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Relationship between Anti-Müllerian hormone, antral follicular population and resumption of cyclicity in postpartum buffaloes

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ABSTRACT

The present study was designed to determine the relationship between Anti-Müllerian hormone (AMH) and antral follicular population (AFP), milk yield (MY), body weight (BW), body condition score (BCS) and resumption of cyclicity (RC) in postpartum buffaloes. For the present study, 20 buffaloes divided into 2 groups: Group I (n=10): buffaloes resuming cyclicity <30 days of calving; Group II (n=10): anestrous buffaloes >90 days postpartum. Blood sampling was carried out in all buffaloes in both groups at day 30 postpartum (day 0 considered as calving day). BW and BCS were monitored fortnightly and MY was recorded every week during first month of lactation. In this study, we found that none of the parameters (AMH, MY, BW and BCS) differed significantly, though RC differed between the two groups based on cyclicity, monitored using transrectal ultrasonography. In addition, AFP between the two groups (A: 19.7±4.95 vs 15.7±5.08) showed a trend in difference, though non-significant. Correlation study between the parameters, i.e. AMH, AFP, MY, BW, BCS and RC in postpartum buffaloes revealed non-significant correlation between AMH with AFP, MY, BW, BCS and RC in postpartum buffaloes. In summary, this study failed to deduce any relationship between AMH with AFP, MY, BW, BCS and RC in postpartum buffaloes.

Keywords: Anti-Müllerian hormone, Antral follicular population, Buffalo, Cyclicity, Postpartum

Ovarian ultrasonic studies in cattle and buffalo have shown that follicular development occurs in a wave like pattern (Rajamahendran and Tayler 1990, Baruselli *et al.* 1997). In cattle, antral follicular population (AFP), a reliable phenotypic biomarker, is positively associated with ovarian function (Jimenez-Krassel *et al.* 2009). AFP is highly repeatable (0.84–0.95) within individuals and this makes it a plausible parameter for classification of animals based on AFP. But, buffaloes differ from cattle in having lower number of primordial (Van Ty *et al.* 1989) and AFP (Baruselli *et al.* 1997, Baldrighi *et al.* 2013). Anti-Müllerian hormone (AMH), a hormone belonging to growth factor-β family, is produced by granulosa cells from healthy growing follicles (La Marca and Volpe 2006) and its expression is higher in small antral follicles and decreases during the follicular growth. A single AMH measurement in young adult heifers is correlated with several AMH measurements during the same or multiple estrous cycles and AFP (Ireland *et al.* 2010) and is sufficient to correlate with follicular population. Study in Murrah heifers, Holstein and Gir heifers showed that buffalo heifers had less AFP and plasma AMH concentration than Gir and Holstein heifers (Baldrighi *et al.* 2014). In addition, AMH has been suggested to predict the fertility of female animals (Ireland *et al.* 2008). Animals with greater ovarian follicular populations have greater circulating AMH. Researchers are focusing their efforts to associate circulating levels of AMH with ovarian population, follicular dynamics and ultimately female fertility (Mossa *et al.* 2012, Visser *et al.* 2012, Guerreiro *et al.* 2014, Souza *et al.* 2014). Hence, single blood sample can be sufficient for evaluation of circulating AMH. But, there are few studies in buffaloes (Baldrighi *et al.* 2014, Liang *et al.* 2016, Kavya *et al.* 2017, 2019) and no studies to observe the relationship between AMH with AFP, resumption of cyclicity (RC), milk yield (MY), body weight (BW) and body condition score (BCS) in postpartum Murrah buffaloes. In this light, the present study was designed to determine the relationship between AMH and AFP, MY, BW, BCS and RC in postpartum buffaloes.

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MATERIALS AND METHODS

Study location: The study was conducted on buffaloes maintained at animal farm section, ICAR-Central Institute for Research in Buffaloes (CIRB), Hisar, Haryana which is located at 29.09°N 75.43°E in western Haryana. The maximum day temperature during the summer varies between 40° to 46°C while minimum temperature during winter ranges between 1.5° to 4°C.

Animals and management: Postpartum buffaloes (n=125) calved between September 2015 to March 2016 were used for present study. Buffaloes were milked twice daily and were screened by ultrasound every 10 days interval from day 30 after calving till 90 for resumption of cyclicity. Among these, multiparous (2–6 parity) which became cyclic within 30 days postpartum with the history of calving interval less than 366±11.7 days (347–379) were selected as group I (n=10) and those remained acyclic more than 90 days of observation and with the previous calving interval more than 519±86 days (437–701) were included as group II (n=10). They were maintained under uniform feeding practices as per ICAR feeding standards (2013). All experimental procedures were carried out following Animal Ethics Committee Guidelines.

Monitoring of ovarian cyclicity: Resumption of cyclicity (RC) was detected using a real time B-mode ultrasound scanner (Just vision 200, Model 320A, Toshiba, Japan) equipped with an intraoperative 7.0 MHz micro convex transducer. Both ovaries were observed for several planes by moving transducer to observe the presence of CL on either of the ovary. Ultrasound scanning was done every 10 days interval from day 30 till day 90. Animals which had corpus luteum (CL) were adjudged as cyclic and those which did not have CL upto day 90 were confirmed as acyclic buffaloes. Blood samples were collected in serum clot activated vacutainer and serum was harvested by centrifuging at 3,000 rpm, 4ºC for 15 min and stored at –20ºC until further hormone estimation.

Hormone estimation, BW,BCS and MY recording: 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 co-efficient of variation was ≤9% with <0.2 ng/ml sensitivity. BW and BCS of the animals were recorded on day 0 and 30. BCS was estimated on a linear scale of 1–5 with an increment of 0.25 as described by Edmonson *et al.* (1989) and Alapati *et al.* (2010). MY in both groups during first four weeks of lactation was recorded based on record of test day of milk recording in the farm.

Statistical analyses: Data analysis was done using SPSS (version 16), using T-test for deducing any difference in AMH,AFP, MY, BW and BCS in both groups. In addition, correlation between AMH with AFP, RC, BW, BCS and MY was carried out using Pearson's correlation coefficient and were considered significant at P<0.05.

RESULTS AND DISCUSSION

This study reports the correlation of AMH with fertility (RC) and production (MY) parameters in buffaloes. It is evident that none of the parameters (AMH, MY, BW and BCS) were comparable, though RC differed between the two groups (Table 1). In this study, we found that group I buffaloes had higher AFP (19.7±4.95 vs 15.7±5.08) and AMH (0.122±0.03 vs 0.109±0.02 ng/mL) as compared to group II buffaloes, though statistically non-significant (P=0.09). The presence of higher AMH and AFP in early cyclers can be attributed to the role of AMH in inhibiting the activation of primordial follicles from entering the follicular wave thereby arresting follicular atresia (Fortune *et al.* 2010). Likewise, our finding of higher AMH and AFP in early cyclers is supported by the fact that circulating AMH is positively correlated with fertility in cattle (Jimenez-Krassel *et al.* 2015), especially ovarian follicular population. It is noteworthy that early cyclers had higher AFP and AMH as compared to their counterparts due to the fact that ovaries with lower AFP consists of more granulosa cells non-responsive to FSH than their counterparts (Ireland *et al.* 2010, Scheetz *et al.* 2012).

Table 1. Anti-Müllerian hormone (AMH), milk yield (MY), body weight (BW), body condition score (BCS) and resumption of cyclicity (RC) in group I and II buffaloes

Parameter	Group I (n=10)	Group II (n=10)	P value
AMH (ng/mL)	0.122±0.03	0.109±0.02	0.38
AFP	19.7±4.95 (14.7–24.5)	15.7±5.08 (10.7–20.5)	0.09
RC (days)	26.1±4.64 (22–30)	–	–
MY (kg)	304.6±23.63 (268–343)	277.2±59.07 (180.6–366.1)	0.27
BW (kg)	557±11.31 (546–568.4)	524±18.34 (505.6–542.3)	0.16
BCS	3.2±0.15 (3.05–3.36)	2.95±0.19 (2.76–3.14)	0.29

Values expressed as mean±SD.

Considering AMH being highly expressed in small healthy follicle responsive to gonadotropins, it becomes an important maker for healthy AFP (Monniaux *et al.* 2012). Similarly, lower AFP deduced in group II is supported by the previous reports of lower AFP relating to lower fertility (Jimenez-Krassel *et al.* 2015). In this study, AMH and AFP were comparable with buffalo heifers and lower than *Bos taurus* and *Bos indicus* breeds as reported by Baldrighi *et al.* (2014) and Gimenes *et al.* (2009) in buffalo heifers.

Presence of lower follicular population in buffalo could be attributed to increased number of atretic follicles, lower follicular growth and intrinsic oocyte properties (cytoplasmic vesicles quantity, mitochondria shape and inner content, zona pellucida deposition and granulosa cells–oocyte junctions) of buffalo species (Mondadori *et al.* 2010). In the present study, no significant correlation was observed between AMH and AFP. This was discrepancy with earlier findings of Baldrighi *et al*. (2014) which might be due to several factor viz. negative energy balance during early fetal life as well as dam-age and lactation status, physiological status of the animals and individual variations (Evans *et al.* 2012, Walsh *et al.* 2014) which determines the AFP in offspring. In addition, studies have shown that AMH remains constant in bovine females during their early stages of reproductive life (Rota *et al.* 2002) as compared to buffaloes which need further investigation.

Furthermore, lower AMH and AFP as compared to cattle can be attributed to species difference and lower AFP buffaloes. In this investigation, the variation in AFP (10–24) was similar to previous reports in *Bos taurus* (Ireland *et al.* 2008), *Bos indicus* (Bastos *et al.* 2010) and buffalo

Table 2. Correlation (r) between Anti-Müllerian hormone (AMH), antral follicle count (AFP), milk yield (MY), body weight (BW), body condition score (BCS) and resumption of cyclicity (RC)in postpartum buffaloes

Parameter	Group I (n=10)	Group II (n=10)	P value
AMH vs AFP	–0.001	–0.042	Group I (0.99); Group II (0.90)
AMH vs RC	–0.298	–	Cyclic (0.40)
AMH vs MY	–0.051	0.097	Group I (0.92); Group II (0.83)
AMH vs BW	0.470	0.137	Group I (0.34); Group II (0.76)
AMH vs BCS	0.620	0.028	Group I (0.18); Group II (0.95)

(Baldrighi *et al.* 2014). In this investigation, AMH was not correlated with BW and BCS. This was similar to the findings of Guerreiro *et al.* (2014) and Jimenez-Krassel *et al*. (2015) in cattle heifers wherein there was significant difference in AFP with within comparable BCS groups. Correlations between the parameters, i.e. AMH, AFP, MY, BW, BCS and RC in postpartum buffaloes is shown in Table 2. Lack of correlation between AMH with MY was in accordance with Jimenez-Krassel *et al.* (2015) in cattle. Lack of correlation between MY, BCS and BW was attributed to the local action of AMH on pre- and early antral follicles in ovaries (Durlinger *et al.* 2002). Since, AFP vary between animals (Jimenez-Krassel *et al.* 2015), breeds (Batista *et al.* 2014), but repeatable within individual (Ireland *et al.* 2007), future investigations on the systemic relation of AMH on MY, BCS, BW and fertility are warranted in buffaloes.

In conclusion, AMH, MY, BW and BCS were comparable between early and late cyclers, though AFP showed a trend in difference between early and late postpartum cyclers. Furthermore, this study failed to establish any relationship between AMH and AFP, MY, BW, BCS and RC in postpartum buffaloes.

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