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Blood metabolites, body condition score, body weight and milk yield in relation to resumption of cyclicity in post-partum buffaloes

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ABSTRACT

This study was designed to decipher the relationship between blood metabolites [glucose, total protein, urea, cholesterol, beta hydroxyl-butryicacic (BHBA), and insulin-like growth factor-1 (IGF-1) and leptin], body condition score (BCS), body weight (BW) and milk yield with resumption of post-partum cyclicity in buffaloes. Pluriparous buffaloes were screened using transrectal ultrasonography from day 0 to day 90 at 10 days interval and divided into two groups: I (n=7; buffaloes ovulated <30 days of calving) and II (n=7; anestrous buffaloes >90 days post-partum). Blood samples were collected in both groups at day 0, 10, 20 and 30 post-partum (day 0 considered as calving day) with BW, BCS and milk production observations during first month of lactation. In cyclic buffaloes, serum albumin was higher as compared to acyclic group. In addition, leptin, BW, BCS and milk yield differed with time. Significant correlation between BHBA, IGF-1, leptin and albumin in group I was observed. In conclusion, blood metabolites (albumin, BHBA, IGF-1 and leptin) were significantly correlated with each other in cyclic post-partum buffaloes, but not in acyclic group.

Key words: Body condition score, Body weight, Buffalo, Cyclicity, Metabolites, Post-partum

Derangement of the sequential interactions between the hypothalmo-pitutary and gonadal axis arising during peri- and postpartum period due to negative energy balance (NEB), adverse season and lactation stress leads to delayed resumption of postpartum cyclicity (DRPC) (Vercouteren *et al.* 2015). Of these factors, energy balance is considered as the prime stimulus and modulator of ovarian rebound during postpartum period in cows and buffaloes. In cows, severe energy deficiency during the transition period results in higher β-hydroxybutyrate (BHBA) with lower insulin, glucose and insulin-like growth factor-1 (IGF-1) (Chandra *et al.* 2011). Studies show that nutritionally-related metabolites (glucose, protein, albumin, urea, IGF-1 and leptin) were positively correlated during early postpartum cyclic cows (Damptey *et al.* 2014). Buffaloes calving during summer showed lower blood plasma urea nitrogen from pre- to postpartum through calving day. This was associated with significant reduction in days to first service and service per conception (Ashmawy *et al.* 2015). Concentrations of blood metabolites, viz. glucose, cholesterol and triglyceride and non-esterified fatty acids (NEFA) remained unaffected by the commencement of cyclicity and milk yield in buffaloes (Hussein *et al.* 2013). In buffaloes, shorter time interval for ovulation was observed with moderate BCS at calving than thin counterparts (Chagas *et al.* 2007). But, studies pertaining to relationship of BCS with blood metabolites, milk yield and resumption of cyclicity in postpartum buffaloes are sparse. In this light, the aim of this study was to investigate the relationship between blood metabolites, BW, BCS and milk yield with resumption of postpartum cyclicity in postpartum buffaloes.

MATERIALS AND METHODS

Location of study and animals: The study was conducted on buffaloes maintained at Animal Farm Section, ICAR-Central Institute for Research on Buffaloes (CIRB), Hisar, Haryana located at 29.09°N 75.43°E in western Haryana. Maximum day temperature during the summer varied between 40° to 46°C, while minimum temperature during winter ranged between 1.5 to 4°C. Postpartum buffaloes calved between September 2015 to March 2016 were used for present study and they were maintained under uniform feeding practices as per ICAR Feeding Standards (2013). Buffaloes were milked twice daily and were screened by ultrasound every 10 days from day 25 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=7) and those remained acyclic more than 90 days postpartum and having calving interval more than 519±86 days (437–701) designated as group II (n=7). All experimental procedures were carried under the

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Table 1. Serum biochemical and hormone profile

Parameter	Day	Group	
		I (n=7)	II (n=7)
Glucose (mg/dl)	0	76.85±4.89[a]	71.00±3.41[a]
	10	60.57±2.06[b]	62.00±1.34[b]
	20	58.71±3.63[b]	58.71±2.21[b]
	30	56.14±3.37[c]	54.42±2.31[c]
Total protein (g/dl)	0	7.92±0.16	7.90±0.46
	10	8.01±0.21	8.38±0.44
	20	8.08±0.20	7.88±0.26
	30	8.25±0.25	7.52±0.34
Albumin (mg/dl)	0	2.70±0.06[A]	1.24±0.06[B]
	10	2.74±0.03[A]	1.37±0.10[B]
	20	2.37±0.28[A]	1.70±0.13[B]
	30	2.82±0.07[A]	1.62±0.18[B]
Urea (mg/dl)	0	49.91±6.43	35.84±6.38
	10	43.57±7.51	28.90±6.57
	20	29.04±5.71	29.48±4.23
	30	33.58±4.59	26.62±4.09
Cholesterol (mg/dl)	0	54.98±3.34	52.44±13.13
	10	60.94±7.96	54.10±12.91
	20	75.05±13.28	68.47±16.21
	30	95.41±4.70	62.11±9.81
BHBA (mg/dl)	0	2.91±0.22	2.70±0.12
	10	2.95±0.26	2.75±0.19
	20	2.88±0.26	2.77±0.20
	30	2.80±0.26	2.47±0.13
IGF-1 (ng/ml)	0	71.45±9.08	84.03±1.99
	10	77.87±5.21	81.94±2.70
	20	81.12±0.81	79.83±2.28
	30	73.18±4.32	76.53±2.54
Leptin (ng/ml)	0	1.73±0.12[a]	1.60±0.12[a]
	10	1.88±0.14[b]	1.74±0.14[b]
	20	1.86±0.13[b]	1.76±0.06[b]
	30	1.72±0.13[a]	1.58±0.08[a]

Values expressed as mean±SEM. [a,b]Values in a column in a group with different lowercase superscript differ significantly (P<0.01). [A,B]Values in a row between groups with different uppercase superscript differ significantly (P<0.01).

Table 2. Body weight and body condition score

Parameter	Day	Group	
		I (n=7)	II (n=7)
Body weight (kg)	0	565.00±22.32[a]	537.87±18.70[a]
	30	549.62±22.10[b]	511.37±21.20[b]
Body condition score	0	3.31±0.10[a]	3.09±0.10[a]
	30	3.09±0.06[b]	2.81±0.10[b]

Values expressed as mean±SEM. [a,b]Values in a column within group with different superscript differ significantly (P<0.01).

Table 3. Milk yield

Parameter	Week post-partum	Group	
		I (n=7)	II (n=7)
Milk yield (kg)	1	66.1±2.90[a]	57.75±6.03[a]
	2	75.8±2.12[b]	63.47±7.12[b]
	3	80.3±2.53[c]	72.80±6.88[c]
	4	82.4±2.24[c]	74.32±6.68[c]

Values expressed as mean±SEM. [a,b]Values in a column within group with different superscript differ significantly (P<0.01).

Table 4. Correlation between blood metabolites, body weight, body condition score in study groups

Variable	Group I	Group II
Glucose	BW (0.82)*	Leptin (0.87)*
Total protein	IGF-1 (–0.88)*; Leptin (–0.86)*; BCS (–0.8)*	-
Albumin	BHBA (0.82)*; IGF-1 (0.8)*; Leptin (0.82)*	-
BHBA	IGF-1 (0.94)*; Leptin (0.95)*	-
IGF-1	Leptin (0.94)*	-
BW	BCS (0.84)*	BCS (0.85)*

(r), *P<0.05. IGF-1, insulin-like growth factor-1; BHBA, beta-hydroxy butyric acid; BW, body weight; BCS, body condition score.

approval of Institutional Animal Ethics Committee (IAEC).

Ultrasound scanning: Resumption of cyclicity was detected using a real time B-mode ultrasound scanner (Just vision 200, Model 320A, Toshiba, Japan) equipped with an intraoperative 7.0 MHz microconvex 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 0 to day 90. Animals which had corpus luteum (CL) were adjudged as cyclic and those which does not have CL upto day 90 days were confirmed as acyclic buffaloes.

Blood sampling, biochemical and hormone estimation: Blood samples (10 ml) were collected from group I and II buffaloes on day 0, 10, 20 and 30 postpartum, considering day 0 as calving day. Blood samples were collected from jugular vein in serum clot activated vacutainer (VACUETTE®). After collection, samples were centrifuged at 3,000 rpm, 4°C for 15 min. Serum was separated and stored at –20°C until analysis.

Serum total protein, albumin, urea and cholesterol concentration were determined using commercial kits (Coral Clinical Systems, India) using automated biochemical analyzer (Coralyzer 200, Tulips Diagnostics, India). Blood glucose estimation was done by using glucometer strips (Accu-Chek®Active) immediately after collection of blood samples. Serum BHBA concentration was estimated using commercially ELISA kits (Sincere Biotech Co., Ltd., Beijing) as per the manufacturer's instructions. The detection range of BHBA was 1.53–100 µg/ml. The intra- and inter-assay co-efficient of variation was ≤9% and ≤15%, respectively for BHBA kits.

Serum BHBA, IGF-1 and leptin concentrations were estimated using ELISA kits (Sincere Biotech Co. Ltd., Beijing) as per instructions. Detection range of BHBA, IGF-1 and leptin kits were 1.53–100 µg/ml, 0.156–10 ng/ml and

0.156–10 ng/ml, respectively. The intra assay and inter-assay co-efficient of variation was ≤9% and ≤15%, respectively for kits used.

Estimation of body weight, BCS and milk yield: Body weight and BCS were recorded on day 0 and 30. BCS was estimated on a scale of 1–5 with an increment of 0.25 as described by Edmonson *et al.* (1989) and Anitha *et al.* (2010). Milk yield (kg) 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: Statistical analyses were carried out using SPSS (version 16) with General Linear model procedure. The correlation (r) between various parameters within a group was carried using Pearson's correlation co-efficient. Data represented as mean±SEM and considered significant at $P<0.05$.

RESULTS AND DISCUSSION

In this study, serum glucose was comparable between the groups and Obese *et al.* (2015) reported that no relation existed between serum glucose and cyclicity resumption in cows. As energy demand for milk production is met through gluconeogenesis, lower blood glucose concentrations during early lactation can be attributed to glucose drain for lactose synthesis. However in contrast, Abdulkareem (2013) found steady plasma glucose both during calving and postpartum. Non-significant difference in serum TP between the groups was similar to that reported by Damptey *et al.* (2014) in cows. In contrast, Guzel and Tanriverdi (2014) found significantly higher TP level in non-cyclic than cyclic cows. Higher serum albumin in cyclic buffaloes was in agreement with the findings of Obese *et al.* (2015). Hence, lower albumin level in acyclic buffaloes might be due to lower protein status at calving that remained low during early lactation as compared to cyclic buffaloes. Blood urea concentration was in agreement with the findings of Jeong *et al.* (2015) who reported it to be lower from 2 to 6 weeks postpartum in non-cyclic cows arising due to reduced dietary protein intake and/or reduced hepatic urea genesis. In both groups, cholesterol remained comparable upto day 20 postpartum. However, higher serum cholesterol in cyclic group was consistent with previous report (Jeong *et al.* 2015) confirming that late-cycling cows have lower serum cholesterol from 2 weeks pre-partum to 4 weeks postpartum. Lower serum cholesterol in anovular buffaloes might be the cause of non-resumption of ovarian activity which needs further investigation. In this study, BHBA showed no difference between the groups which was reported earlier in dairy and beef cows (Castro *et al.* 2012). On the contrary, Jeong *et al.* (2015) reported higher BHBA concentration in non-cyclic cows after calving. From our results, it is evident that buffaloes were not under negative energy balance as they were offered balanced diet and variation in the resumption of cyclicity can be attributed to other factors excluding nutritional causes. In this study, acyclic animals had higher IGF-1 concentration on day 0 with a decreasing trend towards day 30. Similar trends were reported by Ashmawy (2015) in buffaloes.

Furthermore, we found no change in IGF-I level between ovulatory and non-ovulatory cows. This alteration in IGF-1, evident in high yielding dairy cows, but not in buffaloes, might be due to higher lactational demand and negative energy balance in cows (Butler *et al.* 2003). Likewise, we had found non-significant difference in leptin concentration between the two groups. Our results were consistent with Konigsson *et al.* (2008), who observed comparable leptin level between the groups showing difference in ovarian cyclicity resumption. But, it is notable that plasma leptin concentration in cows was reduced by ~50% after parturition and remained depressed during lactation (Hussein *et al.* 2011) which was consistent with our findings in postpartum buffaloes. However, studies on how leptin levels relate to reproductive function at transition period in buffaloes are warranted.

Positive correlation of glucose with BW and leptin was in contrast to results of Guzel and Tanriverdi (2014) in cows. But, significant correlation between leptin and IGF-1 was in agreement with the findings of Hassan *et al.* (2014). In contrast to our results, Hussein *et al.* (2011) found plasma leptin levels showed insignificant positive correlation with TP ($r=0.15$). Though, leptin increased with total cholesterol (Taleb *et al.* 2014). Interestingly, non-significant correlation of BHBA with IGF-1, leptin and albumin in cyclic groups might be attributed to their comparable association in metabolism. Body weight and BCS, in both groups differed with time which was similar to earlier study in cows (Jeong *et al.* 2015). BCS, after parturition, was found to be non-significantly lower in cows that showed delayed resumption of cyclicity (Damptey *et al.* 2014). In addition, BW was correlated with glucose but, BCS was in negative correlation with TP in cyclic animals which need to be further investigated. Likewise, correlation of BCS with BW in both groups was in agreement with the results of Hussein *et al.* (2013). Furthermore, non-significant difference in milk yield among groups was in agreement with the findings of Villa-Godoy *et al.* (1990). In contrast, several studies have found a negative relationship between milk production and several fertility traits (Royal *et al.* 2000) and moreover, Buckley *et al.* (2003) observed a positive association between milk yield variables and reproductive efficiency. In conclusion**,** this study shows that serum albumin was significantly higher in cyclic group and blood metabolites (albumin, BHBA, IGF-1, leptin) were significantly correlated with each other in cyclic postpartum buffaloes as compared to acyclic postpartum buffaloes, besides BW and BCS showing relationship with each other in both groups.

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