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RESEARCH ARTICLE

Postpartum Cyclicity of Holstein-Friesian Crossbred Cows Shows Relation with Serum Biochemical Profiles during 45-60 Days Postpartum

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A B S T R A C T

Reproductive performance of dairy cows is greatly affected by energy, protein and mineral profiles, which is also connected to postpartum anestrus. The present study was conducted during the period from December 2018 to May 2019 on 30 local x Holstein-Friesian crossbred lactating cows with parity 1-5 and body condition score of 2.0-3.5. This study aimed to compare serum glucose, cholesterol, triglycerides, total proteins, urea nitrogen, calcium, phosphorus and magnesium concentrations between anestrus and normal cyclic crossbred cows during 45 to 60 days postpartum. Automated biochemical analyzer was employed to measure these concentrations, using 15 cows in each group. The serum of postpartum anestrus cows contained significantly lower ($P<0.05$) concentrations of glucose, total proteins, calcium and magnesium compared to normal cyclic cows, while urea nitrogen concentration was significantly increased ($P<0.05$) in the postpartum anestrus cows. However, concentrations of other serum biochemical metabolites did not differ between cows of the two groups. These results suggest that blood glucose, total proteins, calcium, magnesium and urea nitrogen might have an effect on the cyclicity of Holstein-Friesian crossbred cows during 45 to 60 days postpartum. However, further studies are recommended to explore the underlying mechanism for the role of these metabolites in the cyclicity of postpartum cows.

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INTRODUCTION

A cow must be bred successfully within 80-85 days postpartum to achieve the desired calving interval of 365 days (Khan *et al.*, 2010). First ovulation within 21 days in postpartum cows is associated with improved reproductive performance and subsequent normal estrous cycles (Cheong *et al.,* 2016). However, the postpartum cows may go through a temporary infertility known as postpartum anestrus, which is commonly influenced by infectious and non-infectious reasons- such as uterine involution, hormonal and nutritional status of the cows (Peter *et al.,* 2009).This problem in crossbred cows is an important barrier for sustainable dairy farming system that caused huge economic losses to the large and small scale dairy farms in Bangladesh (Pariza *et al.,* 2013).

Daily energy balance nadir is considered as major metabolic modulator of postpartum ovarian activity and plays a vital role in anestrus in dairy cows (Beam and Butler, 1999). Lactating cows for a four-month period of postpartum anestrus with inactive ovaries are reported having changes in blood energy metabolites such as reduced level of glucose and increased level of total cholesterol (CHOL-T). Serum total proteins (PRO-T) and blood urea nitrogen (BUN) are responsible for desirable reproductive performance in cows (Puppel and Kuczyriska, 2016). Postpartum anestrus has also been found to be associated with deficiencies of calcium (Kumar *et al.,* 2010), phosphorus (Das *et al.*, 2012) and magnesium (Dutta *et al.,* 2001). However, all these studies have covered a long postpartum period and are unable to visualize the relation of blood biochemical properties with anestrus after uterine involution is completed and before the end of the voluntary waiting period (VWP), that is day 45-60 postpartum.

It is recommended that a minimal VWP of 45 to 60 days postpartum is required, allowing for a complete uterine involution and normal ovarian cyclicity of cows to enhance conception rate after first insemination and to have a shorter calving interval. While, negative energy balance is considered one of the important factors compelling to extend the time between parturition and first postpartum insemination, as well as extending the calving interval, scarce information is available to show its profile at optimal VWP. The lack of prominent expression of estrus behavior may be due to a negative energy balance that may cause failure of observing estruses not before the second and following postpartum estrous cycles. Although, blood proteins and mineral profiles are shown to have relation with postpartum anestrus in cows (Das *et al.*, 2012), their relation in the VWP is unknown. Therefore, the current study was intended to explore the association of serum metabolite profile of energy-glucose (GLU), triglycerides (TAG) and total cholesterol (CHOL-T); proteins- total proteins (PRO-T) and blood urea nitrogen (BUN); and minerals- calcium (Ca), phosphorus (P) and magnesium (Mg) between cyclic and anestrus crossbred cows at 45-60 days postpartum.

MATERIALS AND METHODS

Selection of animals: The present study was conducted for a period of six month from December 2018 to May 2019. Thirty crossbred (Local x Holstein-Friesian) lactating cows, with body weight of 270-380 Kg and parity 1 to 5, were selected from different commercial dairy farms in Sylhet, located in northeast of Bangladesh. The body condition score (scale 1-5, with 0.5 fractions) was estimated for each cow on the basis of deposited fat thickness and bony prominence at the loin and tail head region and it ranged from 2.0 to 3.5. All cows were kept in naturally ventilated housing system with individual stall. Routine deworming (Liver flukes and Round worms) and vaccination schedule (Anthrax, Foot and Mouth Disease, Black Quarter and Haemorrhagic Septicaemia) was maintained properly by farmers. Cows were fed paddy straw, concentrate mixture, green grass and water ad-libitum. The concentrate mixture contained wheat bran, rice bran, rice polish, broken rice, oil cakes and common salt.

Experimental cows were divided into two groups, cyclic and anestrus (n=15 each), on the basis of clinical estrous signs observed by farmers and ovarian examination. Cyclic cows had history of showing clinical estrous signs and had functional corpus luteum on any ovary identified by rectal examination. Postpartum anestrus cows were identified by the absence of estrus signs at 45-60 days postpartum and no palpable corpus luteum on any ovary. The female reproductive tract was routinely examined by per-rectal examination of each cow at seven days interval for up to 65 days post-partum. Cows with any abnormalities in reproductive organs were not included in the study. Both cyclic and anestrus cows were subjected to blood sampling at 45-60 days postpartum.

Serum collection: A vacutainer tube without anticoagulants (Bioson®; Becton, Dickinson and Company, USA) was used to collect about 5 ml of blood from the jugular vein of each cow. Each blood sample was allowed to clot for 1 hour at room temperature (Tuck *et al.*, 2009). The serum was separated by centrifugation at 1000g for 10 minutes, transferred to 1.5 ml micro centrifuge tubes and stored at -20°C until use for biochemical analysis.

Serum analysis: The blood serum was analyzed for energy profile (GLU, TAG & CHOL-T), protein profile (BUN, PRO-T) and minerals (Ca, P & Mg), using commercial kits. Serum and chemical reagents were thawed by keeping at room temperature for about 30 minutes before analysis. The serum samples were vortexed and assayed using automated biochemical analyzer (Humalyzer-3000, Germany) according to the directions provided with commercial kits.

Glucose levels were determined by commercial kit (Catalogue No. 10260, Human Diagnostics, Germany) based on the enzymatic colorimetric principle (Barham and Trinder, 1972). Serum triglycerides were measured by enzymatic hydrolysis with lipases (Trinder, 1969) by using commercial kit (Catalogue No. 10724, Human Diagnostics, Germany). Total cholesterol was estimated by using commercial kit (Catalogue No. 10017, Human Diagnostics, Germany) on the principle of enzymatic hydrolysis and oxidation (Richmond, 1973).

Serum total proteins were determined using commercial kit (Catalogue No. TP245, Randox Laboratories) which worked on the principle of Biuret method (Tietz,1995). Blood urea nitrogen was determined using commercial kit (Catalogue No. 1158010, Linear Chemicals) that followed the principle of colorimetric method (Tietz, 1995).

Calcium was determined using commercial kit (Catalogue No. CA590, Randox Laboratories) based on the principle of colorimetric method (Sarkar and Chauhan, 1967). Phosphorus was measured using commercial kit (Catalogue No. 1149005 Linear Chemicals) that followed the principle of colorimetric method (Tietz, 1995). Magnesium was estimated through commercial kit (Catalogue No. 1144005 Linear Chemicals) which worked on the principle of colorimetric procedure (Tietz, 1995).

Statistical analysis: The data were analyzed using the statistical software program SPSS 22.0 for Windows 7. The normality test of data was done by Kolmogorov-Smirnov test. Mann-Whitney U test was performed to assess the differences between groups. Data was expressed as mean ± standard error (SE). $P<0.05$ was considered as statistically significant.

RESULTS

The comparison of serum energy metabolites revealed that only glucose concentrations were significantly varied ($P<0.001$) between anestrus and cyclic cows and was lower in postpartum anestrus cows than cyclic cows (Table 1). Although serum triglycerides and total cholesterol concentrations were higher in postpartum anestrus cows when compared to cyclic cows, the difference was statistically non-significant (Table 1).

Serum total proteins level was significantly lower ($P<0.05$) in anestrus cows compared to cyclic cows. On the other hand, the concentration of blood urea nitrogen was significantly higher ($P<0.05$) in anestrus than cyclic cows (Table 1).

Table 1: Postpartum serum biochemical profile (mean±SE) of cyclic and anestrus Holstein-Friesian crossbred cows (n=15 in each group)

Parameters		Animal status	Unit	Mean±SE	P Value
Energy profile	Glucose	cyclic	mg/dl	55.98±1.02	0.001
		anestrus		29.31±4.14	
	Triglycerides	cyclic	mg/dl	22.06±2.33	0.838
		anestrus		23.79±4.19	
	Total cholesterol	cyclic	mg/dl	71.68±6.18	0.830
		anestrus		72.07±4.03	
Protein profile	Total protein	cyclic	g/L	61.74±3.96	0.049
		anestrus		45.73±3.20	
	Blood urea nitrogen	cyclic	mmol/L	3.20±0.90	0.032
		anestrus		5.04±1.15	
Mineral profile	Calcium	cyclic	mg/dl	10.55±0.27	0.001
		anestrus		5.22±0.69	
	Phosphorus	cyclic	mg/dl	6.84±0.46	0.305
		anestrus		5.90±0.92	
	Magnesium	cyclic	mg/dl	2.52±0.18	0.045
		anestrus		1.80±0.25	

The concentrations of serum calcium and magnesium were found significantly lower ($P<0.05$) in anestrus cows compared to cyclic cows. Even though the serum phosphorus concentration was observed slightly lower in anestrus cows compared to cyclic cows, the difference was non-significant (Table 1).

DISCUSSION

Energy profiles play crucial role in maintaining uterine physiology, inflammatory status and reproductive performance in cows (Meteer *et al.*, 2015). In the present study, among the energy metabolites, blood glucose concentration was significantly reduced in postpartum anestrus cows compared to the postpartum cyclic cows during day 45 to 60. These results are in agreement with the findings of Das *et al.* (2012), who also found decreased blood glucose levels in postpartum anestrus than cyclic cows. Lower serum glucose indicates the negative energy balance that adversely affects reproductive functions in cows (Wankhade *et al.*, 2017). Negative energy balance during the first 3-4 weeks postpartum is highly correlated with the interval to first ovulation, the timing of the nadir (Beam and Butler, 1999). Deficiency of energy leads to disorders in the function of endometrium, delay in uterine involution, affects utero-immune cell function and ultimately lengthens VWP, which prolongs calving interval (Moyes, 2015; Elmetwally, 2018). The reproductive pathway between negative energy balance and the hypothalamic-pituitary-ovarian axis is very complex and involves several metabolites and hormones. Among the metabolites required for normal ovarian dynamics in dairy cows, glucose has been reported to be most important (Barson *et al.*, 2019). It acts as a vital metabolite for the secretion of GnRH, which is a key hormone for ovarian activity (Crowe *et al.*, 2014). Therefore, low level of blood glucose in postpartum cows after uterine involution in the present study might be one of the important causes of anestrus.

Assessment of protein profile is important, as it acts as a biomarker in energy-protein imbalance and nutritional status of cows (Haile *et al.*, 2014). Estimation of protein profiles revealed that blood urea nitrogen was increased in anestrus cows. Blood urea nitrogen concentrations often fluctuate around calving (Wathes *et al.*, 2007) and are influenced by a wide variety of inter-related parameters, including body requirement and metabolism. Similar to findings of our study, Ahmad *et al.* (2004) and Damptey *et al.* (2014) found that BUN concentrations were higher in postpartum anestrus cows than normal cyclic cows. It is also considered that negative energy balance impairs liver function and reduces the metabolic clearance of urea, which might be the cause of high level of blood urea nitrogen in anestrus cows. The influence of protein intake and the toxic effect of blood urea on dairy cow fertility remains ambiguous and unclear (Jackson *et al.*, 2011).

In the present study, total proteins level was significantly higher in cyclic cows than postpartum anestrus cows. These results are similar to the findings of some previous studies (Pariza *et al.*, 2013; Qureshi *et al.*, 2016). Low serum protein leads to amino acid deficiency which is important in reproductive hormonal regulation and finally leads to ovarian dysfunction (Tedeschi *et al.*, 2015). Protein deficiency has resulted in weak expression, or even cessation, of estrus which leads to lengthening of the voluntary waiting period, as well as repeat breeding (Veena *et al.*, 2015).

Among the measured minerals, calcium and magnesium were significantly decreased in the postpartum anestrus cows during day 45 to 60. Calcium plays a functional role in maintaining the normal reproductive efficiency in dairy cows (Kumar, 2015). Decreased level of calcium has adverse effect on muscle tone in the uterus of postpartum cows, leading to delayed uterine involution and prolongs calving intervals (Bindari *et al.*, 2013). Many authors reported that blood calcium is reduced in postpartum anestrus cows (Dutta *et al.*, 2001; Das *et al.*, 2012; Jayachandran *et al.*, 2013), which is in agreement with the present study results. Calcium alone might not have an effect on the ovarian activity, however, alteration in Ca:P ratio might affect the ovarian activity by impairing hypothalamic-pituitary-ovarian axis. In the present study, we found that while calcium was reduced significantly in anestrus cows, phosphorus did not differ significantly. This is the strong indication of Ca:P imbalance in the postpartum anestrus cows.

Deficiency of magnesium resulted to impaired follicular growth and wave pattern, irregular ovulation, poor conception, infertility and decreased reproductive performance in female cows. Our results regarding serum magnesium levels in anestrus and cyclic cows are in agreement with those of Dutta *et al.* (2001), who suggested that postpartum anestrus cows face the scarcity of the blood concentration of magnesium, which might be one of the key determinants of the ovarian function.

Conclusions: Blood metabolites seem to be the most important indicators of the postpartum cyclicity in dairy cows. In our study, serum biochemical profiles (GLU, T-Proteins, BUN, Ca and Mg) showed significant effect on postpartum cyclicity, in particular after uterine involution of the cows during 45-60 days postpartum. These results suggest that deviation of blood metabolites during postpartum period leads the crossbred dairy cows to anestrus, which in turn renders the animals unable to be inseminated at the end of the optimal voluntary waiting period. These results call for further deliberate research to investigate possible mechanism of role of serum metabolites, as revealed in the present study, in the regulation of ovarian functions.

Authors contribution: AHMMU, MA and MKH conceived and designed the study. AHMMU and MSI executed the Laboratory analysis and acquired data.MA and AHMMU participated in statistical analysis and manuscript writing.MA and MKH critically revised the manuscript.

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