REVIEW ARTICLE

Anti-Mullerian Hormone as a Marker for Enhanced Reproductive Strategies in Buffaloes Vis-à-vis Cattle: A Review

Prachi Sharma, Bhautik N. Saripadiya, Kamlesh, K. Hadiya*, Chirayu N. Patel

Abstract

Buffaloes, comprising the River and Swamp sub-species, hold increasing importance in tropical and subtropical regions due to their ecological adaptability and economic benefits. However, their reproductive physiology, particularly ovarian follicular dynamics, differs from cattle, posing challenges for assisted reproductive technologies (ART). This review explores the role of the anti-Mullerian hormone (AMH) as a potential marker for ovarian reserve, ART outcomes, and fertility in buffaloes. Comparative studies with cattle highlight differences in follicular populations and AMH levels. AMH emerges as a promising marker for predicting superovulatory response, embryo production efficiency, and fertility across different stages of reproductive aging. Molecular insights into the AMH gene shed light on its genetic regulation and evolutionary significance in various domestic species. Understanding the role of AMH in buffalo reproductive biology offers avenues for optimizing ART protocols and genetic improvement strategies, thus enhancing the sustainability and profitability of buffalo breeding programs.

Key words: Anti-Mullerian hormone, ART, Biomarker, Buffalo, OPU-IVEP, Ovarian reserve.

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Introduction

Domestic buffaloes, scientifically referred to as *Bubalus bubalis* and commonly known as water buffaloes, consist of two distinct sub-species: the River buffalo (*B. bubalis ssp. bubalis*; 50 chromosomes) and the Swamp buffalo (*ssp. carabanensis*; 48 chromosomes) (Colli *et al.,* 2022). River buffaloes are primarily distributed across regions such as South Asia, Europe, the Middle East, and the Americas, whereas Swamp buffaloes are predominantly located in Southeast and East Asia (Borghese *et al.,* 2022). In recent times, the significance of buffalo species has grown in tropical and subtropical regions due to their ecological and economic advantages. These include their ability to thrive in challenging environments, their capacity to convert low-quality forage into meat and milk, and their effectiveness in various working roles (Efrain *et al.,* 2016). Buffaloes exhibit higher metabolic efficiency in milk production compared to cattle (Pradhan *et al.,* 1991). Furthermore, buffalo milk boasts higher concentrations of total solids, including protein, fat, and minerals, compared to cow milk, 18-23% versus 13-16%, respectively. Therefore, the buffalo is increasingly recognized as a pivotal animal for the world's potential food supply, playing a central role in human food sustainability. Despite this, buffalo importance has not yet garnered the same level of attention and care as that received by cattle (Borghese, 2005).

The ovarian follicular dynamics in buffaloes closely resemble those observed in cattle. In buffalo, the most prevalent cycle pattern is the 2-wave cycle, accounting for 63.3% (Baruselli *et al.,* 1997). Follicle deviation typically transpires around 2.6 days post-ovulation, coinciding with dominant and largest subordinate follicles reaching diameters of 7.2 mm and 6.4 mm, respectively (Gimenes *et al.,* 2010). Yet, buffalo exhibit a lower count of follicles recruited per wave compared to cattle (Baruselli *et al.,* 1997; Gimenes *et al.,* 2009[a]; Campanile *et al.,* 2010), which may restrict the application of assisted reproductive technologies in this species (Baruselli *et al.,* 2018). Research by Danell *et al.* (1987) revealed that the number of primordial follicles in buffalo ranges from 10,000 to 12,000, significantly lower than the count in cattle as indicated by Manik *et al.* (2002), which is approximately ten times higher. Furthermore, buffalo ovaries

Department of Veterinary Gynaecology & Obstetrics, College of Veterinary Science & animal Husbandry, Kamdhenu University, Anand-388001, Gujarat, India

Corresponding Author: Dr. Kamlesh K. Hadiya, Professor, Department of Veterinary Gynaecology & Obstetrics, College of Veterinary Sciences and Animal Husbandry, KU, Anand-388001, Gujarat, India. e-mail: kamleshhadiya@kamdhenuuni.edu.in

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exhibit a lower count of antral follicles, approximately 47, compared to cattle ovaries, which typically have around 235 follicles (Van Ty *et al.,* 1989). The integration of timed artificial insemination (TAI), superovulation (SOV), *in vivo* embryo production, ovum pick-up (OPU), *in vitro* embryo production (IVEP), and timed embryo transfer (TET) holds significant promise for enhancing reproductive success, propagating desirable genetics, minimizing generation intervals, and expediting genetic advancements in buffalo (Baruselli *et al.,* 2020).

A comprehensive grasp of follicular dynamics in buffaloes is crucial for the advancement of novel reproductive technologies and the enhancement of existing protocols for manipulating the estrous cycle. Trans-rectal ultrasonography-assessed antral follicle count (AFC) has been linked to the subsequent response to superovulation and OPU-IVEP in cattle (Ireland *et al.,* 2008; Sharma *et al.,* 2023). Yet, the reliability of ultrasonographic AFC determination may be compromised by factors such as variations in operator skill levels, types of machines utilized, criteria employed for AFC determination, and the stage of the follicular wave during observation (Burns *et al.,* 2005; Monniaux *et al.,* 2010; Sharma, 2025). Hence, employing an alternative predictor of ovarian response could prove advantageous. Given the inconsistent and variable response observed in buffaloes, along with the considerable expense associated with superovulation or OPU-IVEP treatments, accurately identifying females likely to exhibit a robust and consistent response to such treatments is crucial for safeguarding superior genetics (Redhead *et al.,* 2018). To address this, researchers are investigating the relationship between ovarian response and Anti-Müllerian hormone (AMH). Studies have shown that a single measurement of AMH in young adult heifers correlates with multiple measurements of AMH during the same or across multiple estrous cycles, as well as with antral follicle population (AFP) (Ireland *et al.,* 2010), proving adequate for assessing follicular population. Anti-Müllerian hormone (AMH), a member of the growth factor-β family, is synthesized by granulosa cells within healthy growing follicles (La Marca and Volpe, 2006). Its expression is elevated in small antral follicles and diminishes as follicles mature (Kavya *et al.,* 2017). Recent studies on AMH in buffalo have been reviewed in this article.

Amh as An Ovarian Reserve Marker

Ultrasonographic studies of the ovaries in both cattle and buffalo have revealed a wave-like pattern of follicular development (Rajamahendran and Tayler, 1990; Baruselli *et al.,* 1997). In cattle, the antral follicular population (AFP), a dependable phenotypic biomarker, is positively linked with ovarian function (Jimenez-Krassel *et al.,* 2009; Sharma *et al.,* 2023, 2025; Sharma, 2025). AFP exhibits high repeatability (0.84-0.95) within individuals, rendering it a credible parameter for animal classification based on AFP. However, buffaloes exhibit a lower number of primordial follicles (Van Ty *et al.,* 1989) and AFP compared to cattle (Baruselli *et al.*, 1997; Baldrighi *et al.,* 2013). Researchers are also endeavoring to elucidate the correlation between Anti-Müllerian hormone (AMH) and ovarian reserve in buffalo. In a similar endeavor, Kavya *et al.* (2020) examined two cohorts of buffaloes: one group that resumed cyclicity 30 days postpartum, and another group where animals remained acyclic 90 days postpartum. While they did not detect any statistical significance, they noted that animals in Group 1 exhibited higher levels of AFP and AMH compared to those in Group 2. The elevated levels of AMH and AFP in early cyclers may be attributed to AMH's role in preventing the activation of primordial follicles from entering the follicular wave, thereby halting follicular atresia (Fortune *et al.,* 2010). Similarly, the elevated levels of AMH and AFP in early cyclers find support in the correlation between circulating AMH and fertility in cattle (Jimenez-Krassel *et al.,* 2015), particularly concerning the ovarian follicular population. It's worth mentioning that in the study mentioned above, early cyclers exhibited higher AFP and AMH levels compared to their counterparts, possibly because ovaries with lower AFP contain more granulosa cells that are nonresponsive to FSH, as indicated by Ireland *et al.* (2010) and Scheetz *et al.* (2012). Given that AMH is highly expressed in small, healthy follicles that respond to gonadotropins, it emerges as a significant marker for a healthy AFP, as highlighted by Monniaux *et al.* (2012). Likewise, other investigations have affirmed that reduced AFP correlates with diminished fertility, as noted by Jimenez-Krassel *et al.* (2015).

In a separate investigation, involving postpartum lactating buffaloes treated with the ovsynch protocol, Kekan *et al.* (2019[a]) noted a positive relationship between AMH levels and the count of antral follicles sized 3-5 mm, as well as fertility. Furthermore, it has been noted that buffaloes exhibiting elevated levels of AMH and AFC tend to experience improved fertility outcomes, as demonstrated by higher conception rates (Kekan *et al.,* 2019[a]). Similarly, the study found that AMH exhibits significant individual variation, akin to findings in cattle (Monniaux *et al.,* 2012; Sharma, 2025). However, within an individual during a single estrous cycle, the variation in AMH levels was minor and non-significant, mirroring observations in cattle (Akbarinejad *et al.,* 2017; Sharma *et al.,* 2023, Sharma, 2025). The concentration of AMH serves as a dependable phenotypic marker for forecasting the number of healthy follicles and oocytes in buffalo ovaries, thereby predicting their future potential (Kekan *et al.,* 2019[a]). AMH has also been noted to influence the response of buffaloes to treatments for anestrus. Research indicates that Murrah buffaloes with an AMH level of 200 pg/mL or higher exhibited a positive response to anestrus treatment and were successfully conceived (Kekan *et al.,* 2019[b]). Anestrus in buffaloes can be attributed to various factors, including environmental conditions, nutrition, and management practices (Das and Khan, 2010). Extended

daylight exposure combined with elevated temperatures can induce hyperprolactinemia, which in turn suppresses the secretion of gonadotropins, disrupting ovarian steroid production and follicle development. Hence, according to the findings of Kekan *et al.* (2019[a]), rectifying these factors can lead to conception and sustained pregnancy in anestrus buffaloes with an AMH concentration exceeding 200 pg/mL. Therefore, assessing the AMH concentration in anestrus buffaloes can serve as a fertility indicator, with those surpassing 200 pg/mL potentially exhibiting estrus after addressing various factors (Kekan *et al.,* 2019[b]). The authors suggest that additional research is necessary to determine whether 200 pg/mL can universally serve as the cutoff value for AMH in Murrah buffaloes.

Amh and Art

Various buffalo donors exhibit significant variability in their antral follicular populations (Baruselli *et al.,* 2018). Nevertheless, the count of AFP remains consistent within individual animals as demonstrated by Burns *et al.* (2005) and Ireland *et al.* (2007). Additionally, anti-Müllerian hormone (AMH) serves as a dependable endocrine indicator of ovarian reserve, equivalent to AFP (Ireland *et al.,* 2007, 2008; Monniaux *et al.,* 2012; Sharma, 2025; Sharma *et al.,* 2025). In cattle, the circulating concentration of AMH has been linked to predicting AFP (Ireland *et al.,* 2008; Rico *et al.,* 2009; Batista *et al.,* 2014). Sharma (2025) reported that donor cattle with higher follicle counts and greater oocyte recovery during ovum pick-up (OPU) procedures exhibited significantly higher serum AMH concentrations compared to those with lower antral follicle counts and oocyte yield ($p<0.05$). Further, Sharma *et al.* (2023, 2025) observed that higher AMH concentrations were significantly associated with greater antral follicular counts and oocyte recovery in *Bos indicus* donors ($p<0.05$). In a subsequent study, Sharma *et al.* (2024) proposed a cutoff value of 2020.1 pg/mL ($p<0.05$) for predicting oocyte yield in *Bos indicus*. However, the authors emphasize the need for further large-scale investigations to validate these findings. Additionally, it has shown promise in predicting the response to superovulatory treatments, as evidenced in studies by Rico *et al.* (2009) and Monniaux *et al.* (2010). More recently, Chello (2020) highlighted its potential as a marker for predicting OPU-IVEP-ET and AFP in buffaloes. The correlation between AFP, IVEP, and pregnancy rate in buffalo has recently undergone examination (Baruselli *et al.,* 2018). The quantity of oocytes retrieved per OPU session positively influences the number of blastocysts generated per OPU session in buffaloes. This underscores the significance of pinpointing donors with a higher potential for oocyte retrieval per OPU session to ensure increased success in IVEP (Baruselli *et al.,* 2020). Buffaloes exhibiting elevated plasma AMH levels have demonstrated enhanced IVEP efficacy, characterized by a greater number of visualized follicles during OPU, increased oocyte retrieval, and higher embryo production compared to those with lower AMH levels (Chello, 2020). These findings imply that AMH could serve as an endocrine indicator for forecasting AFP and IVEP outcomes in buffalo donors, like in cattle.

Especially in water buffaloes, the ovarian antral follicular reserve is both low and highly variable among females, constraining the utilization of superovulation and embryo transfer reproductive technologies. Finding a dependable predictive marker for the population of small antral gonadotropin-responsive follicles is crucial to enhancing the superovulatory response (Redhead *et al.,* 2018). The average number of recoverable embryos obtained from superovulatory protocols has been consistently less than 2 in water buffaloes (Carvalho *et al.,* 2002; Drost *et al.,* 2007; Li *et al.,* 2011). Recent research endeavors have sought to address this ongoing challenge by exploring the potential utility of AMH as a biomarker for assessing superovulation response in buffaloes. A positive correlation has been observed between the concentration of AMH and the superovulatory response. Buffaloes with high AMH also exhibit a higher ovulation rate (Redhead *et al.,* 2018). The concentration of AMH in water buffaloes ranges from 110 to 320 pg/mL (Baldrighi *et al.,* 2014). Due to the high repeatability of AMH concentration within individual animals, a single blood sample analysis may predict females with a consistently high response to superovulation treatments. To enhance the low superovulatory response in water buffaloes, employing FSH preparations with reduced LH content is a potential strategy (Redhead *et al.,* 2018). Buffaloes with an AMH concentration exceeding 1 ng/mL, especially those treated with follitropin as a superovulatory drug during the diestrus phase after synchronization with PRID, exhibit a significantly higher incidence of large follicles and total number of follicles, resulting in a higher yield of embryos (Atef *et al.,* 2021). Further investigations on a larger scale are necessary before establishing any definitive threshold value for the utilization of AMH as a biomarker in buffaloes.

Association With Fertility

An important factor contributing to reproductive aging is the gradual decline in ovarian follicular reserve with age (Lahoz *et al.,* 2014). Assessing the reproductive potential of individual females within the herd poses a challenge due to economic considerations. Presently, AMH seems to be the most effective endocrine marker for evaluating the age-related reduction in ovarian reserve in women (van Rooij *et al.,* 2005). The assessment of serum AMH levels has become part of the array of markers for gauging ovarian aging (de Vet *et al.,* 2002; Fanchin *et al.,* 2003), ovarian follicular reserve (Van Rooij *et al.,* 2002), and ovarian response in assisted reproductive technology (Elgindy *et al.,* 2007). The study by Jimenez-Krassel *et al.* (2015) delved into the utilization of AMH to forecast field fertility in cattle. Egyptian buffalo studies have shown that animals conceiving tend to exhibit higher levels of

AMH compared to those that do not. This correlation remains consistent across all age groups, including heifers, and young, and older buffaloes. Furthermore, buffaloes experiencing pregnancy loss have been observed to exhibit lower plasma AMH concentrations compared to those carrying pregnancies to term (Hassan and Gafer, 2021). Plasma AMH levels serve as a key indicator of ovarian follicular reserve, suggesting their potential as an endocrine marker for evaluating individual reproductive status (Sabuncu *et al.,* 2019). The analysis of AMH concentration holds promise as a significant biomarker for reproductive and productive longevity in buffaloes, pending further investigation (Hassan and Gafer, 2021).

AMH and Age

The ovarian reserve of buffaloes comprises a fixed number of primordial follicles established during fetal development, with their quantity diminishing over time (Danell, 1987). AMH levels exhibit a positive correlation with ovarian reserve, which diminishes with age in buffaloes. Specifically, heifers display higher AMH levels compared to older buffaloes (Hassan and Gafer, 2021). In cattle, Sharma *et al.* (2025) found a similar trend where serum AMH levels declined gradually ($p<0.05$) from 6 months (2.53±0.29 ng/mL) to 18 months (1.79±0.14 ng/mL) of age. The query arises regarding the earliest developmental stage at which AMH can be assessed as a fertility indicator. Identifying heifers with low or high fertility at birth or weaning would offer producers an advantage in making management decisions (Sharma, 2025). If an AMH measure at weaning could forecast future fertility, it would not only cut down on replacement heifer expenses but also pinpoint less fertile heifers at an age suitable for marketing them as stocker-feeder cattle at an ideal time. Research indicates that early pubertal heifers exhibit higher AMH levels compared to delayed pubertal heifers (Kavya *et al.,* 2017). Thus, selecting buffaloes with elevated AMH levels at weaning could prove advantageous for extending their fertile lifespan. Recent publications and ongoing studies aim to ascertain whether there exists a correlation between circulating levels of AMH and fertility. Blood samples from buffalo heifers can be collected to forecast the fertility of the animals. Kekan *et al.* (2019[b]) noted that Murrah heifers with AMH concentrations below 200 pg/mL did not conceive, underscoring the significance of early AMH screening when selecting animals for breeding purposes. The diminished AMH levels in heifers could result from a heightened rate of follicular atresia and reduced AFC (Kavya *et al.,* 2017). Heifers exhibiting low AMH concentrations, and consequently failing to conceive, may demonstrate subpar fertility and thus may warrant culling from the herd due to inadequate reproductive performance (Jimenez-Krassel *et al.,* 2015). A sole assessment of AMH levels in young adult dairy heifers could serve as a straightforward diagnostic tool for predicting herd longevity and may stand as a valuable phenotypic marker for enhancing the longevity of dairy buffaloes (Kekan *et al.,* 2019[a]).

Comparative Studies With Cattle

Bubalus bubalis exhibit nuanced distinctions in their reproductive biology in contrast to *Bos taurus* and *Bos indicus* breeds, as documented in various studies (Figueiredo *et al.,* 1997; Bo *et al.,* 2003; Neglia *et al.,* 2003; Escalona *et al.*, 2008; Gimenes *et al.,* 2008; Sartori and Barros, 2011; Baldrighi *et al.,* 2013). Buffalo females exhibit a reduced count of primordial follicles (Van Ty *et al.,* 1989) as well as antral follicles (Baruselli *et al.,* 1997; Gimenes *et al.,* 2010; Baldrighi *et al.,* 2013) in comparison to cattle. While there's considerable variability in the AFP within each genetic background, cattle heifers generally possess a greater number of AFP compared to buffalo heifers (Baldrighi *et al.,* 2014). Likewise, Gimenes and colleagues (2009[b]) discovered that buffalo heifers exhibit a lower number of follicles upon synchronized follicular wave emergence compared to cattle heifers. The reduced follicle count in buffalo may be partially attributed to the higher presence of atretic follicles observed in *Bubalus bubalis* ovaries compared to bovine ovaries, as noted by Mondadori *et al.* (2007, 2010). Possibly, these traits could account for certain functional disparities noted in *Bubalus bubalis* follicular dynamics in comparison to cattle. Buffaloes exhibit significantly lower AMH concentrations ($p<0.01$) than cattle. Although there is a positive association between AFP and plasma AMH concentration in buffaloes, the correlation is stronger in cattle than in buffaloes (Baldrighi *et al.,* 2014).

Potential Applications

The success and profitability of buffalo breeding hinge largely on genetic enhancement, thereby emphasizing the importance of utilizing reproductive biotechnologies. Ever since the inception of producing the first buffalo calf via *in vivo* embryo transfer (Drost *et al.,* 1983), efforts have been concentrated for over three decades on refining superovulation protocols and enhancing embryo recovery techniques. However, despite efforts, the utilization of multiple ovulation and embryo transfer (MOET) techniques continues to yield fewer embryos compared to cattle (Carvalho *et al.,* 2002; Mishra and Tyagi, 2010; Neglia *et al.,* 2010). Therefore, ovum pick-up and *in vitro* embryo production have emerged as the most effective methods to augment the maternal contribution to genetic advancement in buffalo breeding, enabling sustained high embryo yields (Gasparrini, 2002; Gasparrini *et al.,* 2014). The substantial inter-individual variability in the number of embryos produced remains a primary constraint in the widespread adoption of both technologies in buffalo (Gasparrini *et al.,* 2014; Govignon *et al.,* 2000). Furthermore, the inherent lower count of primordial and antral follicles specific to the species (Van Ty *et al.,* 1989; Baruselli *et al.,* 1997) exacerbates

the issue of heightened variability in follicular recruitment in buffaloes compared to cattle. A recent demonstration revealed that follicular recruitment is predetermined within each individual, enabling the identification of both desirable and undesirable donors (Gasparrini *et al.,* 2014; Neglia *et al.,* 2011), similar to observations in cattle (Tamassia *et al.,* 2003). Hence, alongside assessing the antral follicular count, the identification of a dependable marker for selecting quality embryo donors is crucial before incorporating buffaloes into embryo production initiatives, helping to mitigate laboratory expenses (Gasparrini *et al.,* 2014). Identifying dependable markers for selecting embryo donors is crucial for including buffaloes in embryo production programs. Intra-follicular AMH concentration decreases as follicular size increases, with the small follicles (3-5 mm) contributing the most to AMH levels. The notable discovery is the positive correlation observed between the intrafollicular AMH concentration and both the AFC and the number of small follicles, indicating that AMH can serve as a marker for the population of gonadotropin-responsive follicles in buffalo. Effective buffalo donors exhibit greater quantities of small follicles, cumulus-oocyte complexes (COCs), and high-quality Grade A+B COCs in comparison to poor donors. Remarkably, superior donors exhibit elevated concentrations of AMH in the follicular fluid along with increased expression of AMHR2 in the corresponding granulosa cells of small follicles (Liang *et al.,* 2016). Elsewhere, it has been noted that AMH expression is more pronounced in healthy follicles compared to atretic follicles, suggesting a potential role for AMH in inhibiting apoptosis in granulosa cells (Rico *et al.,* 2009; Lehmann *et al*., 2014). The elevated levels of AMH in follicular fluid and the expression of AMHR2 in corresponding granulosa cells observed in good donors indicate a potentially lower rate of atresia in these follicles. The concentration of AMH within follicles displays an inverse relationship with follicular size in buffalo. Additionally, a positive correlation has been identified between the intrafollicular AMH concentration and the AFC, indicating the potential utility of AMH in selecting donors for inclusion in embryo production initiatives (Liang *et al.,* 2016).

Molecular Mechanisms and Genetic Regulation

The AMH protein is encoded by the AMH gene, which spans over 2.75 Kbp and comprises five exons. Its chromosomal location varies across species: on chromosome 7 in cattle, horses, and goats; on chromosome 5 in sheep; on chromosome 9 in buffalo; and on chromosome 2 in pigs (Gao and Womack, 1997; Umer *et al.,* 2019). In Nelore cattle, three synonymous mutations (rs527023314, rs722016629, and rs134387246) were identified in exon 5 of the AMH gene, potentially linked to early pregnancy onset and age at first calving within this breed (Pierucci *et al.,* 2019). Sharma (2025) annotated the top 50 SNPs related to AMH in HFCB cattle in her research. Out of these, two were in the downstream region, three in the upstream region, 20 in the introns, and the rest in the intergenic region. Notably, the majority of these SNPs (11) were located on chromosome 2. Several domestic animals, particularly large and small ruminant species, lack characterization of the AMH gene. Recently, the cDNA of Indian riverine buffalo and goat granulosa cells was used to amplify and sequence the AMH gene, which corresponds to 1728 base pairs (Gautam *et al.,* 2021). In the AMH gene's entire coding sequence, 72% of the DNA is GC. In exon I through exon V, the corresponding GC contents were 69, 69, 73, 71, and 74%. The mature protein's 551 amino acid residues and the 24 residue signal peptides make up the 595 amino acid residues of the AMH protein. The mature peptide's 99 amino acids make up the TGF-β domain. The AMH protein of Indian riverine buffalo consists of 12 conserved cysteine residues. Leucine and proline are the most abundant amino acids in the polypeptide chain, making up 16 and 12% of the total, respectively. Met, Try, Lys, and Ile are the amino acids with the lowest frequency of occurrence. Within the peptide sequences, there are two N-glycosylation sites identified as 78-NGSR and 344-NLSD. The C-terminal region of AMH proteins exhibits greater conservation compared to the N-terminal region. Phylogenetic and syntenic analyses of the AMH gene reveal a close relationship among cloven-hoofed, ruminant vertebrates such as cattle, buffalo, sheep, and goats, suggesting a correlation with evolutionary changes in the relevant biological processes. However, these species have diverged from primates, including humans, as well as from camels, horses, and donkeys. In all these mammals, the AMH gene is situated between the SF3A2 and JSRP1 genes. The dN/dS ratio of the AMH gene indicates the absence of positive selection pressure, implying a consistent and vital physiological role in reproduction across animals within the Bovidae, Cetacea, and Camelidae families (Gautam *et al.,* 2021).

Conclusions

In conclusion, the review underscores the growing significance of buffalo breeding in agricultural landscapes and emphasizes the unique reproductive challenges faced in optimizing their genetic potential. The exploration of AMH as a potential marker for ovarian reserve and fertility presents a promising avenue for improving assisted reproductive technologies in buffaloes. By elucidating the molecular mechanisms and genetic regulation of AMH, we gain insights into its evolutionary significance and its utility as a tool for enhancing reproductive outcomes in buffalo populations. This knowledge not only informs the development of more effective ART protocols but also offers opportunities for genetic selection and improvement strategies aimed at boostering the sustainability and

profitability of buffalo breeding programs. As we continue to deepen our understanding of buffalo reproductive biology, the integration of AMH-based approaches holds promise for driving advancements in buffalo breeding and contributing to global food security efforts.

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