval of the peak significant SNP on chromosome 20 accounted for a significant portion of the genetic variance. Therefore, the joint variance explained by both the regions simultaneously is 13.25 %.

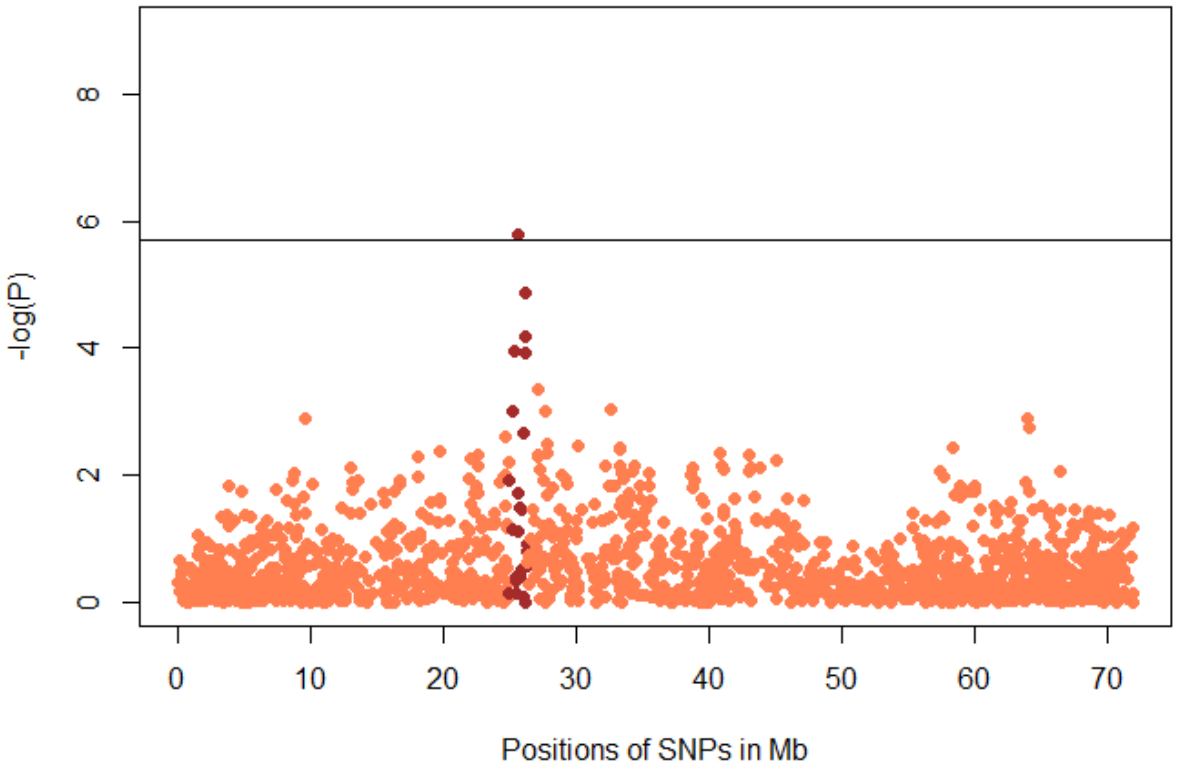

Figure 2.9: Manhattan plot of markers on chromosome 20. Markers with in the 99% confidence interval of the most significant SNP are shown in different color.

LD between significant SNPs

The LD heat map of all the SNPs with in the 99% confidence interval of the peak significant SNP on chromosome 11 and 20 indicated that there is moderate linkage disequilibrium between most of the SNP markers in that region. However, some SNPs were found to be highly correlated with each other with a LD (r^2) value as high as 0.99) The heat map is shown below

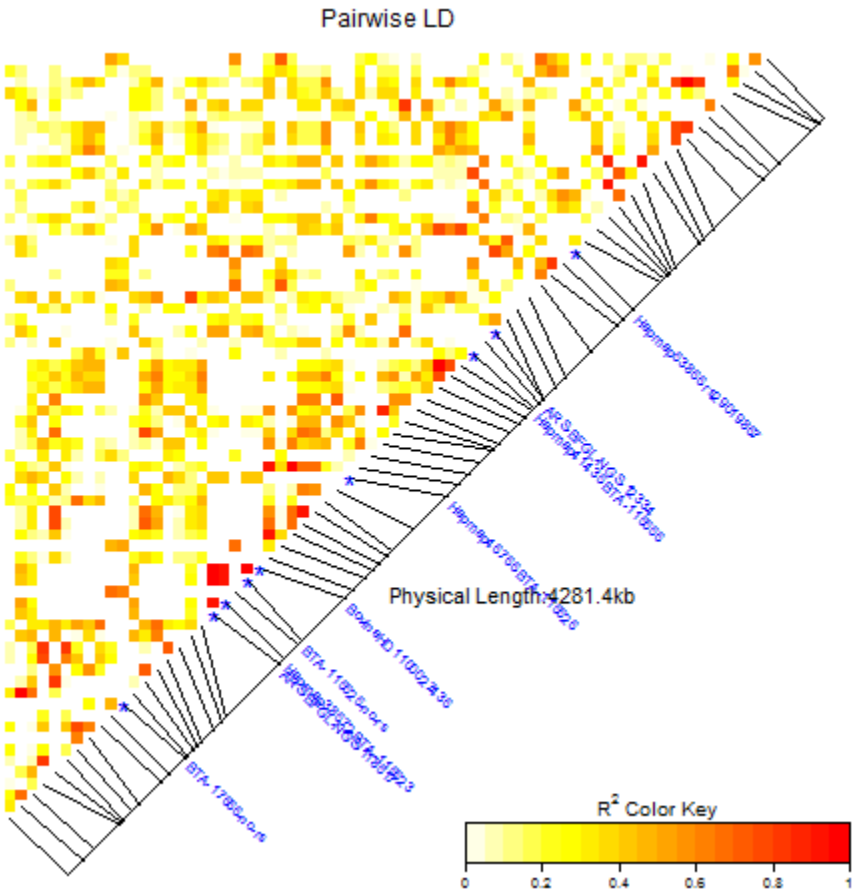

Figure 2.10: LD of all SNPs (n=68) within 99% confidence interval of peak SNP on chromosome 11arranged in increasing order of base pair position. The significant markers are marked in the map.

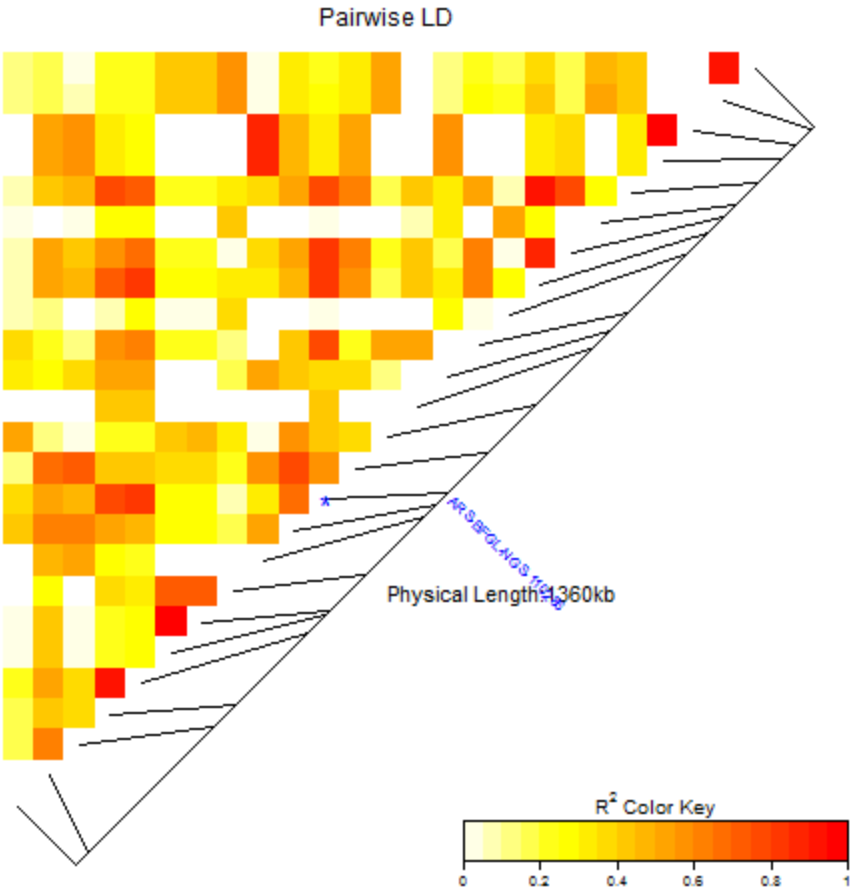

Figure 2.11: LD of all SNPs (n=25) within 99% confidence interval of peak SNP on chromosome 20 arranged in increasing order of base pair position. The significant marker is marked in the map.

Candidate genes

The significant regions on chromosome 11 (92879023 bp - 97160473 bp) and 20 (25002762 bp and 26362752 bp) were searched for candidate genes associated with AMH. Gene annotation information was retrieved from Ensembl genes database v. 88. of the cow genome (v. UMD 3.1). The region identified on chromosome 11 contained 81 genes. The most significant SNP was found within gene DENND1A. The region identified on chromosome 20 contained 9 genes. These genes perform a wide range of functions including maintenance of cell structure, cell

signaling, cell viability, embryonic development of cardiac, gonadal and brain tissues, embryonic survival, oocyte competence, cancer suppression and immunity. All the candidate genes and their functions have been listed in the appendix

DISCUSSION

The genomic heritability of AMH has not been reported for any species. Moreover, the genomic heritability of AMH reported in our study (0.36 ±0.03) is the highest for any trait related to reproduction in female cattle. Since endocrine reproductive traits directly reflect a cow's reproductive physiology and are less likely to be influenced by farm management (Bulman and Lamming, 1978) , Darwash et al., 1999), the heritability of endocrine reproductive traits is expected to be higher (Veerkamp et al., 2000) (Petersson et al., 2007) (Tenghe et al., 2015) relative to direct fertility traits as defined from calving data and insemination records.

The moderate to high heritability of AMH along with a potentially positive association of AMH with fertility in cattle indicates that AMH might be helpful in accurate predictions of breeding values for genetic potential of reproduction. The accuracy of genomic predictions depends on a number of factors including size of the reference population, density of markers, effective population size and most importantly the heritability of the trait under selection. We calculated the accuracy of the breeding values in our study by inverse of the set of linear model equations (the coefficient matrix). The average accuracy of prediction of breeding values in our study using 2905 animals and 54519 markers was 55.7%. Interestingly, the mean accuracy matches what would be expected based on a reference population of 3000 animals and a trait heritability of 0.35 (Hayes et al., 2009).

A number of genome wide association studies have been conducted in the past on a plethora of reproductive traits in dairy and beef cattle. Most of the GWA studies on fertility traits suffer from inadequate statistical power partly due to low heritabilities (Berry et al., 2014). Nonetheless, a number of QTLs have been identified for fertility traits in cattle (Tenghe et al., 2016) (Pryce et al., 2010) (Höglund et al., 2009) (Berry et al., 2012). However, when it comes to AMH, no GWA study has been reported in any livestock species. Perry et al., (2016) found 3 SNP variants associated with log serum AMH in human males which surprisingly had a sex interaction effect i.e. the same SNPs did not appear significant in females. As data in our study consists of female heifers, there is every possibility that a sex interaction effect exists for serum AMH levels in cattle as well. More research is needed in this regard to do a GWA study on a population that includes bulls and cows.

A review of GWA studies in various species revealed that the proportion of genetic variation in complex traits explained by major genes is usually <10% (Visscher et al., 2012). The proportion of genetic variance explained by significant regions mentioned earlier in this study is 13.3%. High density sequencing of the region on Chromosome 11 and 20 might help in identifying the causative QTL. Genes identified from the cow reference genome with in the significant region identified by the 99% confidence interval of the peak significant SNP display a wide range of functions i.e. maintenance of cell structure, cell signaling, cell viability, embryonic development of cardiac, gonadal and brain tissues, embryonic survival, tumor suppression, tumor proliferation, chemo resistance and immunity. Other genes identified in this region belong to olfactory signaling system or micro RNAs. Some of the important candidate genes identified are mentioned below:

The most significant SNP (Hapmap41435-BTA-115556 at 95026013 bp on chr 11) for AMH was located within the DENND1A gene (94526960 bp – 95055931 bp). Welt (2012) found that the most significant SNP for polycystic ovary (PCOS) in European and Chinese human females was also found within the same gene. Females suffering from this condition generally have abnormally high AMH concentrations (Pigny et al., 2003). Durlinger (2001) showed that AMH affects sensitivity of follicles to FSH treatments. Since there is a threshold level of FSH required for follicular growth, different follicles might show different thresholds depending the amount of AMH expressed in them. Therefore, AMH may play a role in the determining which follicles undergo selection and which are removed through atresia.

The gene NR5A1 gene (95514365-95538847)) encodes SF-1 (Steroidogenic factor 1) protein which binds to two different binding sites at the promoter region of AMH gene (Giuili et al., 1997; Watanabe et al., 2000). SF-1 binding site to AMH promoter is essential for sex and cell specific AMH promoter activity (Giuili et al., 1997) and transcription of key genes involved in sexual development and reproduction. Therefore, our analysis can be validated by previously published research findings. Similarly, the NR6A1 gene which is an oocyte specific transcription repressor also belongs to the same family and plays an important role in embryo development and folliculogenesis. NR6A1 is expressed in unfertilized eggs and preimplantation embryos. In adults, NR6A1 is expressed in the oocytes of primary follicles and all subsequent stages of folliculogenesis.

The PTGS1gene (93219287-93245045) has been identified as a candidate gene in a recent GWA study for superovulatory traits (total number of embryos and the number of viable embryos) (Jaton et al., 2015). This study showed that 81% of the significant SNP markers (46/57) for the total number of embryos, were located on Chromosome 11. Jaton et al. (2015) also determined

that for the number of viable embryos, 46 out of 47 significant SNPs associated with that trait were located on chromosome 11. All the significant SNP markers on chromosome 11 in that study were located within the region identified in our study (92879023 bp to 97160473 bp on chr 11). The most significant SNP in that analysis (BovineHD1100027188; 93306002 bp) was located nearby the (60,957 bases) prostaglandin-endoperoxide synthase 1 (PTGS1) gene. This gene is responsible for the conversion of arachidonic acid into different form of prostaglandins such as PGE2 and PGF2α (Arosh et al., 2002). Prostaglandins are well known to play an important role in ovulation (Armstrong, 1981) and are routinely used by veterinarians to treat ovarian cysts in dairy. As AMH is identified as an important marker of superovulation response (Monniaux et al., 2010; Souza et al., 2015), these findings suggest that AMH might have a QTL in common with super ovulatory traits.

The NADH dehydrogenase (ubiquinone) 1 alpha subcomplex (NDUFA8) (93011815-93029730) transfers electrons with a high redox potential from NADH to ubiquinone. This gene is highly expressed in human heart, skeletal muscle, and fetal heart (Triepels et al., 1998). A meta-analysis of studies in humans indicates that patients with PCOS have a 2-fold higher risk of cardiac disease than normal subjects (de Groot et al., 2011). More recently, AMH has been inversely correlated with the ultrasonographic diameters of the distal and mid-infrarenal aorta and high AMH levels have been associated with the absence of cardiovascular disease in men (Dennis et al., 2013).

The Follistatin gene (FST) (25588642-25594057) encodes for Follistatin protein which binds to members of the TGF-β superfamily particularly activin hormone. It was first discovered in the follicular fluid and known to regulate the follicle stimulating hormone (FSH) secretion from pituitary gland (Ying, 1988). Follistatin and activin interaction is widely known as the

autocrine/paracrine regulator of various physiological processes in reproduction including folliculogenesis and maturation of oocytes (Muttukrishna et al., 2004). Furthermore, increased abundance of Follistatin mRNA transcript was found to be positively associated with oocyte competence to fertilize and develop to blastocyst stage (Patel et al., 2007) and bovine early embryogenesis (Lee et al., 2009). Association of AMH with SNP markers adjacent (95027 bp apart) to FST gene indicates there might be a genetic relationship between the two hormones.

CONCLUSION

We have estimated the genomic heritability of AMH in cattle using an animal effects model based on their genotype information using 60 k standard USDA SNP panel. The higher heritability of AMH (0.36 ± 0.02) compared to other reproductive traits and its positive association with fertility can help accurately predict the breeding values of animal for fertility traits. Therefore, it can possibly be used for genetic improvement of reproductive potential in cattle. We also performed a GWA study to identify the QTL involved in AMH regulation. The study resulted in identification of a ~4.28 MB window on chromosome 11 and 1.35 MB window on chromosome 20 of bovine genome that might contain causative QTL of AMH regulation. We also identified potential candidate genes that appeared to have a key role in AMH expression based on previous research. Some of these genes have been previously reported to be involved in maintenance of cell structure, cell signaling, cell viability, embryonic development of cardiac, gonadal and brain tissues, embryonic survival, oocyte competence, cancer suppression and immunity. This might give us an insight to the mechanisms involved in AMH regulation and endocrine role of AMH in reproduction and development. Furthermore, this same region on Chromosome 11 has been recently identified as being associated with super ovulatory response

in an independent population indirectly indicating that some of these candidate genes mediate a role between AMH and super ovulatory response.

APPENDIX

Table 2.2: List of genes identified with in the significant region on chromosome 11 and 20

ENSEMBLE Gene ID	**Gene Name**	**Start position**	**End position**
ENSBTAG00000012827	TTLL11	92737269	92903570
ENSBTAG00000004295	NDUFA8	93011815	93029730
ENSBTAG00000004296	MORN5	93029874	93064357
ENSBTAG00000005525	LHX6	93069014	93090242
ENSBTAG00000016367	RBM18	93101473	93121906
ENSBTAG00000047226	MRRF	93121981	93138808
ENSBTAG00000018426	MRRF	93149379	93158576
ENSBTAG00000006716	PTGS1	93219287	93245045
ENSBTAG00000047610		93268704	93269738
ENSBTAG00000047693		93290982	93291917
ENSBTAG00000046018		93304997	93305947
ENSBTAG00000046137		93313595	93314530
ENSBTAG00000038309		93339271	93340212
ENSBTAG00000038796		93346999	93347931
ENSBTAG00000037739		93401560	93402516
ENSBTAG00000038278		93409030	93409962
ENSBTAG00000039225		93459896	93460798
ENSBTAG00000038726		93563425	93564411
ENSBTAG00000037542		93584334	93585269
ENSBTAG00000000726	OR1J1	93593230	93594171

Table 2.2 (cont'd)

ENSBTAG00000046126	OR1J2	93609988	93610929
ENSBTAG00000040047		93617052	93618040
ENSBTAG00000037767	OR1N1	93638723	93639658
ENSBTAG00000047728		93646064	93647008
ENSBTAG00000047112		93655028	93655969
ENSBTAG00000046536		93670180	93671118
ENSBTAG00000045527		93678033	93678974
ENSBTAG00000045545		93691882	93692865
ENSBTAG00000020660		93702914	93703858
ENSBTAG00000038551	OR1N2	93731042	93731977
ENSBTAG00000046221	OR1Q1	93742434	93743378
ENSBTAG00000045528		93765538	93766494
ENSBTAG00000001549	OR1L1	93797496	93798488
ENSBTAG00000048218	OR1L3	93808260	93809237
ENSBTAG00000038822		93835432	93836470
ENSBTAG00000038941		93853095	93854133
ENSBTAG00000037822		93866953	93868392
ENSBTAG00000038665		93885139	93886080
ENSBTAG00000004667		93900519	93901442
ENSBTAG00000030678		93927186	93927978
ENSBTAG00000030677		93935690	93936631
ENSBTAG00000038249	OR5C1	93960722	93961672

Table 2.2 (cont'd)

ENSBTAG00000030676	OR1K1	93969256	93970206
ENSBTAG00000030675	PDCL	93984123	93991685
ENSBTAG00000023867	RC3H2	94010161	94058169
ENSBTAG00000042329	SNORD90	94037106	94037216
ENSBTAG00000039815	ZBTB6	94072877	94074965
ENSBTAG00000038610	ZBTB26	94081312	94082670
ENSBTAG00000039172	RABGAP1	94103009	94264248
ENSBTAG00000040296	GPR21	94188119	94190302
ENSBTAG00000024604		94221939	94319233
ENSBTAG00000021921	STRBP	94281217	94418835
ENSBTAG00000042085	U6	94384741	94384847
ENSBTAG00000014391	CRB2	94501988	94523379
ENSBTAG00000003610	DENND1A	94526960	95055931
ENSBTAG00000044885	SNORA25	94574530	94574657
ENSBTAG00000023860		94931694	94932632
ENSBTAG00000010990	LHX2	95125308	95145048
ENSBTAG00000019470	NEK6	95314593	95400813
ENSBTAG00000003067	PSMB7	95401692	95458914
ENSBTAG00000017576	ADGRD2	95484105	95508348
ENSBTAG00000009017	NR5A1	95514365	95538847
ENSBTAG00000040585	NR6A1	95551663	95586813
ENSBTAG00000029841	bta-mir-181a-2	95709411	95709520

Table 2.2 (cont'd)

ENSBTAG00000029896	bta-mir-181b-2	95710626	95710714
ENSBTAG00000004848	OLFML2A	95780659	95805530
ENSBTAG00000044811	U6	95816939	95817043
ENSBTAG00000039223	WDR38	95846030	95850595
ENSBTAG00000003205	RPL35	95850116	95854734
ENSBTAG00000015278	ARPC5L	95859894	95867086
ENSBTAG00000015286	GOLGA1	95869270	95911957
ENSBTAG00000015553	SCAI	95925113	95996116
ENSBTAG00000045071	SNORA64	95982801	95982910
ENSBTAG00000008033	PPP6C	96044189	96085315
ENSBTAG00000015098	RABEPK	96092486	96113995
ENSBTAG00000007662	GRP78	96115572	96119306
ENSBTAG00000011544	GAPVD1	96144147	96208779
ENSBTAG00000043246	U6	96242761	96242864
ENSBTAG00000048297	U5	96264484	96264596
ENSBTAG00000010271	MAPKAP1	96278654	96511560
ENSBTAG00000013314	PBX3	96719400	96772227
ENSBTAG00000019834	ARL15	24955390	25026808
ENSBTAG00000003728	NDUFS4	25407689	25523382
ENSBTAG00000003329	FST	25588642	25594057
ENSBTAG00000046997	NA	25631428	25632999
ENSBTAG00000005380	MOCS2	25965175	25975522

Table 2.2 (cont'd)

ENSBTAG00000019289	ITGA2	25984555	26089593
ENSBTAG00000016525	ITGA1	26116747	26227530
ENSBTAG00000046575	NA	26174067	26174830
ENSBTAG00000003268	PELO	26278617	26280571

LITERATURE CITED

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Armstrong, D.T. 1981. Prostaglandins and follicular functions.. J. Reprod. Fertil. 62:283–91.

Arosh, J. a, J. Parent, P. Chapdelaine, J. Sirois, and M. a Fortier. 2002. Expression of cyclooxygenases 1 and 2 and prostaglandin E synthase in bovine endometrial tissue during the estrous cycle.. Biol. Reprod. 67:161–169. doi:10.1095/biolreprod67.1.161.

Baarends, W.M., J.T.J. Uilenbroek, P. Kramer, J.W. Hoogerbrugge, Axel P. N. Themmen, C.M.V.L. Elisabeth, and J.A. Grootegoed. 1995. Anti-Mullerian Hormone and Anti-Mullerian Hormone Type II Receptor Messenger Ribonucleic Acid Expression in Rat Ovaries during Postnatal Development, the Estrous Cycle, and Gonadotropin-Induced Follicle Growth. Endocrinology 136.

Batista, E.O.S., G.G. Macedo, R. V. Sala, M.D.D. V Ortolan, M.F. Sá Filho, T.A. Del Valle, E.F. Jesus, R.N.V.R. Lopes, F.P. Rennó, and P.S. Baruselli. 2014. Plasma antimullerian hormone as a predictor of ovarian antral follicular population in bos indicus (nelore) and bos taurus (holstein) heifers. Reprod. Domest. Anim. 49:448–452. doi:10.1111/rda.12304.

Berry, D.P., J.W.M. Bastiaansen, R.F. Veerkamp, S. Wijga, E. Wall, B. Berglund, and M.P.L. Calus. 2012. Genome-wide associations for fertility traits in Holstein–Friesian dairy cows using data from experimental research herds in four European countries. Animal 6:1206–1215. doi:10.1017/S1751731112000067.

Berry, D.P., E. Wall, and J.E. Pryce. 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8 Suppl 1:105–121. doi:10.1017/S1751731114000743.

Bulman, D.C., and G.E. Lamming. 1978. Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows.. J. Reprod. Fertil. 54:447–458. doi:10.1530/jrf.0.0540447.

Butler, W.R. 1998. Review: Effect of Protein Nutrition on Ovarian and Uterine Physiology in Dairy Cattle. J. Dairy Sci. 81:2533–2539. doi:10.3168/jds.S0022-0302(98)70146-8.

Casiró, S., D. Velez-Irizarry, C.W. Ernst, N.E. Raney, R.O. Bates, M.G. Charles, and J.P. Steibel. 2017. Genome-wide association study in an F2 Duroc x Pietrain resource population for economically important meat quality and carcass traits. J. Anim. Sci.. doi:10.2527/jas.2016.1003.

Cate, R.L., R.J. Mattaliano, C. Hession, R. Tizard, N.M. Farber, A. Cheung, E.G. Ninfa, A.Z. Frey, D.J. Gash, E.P. Chow, R.A. Fisher, J.M. Bertonis, G. Torres, B.P. Wallner, K.L. Ramachandran, R.C. Ragin, T.F. Manganaro, D.T. MacLaughlin, and P.K. Donahoe. 1986. Isolation of the bovine and human genes for müllerian inhibiting substance and expression of the human gene in animal cells. Cell 45:685–698. doi:10.1016/0092-8674(86)90783-X.

Cohen-Zinder, M., E. Seroussi, D.M. Larkin, J.J. Loor, A. Everts-Van Der Wind, J.H. Lee, J.K.

Drackley, M.R. Band, A.G. Hernandez, M. Shani, H.A. Lewin, J.I. Weller, and M. Ron. 2005. Identification of a missense mutation in the bovine ABCG2 gene with a major effect on the QTL on chromosome 6 affecting milk yield and composition in Holstein cattle. Genome Res. 15:936–944. doi:10.1101/gr.3806705.

Cole, J.B., P.M. VanRaden, J.R. O'Connell, C.P. Van Tassell, T.S. Sonstegard, R.D. Schnabel, J.F. Taylor, and G.R. Wiggans. 2009. Distribution and location of genetic effects for dairy traits. J. Dairy Sci. 92:2931–2946. doi:10.3168/jds.2008-1762.

Cong;, L., F.A. Ran;, D. Cox;, S. Lin;, R. Barretto;, N. Habib;, P.D. Hsu;, X. Wu;, W. Jiang;, L.A. Marraffini;, and F. Zhang; 2013. Multiplex Genome Engineering Using CRISPR/Cas Systems. Science (80-.). 339:819–823. doi:10.1126/science.1231143.

Daetwyler, H.D., B. Villanueva, and J.A. Woolliams. 2008. Accuracy of predicting the genetic risk of disease using a genome-wide approach. PLoS One 3. doi:10.1371/journal.pone.0003395.

Dematawewa, C.M.B., and P.J. Berger. 1998. Genetic and Phenotypic Parameters for 305-Day Yield, Fertility, and Survival in Holsteins. J. Dairy Sci. 81:2700–2709. doi:10.3168/jds.S0022-0302(98)75827-8.

Dennis, N.A., G.T. Jones, Y.H. Chong, A.M. van Rij, and I.S. McLennan. 2013. Serum anti-Mullerian hormone (AMH) levels correlate with infrarenal aortic diameter in healthy older men: Is AMH acardiovascular hormone?. J. Endocrinol. 219:13–20. doi:10.1530/JOE-13-0125.

Dijkhuizen, A.A., J. Stelwagen, and J.A. Renkema. 1984. Economic Aspects of Reproductive Failure in Dairy Cattle. I. Financial Loss At Farm Level. Elsevier Scmnce Publ. B V 385:251–263.

Durlinger, A.L.L., M.J.G. Gruijters, P. Kramer, B.A.S. Karels, H.A. Ingraham, M.W. Nachtigal, J.A.N.T.H.J. Uilenbroek, J.A. Grootegoed, A.P.N. Themmen, A.L.L.D. Reproduction, J.J.U. Th, and F. Medicine. 2002. Anti-Mullerian Hormone Inhibits Initiation of Primordial Follicle Growth in the Mouse Ovary. Endocrinology 143:1076–1084.

Durlinger, A.L.L., M.J.G. Gruijters, P. Kramer, B. Karels, T.R. Kumar, M.M. Matzuk, U.M. Rose, F.H. De Jong, J.T.J. Uilenbroek, J.A. Grootegoed, and A.P.N. Themmen. 2001. AMH attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology 142:4891–4899. doi:10.1210/en.142.11.4891.

Durlinger, A.L.L., P. Kramer, B. a S. Karels, F.H.D.E. Jong, J. a N.T.H.J. Uilenbroek, J.A. Grootegoed, A.P.N. Themmen, N. di Clemente, S. Ghaffari, R.B. Pepinsky, C. Pieau, N. Josso, R.L. Cate, and B. Vigier. 1999. Control of Primordial Follicle Recruitment by Anti-Mullerian Hormone in the Mouse Ovary. Endocrinology 140:5789–5796. doi:10.1210/en.140.12.5789.

Fricke, P.M., and M.C. Wiltbank. 1999. Effect of milk production on the incidence of double ovulation in dairy cows. Theriogenology 52:1133–1143. doi:10.1016/S0093-691X(99)00205-8.

Gamarra, G., C. Ponsart, S. Lacaze, B. Le Guienne, P. Humblot, M.C. Deloche, D. Monniaux, and A.A. Ponter. 2015. Dietary propylene glycol and in vitro embryo production after ovum pick-up in heifers with different anti-Müllerian hormone profiles. Reprod. Fertil. Dev. 27:1249–1261. doi:10.1071/RD14091.

García-Ruiz, A., J.B. Cole, P.M. VanRaden, G.R. Wiggans, F.J. Ruiz-López, and C.P. Van Tassell. 2016. Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection. Proc. Natl. Acad. Sci. 113:E3995–E4004. doi:10.1073/pnas.1519061113.

Garcia, J.F., R. V. Alonso, Y.T. Utsunomiya, and A.S. Carmo. 2013. Genomic selection and assisted reproduction technologies to foster cattle breeding. Anim. Reprod. 297–301.

Giuili, G., W.H. Shen, and H. a Ingraham. 1997. The nuclear receptor SF-1 mediates sexually dimorphic expression of Mullerian Inhibiting Substance, in vivo.. Development 124:1799–807.

Goddard, M. 2009. Genomic selection: Prediction of accuracy and maximisation of long term response. Genetica 136:245–257. doi:10.1007/s10709-008-9308-0.

Grisart, B., F. Farnir, L. Karim, N. Cambisano, J.-J. Kim, A. Kvasz, M. Mni, P. Simon, J.-M. Frère, W. Coppieters, and M. Georges. 2004. Genetic and functional confirmation of the causality of the DGAT1 K232A quantitative trait nucleotide in affecting milk yield and composition.. Proc. Natl. Acad. Sci. U. S. A. 101:2398–2403. doi:10.1073/pnas.0308518100.

de Groot, P.C.M., O.M. Dekkers, J.A. Romijn, S.W.M. Dieben, and F.M. Helmerhorst. 2011. PCOS, coronary heart disease, stroke and the influence of obesity: A systematic review and meta-analysis. Hum. Reprod. Update 17:495–500. doi:10.1093/humupd/dmr001.

Gualdrón Duarte, J.L., R.J.C. Cantet, R.O. Bates, C.W. Ernst, N.E. Raney, and J.P. Steibel. 2014. Rapid screening for phenotype-genotype associations by linear transformations of genomic evaluations.. BMC Bioinformatics 15:246. doi:10.1186/1471-2105-15-246.

Guerreiro, B.M., E.O.S. Batista, L.M. Vieira, M.F. Sá Filho, C.A. Rodrigues, A.C. Netto, C.R.A. Silveira, B.M. Bayeux, E.A.R. Dias, F.M. Monteiro, M. Accorsi, R.N.V.R. Lopes, and P.S. Baruselli. 2014. Plasma anti-Mullerian hormone: an endocrine marker for in vitro embryo production from Bos taurus and Bos indicus donors. Domest. Anim. Endocrinol. 49:96–104. doi:10.1016/j.domaniend.2014.07.002.

Habier, D., R.L. Fernando, and J.C.M. Dekkers. 2007. The impact of genetic relationship information on genome-assisted breeding values. Genetics 177:2389–2397.

doi:10.1534/genetics.107.081190.

Hansen, L.B. 2000. Consequences of Selection for Milk Yield from a Geneticist's Viewpoint. J. Dairy Sci. 83:1145–1150. doi:10.3168/jds.S0022-0302(00)74980-0.

Hirobe, S., W.W. He, M.M. Lee, and K. Donahoe. 1992. Mullerian Inhibiting Substance Messenger Ribonucleic Acid Expression in Granulosa and Sertoli Cells coincides with Their Mitotic Activity. Endocrinology 131:854–862.

Höglund, J.K., a J. Buitenhuis, B. Guldbrandtsen, G. Su, B. Thomsen, and M.S. Lund. 2009. Overlapping chromosomal regions for fertility traits and production traits in the Danish Holstein population.. J. Dairy Sci. 92:5712–5719. doi:10.3168/jds.2008-1964.

Ireland, J.J., G.W. Smith, D. Scheetz, F. Jimenez-Krassel, J.K. Folger, J.L.H. Ireland, F. Mossa, P. Lonergan, and A.C.O. Evans. 2011. Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-Mllerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in . Reprod. Fertil. Dev. 23:1–14. doi:10.1071/RD10226.

Ireland, J.J., A.E. Zielak-Steciwko, F. Jimenez-Krassel, J. Folger, A. Bettegowda, D. Scheetz, S. Walsh, F. Mossa, P.G. Knight, G.W. Smith, P. Lonergan, and A.C.O. Evans. 2009. Variation in the Ovarian Reserve Is Linked to Alterations in Intrafollicular Estradiol Production and Ovarian Biomarkers of Follicular Differentiation and Oocyte Quality in Cattle. Biol. Reprod. 80:954–964. doi:10.1095/biolreprod.108.073791.

Ireland, J.L.H., D. Scheetz, F. Jimenez-Krassel, a P.N. Themmen, F. Ward, P. Lonergan, G.W. Smith, G.I. Perez, a C.O. Evans, and J.J. Ireland. 2008. Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle.. Biol. Reprod. 79:1219–1225. doi:10.1095/biolreprod.108.071670.

Jaton, C., M. Sargolzaei, F. Miglior, C.A. Price, and A. Koeck. 2015. Genome-wide association study of superovulatory response traits in Canadian Holsteins (preliminary study) 1–10.

Jimenez-Krassel, F., D.M. Scheetz, L.M. Neuder, J.L.H. Ireland, J.R. Pursley, G.W. Smith, R.J. Tempelman, T. Ferris, W.E. Roudebush, F. Mossa, P. Lonergan, A.C.O. Evans, and J.J. Ireland. 2015. Concentration of anti-Müllerian hormone in dairy heifers is positively associated with productive herd life.. J. Dairy Sci. 98:3036–45. doi:10.3168/jds.2014-8130.

Kevenaar, M.E., M.F. Meerasahib, P. Kramer, B.M.N. Van De Lang-Born, F.H. De Jong, N.P. Groome, A.P.N. Themmen, and J.A. Visser. 2006. Serum anti-Mullerian hormone levels reflect the size of the primordial follicle pool in mice. Endocrinology 147:3228–3234. doi:10.1210/en.2005-1588.

Lahoz, B., J.L. Alabart, D. Monniaux, P. Mermillod, and J. Folch. 2012. Anti-Müllerian hormone plasma concentration in prepubertal ewe lambs as a predictor of their fertility at a young age. BMC Vet. Res. 8:118. doi:10.1186/1746-6148-8-118.

Lawrence, M., W. Huber, H. Pagès, P. Aboyoun, M. Carlson, R. Gentleman, M.T. Morgan, and V.J. Carey. 2013. Software for Computing and Annotating Genomic Ranges. PLoS Comput. Biol. 9:1–10. doi:10.1371/journal.pcbi.1003118.

Lee, K.B., A. Bettegowda, G. Wee, J.J. Ireland, and G.W. Smith. 2009. Molecular determinants of oocyte competence: Potential functional role for maternal (oocyte-derived) follistatin in promoting bovine early embryogenesis. Endocrinology 150:2463–2471. doi:10.1210/en.2008-1574.

Lee, M.M., A. Noto, D. Schoenfeld, D.T. Maclaughlin, G. Mol-rr-, N.C. Grant, and L.E.E.E.T. Al. 2008. Substance in Humans : Normal 81:2–7.

Lourenço, D., R. Brauner, L. Lin, A.D. Perdigo, G. Weryha, M. Muresan, R. Boudjenah, K.M. Guerra-Junior, A. T. Maciel-Guerra, J. C. Achermann, and A. Bashamboo. 2009. Mutations in NR5A1 Associated with Ovarian Insufficiency. N. Engl. J. Med. 360:10. doi:10.1083/jcb.200402157.

Lucy, M.C. 2001. Reproductive Loss in High-Producing Dairy Cattle: Where Will It End?. J. Dairy Sci. 84:1277–1293. doi:10.3168/jds.S0022-0302(01)70158-0.

Lucy, M.C., W.J. Weber, L.H. Baumgard, B.S. Seguin, A.T. Koenigsfeld, L.B. Hansen, H. Chester-Jones, and B.A. Crooker. 1998. Reproductive endocrinology of lactating dairy cows selected for increased milk production. J. Anim. Sci. 76:246.

Luo, X., Y. Ikeda, and K.L. Parker. 1994. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell 77:481–490. doi:10.1016/0092-8674(94)90211-9.

Macmillan, K., I. Lean, and C. Westwood. 1996. The effects of lactation on the fertility of dairy cows. Aust. Vet. J. 73:141–147. doi:10.1111/j.1751-0813.1996.tb10007.x.

La Marca, A., R. Orvieto, S. Giulini, V.M. Jasonni, A. Volpe, and V. De Leo. 2004. Mullerian-inhibiting substance in women with polycystic ovary syndrome: relationship with hormonal and metabolic characteristics.. Fertil. Steril. 82:970–2. doi:10.1016/j.fertnstert.2004.06.001.

Marion, G.B., and H.T. Gier. 1967. Factors Affecting Bovine Ovarian Activity After Parturition. J. Anim. Sci. 1621–1626.

McAllister, J.M., R.S. Legro, B.P. Modi, and J.F. Strauss. 2015. Functional genomics of PCOS: From GWAS to molecular mechanisms. Trends Endocrinol. Metab. 26:118–124. doi:10.1016/j.tem.2014.12.004.

Meuwissen, T.H. 2009. Accuracy of breeding values of "unrelated" individuals predicted by dense SNP genotyping. Genet. Sel. Evol. 41:35. doi:10.1186/1297-9686-41-35.

Meuwissen, T.H.E., B.J. Hayes, and M.E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829. doi:11290733.

Miglior, F., B.L. Muir, and B.J. Van Doormaal. 2005. Selection indices in Holstein cattle of various countries.. J. Dairy Sci. 88:1255–1263. doi:10.3168/jds.S0022-0302(05)72792-2.

Monniaux, D., S. Barbey, C. Rico, S. Fabre, Y. Gallard, and H. Larroque. 2010. Anti-Mllerian hormone: A predictive marker of embryo production in cattle?. Reprod. Fertil. Dev. 22:1083–1091. doi:10.1071/RD09279.

Mossa, F., S.W. Walsh, S.T. Butler, D.P. Berry, F. Carter, P. Lonergan, G.W. Smith, J.J. Ireland, and A.C.O. Evans. 2012. Low numbers of ovarian follicles ≥3mm in diameter are associated with low fertility in dairy cows. J. Dairy Sci. 95:2355–2361. doi:10.3168/jds.2011-4325.

Munsterberg, A., and R. Lovell-Badge. 1991. Expression of the mouse anti-mullerian hormone gene suggests a role in both male and female sexual differentiation.. Development 113:613–624.

Muttukrishna, S., D. Tannetta, N. Groome, and I. Sargent. 2004. Activin and follistatin in female reproduction. Mol. Cell. Endocrinol. 225:45–56. doi:10.1016/j.mce.2004.02.012.

Patel, O. V., A. Bettegowda, J.J. Ireland, P.M. Coussens, P. Lonergan, and G.W. Smith. 2007. Functional genomics studies of oocyte competence: Evidence that reduced trascript abundance for follistatin is associated with poor developmental competence of bovine oocytes. Reproduction 133:95–106. doi:10.1530/rep.1.01123.

Perry, J.R.B., G. McMahon, F.R. Day, S.M. Ring, S.M. Nelson, and D.A. Lawlor. 2016. Genome-wide association study identifies common and low-frequency variants at the AMH gene locus that strongly predict serum AMH levels in males. Hum. Mol. Genet. 25:382–388. doi:10.1093/hmg/ddv465.

Petersson, K.J., B. Berglund, E. Strandberg, H. Gustafsson, A.P.F. Flint, J.A. Woolliams, and M.D. Royal. 2007. Genetic Analysis of Postpartum Measures of Luteal Activity in Dairy Cows. J. Dairy Sci. 90:427–434. doi:http://dx.doi.org/10.3168/jds.S0022-0302(07)72644-9.

Pigny, P., E. Merlen, Y. Robert, C. Cortet-Rudelli, C. Decanter, S. Jonard, and D. Dewailly. 2003. Elevated Serum Level of Anti-Mullerian Hormone in Patients with Polycystic Ovary Syndrome: Relationship to the Ovarian Follicle Excess and to the Follicular Arrest. J. Clin. Endocrinol. Metab. 88:5957–5962. doi:10.1210/jc.2003-030727.

Pryce, J.E., S. Bolormaa, a J. Chamberlain, P.J. Bowman, K. Savin, M.E. Goddard, and B.J. Hayes. 2010. A validated genome-wide association study in 2 dairy cattle breeds for milk production and fertility traits using variable length haplotypes.. J. Dairy Sci. 93:3331–3345. doi:10.3168/jds.2009-2893.

Redhead, A.K.R., M. Knights, D. Ph, I. Holaskova, D. Ph, J. Moritz, D. Ph, K. Shaffer, D. Ph, M. Wilson, and D. Ph. 2017. The Use of Concentration of Anti-Mullerian Hormone (AMH) as an Indicator of Reproductive Performance in Livestock Species Adam Karl Russell Redhead Dissertation submitted To the Davis College of Agriculture , Natural Resources and Design At West Virgini.

Ribeiro, E.S., R.S. Bisinotto, F.S. Lima, L.F. Greco, A. Morrison, A. Kumar, W.W. Thatcher, and J.E.P. Santos. 2014. Plasma anti-Müllerian hormone in adult dairy cows and associations with fertility. J. Dairy Sci. 97:6888–6900. doi:http://dx.doi.org/10.3168/jds.2014-7908.

Rico, C., L. Drouilhet, P. Salvetti, R. Dalbìs-Tran, P. Jarrier, J.L. Touź, E. Pillet, C. Ponsart, S. Fabre, and D. Monniaux. 2012. Determination of anti-Mullerian hormone concentrations in blood as a tool to select Holstein donor cows for embryo production: From the laboratory to the farm. Reprod. Fertil. Dev. 24:932–944. doi:10.1071/RD11290.

Rico, C., S. Fabre, C. Medigue, N. d. Clemente, F. Clement, M. Bontoux, J.-L. Touze, M. Dupont, E. Briant, B. Remy, J.-F. Beckers, and D. Monniaux. 2009. Anti-Mullerian Hormone Is an Endocrine Marker of Ovarian Gonadotropin-Responsive Follicles and Can Help to Predict Superovulatory Responses in the Cow. Biol. Reprod. 80:50–59. doi:10.1095/biolreprod.108.072157.

Rico, C., C. Médigue, S. Fabre, P. Jarrier, M. Bontoux, F. Clément, and D. Monniaux. 2011. Regulation of Anti-Müllerian Hormone Production in the Cow: A Multiscale Study at Endocrine, Ovarian, Follicular, and Granulosa Cell Levels1. Biol. Reprod. 84:560–571. doi:10.1095/biolreprod.110.088187.

Sartori, R., G.J.M. Rosa, and M.C. Wiltbank. 2002. Ovarian Structures and Circulating Steroids in Heifers and Lactating Cows in Summer and Lactating and Dry Cows in Winter. J. Dairy Sci. 85:2813–2822. doi:10.3168/jds.S0022-0302(02)74368-3.

Smidt, D., and H. Niemann. 1999. Biotechnology in genetics and reproduction. Livest. Prod. Sci. 59:207–221. doi:10.1016/S0301-6226(99)00028-7.

Souza, A.H., P.D. Carvalho, A.E. Rozner, L.M. Vieira, K.S. Hackbart, R.W. Bender, A.R. Dresch, J.P. Verstegen, R.D. Shaver, and M.C. Wiltbank. 2015. Relationship between circulating anti-Müllerian hormone (AMH) and superovulatory response of high-producing dairy cows. J. Dairy Sci. 98:169–178. doi:10.3168/jds.2014-8182.

Stojsin-Carter, A., K. Mahboubi, N.N. Costa, D.J. Gillis, T.F. Carter, M.S. Neal, M.S. Miranda, O.M. Ohashi, L.A. Favetta, and W.A. King. 2016. Systemic and local anti-Mullerian hormone reflects differences in the reproduction potential of Zebu and European type cattle. Anim. Reprod. Sci. 167:51–58. doi:10.1016/j.anireprosci.2016.02.003.

Storey, J.D., and R. Tibshirani. 2003. Statistical significance for genomewide studies.. Proc. Natl. Acad. Sci. U. S. A. 100:9440–5. doi:10.1073/pnas.1530509100.

Tenghe, A.M.M., A.C. Bouwman, B. Berglund, E. Strandberg, J.Y. Blom, and R.F. Veerkamp. 2015. Estimating genetic parameters for fertility in dairy cows from in-line milk progesterone profiles.. J. Dairy Sci. 98:5763–5773. doi:10.3168/jds.2014-8732.

Tenghe, A.M.M., A.C. Bouwman, B. Berglund, E. Strandberg, D.J. de Koning, and R.F. Veerkamp. 2016. Genome-wide association study for endocrine fertility traits using single nucleotide polymorphism arrays and sequence variants in dairy cattle. J. Dairy Sci. 99:5470–5485. doi:10.3168/jds.2015-10533.

Tiftik, A.M., A. Kelimeler, and O. Kisti. 2016. Anti-Müllerian Hormone (AMH) in Adults with Polycystic Ovary Syndrome and Pregnants. Ann. Eurasian Med. 4:65–70. doi:10.4328/AEMED.92.

Triepels, R., L. Van den Heuvel, J. Loeffen, R. Smeets, F. Trijbels, and J. Smeitink. 1998. The nuclear-encoded human NADH:ubiquinone oxidoreductase NDUFA8 subunit: CDNA cloning, chromosomal localization, tissue distribution, and mutation detection in complex-I-deficient patients. Hum. Genet. 103:557–563. doi:10.1007/s004390050869.

Tsepelidis, S., F. Devreker, I. Demeestere, A. Flahaut, C. Gervy, and Y. Englert. 2007. Stable serum levels of anti-Mullerian hormone during the Stable serum levels of anti-Mu menstrual cycle : a prospective study in normo-ovulatory women. Hum. Reprod. 22:1837–1840.

VanRaden, P.M. 2008. Efficient methods to compute genomic predictions.. J. Dairy Sci. 91:4414–23. doi:10.3168/jds.2007-0980.

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. 87:2285–92. doi:10.3168/jds.S0022-0302(04)70049-1.

Veerkamp, R.F., E.P.C. Koenen, and G. De Jong. 2001. Genetic Correlations Among Body Condition Score, Yield, and Fertility in First-Parity Cows Estimated by Random Regression Models. J. Dairy Sci. 84:2327–2335. doi:10.3168/jds.S0022-0302(01)74681-4.

Veerkamp, R.F., J.K. Oldenbroek, H.J. Van Der Gaast, and J.H.J. Van Der Werf. 2000. Genetic Correlation Between Days Until Start of Luteal Activity and Milk Yield, Energy Balance, and Live Weights. J. Dairy Sci. 83:577–583. doi:10.3168/jds.S0022-0302(00)74917-4.

Vernunft, A., M. Schwerhoff, T. Viergutz, M. Diederich, and A. Kuwer. 2015. Anti-Muellerian hormone levels in plasma of Holstein-Friesian heifers as a predictive parameter for ovum pick-up and embryo production outcomes. J. Reprod. Dev. 61:1–6.

Visscher, P.M., M.A. Brown, M.I. McCarthy, and J. Yang. 2012. Five years of GWAS discovery. Am. J. Hum. Genet. 90:7–24. doi:10.1016/j.ajhg.2011.11.029.

Visser, J.A., and A.P.N. Themmen. 2005. Anti-Mullerian hormone and folliculogenesis. Mol. Cell. Endocrinol. 234:81–86. doi:10.1016/j.mce.2004.09.008.

Van Vleck, L.D. 1993. Selection Index and Introduction to Mixed Model Methods. CRC Press, Boca Raton, FL.

de Vries, M.J., and R.F. Veerkamp. 2000. Energy Balance of Dairy Cattle in Relation to Milk Production Variables and Fertility. J. Dairy Sci. 83:62–69. doi:10.3168/jds.S0022-0302(00)74856-9.

Watanabe, K., T.R. Clarke, A.H. Lane, X. Wang, and P.K. Donahoe. 2000. Endogenous expression of Mullerian inhibiting substance in early postnatal rat Sertoli cells requires multiple steroidogenic factor-1 and GATA-4-binding sites. Proc. Natl. Acad. Sci. 97:1624–1629. doi:10.1073/pnas.97.4.1624.

Weller, J.I., and M. Ron. 2011. Invited review: Quantitative trait nucleotide determination in the era of genomic selection. J. Dairy Sci. 94:1082–1090. doi:10.3168/jds.2010-3793.

Welt, C.K., U. Styrkarsdottir, D.A. Ehrmann, G. Thorleifsson, G. Arason, J.A. Gudmundsson, C. Ober, R.L. Rosenfield, R. Saxena, U. Thorsteinsdottir, W.F. Crowley, and K. Stefansson. 2012. Variants in DENND1A are associated with polycystic ovary syndrome in women of European ancestry. J. Clin. Endocrinol. Metab. 97:1342–1347. doi:10.1210/jc.2011-3478.

Ying, S.Y. 1988. Inhibins, activins, and follistatins: Gonadal proteins modulating the secretion of follicle-stimulating hormone. Endocr. Rev. 9:267–293. doi:10.1210/edrv-9-2-267.

CHAPTER THREE

Relationship of Anti Mμllerian Hormone with Reproductive Traits in Holstein Heifers

ABSTRACT

AMH is an endocrine marker of reproductive potential in various species of animals. Superovulation response and some traits of reproductive performance in dairy cows like conception rate and herd longevity are positively correlated with AMH concentration. The objective of this study was to look at the relationship of dairy heifer fertility with AMH concentrations using 3071 Holstein dairy heifers. Measures of reproductive performance analyzed were: first service conception rate of animals subjected to artificial insemination via regular or sexed semen, conception rate following embryo transfer, overall probability of conception, probability of abortion, number of services per conception, age at first calving and number of days open at first lactation. Results indicated that the probability of conception has a moderately significant (P=0.03) linear association with AMH concentration in animals within the first quartile of AMH, suggesting a possible threshold effect of AMH. However, no phenotypic relationship was found between AMH and the other measures of reproductive performance in heifers. Future studies will evaluate whether AMH concentration is correlated with measures of reproductive performance in later stages of productive life when animals are more prone to reproductive problems.

INTRODUCTION

Efficiency of reproduction is one of the primary determinants of dairy farm profitability. Reproductive losses on a dairy farm translate into a number of short term and long term economic losses such as longer calving intervals, shorter herd life, higher replacement costs, higher costs of veterinary treatment and drugs, etc. Total losses due to reproductive failure equals about 2% of the gross production value or 10% of an average farmer's income (Dijkhuizen et al., 1984). Therefore, in addition to optimizing reproductive management of animals on the farm, identification and selection of animals with higher genetic potential for reproduction and successful application of assisted reproductive technologies (ART) are equally important to maximize fertility. However, the rate of genetic gain depends on the heritability of reproductive traits while the success of ART depends on successful identification of animals with superior genetic potential for super ovulatory traits.

AMH is a novel endocrine indicator of reproductive potential in animals. We have established AMH concentration in Holstein heifers (n = 2905) is highly heritable compared with other reproductive traits (h=0.36±0.02). AMH has already been reported to be positively associated with antral follicle count (AFC) (Ireland et al., 2008; Rico et al., 2009; Batista et al., 2014), superovulation response (Monniaux et al., 2010; Souza et al., 2015), in-vitro embryo production (IVEP) (Guerreiro et al., 2014; Gamarra et al., 2015; Vernunft et al., 2015), dairy herd longevity (Jimenez-Krassel et al., 2015), maintenance of pregnancy, and pregnancy rate in cows bred on estrus (Ribeiro et al., 2014). Moreover, AFC which is highly positively correlated with AMH concentration, is also positively correlated with number of morphologically healthy oocytes, progesterone and testosterone production during estrous cycles, responsiveness of granulosa and thecal cells to gonadotropins in vitro and responsiveness to superovulation. Taken together,

circulating AMH concentration has the potential to be a unique phenotypic marker that may be useful to genetically improve reproduction in dairy cattle. The present study used a large number of Holstein heifers (n = 3259) to test the hypothesis that AMH concentration is positively correlated with fertility. To test this hypothesis, we examined the association of AMH concentration with the following reproductive traits:

- Probability of conception to first service
- Probability of conception in embryo recipients
- Probability of conception after multiple services
- Number of services per conception
- Probability of abortion
- Days open in first lactation animals
- Age at first calving
- Differences in AMH concentrations between pregnant and non-pregnant animals

MATERIALS AND METHODS

All experiments involving cattle were approved by the IACUC at Michigan State University. Adult Holstein heifers (n = 3259, 11-15 months old, located on Green Meadow Farms Inc, Elsie, MI) were subjected *once* to two 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 per month for a total number of 29 groups. 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 and establish relative size of the ovarian reserve. Blood samples were taken beginning on 4/18/2014 and ending on 12/4/2015. Follicle hair samples were collected at this times for

genotypic analyses. Animals that were considered freemartins (n=144) and animals that were sold as replacements to other farmers (n=243) were not included in statistical analyses of reproductive performance as lactating animals. After completion of the $PGF_{2\alpha}$ -induced estrous cycle, heifers were subjected to artificial insemination (AI) at the next detected "standing" estrus or the next morning after standing estrus, and palpation of uterine contents 45 to 60 days after AI was used to diagnose pregnancy. Heifers not diagnosed pregnant after AI were subjected to AI, as just explained (except $PGF_{2\alpha}$ was not used), up to 5 to 6 times. After calving, and starting at 45 DIM, cows were subjected to AI at standing estrus after either of two injections of $PGF_{2\alpha}$ or at fixed-time AI (using Ovsynch technology) at discretion of farm personnel and pregnancy determined 35 d after AI (via palpation or ultrasonography). Cows exhibiting estrous behavior before pregnancy diagnosis were inseminated at standing estrus while cows diagnosed not pregnant were given a single $PGF_{2\alpha}$ injection if a corpus luteum was present and inseminated at the next standing estrus. Cows without CL were given two $PGF_{2\alpha}$ injections spaced 11 d apart as explained for heifers. This breeding regimen in cows continued up to 4 to 5 times.

Records on reproductive performance, level of milk production, health, and reasons for culling of each individual animal at Green Meadow Farms were maintained in an on-the-farm computer using the commercial DairyCOMP 305 software program. Relevant information for each cow was recorded daily into DairyCOMP 305 by Green Meadow Farms' managers and selected employees.

Statistical analysis

Logistic regression was used with each model including a linear coefficient on serum concentration of AMH to assess the effect of AMH on first service conception rate of animals subjected to AI via regular or sexed semen, conception rate in embryo recipients, overall

conception rate and abortion rate. The same phenotypes were also examined by using AMH quartiles as a factor with four levels followed by using nesting linear coefficients on AMH within each quartile separately. This was pursued to allow for the possibility of threshold effects; i.e., a linear relationship with AMH may only exist before or beyond a certain quartile. Quartile means were separated using the Tukey test. The cutoff values for AMH quartiles are given below:

Table 3.1: Cut off values of quartiles for AMH concentration

	Quartile 1 (pg/ml)	**Quartile 2 (pg/ml)**	**Quartile 3 (pg/ml)**	**Quartile 4 (pg/ml)**
Cut off values	<198.96	>198.96 and <323.17	>323.17 and <502.88	>502.88

Furthermore, we examined differences in AMH concentrations for binary traits i.e. pregnant vs non-pregnant on first service for each of regular semen, sexed semen and embryo transfer recipients, pregnant vs non-pregnant for all animals that were bred at least once, and aborted vs non-aborted animals using a one-way ANOVA with AMH concentration as a dependent variable.

A Poisson regression model was used to study the effect of AMH concentration on the number of services per conception. In a similar manner with the other traits, we also used AMH quartiles and with separate linear coefficients on AMH within each quartile in separate analysis to model the number of services per conception.

A simple linear regression model was also used to study the effect of AMH on age at first calving and days open in the first lactation.

All analyses were done using the ‘glm’ function for Poisson and logistic regression and the ‘lm’ function for linear regression in R programming software version 3.3.1.

RESULTS

Probability of conception in embryo recipients

No overall relationship (P>0.3) between linear AMH concentration and probability of conception was determined for embryo recipients using a classical logistic regression analysis. There was a marginally significant relationship (P=0.03) between probability of conception in embryo recipients and their AMH concentration with in the first quartile. However, no such relationship was found for animals within the 2^{nd}, 3^{rd} and 4^{th} quartiles. There was no significant difference in conception rates between the quartiles separated using the Tukey test. Least squares mean AMH concentration of animals that were successfully pregnant (336.97±11.76) on embryo transfer were not significantly different from animals that failed to become pregnant (314.19±12.57).

Probability of conception to first service in artificial insemination recipients

No relationship was found between linear AMH concentration and probability of conception in AI recipients using regular semen (P>0.9) and sexed semen (P>0.7). There was no significant difference of probability of conception for regular and sexed semen recipients between the quartiles separated using Tukey HSD.Least squares mean AMH concentration of animals that were successfully pregnant to first service using regular AI semen was 293.67±9.38verses 298.74±10.08for non-pregnant animals respectively.

Least squares mean AMH concentration of animals that were successfully pregnant to first service using sexed semen was 309.55±14.05verses 305.40±13.43for non-pregnant animals respectively.

Table 3.2: Least square geometric mean AMH concentrations for different reproductive events

Event	**Yes**	**No**	**P-value**
Conception to Embryo transfer	336.97±11.76	314.19±12.57	0.21
Conception on first AI (regular semen)	293.67±9.38	298.74±10.08	0.71
Conception on first AI (sexed semen)	309.55±14.05	305.40±13.43	0.83
Conceived at least once	307.74±4.83	293.07±24.25	0.56
Abortion	307.54±21.76	307.68±4.95	0.99

Table 3.3: Relationship of AMH with probability of conception nested within quartiles

Event	**Logistic regression P-values**			
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**
Conception to Embryo transfer	0.03 n=221	0.13 n=226	0.10 n=221	0.30 n=256
Conception on first AI (regular semen)	0.06 n=378	0.757 n=378	0.203 N=391	0.89 n=343
Conception on first AI (sexed semen)	0.85 n=169	0.89 n=164	0.84 n=155	0.69 n=169

Overall probability of conception

No relationship (P>0.4) was found between probability of conception and linked linear function of AMH concentration. 2964 animals were pregnant out of 3072 animals that were bred at least once. Least squares mean AMH concentration in animals that conceived (307.74±4.83) verses those who never conceived (293.07±24.25) were not different from each other.

Probability of abortion

No relationship (P>0.5) was found between probability of abortion and linked linear function of AMH concentration. 146 animals aborted out of 2964 that were confirmed pregnant. Least

squares mean AMH concentration in aborted (307.54±21.76) verses non aborted (307.68±4.95) were not different from each other.

Days open

Number of days open after first lactation had a near-zero correlation with AMH (r=-0.02) such that there was no statistically significant relationship (P>0.39) between them.

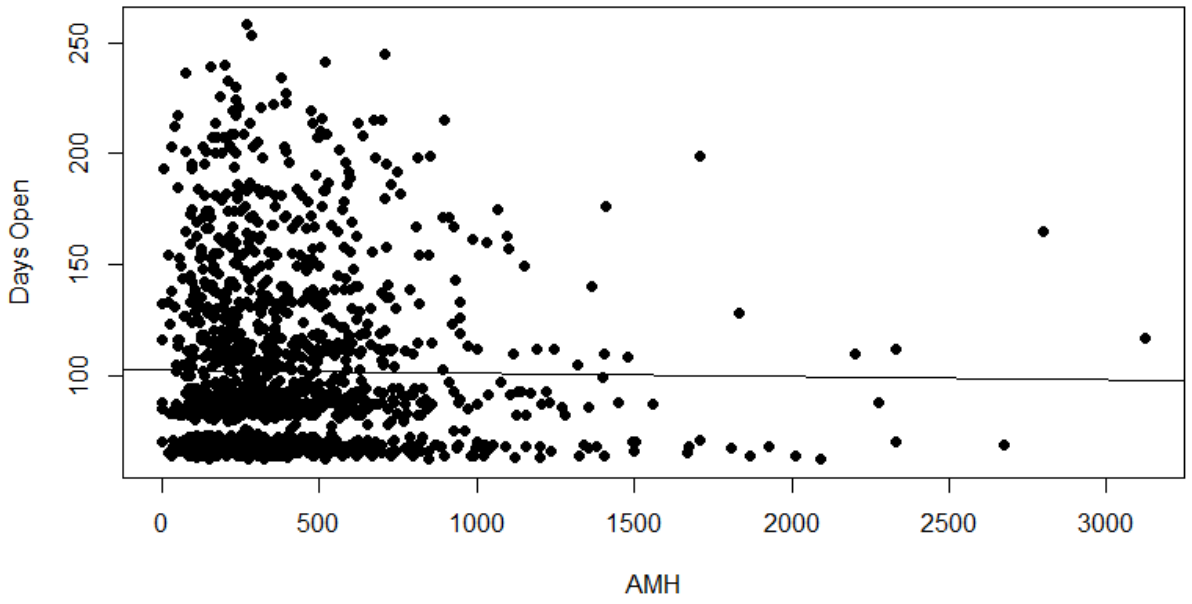

Figure 3.1: Plot of number of days open after first lactation and serum AMH concentration

Age at first calving

Age at first calving had a near-zero correlation with AMH (r=-0.004) such that there was no statistically significant relationship (p>0.8) between the age at first calving and AMH concentration.

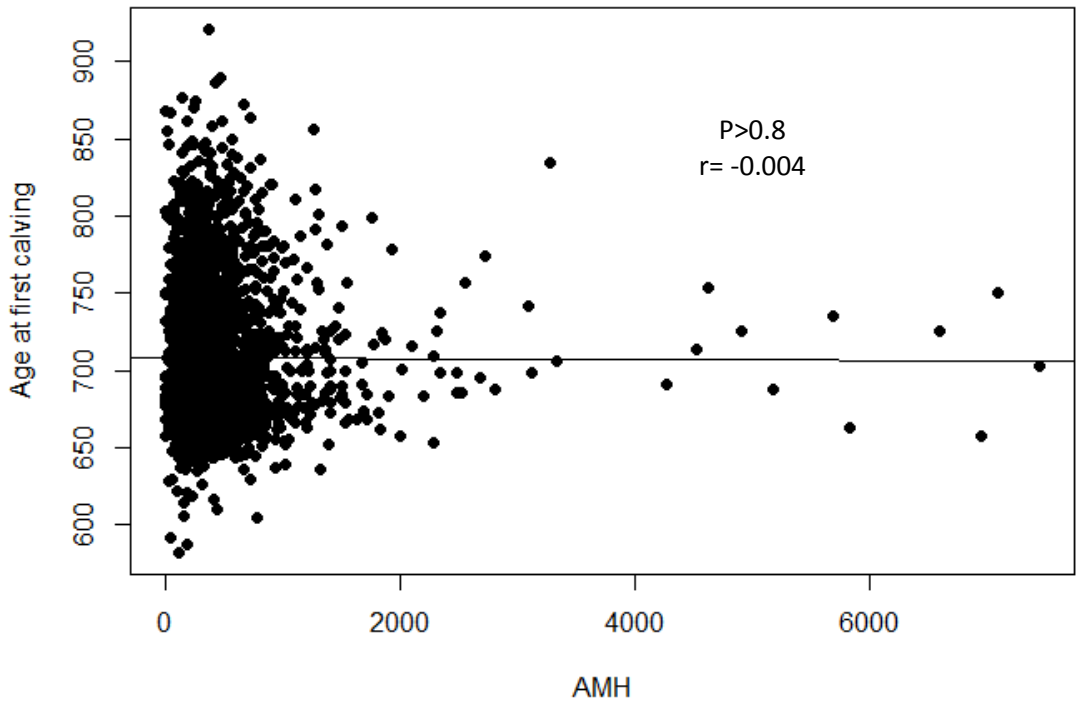

Figure 3.2: Plot of age at first calving and serum AMH concentration

Number of services per conception

Number of services per conception had a low negative correlation (r=0.019) with AMH which was not statistically significant (P> 0.2) when analyzed using Poisson regression model assuming quasi-Poisson distribution. When the AMH covariate was divided into quartiles, there was no difference in number of services per conception between the quartiles.

DISCUSSION

Correlation between AMH with fertility traits has been studied in a number of different ways. Redhead et al., (2017) placed animals into LOW, MEDIUM and HIGH AMH groups, respectively. Jimenez-Krassel et al., (2015) divided the animals into quartiles based on AMH concentrations as 11 to 12 months old heifers and examined differences in fertility traits between quartiles. They concluded that the herd longevity was higher for second and third quartile than the first and fourth quartile suggesting a quadratic relationship. Therefore, we adopted a set of different strategies: first looking at overall linked linear relationships of fertility traits with AMH, followed by linear coefficients on AMH within each quartile and between quartile comparisons.

Our results indicate that AMH appears to be moderately associated with probability of conception in embryo recipients within the first quartile. However, no such relationship appears to exist for animals in the other AMH quartiles, suggesting that there might be a threshold level of AMH concentration required for successful pregnancy. That is, once this threshold is achieved, higher AMH concentrations are not associated with higher probability of conception. Lahoz et al., (2012) reported a similar observation in Rasa Aragonesa sheep, and suggested the cut off value of 97 pg/ml to distinguish between females with low and high fertility.

We found no evidence of a relationship between AMH and probability of conception to first service for heifers subjected to timed AI using sexed or regular semen and embryo recipients. Ribeiro (2014) presented similar results that no relationship existed between AMH and pregnancy for cows bred on timed AI protocol. However, cows with low AMH concentration had lower pregnancy rates after first service for cows bred after detection of estrus. This is probably due to the fact that estrus synchronization procedure might have optimized the follicle

development and ovulation process such that variation in AMH concentrations was no longer associated with pregnancy.

We determined no evidence that overall probability of conception of heifers subjected to multiple fixed time AI, and probability of abortion in heifers was associated with AMH. Furthermore, no relationship was found between AMH with days open, number of services per conception, age at first calving and probability of abortion in heifers. Jimenez-Krassel (2015) showed similar results with a much smaller dataset. Age at first AI, at first conception, and at each calving did not differ among the different AMH quartiles. This can possibly be explained by considering the fact that AMH is correlated with the number of follicles but not the quality of oocytes. Heifers being mono ovulatory animals, need only a single oocyte per successful pregnancy. Therefore, their probability of conception may be independent of the number of follicles in the ovary.

In contrast to heifers, dairy cows of mixed ages (up to 8 parities) with a low AFC, which is correlated positively with AMH , had a significantly lower conception rate to first AI, required a greater number of AI to conceive, and had a greater calving interval compared with cows with an intermediate or higher AFC (Mossa et al., 2012). Cows with low AFC and AMH have abnormal changes in endometrial thickness during the estrous cycle (Jimenez-Krassel et al., 2009), which suggest that the endometria of such cows are less capable of conceptus implantation and maintenance of pregnancy. Moreover, cows with low AFC also have a lower concentration of progesterone (Jimenez-Krassel et al., 2015) which is required to play an important role in maintenance of pregnancy. Ribeiro (2014) showed that lactating cows with low AMH had greater risk of pregnancy loss than cows with intermediate or high AMH. Perhaps, compared with heifers, fertility in cows with low compared with higher AMH is more sensitive

to the physiological stress related to lactation and decreased progesterone concentration (Sartori et al., 2002). Therefore, heifers may not be the ideal model to study the effect of AMH on fertility.

CONCLUSION

In summary, AMH concentration shows some tendency to predict the probability of conception in embryo recipient heifers within the first quartile of AMH. Hence, there may be a threshold level of AMH required for optimum probability of conception in embryo recipients as the relationship only exists in the first quartile animals. However, other reproductive parameters in heifers such as age at first calving, days open, conception rates, and probability of abortion are not related with serum AMH concentrations.

OVERALL CONCLUSIONS AND FUTURE DIRECTIONS

Anti Mullerian hormone (AMH) is considered to be an endocrine marker of fertility and super ovulatory response traits in cattle. We set out to estimate the genomic heritability of AMH in Holstein heifers based on their genotype information using 60 k standard USDA SNP panel. The heritability of AMH (0.36 ± 0.02) that we estimated is relatively large compared to conventional but economically relevant reproductive traits. Furthermore, its well documented association with super ovulatory response hints at the possible use of AMH for animals that respond better to superovulation. We also performed a GWA study to identify the QTL involved in AMH regulation. The study resulted in identification of a ~4.28 MB window on chromosome 11 and a 1.35 MB window on chromosome 20. These regions likely contain a causative QTL for AMH regulation given that we also identified potential candidate genes in those regions that appeared to have a key role in AMH expression based on previous research. Some of these genes have been previously reported to be involved in maintenance of cell structure, cell signaling, cell viability, embryonic development of cardiac, gonadal and brain tissues, embryonic survival, oocyte competence, cancer suppression and immunity.

We also looked at the correlations of AMH with reproductive traits in heifers. AMH concentration shows some tendency (P=0.03) to predict the probability of conception in embryo recipient heifers. There might be a threshold level of AMH required for optimum probability of conception in embryo recipients as the relationship only exists in animals having low AMH. Other reproductive parameters in heifers and first lactation cows like age at first calving, days open, probability of conception, probability of abortion, and number of services per conception did not appear to be related with serum AMH concentrations. However, it seems that these associations should be studied in later parities as these heifers continue to mature.

Further research needs to be done on the candidate genes identified in this study. Some of these genes have been studied extensively in the past. For example NR5A1 knock out mice showed complete adrenal and gonadal agenesis (Luo et al., 1994), and mutations in this gene caused ovarian insufficiency. (Lourenço et al., 2009). However, validation of other genes should be done by a series of experiments. Firstly, fine sequencing should be done in the significant genomic region to detect the causative QTL. These genes can be studied by differential gene expression studies and knockout experiments to confirm the role of genes in that region in regulation of AMH.

Secondly, wide spread application of AMH as a diagnostic tool to select superior dairy animals for reproductive traits requires further research involving different species of livestock in different stages of productive life and in different management conditions

In conclusion, AMH can be used to select animals for larger gonads, more number of follicles, super-ovulatory response and herd longevity, but not for direct measures of fertility which are considered to be economically important for a dairy farm.

LITERATURE CITED

LITERATURE CITED

Batista, E.O.S., G.G. Macedo, R. V. Sala, M.D.D. V Ortolan, M.F. Sá Filho, T.A. Del Valle, E.F. Jesus, R.N.V.R. Lopes, F.P. Rennó, and P.S. Baruselli. 2014. Plasma antimullerian hormone as a predictor of ovarian antral follicular population in bos indicus (nelore) and bos taurus (holstein) heifers. Reprod. Domest. Anim. 49:448–452. doi:10.1111/rda.12304.

Dijkhuizen, A.A., J. Stelwagen, and J.A. Renkema. 1984. Economic Aspects of Reproductive Failure in Dairy Cattle. I. Financial Loss At Farm Level. Elsevier Scmnce Publ. B V 385:251–263.

Gamarra, G., C. Ponsart, S. Lacaze, B. Le Guienne, P. Humblot, M.C. Deloche, D. Monniaux, and A.A. Ponter. 2015. Dietary propylene glycol and in vitro embryo production after ovum pick-up in heifers with different anti-Müllerian hormone profiles. Reprod. Fertil. Dev. 27:1249–1261. doi:10.1071/RD14091.

Guerreiro, B.M., E.O.S. Batista, L.M. Vieira, M.F. Sá Filho, C.A. Rodrigues, A.C. Netto, C.R.A. Silveira, B.M. Bayeux, E.A.R. Dias, F.M. Monteiro, M. Accorsi, R.N.V.R. Lopes, and P.S. Baruselli. 2014. Plasma anti-Mullerian hormone: an endocrine marker for in vitro embryo production from Bos taurus and Bos indicus donors. Domest. Anim. Endocrinol. 49:96–104. doi:10.1016/j.domaniend.2014.07.002.

Ireland, J.L.H., D. Scheetz, F. Jimenez-Krassel, a P.N. Themmen, F. Ward, P. Lonergan, G.W. Smith, G.I. Perez, a C.O. Evans, and J.J. Ireland. 2008. Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle.. Biol. Reprod. 79:1219–1225. doi:10.1095/biolreprod.108.071670.

Jimenez-Krassel, F., D.M. Scheetz, L.M. Neuder, J.L.H. Ireland, J.R. Pursley, G.W. Smith, R.J. Tempelman, T. Ferris, W.E. Roudebush, F. Mossa, P. Lonergan, A.C.O. Evans, and J.J. Ireland. 2015. Concentration of anti-Müllerian hormone in dairy heifers is positively associated with productive herd life.. J. Dairy Sci. 98:3036–45. doi:10.3168/jds.2014-8130.

Lahoz, B., J.L. Alabart, D. Monniaux, P. Mermillod, and J. Folch. 2012. Anti-Müllerian hormone plasma concentration in prepubertal ewe lambs as a predictor of their fertility at a young age. BMC Vet. Res. 8:118. doi:10.1186/1746-6148-8-118.

Monniaux, D., S. Barbey, C. Rico, S. Fabre, Y. Gallard, and H. Larroque. 2010. Anti-Mllerian hormone: A predictive marker of embryo production in cattle?. Reprod. Fertil. Dev. 22:1083–1091. doi:10.1071/RD09279.

Mossa, F., S.W. Walsh, S.T. Butler, D.P. Berry, F. Carter, P. Lonergan, G.W. Smith, J.J. Ireland, and A.C.O. Evans. 2012. Low numbers of ovarian follicles ≥3mm in diameter are associated with low fertility in dairy cows. J. Dairy Sci. 95:2355–2361. doi:10.3168/jds.2011-4325.

Redhead, A.K.R., M. Knights, D. Ph, I. Holaskova, D. Ph, J. Moritz, D. Ph, K. Shaffer, D. Ph,

M. Wilson, and D. Ph. 2017. The Use of Concentration of Anti-Mullerian Hormone (AMH) as an Indicator of Reproductive Performance in Livestock Species Adam Karl Russell Redhead Dissertation submitted To the Davis College of Agriculture , Natural Resources and Design At West Virgini.

Ribeiro, E.S., R.S. Bisinotto, F.S. Lima, L.F. Greco, A. Morrison, A. Kumar, W.W. Thatcher, and J.E.P. Santos. 2014. Plasma anti-Müllerian hormone in adult dairy cows and associations with fertility. J. Dairy Sci. 97:6888–6900. doi:http://dx.doi.org/10.3168/jds.2014-7908.

Rico, C., S. Fabre, C. Medigue, N. d. Clemente, F. Clement, M. Bontoux, J.-L. Touze, M. Dupont, E. Briant, B. Remy, J.-F. Beckers, and D. Monniaux. 2009. Anti-Mullerian Hormone Is an Endocrine Marker of Ovarian Gonadotropin-Responsive Follicles and Can Help to Predict Superovulatory Responses in the Cow. Biol. Reprod. 80:50–59. doi:10.1095/biolreprod.108.072157.

Sartori, R., G.J.M. Rosa, and M.C. Wiltbank. 2002. Ovarian Structures and Circulating Steroids in Heifers and Lactating Cows in Summer and Lactating and Dry Cows in Winter. J. Dairy Sci. 85:2813–2822. doi:10.3168/jds.S0022-0302(02)74368-3.

Souza, A.H., P.D. Carvalho, A.E. Rozner, L.M. Vieira, K.S. Hackbart, R.W. Bender, A.R. Dresch, J.P. Verstegen, R.D. Shaver, and M.C. Wiltbank. 2015. Relationship between circulating anti-Müllerian hormone (AMH) and superovulatory response of high-producing dairy cows. J. Dairy Sci. 98:169–178. doi:10.3168/jds.2014-8182.

Vernunft, A., M. Schwerhoff, T. Viergutz, M. Diederich, and A. Kuwer. 2015. Anti-Muellerian hormone levels in plasma of Holstein-Friesian heifers as a predictive parameter for ovum pick-up and embryo production outcomes. J. Reprod. Dev. 61:1–6.