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# ANTI MULLERIAN HORMONE: A PUTATIVE ENDOCRINE MARKER FOR PREDICTION OF SUPEROVULATION RESPONSE IN CATTLE

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**Abstract:** Anti-Müllerian Hormone (AMH) is a dimeric glycoprotein molecule with a molecular weight of 140-kDa linked to the Transforming Growth Factor-β (TGF-β) superfamily. Research on the use of AMH in livestock has gained momentum in recent years. In particular, it is now widely used in cattle breeding, where embryo transfer technology is used to obtain more offspring from genetically superior females. One of the most important factors that increase the success of embryo transfer is the response of the selected donor to the superovulation protocol. AMH has been successfully used as a biomarker in predicting superovulation response in cattle and in estimating the numbers of oocytes collected by ovum pick up (OPU). AMH plasma concentrations are positively and highly correlated with antral follicle count (AFC) in cattle and can also be used as a marker of ovarian reserve. In addition, AMH was also positively and highly correlated with the number of corpus luteum (CL) and total embryos after superovulation in several studies. It has been also reported via Genome-Wide Association Studies (GWAS) that plasma AMH level is an inherited trait in cattle and can be improved through genomic selection. In this study, we aimed to evaluate the relationship between plasma AMH levels and superovulation response in cattle by compiling the data obtained from various studies in light of current scientific literature.

**Keywords:** Biomarker, Superovulation, Embryo transfer, Cattle

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## 1. Introduction

In mammals, for normal sexual development to occur, one of the Wolffian or Müllerian ducts found in mammalian embryos must develop while the other regresses. The Wolffian duct differentiates into male reproductive organs, while the Müllerian duct differs into the female reproductive tract (oviduct, uterus, cervix and vagina). While the Wolffian duct differentiates with stimulation of testosterone produced from fetal leydig cells in males, Anti-Müllerian Hormone (AMH) expressed from sertoli cells of fetal testes activates the regression of the Müllerian duct through apoptosis of the epithelial cell. In females, it is secreted by the granulosa cells of the developing follicles and plays an inhibitory role on the primordial follicles in folliculogenesis (Jost, 1953; Josso et al., 1993; Behringer et al., 1994). Also it has been used as a marker of controlled ovarian stimulation response for in-vitro fertilization (IVF) administration especially in the treatment of infertility in women. Recently, plasma concentrations of AMH have been utilized in ovarian pathological conditions such as menopause prediction, ovarian tumours, polycystic ovary syndrome (PCOS) and premature ovarian failure in women (Leader and Baker, 2014).

Multiple ovulation and embryo transfer (MOET) a technology that has the potential to increase the genetic progress and production of beef and dairy breeds, has been applied in cattle for many years. Even though there have been improvements in MOET technology it is still difficult to predict superovulation response to follicle stimulating hormone (FSH) treatment which varies widely between individuals in cattle (Hasler, 2014). One of the most important factors that increase the success of embryo transfer is the response of the selected donor to the superovulation protocol. In addition to determining healthy animals with superior genetic characteristics, the most important and desired criterion in the selection of donor cattle for an economical and efficient assisted reproduction technology is obtaining a large number of transferable embryos per donor (Sağırkaya, 2009). It is important to estimate the superovulation response in cattle breeding, where embryo transfer technology is used to obtain more offspring from genetically superior females. For this purpose, estimating the superovulation response of cattle and thus the selection of suitable donors is an important research area. In recent years, researchers have shown that plasma AMH levels can be used as an endocrine marker for the prediction of superovulation response (Rico et al., 2009).

## 2. Signalling pathways and the role of AMH in granulosa cells

AMH is a dimeric glycoprotein molecule with a molecular weight of 140-kDa composed of 551 amino acids and linked to the Transforming Growth Factor-β (TGF-β) superfamily (Jost, 1953; Cate et al, 1986). It is encoded by the gene on chromosome 7 in cattle (Gao and Womack, 1997) and chromosome 19 in women (Picard et al., 1986). AMH uses a heteromeric receptor system consisting of a single membrane encompassing serine, threonine kinase receptors (termed type I and type II). The type II receptor (AMHRII) conferred ligand binding specificity, while the type I receptor mediates the downstream signal. AMH is secreted by primary and preantral follicles in the ovary and inhibits initial follicle recruitment and FSH-stimulated follicular growth. The study conducted by Durlinger et al. (2002) reported a decrease in the sensitivity of the follicles to FSH following AMH binding, causing an inhibitory effect on the recruitment of primordial follicles into the growing follicle pool in mice. AMH acts as a negative regulator of the early stages of follicular development (Figure 1) (Durlinger et al., 2002; La Marca&Volpe, 2006; Umar et al. 2019).

## 3. Variations in plasma AMH levels

Various studies indicate that plasma AMH levels in cattle show very small changes throughout the oestrous cycle. Souza et al. (2015) found significant positive correlations between AMH concentrations in cows at different stages of the oestrous cycle (random&proestrus: r=0.77, P<0.01; proestrous & diestrous: r=0.79, P<0.01; random & diestrous: r=0.76, P<0.01) and reported the repeatability of plasma AMH as 0.91 (Figure 2). This stability of AMH allows the determination of plasma levels by a single measurement at any stage of the oestrous cycle.

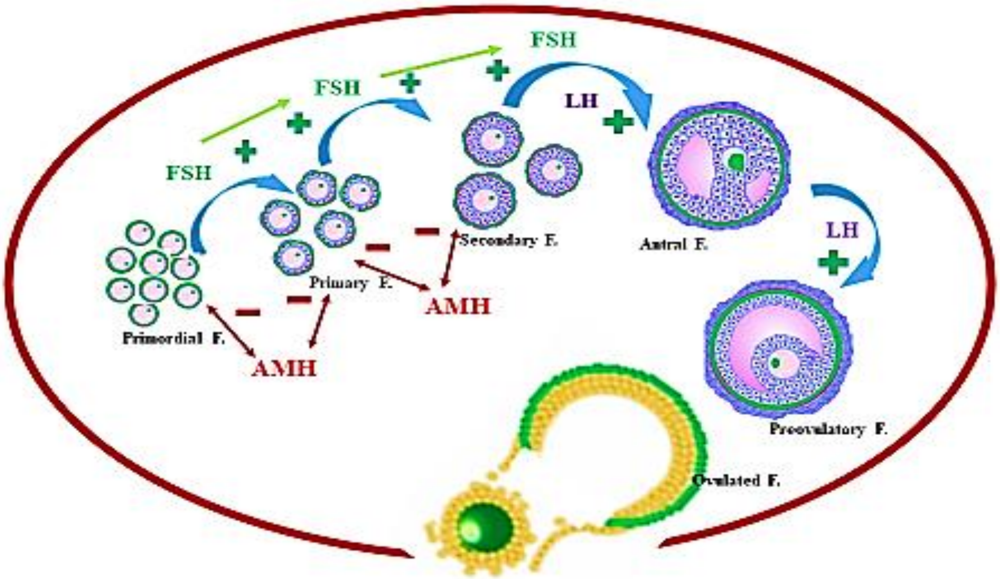


**Figure 1.** Inhibitory effect of AMH on growing follicles.

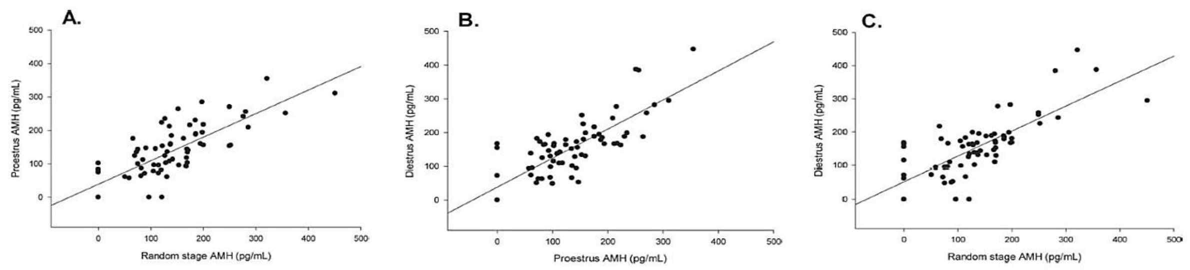


**Figure 2.** Comparison of AMH measurements at different times of the estrous cycle in cows (Souza et al. 2015).

While AMH concentrations in an adult cow show high repeatability, there is a high variation in AMH concentrations among cows (Table 1). In a study conducted by Ribeiro et al. (2014) on 1200 cows, it was found that AMH levels ranged from 10 to 3,198 pg/ml. In the same study, the average AMH concentration was found as 320.3±251.1 pg/ml. In addition, numerous studies show that in-herd AMH concentrations range from 0.01-400 pg/ml and only a few cows reach levels above 400 pg/ml (Rico et al., 2009; Souza et al., 2015). Due to the wide variation between herds and individuals, the reference AMH concentration range for a cow could not be determined. Therefore for the selection of the most suitable donor cows for embryo production, it is more appropriate to select those with high plasma AMH levels among the animals with high genetic value in the herd.

**Table 1.** Variations of plasma AMH concentrations among cows

| Literature | Plasma AMH concentrations | Material |
|---|---|---|
| Rico et al. (2009) | 25 to 228 pg/ml | Holstein Dairy Cows |
| Ribeiro et al. (2014) | 10 to 3,198 pg/ml | North American Holstein Cows |
| Souza et al. (2015) | 0 to 400 pg/mL | Holstein Cows |
| Jimenez-Krassel et al. (2015) | 6 to 440 pg/mL | Holstein Heifers |
| Hirayama et al. (2017) | 32 to 1,992 pg/mL | Japanese Black Cows |
| Gobikrushanth et al. (2018) | 14 to 1,879 pg/mL | Canadian Holstein Cows |
| Nawaz et al. (2018) | 2 to 2,000 pg/mL | Holstein Heifers |
| Akbarinejad et al. (2019) | 98 to 2110 pg/mL | Holstein Dairy Cows |
| Sevgi et al. (2019) | 233 to 2531 pg/mL | Simental Cows |
| Gobikrushanth et al. (2019) | 15 to 2,863 pg/ml | Irish Holstein Cows |
| Akbarinejad et al. (2020) | 46 to 2089 pg/mL | Holstein Dairy Cows |

Also the effect of the breed of cows and lactation number on AMH levels was described. Many studies indicate that AMH concentrations were lower in dairy cattle than those in beef. Mossa et al. (2017) showed that plasma AMH concentrations, follicle numbers and ovary size were lower (P<0.01) in dairy heifers compared with beef heifers. The analyses of the literature suggested that plasma AMH levels vary not only between dairy or beef breeds but also within individuals in the same breed.

Souza et al. (2015) demonstrated the relationship between circulating AMH levels and the number of corpus luteum in primiparous and multiparous cows (Figure 3) and reported a significant (primiparous: r = 0.67; P<0.01; multiparous: r = 0.63; P < 0.01).

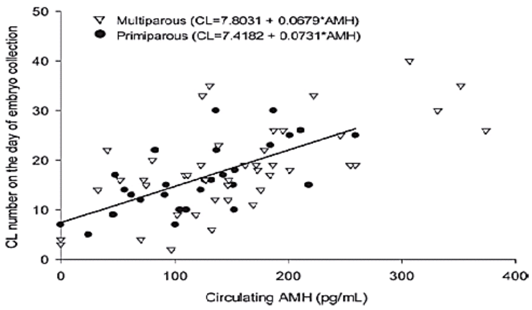


**Figure 3.** Scatterplot showing correlation between CL number and AMH levels in multiparous and primiparous cows (Souza et al., 2015).

The recent studies conducted to show the variations of AMH plasma levels in dairy female calves from birth to puberty shows that AMH concentrations increase in the first 2 months of age, decrease at 5 months of age, and are stable at approximately 8-9 months of age (onset of puberty). Similar results have been reported in beef calves (Mossa et al. 2017). This evidence proves that AMH concentrations start to increase in the first months of life in female calves and decrease before puberty and remain stable during the sexual cycle after the first ovulation (Figure 4).

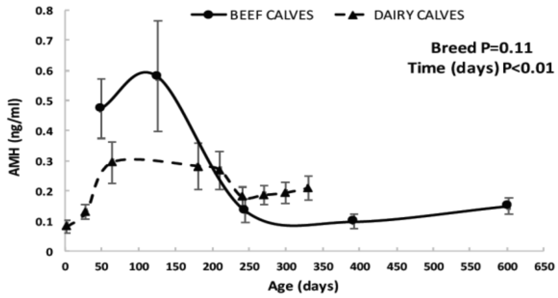


**Figure 4.** Circulating AMH concentrations in dairy and beef calves (Mossa et al., 2017).

## 4. Relationship between AMH and superovulation response

In recent decades, there has been a great deal of literature examining the relationship between plasma Anti-Mullerian Hormone level and fertility in humans and also many studies reported a positive and strong correlation between plasma AMH levels and ovarian reserve and activity in women (de Vet et al. 2002; Van Rooij et al., 2002; Mulders et al. 2004; Tremellen et al., 2005; Broekmans et al., 2006; Visser et al., 2006; Helden and Weiskirchen, 2017). The average number of transferable embryos in the bovine embryo transfer industry is reported to have remained virtually unchanged over the past 40 years, with approximately 6 transferable embryos per superovulation and embryo collection. Consistently some females produce above-average embryos, while others of similar age, breed and management perform worse (Hasler, 2014). Superovulation aims to stimulate the growth and maturation of small antral follicles, resulting in multiple ovulation. Therefore, the small pool of antral follicles available for stimulation is crucial in predicting superovulation response (Sevgi et al., 2019).

AMH plasma concentrations are positively correlated with antral follicle count (AFC) in cattle and can also be used as a marker of ovarian reserve (Center et al. 2018; Mossa et al. 2017). Rico et al. (2009) found that plasma AMH concentration was highly correlated with the numbers of 3 to 7 mm antral follicles detected before FSH treatment (r = 0.79, P<0.001) and the numbers of ovulations after treatment (r= 0.64, P<0.01). In addition, AMH was also positively and highly correlated with the number of corpus luteum (CL) and total embryos after superovulation in many studies (Hirayama et al. 2017; Souza et al. 2015; Sevgi et al. 2019; Monniaux et al. 2010). Center et al. (2018), classified cows into quartiles according to their plasma AMH levels and found that there was a 5-fold difference between AMH concentrations in Q1 (44.9 pg/mL) and Q4 (243.1 pg/mL) and a 2-fold difference (P < 0.01) in CL numbers between Q1 (12.0) and Q4 (25.6) (Table 2).

**Table 2.** The quartile categorization of plasma AMH concentrations and the relationship between superovulation response in beef cattle (Center et al., 2018).

| Item | Quartile of AMH concentration | | | | |
|---|---|---|---|---|---|
| AMH, ng/ml | Q1<br>0.013 – 0.168 | Q2<br>0.169 – 0.263 | Q3<br>0.264 – 0.363 | Q4<br>0.364 – 0.898 | P-value |
| No of donors/collections | 26 | 23 | 24 | 24 | 0.001 |
| No of follicles | 11.62±1.54 | 16.68±1.67 | 16.79±0.94 | 19.33±0.94[a] | 0.001 |
| No of CL | 11.62±1.54 | 13.68±1.67 | 17.58±1.60 | 20.54±1.60 | 0.001 |
| No of embryos | 9.77±1.76 | 9.36±1.91 | 15.50±1.83 | 20.13±1.83 | 0.001 |

AMH= anti-müllerian hormone, CL= corpus luteum

Batista et al. (2014) found a positive correlation between plasma AMH levels and the number of ovarian follicles detected by ultrasonography in Bos indicus (Nelore) and Bos taurus (Holstein) heifers. In another study carried out with Japanese Black heifers, a positive correlation reported between plasma AMH levels of heifers and the total number of follicles (r=0.647, P<0.01) and embryos (r=0.681, P<0.01). However, the researchers didn't find any correlation between AMH and the total number of transferable embryos in the same study (Fushimi et al. 2020).

In a recent study conducted using 46 Simental donor cows, researchers found a positive correlation between plasma AMH levels and the number of CL and total embryos (P<0,05) (Figure 5). Also they reported that every 200 pg/ml increase in serum AMH level leads to approximately 1 piece increase in corpus luteum (CL) number (r=0,68, P<0.05) (Sevgi et al. 2019). These results increase the interest in AMH as a reliable endocrine marker that provides accurate estimation to select the most suitable donor cows for MOET technology.

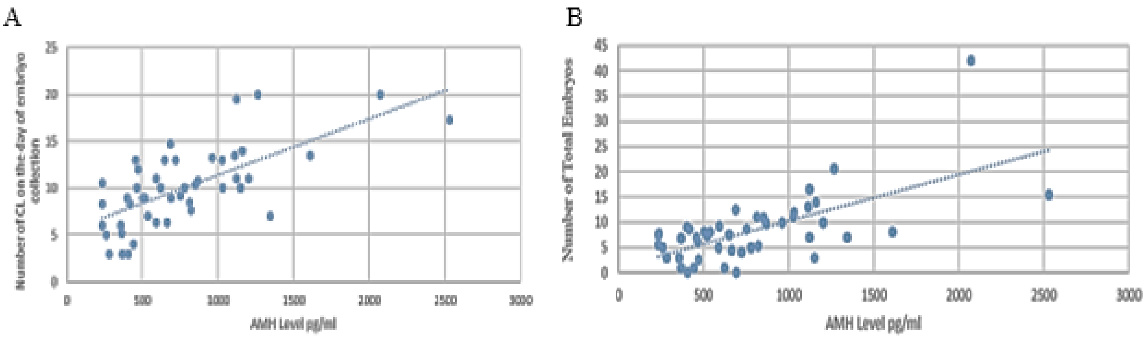


**Figure 5.** The relationship between plasma AMH levels and the number of the CL (A) and the number of total embryos (B) after superovulation therapy (Sevgi et al., 2019).

## 5. Genomic heritability of AMH

A meta-analysis of the literature reported that the heritability of the economically important female reproductive traits in dairy and beef cattle tends to be low (0.02 to 0.04) (Berry et al. 2014). There is currently few research articles on the Genome-Wide Association Study (GWAS) that identifies potential quantitative trait locus associated with phenotypic variation in AMH concentrations and on the genomic heritability of AMH in cattle (Table 3).

**Table 3.** Heritability of AMH and genomic regions associated with plasma AMH concentrations

| Literature | Genomic Heritability | Pedigree based heritability | Significant Genomic Regions (Position) | Material (n) |
|---|---|---|---|---|
| Nawaz et al. (2018) | 0.36 ± 0.03 | 0.43±0.07 | BTA11 (92.8 to 97.1 Mb) BTA20 (25.0 to 26.3 Mb) | Holstein heifers (n=2905) |
| Gobikrushanth et al. (2018) | 0.46 ± 0.31 | ---- | BTA11 (14-Mb) | Canadian Holstein cows (n=198) |
| Gobikrushanth et al. (2019) | 0.45 ± 0.05 | 0.40±0.06 (n=2628) | BTA7 (21.359 to 21.886 Mb) BTA11 (92.051 to 101.918 Mb) | Irish Holstein cows (n=1725) |
| Grigoletto et al. (2020) | 0.28 ± 0.07 | ---- | BTA11 (6 Mb) | Nellore cattle (n=944) |

Nawaz et al. (2018) carried out a study to estimate the genomic heritability of AMH from pedigree and genomic information and determine genomic regions associated with AMH production via genome-wide association studies (GWAS). To determine plasma AMH levels, 3259 Holstein heifers were used and 2905 of them were genotyped for SNP (single-nucleotide polymorphism) markers. Pedigree information of the last four generations was also evaluated for estimation of heritability of AMH. They reported the pedigree-based heritability of AMH as 0.43±0.07 and the genomic heritability of AMH as 0.36 ± 0.03 (Nawaz et al. 2018). In another study, the estimation of genomic heritability of AMH in Nellore cattle (n=944) was reported as 0.28 ± 0.07 (Grigoletto et al. 2020). Gobikrushanth et al. (2018) indicated a high (0.46 ± 0.31) heritability estimate for AMH in Holstein cows (n=198). These reports suggest that the heritability estimates of AMH were higher compared with the heritability of the most economically important female reproductive traits.

Nawaz et al. (2018) also reported significant genomic regions on BTA11 (92.8 to 97.1 Mb) and BTA20 (25.0 to 26.3 Mb). Through GWA analysis, they concluded that there were significant associations between AMH levels and the 11 SNP markers on chromosome 11 and 1 SNP marker on chromosome 20 (Figure 6). In another study, the strongest associations with the AMH were found in BTA11 (513 SNPs in the 14-Mb) (Gobikrushanth et al., 2018).

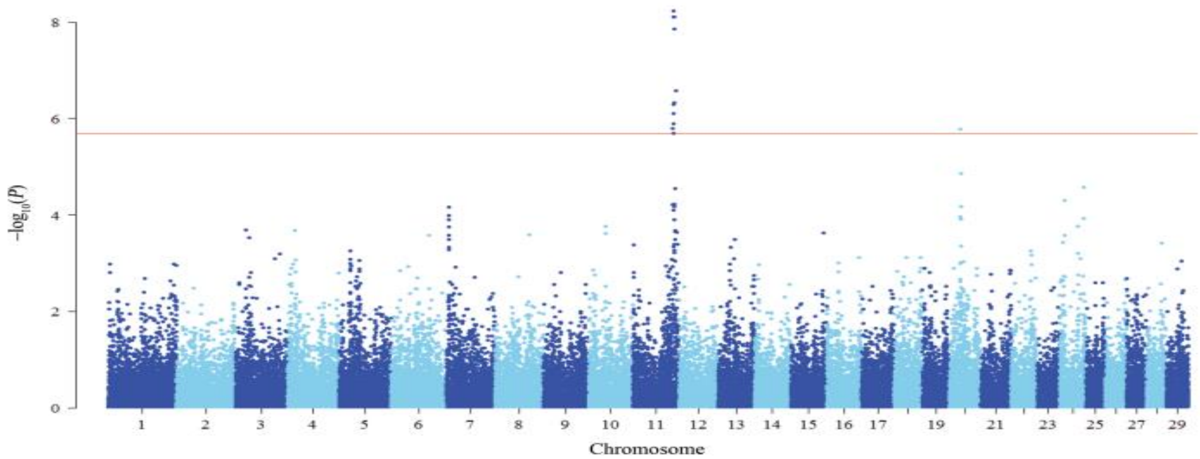


**Figure 6**. Manhattan plot of −log10 P-values for plasma AMH concentrations in dairy Holstein heifers (Nawaz et al., 2018).

## 6. Conclusions

Researches on the use of AMH in cattle have gained momentum in recent years. Evidence from many studies indicates that;

- Because of its stability, it is possible to measure plasma AMH concentration with a single sampling at any stage of the oestrous cycle.
- Plasma AMH concentration is positively and highly correlated with the number of corpus luteum (CL) and total embryos after superovulation.
- AMH concentration in dairy cattle is lower than in beef cattle.
- The increase in plasma AMH concentrations in females starting from the first month after birth and continues until puberty.
- The findings suggest that plasma AMH level is an inherited trait in cattle and can be improved through genomic selection.

Thanks to the intensive studies in recent years, considering its easy applicability and cost-benefit status, the AMH test has become a valuable and practical method to predict ovarian stimulation response in cattle to be selected for embryo production and to increase the efficiency of embryo transfer technology.

**Author Contributions**

All tasks have been performed by single author.

**Conflict of Interest**

The author declares that there is no conflict of interest.

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