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Association of serum anti-mullerian hormone concentration with fertility status of crossbred (*Vrindavani)* cows

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ABSTRACT

Anti-Mullerian hormone (AMH) is considered a biomarker of ovarian follicular reserve and fertility in humans and farm animals. A study was carried out to evaluate the peripheral AMH concentration in high and low fertile crossbred cows. *Vrindavani* crossbred cows (n=60) at the institutional dairy farm were used for the study. The age-specific decline in AMH concentration was evident, with the highest concentration at 4-6 years (134.8±17.74 pg/mL) corresponding to second to third parity. Retrospective assessment of fertility using 217 lactation records in crossbred cows revealed that circulatory AMH was higher by 69 pg/mL in the high fertile cows than those of low fertile cows. It was concluded that the serum AMH concentration was influenced by age and parity. Further, serum AMH concentrations were found to be higher in the group of cows that had superior fertility records in comparison to the group with low-performing animals.

Keywords: Anti-Mullerian hormone, Crossbred cow, Estrus, Fertility

Fertility marker of ovarian origin has the potential for the propagation of genetically elite animals. Anti-Mullerian hormone (AMH), which is released into the circulation from the granulosa cells of preantral and antral follicles, objectively reflects the ovarian follicular reserve in the cow (Rico *et al.* 2009). The circulatory concentration of AMH is low at birth, on peak at puberty, and declines throughout the reproductive life of the animal (Alward *et al.* 2020). Cows with a larger pool of small and medium-sized antral follicles have higher plasma AMH concentrations (Rico *et al.* 2009). Anti-Mullerian hormone reduces the sensitivity of pre-antral and small antral follicles to FSH, thereby restricting primordial follicular recruitment and preantral follicular growth in a time-dependent manner (Almeida *et al.* 2018). In the absence of AMH, follicles are highly sensitive to FSH resulting in early depletion of the follicular reserve (Durlinger *et al.* 2001). Significantly higher AMH concentrations are present in the healthy follicles than in those of atretic follicles in the cows (Monniaux *et al.* 2013), hence, threshold AMH concentration is crucial for follicular growth and viability as evident from a high rate of follicular atresia and oocyte degeneration in AMH null mice (Visser *et al*. 2007).

Unlike ovarian steroids, peripheral AMH concentrations are least fluctuated by hypothalamo-hypophyseal-gonadal axis (Ireland *et al.* 2009), ovarian follicular wave dynamics (Rico *et al.* 2009), stage of the estrous cycle (Souza *et al.* 2015), and production status (Monniaux *et al.* 2013) in the dairy cows. Some of the reported studies on the clinical application of measuring AMH concentration in determining reproductive success include having a positive association with ovarian weight, size (Ireland *et al.* 2010) super ovulatory response (Souza *et al.* 2015), and longevity of productive life in the cow (Jimenez-Krassel *et al.* 2015), gestation length in the pigs (Steel *et al.* 2018), mares for *in vitro* embryo production (Papas *et al.* 2021), litter size in bitch (Hollinshead *et al.* 2017) and superior semen quality in dogs (Domain *et al.* 2022). In contrast, a few reports indicate a little relationship between the circulating AMH and fertility (Gobikrushanth *et al.* 2019).

Breed-specific studies in cattle revealed serum AMH concentration has moderate heritability ranging from 0.36 to 0.45 (Gobikrushanth *et al.* 2018, Nawaz *et al.* 2018) which is higher than that of classical reproductive parameters (0.02 to 0.17), such as calving interval, days open, number of services, days in milk, and age at first calving (Berry *et al.* 2014). As crossbreds have a heterogeneous genetic makeup and different levels of exotic inheritance, this study was carried out to test the effect of fertility status on circulating AMH concentration in crossbred cows.

MATERIALS AND METHODS

The present study was conducted on *Vrindavani* crossbred cows (n=60) at the institutional dairy farm,

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Livestock Production and Management section, Indian Veterinary Research Institute, Izatnagar. The experiment was performed following the review and approval of the Institutional Animal Ethics Committee.

Management of experimental cows: Apparently healthy parous *Vrindavani* crossbred cows (synthetic crossbred having exotic inheritance up to 75% of Friesian, Jersey, and Brown Swiss and indigenous Haryana inheritance of 25% maintained under loose housing system were included in the study. Cows were fed as per the recommendations of the National Research Council (NRC 2001). The records pertaining to breeding, and reproductive performance were retrieved from the farm database.

Blood sampling and hormone estimation: Blood samples were collected randomly from the parous lactating *Vrindavani* crossbred cows (n=48) by jugular venipuncture in clot activator tubes (BD Vacutainer ® UK). At the time of blood collection, the cows were at estrus and presented for artificial insemination. Serum AMH concentration was assayed using a commercial ELISA kit (Bovine Anti-Mullerian Hormone ELISA kit, Cat# ITE05241, Geno Technology Inc., USA). The detection range of AMH was 3–700 pg/mL; intra-assay and inter-assay coefficient of variations were within 8-10%.

Categorization based on the fertility indices: A total of 217 lactation records of 48 *Vrindavani* cows were used to categorize the cows into high and low fertile categories considering classical reproductive traits such as age at first estrus (AFE), age at first conception (AFS), age at first calving (AFC) and service per conception (SC), calving to conception interval (CCI) and inter-calving interval (ICI). These parameters were computed as an average of each reproductive index considering the overall breeding performance of the cow (Table 1). The median of each reproductive trait was calculated for the population to categorize into high fertile group when a particular cow qualifies for at least four or more reproductive traits. The rest were clubbed into the low fertile group.

Statistical analysis: Frequency distribution was plotted to find the pattern of the circulatory AMH concentration dynamics across the herd. Age, parity, and lactation effect on the AMH dynamics was analyzed by one-way analysis of variance with Tukey's post-hoc test. The effect of two fertility groups on the AMH was analyzed by independent t-test. The level of significance was set at $P<0.05$. Data was analyzed by SPSS 16.0 and Graph Pad Prism 9.0 was used for chart representation.

RESULTS AND DISCUSSION

Serum AMH concentration was characterized by high variability in the population of *Vrindavani* crossbred cows. It ranged from 8.0 to 510.5 pg/mL with a mean concentration of 122.9±14.0 pg/mL (Fig. 1). Reproducible ideal marker to be incorporated in genetic selection programs should elicit measurable individual variability, possessing an acceptable association with productive or reproductive outcome and must pass on to the next generation (Gobikrushanth *et al.* 2019). High variability in AMH physiological values across the individuals is a constantly noticeable feature of the reported findings. However, the wide variance in the present study was comparatively lower than the values reported in Japanese black (Ali *et al.* 2017), Jersey (Gobikrushanth *et al.* 2019), Holstein cows (Ribeiro *et al.* 2014, Gobikrushanth *et al.* 2018, Nawaz *et al.* 2018, Akbarinejad *et al.* 2020). Among the bovine species, *Bos indicus* cows possess higher AMH concentrations than *Bos taurus* (Baldrighi *et al.* 2014).

The present study demonstrated the age-specific decline of serum AMH, with the highest concentrations of 134.8±17.74 pg/mL in the age group of 4 to 6 years and the lowest concentration in 9 to 12 years (77.60±9.08 pg/mL) in the *Vrindavani* cows (Table 2). The age-specific decline in AMH concentrations is also reported in the doe (Haldar *et al.* 2019), mare (Claes *et al.* 2014) and buffalo (Haldar *et al.* 2020) with the highest values recorded in the age group of 3 to 7 years. Age-related decrease in the AMH levels is consistent with a general decline in the biological activity of the cow. It is reported that a decrease in the

Table 1. Comparative reproductive indices of high and low fertile crossbred cows

Fertility index	Threshold value (median) for categorization	High fertile (n=28)	Low fertile (n=20)	Mean difference	P-value
Age at first estrus (AFE, months)	18	17.98±0.42 (14.30 –24.60)	19.90± 0.64 (16.10 – 25.90)	1.92	0.01
Age at first conception (AFS, month)	20	19.65± 0.40 (14.30-26)	22.38±0.64 (18.77-29.20)	2.73	0.0004
Age at first calving (AFC, month)	31	29.32±0.72 (23.9 – 45.1)	35.91±1.6 (28.6- 57.30)	6.59	0.0002
Service per conception (SC)	1.6	1.62±0.09 (1 – 3)	2.18±0.13 (1 – 3.33)	0.56	0.001
Calving to conception interval (days, CCI)	120	117.3±7.204 (71.60 – 229.0)	180±21.85 (81.0 – 482.0)	62.7	0.003
Inter-calving interval (days, ICI)	408	407±9.78 (338.7 – 585)	466 ± 23.96 (262.0 – 762)	59	0.014

Values in the parenthesis indicate range.

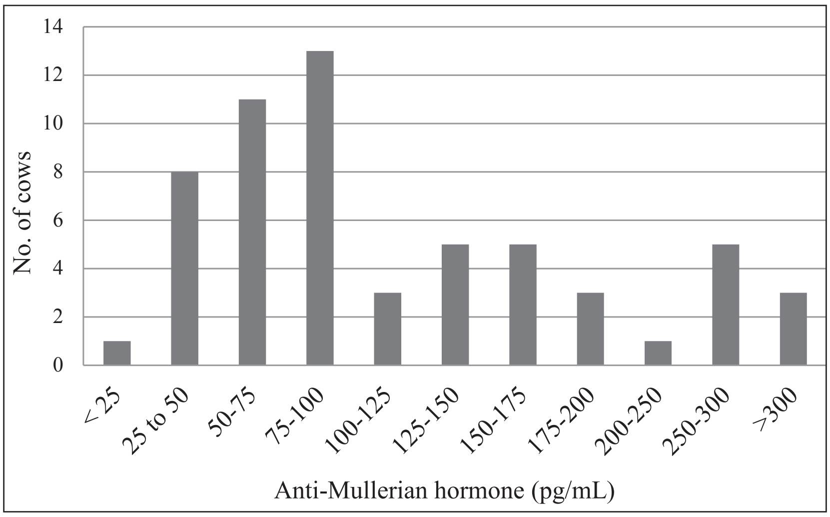

Fig.1. Frequency distribution of circulatory anti-Mullerian hormone concentration (pg/mL) in the crossbred cows (n=60).

AMH is correlated with a decrease in the number of follicles, and is considered an endocrine marker of ovarian follicular reserve (Amanvermez *et al.* 2016). The demarcating decline of AMH as a function of age without being affected directly by the loop of the HPG axis makes it an endocrine marker in assessing ovarian pool in humans and animals (Kevenaar *et al.* 2006, Souza *et al.* 2015). However, baseline data of AMH specifying age-related decline needs to be validated in a longitudinal study covering birth to senility since the present study is based on single point sampling. In addition, serum AMH was observed to be associated with the parity of the cows (Table 2). Cows at second to third parity had higher serum AMH concentration of 172.6±24.98 pg/mL than other groups supported by earlier reports (Ribeiro *et al.* 2014, Gobikrushanth *et al.* 2019). The age of the animal in second to third parity falls between 4 to 6 years when the cows are at peak production and reproductive capacity. Thus, AMH profiling would help us to select a potential donor for superovulation and also in the regular replacement of cows into the herd.

Crossbred cows in the present study were grouped into high and low-fertile categories based on the classical reproductive parameters. The AFE, AFS, and AFC of the high-fertile group were significantly higher than those of low-fertile cows (Table 1). On the contrary, the SC, CCI, and ICI were significantly lower in high fertile than the low fertile cows. Estimation of serum AMH concentration with respect to the fertility status revealed a higher mean circulatory AMH concentrations in the high fertile group than those of the low fertile group by 69 pg/mL ($P<0.05$, Table 2). In a recent study, cows with low AMH concentrations had longer days-open period, extended calving to conception interval, high repeat breeding incidence, while poor association with first service conception rate and number of services per conception (Akbarinejad *et al.* 2020).

Table 2. Association of age, parity, and fertility status of the animal with serum AMH concentration in crossbred cows

Attribute		AMH (pg/mL)	P-value
Age (years)	2-3	94.23± 10.78^{a}	0.003
	4-6	134.8±17.74^{b}	
	7&8	80.54±13.04^{a}	
	> 9	77.60±9.083^{a}	
Parity	1	99.80 ± 13.29^{a}	0.0013
	2nd & 3rd	172.6 ± 24.98^{b}	
	4th-6th	96.95 ± 19.78^{a}	
	7th-9th	80.96 ±14.52^{a}	
Fertility- status	High-fertile	162.6 ± 24.04^{a}	0.03
	Low-fertile	93.90 ± 15.06^{b}	

Data represented as mean±SEM and mean values with different superscripts (a, b) within the columns differ significantly ($P<0.01$).

Low fertility in cows was explained in terms of lower chances of conception and proportionally lower antral follicle count in the ovary at any time point. In contrast, the association after sorting the animals into high and low-fertile categories was also studied by considering their overall reproductive performance from the previous breeding records. Nonetheless, several works stressed the fertility performance of animals after grouping into quartiles where the performance was reported to be higher in the intermediate group than in the very high AMH group (Jimenez-Krassel *et al.* 2015, Ribeiro *et al.* 2014, Gobikrushanth *et al.* 2019). Suboptimal fertility in animals with very low and very high AMH concentrations can be related to the antral follicle model and associated hormonal milieu that disrupts the growth of follicles and oocytes (Mossa *et al.* 2010). Cows with lower AMH possess low expected progesterone at all stages of oestrous cycle (Jimenez-Krassel *et al.* 2009). Further, lower progesterone concentration in diestrus is associated with high rates of embryonic mortality (Morris *et al.* 2008). These variable results might be due to different grouping methods and the effect of sample size accompanied by complexly interlinked neuro, and somato-endocrine governing mechanisms involving both foetal and maternal aspects in pregnancy maintenance (Spencer *et al.* 2004).

In conclusion, the circulatory AMH concentration showed a decreasing trend with increasing age and parity, and a higher serum AMH in high fertile crossbred cows suggested the putative role of AMH as a female fertility indicator.

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