



## Higher antral follicular count is associated with body weight in peri-pubertal Murrah buffalo heifers

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Anti-Müllerian hormone (AMH), a member of the transforming growth factor- $\beta$  family, is produced from the granulosa cells of growing follicles. A single AMH measurement is highly correlated with multiple AMH measurements during different days of the same or multiple estrous cycles (Ireland *et al.* 2007) and henceforth depicts the follicular population (Redhead *et al.* 2018). Study showed that buffalo heifers had lower AFC and plasma AMH concentration than Gir and Holstein counterparts (Baldrighi *et al.* 2014). Antral follicular count, a reliable phenotypic biomarker, is positively associated with ovarian function in cattle (Ireland *et al.* 2009) and is highly repeatable within individuals (Silva-Santos *et al.* 2011). Likewise, serum AMH concentration is highly correlated with AFC in cattle (Batista *et al.* 2014) and buffalo (Baldrighi *et al.* 2014). Also, bovine females with high AFC (>25 follicles) have higher circulating AMH level (Ireland *et al.* 2008). A study in early and delayed peri-pubertal buffalo heifers showed that peripheral AMH levels had no correlation with AFC, but body weight was significantly correlated with AFC, irrespective of the onset of puberty (Kavya *et al.* 2017). But, this relation between the AMH, AFC in peri-pubertal buffalo heifers with varied follicular population and body weight has not been studied. Hence, the present study was designed to test the hypothesis of any relationship between serum AMH, BW and AFC in peri-pubertal Murrah buffalo heifers with varied follicular population.

For the study, peri-pubertal Murrah buffalo heifers (21; aged 2–3 years) were used. They were maintained under uniform management practices with feeding regime as per Nutrient Requirement of Cattle and Buffalo, 2013, ICAR. All the experimental procedures were carried with the approval of Institutional Animal Ethical Committee (IAEC). All the heifers underwent ultrasound scanning on a fixed day once a month by single operator using trans-rectal real time ultrasound scanner (Model 320A, Toshiba) equipped with an intraoperative 7.0 MHz micro convex transducer.

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Antral follicular count was recorded by observing both the ovaries at different planes by moving transducer and the total number of visible antral follicles >1 mm were counted. Onset of puberty was adjudged by the detection of a corpus luteum (CL) on any one of the ovary in the study animals. Based on the AFC, the peri-pubertal buffalo heifers were divided into two groups, viz. high follicular group (HFG) (AFC>18, n=11) and low follicular group (LFG) (AFC $\leq$ 18, n=10). From the study groups, a single blood sample was collected in serum clot activated vacutainer and serum was harvested following centrifuging at 3,000 rpm at 4°C for 15 min and the collected serum was stored at -20°C until further hormone estimation. Body weight of the heifers was measured for three continuous days once a month and age was calculated from the farm records. Serum AMH concentration was estimated using commercially available ELISA kit (Sincere Biotech Co, Ltd., Beijing, China) as per the manufacturer's instructions. The intra-assay and inter-assay coefficient of variation were  $\leq$  9% and  $\leq$  15%, respectively with sensitivity <0.2 ng/ml. Data were analysed by T-test to compare the difference in AMH, AFC and BW between the two groups using SPSS (version 16). Correlation between AMH, AFC and BW was carried out using Pearson's correlation coefficient. Results were considered significant at P<0.05.

Mean AMH concentration (ng/ml) was comparable between HFG (0.202 $\pm$ 0.049) and LFG (0.162 $\pm$ 0.016). Also, the mean AFC of HFG and LFG were 22.68 $\pm$ 3.23 and 16 $\pm$ 1.37, respectively. Heifers with high AFC had higher AMH concentration, but it was statistically nonsignificant. Furthermore, no relation between AMH and AFC was observed, but significant correlation (P=0.04; r=0.65) was observed between BW and AFC in HFG peri-pubertal heifers (Table 1).

This study indicates that peri-pubertal heifers with higher AMH showed more AFC, though statistically nonsignificant. Nonsignificant difference in peripheral AMH level between the study groups with varied follicular population can be attributed to the species difference and age group which needs to be further investigated. Nonetheless, the difference in AFC between the two groups (22.68 $\pm$ 3.23 vs 16 $\pm$ 1.37) was in consonance with earlier reports (Baldrighi *et al.* 2014,

Table 1. Anti-Müllerian hormone (AMH) concentration, body weight (BW) and antral follicular count (AFC) of heifers with high and low follicular count groups

Parameter	High follicular group (HFG, n=11)	Low follicular group (LFG, n=10)	P value
Mean AFC (range)	22.68±3.23 (20-30)	16±1.37 (13-18)	<0.0001
AMH (ng/ml)	0.202±0.049	0.162±0.016	0.4661
BW (kg)	303.9±9.81	304.8±5.4	0.9006
Correlation (r)			
AMH vs AFC	0.07	0.36	HFG (0.56); LFG (0.30)
AMH vs BW	0.07	0.03	HFG (0.86); LFG (0.93)
BW vs AFC	0.65	0.02	HFG (0.04); LFG (0.94)

Values expressed in mean±SE.

Batista *et al.* 2014). Also, AFC had been reported to vary between species, breed and stage of estrous cycle, but remains highly repeatable within individuals (Ireland *et al.* 2007). A previous study had shown that bovine females (taurine and indicus) with high AFC (>25 follicles) had higher circulating AMH concentrations in comparison to their lower counterparts (Ireland *et al.* 2008), which was evident in our results, though nonsignificant, paving way for future investigation. In this investigation, in contrast to Baldrighi *et al.* (2014), we observed no significant correlation between AMH and AFC or AMH and BW in both the groups. The difference can be attributed to physiological stages of the study animals and size of the follicles, i.e. > 1 mm included in this investigation. It is interesting to note that though all heifers were maintained under similar feeding and management conditions, the difference in the AFC may be inherent which needs to be investigated. Our results showed that AFC was correlated ( $r=0.65$ ;  $P=0.04$ ) with BW in high follicular group, being in accordance with Cushman *et al.* (2009) and (Summers *et al.* 2013) as higher AFC heifers had more BW and hence higher average daily gain. This sounds true as selection for twinning rate and mature BW had a positive genetic correlation with ovulation rate in sheep (Guan *et al.* 2007). The present results hint the plausible association of genes controlling growth and ovarian reserve development as reported earlier (Summers *et al.* 2009, Kavya *et al.* 2017).

#### SUMMARY

AMH had no correlation with AFC and BW, but AFC was correlated with BW in high antral follicular group heifers. Furthermore, this study shows that AMH exhibited a trend with AFC in peri-pubertal buffalo heifers.

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#### REFERENCES

- Baldrighi J M, Sá Filho M F, Batista E O, Lopes R N, Visintin J A, Baruselli P S and Assumpção M E. 2014. Anti-Müllerian hormone concentration and antral ovarian follicle population in Murrah heifers compared to Holstein and Gyr kept under the same management. *Reproduction in Domestic Animals* **49**(6): 1015–20.
- Batista E, Macedo G, Sala R, Ortolan M, Sá Filho M, Del Valle T, Jesus E, Lopes R, Rennó F and Baruselli P. 2014. Plasma anti-müllerian hormone as a predictor of ovarian antral follicular count in *Bos indicus* (Nelore) and *Bos taurus* (Holstein) heifers. *Reproduction in Domestic Animals* **49**: 448–52.
- Cushman R A, Allan M F, Kuehn L A, Snelling W M, Cupp A S and Freetly H C. 2009. Evaluation of antral follicle count and ovarian morphology in crossbred beef cows: investigation of influence of stage of the estrous cycle, age, and birth weight. *Journal of Animal Science* **87**: 1971–80.
- Guan F, Liu S R, Shi G Q and Yang L G. 2007. Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. *Animal Reproduction Science* **99**: 44–52.
- Ireland J J, Ward F, Jimenez-Krassel F, Ireland J L, Smith G W, Lonergan P and Evans A C. 2007. Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good quality embryos after ovarian stimulation in cattle. *Human Reproduction* **22**: 1687–95.
- Ireland J J, Zielak A E, Jimenez-Krassel F, Folger J, Bettgowda A, Scheetz D, Walsh S, Mossa F, Knight P G, Smith G W, Lonergan P and Evans A C O. 2009. Variation in the ovarian reserve is linked to alterations in intra-follicular estradiol production and ovarian biomarkers of follicular differentiation and oocyte quality in cattle. *Biology of Reproduction* **80**: 954–64.
- Ireland J L, Scheetz D, Jimenez-Krassel F, Themmen A P, Ward F, Lonergan P, Smith G W, Perez G I, Evans A C O and Ireland J J. 2008. Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. *Biology of Reproduction* **79**: 1219–25.
- Kavya K M, Sharma R K, Jerome A, Phulia S K and Singh I. 2017. Anti-Müllerian hormone and antral follicular count in early and delayed pubertal Murrah buffalo heifers. *Livestock Science* **198**: 89–92.
- Redhead A K, Siew N, Lambie N, Carnarvon D, Ramgattie R and Knights M. 2018. The relationship between circulating concentration of AMH and LH content in the follicle stimulating hormone (FSH) preparations on follicular growth and ovulatory response to superovulation in water buffaloes. *Animal Reproduction Science* **188**: 66–73.
- Silva-Santos K C, Santos G M G, Siloto L S, Hertel M F, Andrade E R, Rubin M I B, Sturion L, Melo-Sterza F A and Seneda M M. 2011. Estimate of the population of pre-antral follicles in the ovaries of *Bos taurus indicus* and *Bos taurus taurus* cattle. *Theriogenology* **76**:1051–57.
- Summers F A, Cushman R A, Moline K V, Bergman J W and Cupp A S. 2013. Heifers with low antral follicle counts have low birth weights and produce progeny with low birth weights. *Nebraska Beef Cattle Report*, 11–12.