RESEARCH ARTICLE

Age-Related Dynamics of Anti-Müllerian Hormone and its Correlation with Antral Follicle Count in Growing Heifers

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Abstract

The profitability of the dairy industry relies heavily on reproductive efficiency and genetic improvement, particularly in countries where dairy farming is vital for the economy. This study aimed to evaluate the relationship between Anti-Müllerian hormone (AMH) levels and antral follicle count (AFC) in female calves as potential biomarkers for predicting reproductive success in advanced breeding programs. A total of 31 female calves of indigenous and crossbred cattle breeds, aged six months, were selected and monitored over one year, with blood samples collected at 6, 12, and 18 months to assess AMH levels. Upon reaching 18 months, ultrasound examinations were performed to determine AFC. Based on the median follicle count of 34.40, irrespective of genetic makeup, the animals were divided into High AFC (n=15, 50.37±3.08) and Low AFC (n=16, 27.25±1.52) groups. Results indicated a significant decline in AMH levels over age. Mean AMH level decreased from 2.53±0.29 ng/mL at 6 months to 1.79±0.14 ng/mL at 18 months ($p<0.05$). Notably, higher AMH concentrations were consistently associated with greater AFC throughout the age groups ($p<0.05$), highlighting AMH's potential as an early predictor of reproductive performance. The findings underscore the importance of AMH as a valuable biomarker in selecting heifers for *in vitro* embryo production programs at an early age, ultimately enhancing the efficiency of breeding strategies in the dairy industry.

Key words: Age-related dynamics, Anti-Müllerian hormone, Antral follicle count, Correlation, Growing heifers.

Ind J Vet Sci and Biotech (2025): 10.48165/ijvsbt.21.6.05

Introduction

The profitability of the dairy industry is deeply dependent on several key factors, including milk yield, genetic selection, and reproductive efficiency. Among these, reproductive efficiency and genetic improvement have become central to the industry's success, especially in countries like India where the dairy sector plays a crucial role in the economy and rural livelihoods. To achieve higher productivity, dairy cattle's genetic traits and ability to reproduce efficiently must be optimized. Recent advancements in reproductive technologies, such as ovum pick-up (OPU) and *in vitro* embryo production (IVEP), have dramatically accelerated the process of genetic improvement (Layek *et al.*, 2022; Sharma *et al.*, 2025). These technologies allow breeders to enhance the genetic qualities of both male and female lineages, offering a faster route to achieving superior livestock.

However, the successful implementation of these technologies is not without challenges. One of the primary obstacles is the variability in donor animals' responses to IVEP, which can significantly affect the overall efficiency of breeding programs (Monteiro *et al.*, 2017; Yusuf, 2024). Studies by Merton *et al.* (2003) and Pontes *et al.* (2011), point to significant differences in how individual donor animals produce oocytes and embryos. This inconsistency has hindered the widespread adoption of IVEP technologies, as the outcomes can be unpredictable. Identifying ways to minimize this variability remains a key focus of current research, with scientists exploring potential biomarkers and traits that could help predict an animal's response to IVEP. Physiological traits such as the ovarian antral follicle population, number of oocytes retrieved during each OPU session (Merton *et al.*, 2003), and oocyte competence are strongly associated with successful embryo production (Pontes *et al.*, 2011; Silva-Santos *et al.*, 2014; Sharma *et al.*, 2025). The quality and number of oocytes available for collection are critical determinants of the overall efficiency

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How to cite this article: Sharma, P., Hadiya, K. K., Layek, S. S., Koringa, P. G., Patil, S. P., Raval, K. B., & Saripadiya, B. N. (2025). Age-Related Dynamics of Anti-Müllerian Hormone and its Correlation with Antral Follicle Count in Growing Heifers. Ind J Vet Sci and Biotech, 21(6), 27-31.

Source of support: Nil

Conflict of interest: None

Submitted 04/09/2025 **Accepted** 17/09/ 2025 **Published** 10/11/2025

of OPU-IVEP programs. This makes it essential to predict which donor animals will yield the most oocytes, as selecting high-oocyte producers will optimize the efficiency of the entire process. Researchers like Rico *et al*. (2012) have stressed the importance of early identification of animals with poor oocyte yield potential, as excluding these animals from breeding programs can prevent wastage of resources and improve results.

One promising biomarker that has gained attention for its predictive value is Anti-Müllerian hormone (AMH). AMH is secreted by granulosa cells in the ovarian follicles and serves as an indicator of the ovarian reserve (Kekan *et al*., 2023). Studies by Ireland *et al*. (2008), Rico *et al*. (2012), and Batista *et al*. (2014) have demonstrated that circulating AMH concentrations are closely linked to the size of the ovarian follicle reserve in both *Bos indicus* and *Bos taurus* cattle. Higher AMH levels typically indicate a larger pool of follicles and a greater reproductive potential, whereas lower AMH levels may suggest a reduced ovarian reserve, potentially limiting fertility (Sharma *et al.,* 2025). The ability to measure AMH levels early in an animal's life, even in calves, provides a valuable opportunity to predict future reproductive potential. Animals with higher AMH levels are more likely to have a larger pool of follicles available for ovulation, making them ideal candidates for reproductive technologies like IVEP (Ireland *et al*., 2008; Monniaux *et al*., 2012; Guerreiro *et al*., 2014; Souza *et al*., 2015). They also tend to respond better to ovarian stimulation, yielding more oocytes for retrieval and ultimately producing more embryos during IVEP cycles (Gamarra *et al*., 2014; Vernunft *et al*., 2015). By identifying these patterns early in life, breeders can make more informed decisions about which animals to include in IVEP programs, thereby improving efficiency, reducing costs, and maximizing the genetic potential of herds. Hence, this study was planned to evaluate age-related dynamics of Anti-Müllerian hormone and its correlation with antral follicle count in growing heifers.

MATERIALS AND METHODS

The experimental protocol (F. NO. 383/VGO/2022) received approval from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), based on recommendations from the Institutional Animal Ethics Committee (IAEC), and all animals were handled as per standard guidelines.

Selection of Animals

Thirty-one female calves of six months old were randomly selected for enrollment in a year-long trial. These animals were born and raised on dairy farms located in Gujarat, India. The group represented a diverse genetic mix, comprising crossbreds and indigenous breeds. Specifically, it included Holstein and Jersey crossbreds, Red Sindhi and Sahiwal breeds. All calves were reared under standard management conditions at their respective farms. Blood samples were collected from these calves at three critical age points: 6 months, 12 months, and 18 months. These time intervals were chosen to capture the developmental changes in AMH levels during different stages of the heifers' reproductive maturation. Upon reaching 18 months of age, the animals underwent USG screening to assess their antral follicle count (AFC). The AMH levels were calculated for each age group and expressed as mean ± SE to provide a clear picture of hormonal fluctuations over time. The AMH concentrations were then correlated with the AFC to examine the relationship between them. Additionally, the study aimed to identify which age point, 6, 12, or 18 months, provided the strongest correlation between AMH levels and AFC, to determine the most appropriate age for using AMH as a predictive biomarker for AFC.

Antral Follicle Count via Ultrasonography

The heifers at the age of 18 months were securely placed in a chute to facilitate the examination procedure. Before scanning, the rectum was cleared using a lubricated, gloved hand to ensure unobstructed access. A thorough palpation was conducted to accurately locate the ovaries. A lubricated probe was then carefully inserted into the rectum, oriented with the 'faceplate' facing downward to enhance visualization. The heifers were examined trans-rectally with a linear array rectal transducer, which was covered with a transparent plastic sheath to maintain hygiene. Contact gel was applied between the transducer and the sheath before insertion to improve clarity during imaging. Once prepared, the transducer was gently positioned on the cervix and aligned over the ovary. Imaging was paused to count the follicles, allowing for a detailed assessment of the ovarian structures.

Blood Collection and Processing for AMH Analysis

Blood was collected via jugular venipuncture into clot activator vacutainer tubes for serum AMH analysis. After collection, samples were allowed to clot, transported to the lab, and centrifuged at 1000 × g for 20 min to separate serum, which was stored at –20°C until assayed. Serum AMH concentrations were assayed using an Enzyme-linked Immunosorbent Assay (ELISA) kit (CEA228Bo; Cloud-Clone Corp. USA). The kit is specific for bovines, and the sensitivity of this kit is less than 25.3 pg/mL with intra- and inter-assay coefficients of variation as <10% and <12%, respectively.

Statistical Analysis

AMH levels in each group were expressed as mean ± SEs. Statistical comparisons were performed using Student's t-test and ANOVA to assess differences between groups. Additionally, the Pearson correlation coefficients (r) were calculated to evaluate the relationship between AMH levels and AFC in heifers as well as for AMH levels within age groups.

RESULTS AND DISCUSSION

The mean follicle count across all animals was 38.44±2.68, with a range of 14.4 to 81.4. Based on the median follicle count of 34.40, the animals were divided into High and Low AFC groups, irrespective of genetic makeup. In the High group (n=15), the mean follicle count was 50.37±3.08, while the Low group (n=16) had a mean count of 27.25±1.52. The difference between groups was statistically significant ($p<0.01$).

AMH Levels over Age

AMH levels declined progressively from 6 to 18 months, indicating depletion of ovarian reserve as calves matured. Levels at 6 months were significantly higher than at 18 months ($p<0.05$), while no significant differences were observed at 12 months compared to either 6 or 18 months, suggesting a stabilization phase during this transitional period (Table 1). This decline reflects the physiological process of follicular recruitment, where some follicles are selected for further growth while others undergo atresia (Roche *et al*., 1998). AMH, primarily expressed in granulosa cells of preantral and small antral follicles, decreases as follicles develop (Visser *et al*., 2006). The ovarian reserve is closely tied to reproductive aging and the number of visible antral follicles (Erickson, 1966), explaining why calves generally exhibit higher preantral and antral follicle counts compared to cycling heifers (Batista *et al*., 2016).

Several studies corroborated these findings. Monniaux *et al*. (2012) reported high AMH levels in Maine-Anjou heifers from 1 to 6 months, followed by decline. Batista *et al*. (2016) observed higher AMH in calves than heifers across Holstein and Nellore breeds (2.5±0.8 vs 1.1±0.2 ng/mL). Mossa and Ireland (2019) noted rising AMH until five months in Holsteins, stabilizing by 8-9 months with onset of ovulation. El-Sheikh Ali *et al*. (2017) documented minimal levels at one week, peaking at 10 weeks, then stabilizing before puberty. These studies highlight early AMH dynamics as reliable indicators of ovarian reserve, useful for guiding donor selection and assisted reproductive technologies such as IVEP.

Table 1: Mean (±SE) AMH levels (ng/mL) in high and low AFC groups at 6, 12, and 18 months of age

Age	High AFC group	Low AFC group	Overall
6 months	3.23 ± 0.47^{a}	1.87 ± 0.25^{b}	2.53 ± 0.29^{A}
12 months	2.62 ± 0.27^{a}	1.69 ± 0.19^{b}	2.14 ± 0.18^{AB}
18 months	2.16 ± 0.22^{a}	1.44 ± 0.16^{b}	1.79 ± 0.14^{B}

The superscripts with lower case (a, b) between AFC groups, and those with upper case (A, B) indicate significant differences between age groups ($p<0.05$).

Correlation between AMH Levels at Different Ages

The correlation analysis indicated significant relationships between AMH levels at different ages, reflecting consistency in ovarian reserve trends during development. Strong positive correlations were observed between 6 and 12 months ($r=0.74$, $p<0.01$) and 12 and 18 months ($r=0.72$, $p<0.01$), while the 6–18 month correlation was lower ($r=0.57$, $p<0.01$), indicating greater fluctuation across this longer interval. These findings reinforce AMH's reliability as a marker of ovarian function and its potential as a diagnostic tool in reproductive management.

Importantly, AMH levels at 18 months correlated with mean follicle count ($r=0.61$), directly linking circulating AMH with ovarian population size at this stage. Early AMH at 6 months also predicted later follicle counts, highlighting its utility for early donor selection in breeding programs (Mikkola *et al*., 2024). Batista *et al*. (2016) similarly reported strong correlations between AMH and follicle count in *Bos indicus* and *Bos taurus* using laparoscopic OPU (r=0.86, 0.78; $p<0.0001$). Estimating AMH at 6 months appears particularly effective, as levels are elevated and reflective of a larger ovarian reserve, but incorporating both 6- and 12-month measurements could provide a more comprehensive assessment and improve donor selection accuracy, ultimately enhancing breeding outcomes.

AMH as a Predictor of Ovarian Function

At 6 months, calves in the high AFC group exhibited significantly higher AMH levels compared to those in the low AFC group ($p<0.05$), clearly distinguishing ovarian reserve status (Table 1). A positive correlation was observed between AMH and mean follicle count ($r=0.36$, $p<0.05$; Fig. 1). This trend persisted at 12 months, with the high AMH group maintaining significantly elevated levels and showing a positive correlation with follicle count ($r=0.41$, $p<0.05$; Fig. 2). By 18 months, the high group continued to show higher AMH levels, with a strong correlation to mean follicle count ($r=0.61$, $p<0.01$; Fig. 3). These consistent differences across all ages underscore AMH's value as a biomarker of ovarian reserve and reproductive potential (Koca *et al*., 2024).

The present findings aligned with earlier research demonstrating robust associations between AMH and follicle count. Ireland *et al*. (2008) reported circulating AMH concentrations six times higher in cattle with high AFC and twice as high in those with intermediate AFC compared to low AFC animals ($p<0.01$). Guerreiro *et al*. (2014) found strong correlations in Holstein and Nellore donors (r=0.61, 0.84), while Vernunft *et al*. (2015) observed a correlation of 0.45 in Holstein-Friesians, with higher AMH linked to more aspirated follicles. Similarly, Ghanem *et al*. (2016) reported that Korean beef cows with higher AMH had significantly greater AFCs ($p \leq 0.05$) and more COCs recovered ($r=0.78$). Fushimi *et al*. (2020) noted a weaker but positive correlation ($r=0.261$) in Japanese Black cattle, and Shehabeldin and El-Keraby (2021) documented higher follicle counts in Holsteins with high AMH (7.8 Vs. 3.6). More recently, Feres *et al*. (2024) reported a correlation of 0.516 ($p<0.0001$) in Gir cattle. Collectively, these studies reinforce AMH as a reliable marker of ovarian reserve and oocyte recovery.

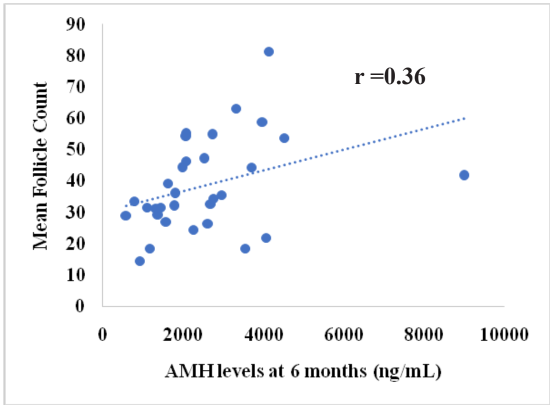

Fig. 1: Scatter plot showing the correlation between serum AMH levels (ng/mL) at 6 months and average follicle count (r=0.36, p<0.05).

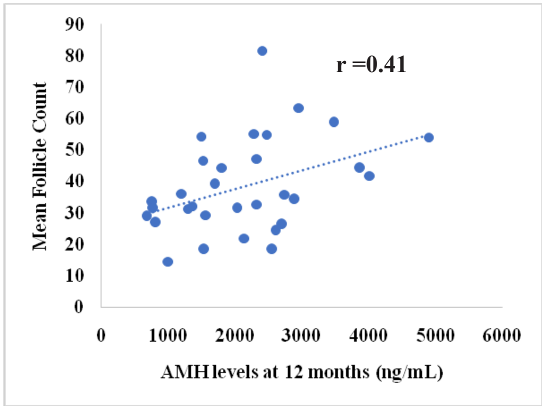

Fig. 2: Scatter plot showing the correlation between serum AMH levels (ng/mL) at 12 months and average follicle count (r=0.41, p<0.05).

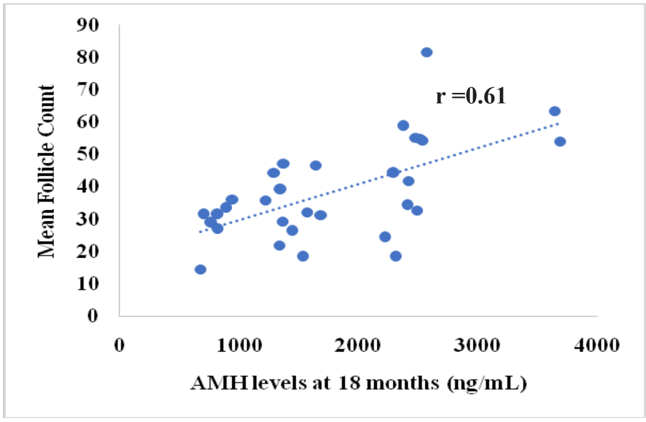

Fig. 3: Scatter plot showing the correlation between serum AMH levels (ng/mL) at 18 months and average follicle count (r=0.61, p<0.01).

Despite the strong evidence, validation through larger and more diverse datasets is essential to ensure broader applicability. The calves in this study belonged to a heterozygous, high-pedigree group raised under optimal management, the factors likely influencing AMH expression. Future studies should include varied breeds, management systems, and longitudinal tracking of AMH dynamics to better contextualize ovarian reserve variability and strengthen donor selection strategies.

Conclusion

The present study demonstrated that AMH levels are strongly associated with follicle count and can serve as a reliable biomarker of ovarian reserve in cattle. Calves in the high AFC group consistently exhibited higher AMH concentrations across all ages, confirming its predictive value for reproductive potential. AMH levels declined with age, particularly between 6 and 18 months, reflecting physiological depletion of the ovarian reserve, though a stabilization phase was evident around 12 months. Strong correlations between AMH levels at different ages and with follicle count highlight its utility for early donor selection in breeding programs. Measuring AMH at 6 months proved especially informative, while combining 6- and 12-month data may enhance accuracy. These findings reinforce AMH's role as a diagnostic tool in reproductive management. However, validation through larger, multi-breed studies under diverse management conditions is essential to strengthen its broader applicability.

Acknowledgment

We acknowledge and thank the members of our research team and the NDDB, Dept. of Gynaecology and Obstetrics of the College (KU, Anand), Sabarmati Ashram Gaushala (Bidaj), and Amul Dairy Farm (Mogar) staff for their support and assistance throughout this study.

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