



Evaluation of anti-Mullerian hormone in regular and repeat breeding Murrah buffaloes during estrous cycle

P M KEKAN^{1*}, S D INGOLE², A S NAGVEKAR², S V BHARUCHA² and S D KHARDE²

Bombay Veterinary College, Mumbai, Maharashtra 400 012 India

Received: 6 December 2019; Accepted: 25 February 2020

Keywords: Anti-Mullerian hormone, Buffalo, Estrous cycle, Regular breeding, Repeat breeding

Anti-Mullerian hormone (AMH), also known as Mullerian Inhibiting Substance, is a glycoprotein of 140 kDa belonging to the transforming growth factor 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 up to 4 to 5 mm diameter and reflects the total number of healthy follicles within the ovaries. The function of AMH in females is to regulate or limit the recruitment of primordial follicles into folliculogenesis, by reducing the responsiveness to these follicles to follicle stimulating hormone. Anti-Mullerian hormone production decreases after antral stage follicles reach the 4–5 mm stage, allowing these follicles to regain responsiveness to follicle stimulating hormone and undergo final maturation (Visser *et al.* 2006).

A typical estrous cycle consists of either one or two waves, with wave emergence detected on day 0 (day of ovulation) and day 10, or days 0, 9, and 16. Kekan *et al.* (2019b) reported 2 and 3 follicular waves in Murrah buffaloes. Follicular waves are not exclusive to cyclicity, but also occur prior to puberty, during pregnancy, and anestrus. However, these waves do have dominant follicles to produce enough estradiol (for various reasons) for ovulation and estrus (Chachere 2015).

Presently, AMH is the best endocrine marker of the ovarian follicular reserve in human, in mouse, and recently the AMH is also a reliable endocrine marker of the population of small antral gonadotropin responsive follicles in the cow (Rico *et al.* 2009).

Anestrus and repeat breeding is one of the most commonly occurring reproductive problems in cattle and buffalo in India, affecting livestock productivity and economics to a great extent. The problem is more severe in sub-urban and rural areas of the country. Anestrus is a functional disorder of the reproductive cycle which is characterized by absence of overt signs of estrus manifested either due to lack of expression of estrus or failure of its

detection (Kekan *et al.* 2019a). They further reported lowest AMH concentration in anestrus buffaloes but the animals showed the signs of estrus after correcting the various factors of anestrus. Likewise, repeat breeding is also major problem of infertility in dairy animals. Therefore, the objective of the study is to determine the AMH concentration in regular and repeat breeder Murrah buffaloes during estrous cycle.

All the buffaloes were selected on the basis of record and per rectal. The buffaloes which were having normal genitalia but failed to conceive were checked by per rectal palpation. The buffaloes inseminated for more than three times but failed to conceive were designated as repeat breeder buffaloes. Six animals in each group were selected for the study. All the animals were synchronized as per standard protocol (Table 1). The blood was collected by jugular vein puncture from each animal of both the group on 7th, 14th and 21st day of estrous cycle to determine the AMH concentration, serum was separated by centrifugation and stored at –20°C. Serum AMH concentration was evaluated 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 coefficient of variation (CV) was <5%. The data was analyzed by Students T-Test using WASP-2 (Web Agri Stat Package), ICAR.

Table 1. Synchronization protocol for onset of estrous cycle in buffaloes

0 Day	GnRH - 5 ml (I/M)
7 th Day	PGF2 α - 2 ml (I/M)
9 th Day	GnRH - 5 ml (I/M)
10 th Day onwards	Three times observation of estrus cycle

The analysis of data revealed non significantly higher mean values of AMH in cyclic buffaloes than repeat breeders on 7th 14th and 21st day. The AMH concentration was lowest on the day 7th which gradually increased on 14th day and reached a high concentration on 21st day of estrus cycle in the cyclic buffaloes. In repeat breeding buffaloes highest AMH concentration was recorded on 21st day and lowest concentration on 14th day (Table 2). No

Present address: ¹College of Veterinary and Animal Sciences, Parbhani 431 402; ²Department of Veterinary Physiology, Bombay Veterinary College, Mumbai, Maharashtra.
*Corresponding author e-mail: drprakash73 @gmail.com

Table 2. Mean + SE for AMH concentration in cyclic and repeat breeding buffaloes

Day	Buffaloes	Mean	t Stat	t table
7 th	Cyclic	380.00±78.20	0.639 ^{NS}	2.20
	Repeat breeder	291.00±111.31		
14 th	Cyclic	415.00±68.64	1.10 ^{NS}	
	Repeat breeder	271.66±107.60		
21 st	Cyclic	487.14±95.24	1.254 ^{NS}	
	Repeat breeder	298.33±110.54		

NS, Non-significant difference.

specific trend of gradual increase in AMH concentration was observed in regular breeding buffaloes as compared to repeat breeding buffaloes.

No reports are available in literature in which comparison has been made regarding AMH concentration in repeat breeding animals of any species. The cause of infertility in repeat breeding might be due to various factors which affects the fertility. In the present study the mean values of AMH concentration in repeat breeding buffaloes is similar to the heifer buffaloes which is in agreement with the work done by Kekan *et al.* (2019a). Repeat breeding animals may conceive but, it is essential to find out the cause of repeat breeding condition. Brunner (1984) also suggested to diagnose the cause of failure of conception. He further stated that the cause may be a herd problem or a variety of individual cow problems. He elaborated the common causes of repeat breeding which includes, inadequate estrous detection, resulting in improper timing of insemination in relation to the onset of standing estrus, cows being inseminated that have not actually been in estrus, inadequate semen quality, insufficient numbers of sperm, improper insemination techniques, endocrine (hormonal) disorders like, cystic ovaries (may also cause irregular or short cycles), delayed ovulation, ovulation disorders (these may also be hormonal), obstructed oviducts, defective ova, anatomical defects of the reproductive tract, early embryonic death (may also cause abnormally long cycles).

The different phases of reproductive cyclicity are regulated by intricate sequential events and interactions between hormone releasing factors from pituitary and sex steroids. Therefore, lack of interaction or synchronization and endocrine imbalances at any phase of sequence may result in reproductive failure. High progesterone levels in some repeat breeders and lack of estradiol 17 β and LH peak might be one of the reasons of reproductive failure. Alternatively, it may be possible that the animals were inseminated too late, i.e. after ovulation and CL formation

had occurred. Another reason of inaccurate detection of estrus may be due to poor symptoms particularly in summer season (Arora and Pandey 1982).

SUMMARY

The objective of the study was to evaluate AMH concentration in repeat breeding Murrah buffaloes. Two groups of regular and repeat breeding were formed, each group consist of six buffalo animals. Blood samples were collected on 7th, 14th and 21st day of estrous cycle and after centrifugation serum was stored in refrigerator at -20°C until analysis of AMH. The result showed non-significantly higher mean values of AMH in cyclic buffaloes than repeat breeders on 7th, 14th and 21st day of estrous cycle. Therefore, it is concluded that AMH may not be the cause of infertility in repeat breeders. Repeat breeding buffaloes may conceive but after correcting the various factors of infertility.

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