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Short communication

Anti-Müllerian hormone and antral follicular count in early and delayed pubertal Murrah buffalo heifers

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ABSTRACT

The present study was designed to determine the relationship between Anti-Müllerian hormone (AMH), antral follicular count (AFC) and body weight (BW) in early and delayed pubertal buffalo heifers. Out of 72 heifers screened ultrasonographically, 19 heifers were selected based on age, BW and attainment of puberty for the present investigation. They were divided into two groups (Group I, n=12; Group II, n=7). Group I heifers (age: 30–40 months; BW: 370–468 kg) showed no signs of puberty whereas Group II heifers (age: 18–29 months, BW: 279–318 kg) attained puberty. Total number of all visible antral follicles >1 mm were counted and cyclicity status was monitored at monthly interval. Single blood sample was collected for estimation of serum AMH concentration once the heifers were adjudged cyclic by the presence of corpus luteum. We found lower AMH concentration in delayed pubertal heifers (0.099 ± 0.006 ng/ml) as compared to early pubertal heifers (0.17 ± 0.03 ng/ml) but, difference was not significant (p=0.0788). Likewise, AMH showed non-significant correlation with AFC and BW in both groups. But, there was significant relationship between BW and AFC in delayed (r=0.79; p=0.00) and early (r=0.86; p=0.01) pubertal heifers. In conclusion, this study indicates non-significant difference between AMH and AFC in early and delayed pubertal heifers. Moreover, AMH had no correlation with AFC and BW, but BW was highly correlated with AFC in both study groups.

1. Introduction

Attainment of puberty in buffalo heifers depends on several factors *viz.* genotype, nutrition, management, social environment, climate, year and season of birth (Chaudhry et al., 1988; Bashir, 2006). Buffalo heifers attain puberty at about 55–60% of their adult BW, but the age of onset of puberty is highly variable, ranging from 18 to 46 months (Jainudeen and Hafez, 2000). Borghese (2005) showed that there is difference in onset of puberty between riverine and swamp buffalo heifers (15–18 *vs* 21–24 months). Attainment of BW for puberty onset is strongly influenced by genotype and breed. It ranges from 200 to 300 kg in swamp and 250–400 kg in riverine buffalo (Borghese, 2005). Studies have shown that buffalo heifers can be bred once they attain 60% of their adult BW, thereby contributing to longer productive life (Warriach et al., 2015).

Buffalo ovary consists of fixed number of primordial follicles pool formed during fetal life with their numbers depleting with age (Danell, 1987). In cattle, ovarian ultrasonic studies have demonstrated that follicular development occurs in a wave-like pattern (Savio et al., 1988), involving continuous turnover of follicles even during anovulatory periods such as gestation (Ginther et al., 1996), post-partum involution period (Rajamahendran and Taylor, 1990), and in calves as young as 2-week-old (Evans et al., 1994). In cattle, AFC is a reliable phenotypic biomarker positively associated with ovarian function (Ireland et al., 2009; Jimenez-Krassel et al., 2009). Antral follicular count is highly repeatable (0.84–0.95) within individuals and this consistency becomes a strategic resource for possible classification of animals based on AFC with a single ultrasound examination. Buffaloes have subtle differences in their reproductive biology compared to cattle (Baldrighi et al., 2013). One of the major differences was, being lower number of primordial (Van et al., 1989) and antral follicles (Baruselli et al., 1997; Baldrighi et al., 2013) as compared to cattle.

Anti-Müllerian hormone, a hormone belonging to transforming growth factor–β family, is produced by granulosa cells from healthy growing follicles (La Marca and Volpe, 2006). Its expression is elevated in granulosa cells of small antral follicles and decreases during the follicular growth. Greater concentration of AMH is positively associated with number of follicles in mice (Durlinger et al., 2002) and women (Fanchin et al., 2005) ovaries population. A single AMH measurement in young adult heifers is highly correlated (r=0.97) with

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the average for multiple AMH measurements during different days of the same or multiple estrous cycles and with ovary size and AFC (Ireland et al., 2011). Hence single estimation of AMH is sufficient to correlate with follicular population. Study in Murrah, Holstein and Gir heifers showed that buffalo heifers had less AFC and plasma AMH concentration than Gir and Holstein heifers (Baldrighi et al., 2014). This lower plasma AMH concentration in Murrah heifers might be due to higher rates of follicular atresia and lower AFC which need to be further investigated. But, the relation between the AMH, AFC and BW with attainment of puberty has not yet been studied in buffaloes. Therefore, this study was designed to find out the association, if any between AMH concentration and attainment of puberty in buffalo heifers.

2. Materials and methods

2.1. Study location and management

The experiment was conducted at animal farm section, ICAR-Central Institute for Research in Buffaloes, Hisar, located at 29.09°N 75.43°E in western Haryana, India during breeding season (October to February 2016). Buffalo heifers were maintained under uniform management practices with feeding regime per Nutrient Requirement of Cattle and Buffalo, ICAR (2013) in this study.

2.2. Animals and ovarian ultrasonography

For the present study, peri-pubertal Murrah buffalo heifers (n=72) maintained at the animal farm section were selected and monitored for cyclicity at 10 days interval using transrectal ultrasonography. Out of 72 heifers, 19 heifers were selected (based on age, BW and attainment of puberty) and divided into two groups: Group I (n=12): heifers aged between 30 and 40 months, with BW ranging between 370 and 468 kg showing no signs of puberty. Group II (n=7): heifers aged between 18 and 29 months, with a BW ranging between 279–318 kg and have attained puberty. All the heifers underwent fixed day ultrasound at monthly interval by single operator using trans-rectal real time ultrasound scanner (Model 320 A, Toshiba) equipped with an intra-operative 7.0 MHz micro convex transducer. Heifers were declared pubertal if corpus luteum (CL) were detected following transrectal ultrasonography. Antral follicular count was recorded by observing both the ovaries at different planes by moving transducer and total number of all visible antral follicles > 1 mm were counted. Stage of estrous cycle group was determined by method of Cushman et al. (2009).

2.3. Blood sampling and AMH estimation

Blood samples (5 ml) were collected from early pubertal heifers once CL was detected and simultaneously from delayed pubertal heifers on confirming acyclicity with ultrasound examination. From each heifer in both groups, single blood sample was collected in serum clot activated vacutainer and serum was harvested by centrifuging at 3000 rpm, 4 °C for 15 min and stored at −20 °C until further hormone analysis. In this study, heifers' BW was measured for three continuous days and age was calculated from the farm records. Serum AMH concentration was estimated in duplicates using bovine AMH ELISA kit (Sincere Biotech Co., Ltd, China) as per the manufacturer's instructions. The intra assay co-efficient of variation was ≤9% and the assay's sensitivity was 0.093 ng/ml with correlation co-efficient (r) of linear regression of more than 0.92.

2.4. Statistical analyses

Statistical analyses were done (SPSS, version 16, 2007) for finding the difference in AMH, AFC and BW in study groups using General linear model (GLM). Correlation between AFC, BW and AMH in both groups was carried out using Pearson's correlation coefficient and results were considered significant at $p < 0.05$.

Table 1
Anti-Müllerian hormone (AMH) concentration, antral follicles count (AFC), body weight (BW) and age in group I and II heifers.

Parameter	Group I	Group II	P-value
AMH (ng/ml)	0.099 ± 0.006	0.17 ± 0.03	0.0788
AFC (n)	19.92 ± 1.14 (15–30)	17.14 ± 1.34 (14–23)	0.1363
BW (kg)	403.5 ± 7.9 (370–468)	301.3 ± 4.79 (279–318)	< 0.0001
Age (months)	34.93 ± 0.72 (30–40)	23.93 ± 1.30 (18–29)	< 0.0001

(Values expressed as mean ± SD)

3. Results and discussion

In this study, mean age and BW of Group I heifers was 34.93 ± 0.72 months and 403.5 ± 7.9 kg, respectively whereas in Group II, mean age and BW was 23.93 ± 1.30 months and 301.3 ± 4.79 kg, respectively. Peripheral AMH concentration was low (p=0.0788) in delayed pubertal heifers (0.099 ± 0.006 ng/ml) as compared to early pubertal heifers (0.17 ± 0.03 ng/ml) (Table 1). Similarly, there was no significant correlation between AMH and AFC or AMH and BW in both the groups (Table 2). Lahoz et al. (2012) suggested that plasma AMH concentration prior puberty could be used as a predictor of the fertility of adult ewes at first mating. In addition, AFC vary among species, breed and stage of estrous cycle, but remains highly repeatable within individuals (Burns et al., 2005; Ireland et al., 2007). A highly positive association of AFC with ovary size, total number of morphologically healthy follicles, ovarian reserve (Ireland et al., 2008), fertility (Cushman et al., 2009; Mossa et al., 2012) and birth weight (Cushman et al., 2009) has also been reported. However, other factors such as genetics (Walsh et al., 2014), maternal environment, nutritional status and health (Ireland et al., 2011; Evans et al., 2012) also appear to influence the AFC. Though, it has been documented that reproductive performance is inferior in dairy cows with relatively low AFC as compared to higher counterparts (Mossa et al., 2012), AFC remained comparable between Group I (19.92 ± 1.14) and Group II (17.14 ± 1.34) in this study.

In this study, we observed a significant positive correlation of AFC with BW in early and delayed pubertal heifers. It has been documented that BW had a meagre, but positive influence on AFC, such that the total follicle number in the ovarian cortex increased as birth weight increased (Cushman et al., 2009; Da Silva et al., 2002, 2003). Summers et al. (2013) reported that high AFC heifers have increased BW through pre-breeding, improved average daily gain prior to development, and give birth to larger heifer calves compared to low AFC heifers. Cattle selected for increased twinning rate and birth weight, it has been reported that mature BW had a positive genetic correlation with ovulation rate, indicating a link between BW and ovarian function (Gregory et al., 1997). The association between BW and ovarian reserve is supported by study linking Booroola genotype positively to BW in sheep (Guan et al., 2007). Taken together, these data indicate possible

Table 2
Correlation between Anti-Müllerian hormone (AMH), antral follicle count (AFC) and body weight (BW) in group I and II heifers.

Correlation (r)	Group I (GI)	Group II (G II)	P-value
AMH *vs* AFC	0.00	0.53	G1 (0.99); G2 (0.22)
AMH *vs* BW	−0.12	0.52	G1 (0.71); G2 (0.23)
BW *vs* AFC	0.79	0.86	G1 (0.00); G2 (0.01)