



Evaluation of anti-mullerian hormone in heifers and anestrus Murrah buffaloes

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ABSTRACT

An experiment was conducted to quantify the anti-mullerian hormone (AMH) concentration in heifers and anestrus Murrah buffaloes during fortnight blood collections for 3 months. Two groups of heifer and anestrus Murrah buffaloes were formed on the basis of record and actual per rectal examination. Serum AMH concentration was determined by ELISA method, using bovine AMH ELISA kit. Nonsignificant difference was recorded in AMH concentration (pg/ml) in heifer and anestrus buffaloes during fortnight collection (0, 15, 30, 45, 60 and 75). Highly significant individual variation was observed in heifer and anestrus buffaloes in this study. Heifers (6) with AMH concentration above 200 pg/ml were pregnant and 4 heifers with AMH concentration below 200 pg/ml were non pregnant. AMH concentration is a reliable phenotypic marker to predict the number of healthy follicles and oocytes, in ovaries and predict the future potential of heifers and young adult buffaloes. The cut-off value of AMH concentration in Murrah heifers buffaloes as a marker of fertility may be 200 pg/ml. However, study with more number of buffaloes is required to determine the accurate cut-off value of AMH concentration.

Key words: Anestrus, Anti-Mullerian hormone, Buffaloes, Cattle, Heifer, Mullerian inhibiting substance

Anti-Mullerian hormone (AMH), also known as Mullerian inhibiting substance, is the transforming growth factor of beta family that is expressed only in the gonads (Cate *et al.* 1986). Anti-Mullerian hormone is produced by granulosa cells of all primordial, primary, secondary follicles, as well as antral follicles and reflects the total number of healthy follicles within the ovaries. Its function is to regulate or limit the recruitment of primordial follicles into folliculogenesis by reducing the responsiveness of these follicles to FSH. AMH production decreases after antral stage follicles reach the 4 to 5 mm stage, allowing these follicles to regain responsiveness to FSH and undergo final maturation (Visser *et al.* 2006).

However, the question arises as to how early in development, AMH can be measured as an indicator of fertility. Identification of heifers with low or high fertility at birth or weaning would be advantageous to producers for making management decisions. If measure of AMH at weaning could predict subsequent fertility, this would not only reduce replacement heifer costs, but also identify less fertile heifers at an age that would allow their marketing as stocker-feeder cattle at a more optimal time. Recent publications and ongoing studies are in a pursuit to determine whether circulating levels of AMH are correlated with fertility. This experiment summarizes recent

information concerning antral follicle population and its association with AMH, and the possibility of utilizing AMH as a marker for reproductive technologies and ultimately to enhance cattle fertility (Baruselli *et al.* 2015).

Anestrus, one of the most commonly occurring reproductive problems in cattle and buffalo in India, is affecting livestock productivity and economics to a great extent. The problem is more severe in sub urban and rural areas of the country. Clinical studies showed that, low plasma AMH concentrations are indicative of ovarian ageing (Fallat *et al.* 1997). AMH is also a reliable endocrine marker of the population of small antral gonadotropin responsive follicles in the cow (Rico *et al.* 2009).

The objective of this study was to evaluate the AMH concentration in Murrah heifers before puberty, which will help to predict the fertility at the young stage.

MATERIALS AND METHODS

The experiment was conducted in summer season (February to May 2017) at a private farm.

Group 1: Total 10 Murrah buffalo heifers aged between 25–30 months were selected. Blood was collected fortnightly from all the heifers for 3 months to study anti-mullerian hormone concentration at heifer stage (before attainment of puberty). Before selecting the heifers, they were checked by per rectal palpation to confirm the physiological status and activity of the ovary. The animals were synchronized after attainment of puberty as per the standard ovsync protocol GnRH-PGF2 α -GnRH. About

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5 ml (I/M) of GnRH was injected (-0 day) followed by 2 ml (I/M) PGF2 α (-7 day) and again 5 ml (I/M) of GnRH (-9 day), three times observation of estrus was done from next day (-10 day). The artificial insemination (AI) of all the 10 animals was done on the day of estrus (0 day) to know the fertility. The pregnancy was checked after 45 days and confirmed after 3 months by per rectal examination.

Group 2: Ten postpartum anestrus Murrah buffaloes were selected. The anestrus buffaloes were selected on the basis of record and actual per rectal examination. The buffaloes selected in the study did not show any sign of estrus after calving for at least 6 months and above, and were designated as anestrus buffaloes. Blood was collected fortnightly for 3 months to study AMH concentration.

Management and feeding of animals: All the selected animals were housed in shed having concrete floor with asbestos roof. Standard daily management practices were followed throughout the experimental period. Cotton seed cake (2 kg) was offered to the experimental animals on daily basis, throughout the experimental period. Mineral mixture was given @ 20–25 g/day/head in the morning and evening. To meet the dry matter requirement as well as to satisfy the bulk, *ad lib.* green and dry fodder consisting of *arharchunni*, chaffed green maize, chaffed green *bajra*, paddy straw and sorghum stover was provided to all the animals on daily basis. Animals were provided free access to drink clean water.

Estimation of AMH: Serum AMH concentration was estimated by using bovine AMH ELISA kit (AL-114; Ansh Labs, Webster, TX, USA). The sensitivity of the AMH assay was 11 pg/ml and intra-assay coefficients of variation (CV) was <5%. The absorbance of the solution in the wells was read within 20 min, using a microplate reader set to 450 nm, using ELISA reader.

Statistical analysis: The data was analyzed by CRD using WASP – 2 (Web Agri Stat Package), ICAR.

RESULTS AND DISCUSSION

Group 1: The variation in the mean values of AMH in heifers was nonsignificant (Table 1) from the day of blood collection (day 0) till day 75. The highest concentration was observed on day 0. Further, it decreased till day 45 and then again increased on day 60 and remained almost similar till day 75. The blood samples were collected at unknown stages of follicular phase, this could be attributed due to the high incidence of the 2 or 3 waves of follicular activity might be based on presence of 2 or 3 peaks of gonadotrophic hormones, particularly FSH. The peaks of FSH were also related to lower estrogen concentration, which in turn depended on regression in follicular size. Genetic predisposition or uncontrolled environmental conditions may play an important role in regulation of incidence of the 2 or 3 follicular waves within one estrous cycle, through influences on follicular development and the level of their estrogen secretion (Nosier 2003). The mean values obtained in the present study are comparable with the findings reported by Jimenez-Krassel *et al.* (2015), Souza *et al.*

Table 1. AMH concentration (Mean $\pm$ SE) in heifers and anestrus buffaloes at fortnight interval

Days of blood collection	AMH Concentration (pg/ml)	
	Heifers (n=10)	Anestrus (n=10)
0	309.50 $\pm$ 66.47	168.60 $\pm$ 23.55
15	257.50 $\pm$ 34.36	162.10 $\pm$ 24.72
30	220.00 $\pm$ 38.28	150.80 $\pm$ 7.75
45	226.50 $\pm$ 31.53	161.40 $\pm$ 22.04
60	243.20 $\pm$ 36.06	153.40 $\pm$ 21.58
75	250.50 $\pm$ 39.02	154.40 $\pm$ 23.17

(2015), Silva *et al.* (2016) and Ali *et al.* (2017) in heifers; Center *et al.* (2018) in cows.

There was highly significant difference ($P<0.01$) between the mean AMH concentration of heifers before puberty (Table 2). The AMH concentration ranged between 132.00 $\pm$ 7.12 to 537.50 $\pm$ 66.21 pg/ml. Total four heifers out of 10 with AMH concentration below 200 pg/ml remained non-pregnant. These result are in accordance with Batista *et al.* (2016), Hirayama *et al.* (2017), Ali *et al.* (2017), Kavaya *et al.* (2017) and Center *et al.* (2018) in cattle.

The low AMH concentration in heifers might be due to higher rate of follicular atresia and lower AFC (Kavaya *et al.* 2017). The heifers with low AMH concentration (which did not conceive) may have suboptimal fertility and can be removed from herd due to poor reproductive performance (Jimenez-Krassel *et al.* 2015). The heifers having lower AMH have a shorter productive herd life as compared to those with higher AMH (Jimenez-Krassel *et al.* 2015). A single determination of AMH concentration in young adult dairy heifers may be a simple diagnostic method to predict herd longevity, and AMH can be a useful phenotypic marker to improve longevity of dairy buffaloes.

Among non-pregnant, one heifer buffalo (tag no. 7) had AMH concentration of 198.33 $\pm$ 9.93 which was slightly lower than 200 pg/ml. This heifer did not conceive during the experimental period, but may conceive at later estrous cycle. The heifers with moderate or low AMH

Table 2. AMH concentration (mean $\pm$ SE) and pregnancy status of individual buffalo heifers

Buffalo Tag No.	AMH (pg/ml) (n=10)	Pregnancy status
2	286.33 $\pm$ 14.91 ^c	Pregnant
3	367.50 $\pm$ 17.76 ^b	Pregnant
4	132.00 $\pm$ 7.12 ^e	Non pregnant
5	537.50 $\pm$ 66.21 ^a	Pregnant
6	214.17 $\pm$ 18.71 ^d	Pregnant
7	198.33 $\pm$ 9.93 ^{de}	Non pregnant
8	246.67 $\pm$ 22.44 ^{cd}	Pregnant
9	138.17 $\pm$ 12.59 ^e	Non pregnant
11	250.00 $\pm$ 5.34 ^{cd}	Pregnant
14	141.33 $\pm$ 15.41 ^e	Non pregnant

Mean values within a column with no common superscript differed significantly ($P<0.01$).

concentration, i.e. below 150 pg/ml may conceive at later estrous cycle after multiple artificial insemination (Rorie *et al.* 2016). Further, Rico *et al.* (2012) suggested that the animals having low AMH concentration below cut-off level by determining single blood sample should be culled. However, as the cut-off value may differ breed wise (Chachere 2015), utmost precautions should be taken before culling of valuable animal.

Our results reinforce that the measurement of AMH concentration in blood of Murrah heifer buffaloes can help to predict their capacity for embryo production. Furthermore, they give new practical information about the use of AMH concentration measurement as a tool for discarding the lowest-responding buffalo heifers. A cut-off value of 200 pg/ml as a single evaluation of AMH in Murrah heifer buffaloes should provide sufficient information to establish a reasonable estimate of ovarian reserve.

Group 2: The AMH concentration at fortnightly intervals differed nonsignificantly in anestrus Murrah buffaloes (Table 1). Unlike heifer, no specific trend was noticed in AMH concentrations in anestrus buffaloes and minor numerical fluctuation was observed. The statistical analysis of data revealed highly significant ($P < 0.01$) difference among the individual anestrus buffaloes (Table 3).

The low and moderate AMH concentration recorded in buffalo tag no. (4 to 10) are in accordance with Rico *et al.* (2012). These values were comparable with the AMH concentration recorded in buffalo heifers of present study, which remained non-pregnant. Similarly, the high AMH concentration in buffalo tag no. (1 to 3) were comparable with the AMH concentration recorded in buffalo heifers (200 pg/ml) of the present study, which became pregnant. Considering this, buffalo tag no. (1 to 3) have more chances of conception and pregnancy on correction of the factors affecting pregnancy. There are several factors like environmental, nutritional and management causing anestrus in buffaloes (Das and Khan 2010). Increased day length with high environment temperature causes hyper-prolactinaemia, suppressing the secretion of gonadotrophins, which leads to alteration in ovarian steroidogenesis and folliculogenesis.

Table 3. AMH concentration (mean $\pm$ SE) of individual anestrus buffaloes

Buffalo tag No.	AMH (pg/ml) (n=10)
1.	216.16 $\pm$ 9.77 ^a
2.	248.33 $\pm$ 14.98 ^a
3.	213.33 $\pm$ 16.68 ^a
4.	135.83 $\pm$ 4.18 ^b
5.	98.00 $\pm$ 15.12 ^b
6.	142.83 $\pm$ 13.42 ^b
7.	197.00 $\pm$ 38.45 ^a
8.	134.50 $\pm$ 23.09 ^b
9.	98.50 $\pm$ 13.56 ^b
10.	100.00 $\pm$ 14.02 ^b

Mean values within a column with no common superscript differed significantly ($P < 0.01$).

Thus on correcting these factors, the anestrus buffaloes having more than 200 pg/ml of AMH concentration will conceive and remain pregnant. No reports are available in literature in which comparison has been made regarding AMH concentration in anestrus animals of any species.

Thus evaluation of AMH concentration in anestrus buffaloes can also be utilized as a marker for fertility, wherein the buffaloes with more than 200 pg/ml can show estrus on correcting various factors. According to Akbarinejad *et al.* (2017), AMH is a reliable marker of ovarian reserve. Besides this, there is postpartum anestrus which is variable and for short period (Kumar *et al.* 2014), but this is longer in buffaloes as compared to cattle.

It is concluded that, the cut-off value of AMH concentration in Murrah heifers is 200 pg/ml. This is a first study in Murrah heifers before puberty. Study at a large scale with more number of animals is required, which will help to estimate the cut-off value of AMH concentration.

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