SHORT COMMUNICATION

Changes in hormones of the somatotrophic axis associated with postpartum reproductive infections in Murrah buffaloes (*Bubalus bubalis*)

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Received: 12 August 2021 / Accepted: 08 September 2021 / Published online: 31 October 2021
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Abstract: This study was designed to investigate the risk association of hormones of the somatotrophic axis with postpartum infections/ disorders in Murrah buffaloes. A total of six (n=6) healthy pregnant Murrah buffaloes and 23 symptomatic buffaloes with symptoms indicative of metritis (n=5), endometritis (n=6) mastitis (n=8), and retained placenta (RP) (n=4) were selected from the ICAR-NDRI Cattle herd. Blood samples were collected from each healthy buffalo on days -21, -14, -7, 0, +7, +14, +21 relative to calving. Blood samples were collected from unhealthy buffaloes twice on alternate days as and when the symptoms of reproductive abnormalities were noticed. The results revealed that the plasma growth hormone was significantly ($P<0.05$) elevated in buffaloes exhibiting symptoms of metritis (7.40 ± 0.96 ng/mL), mastitis (9.23 ± 1.60 ng/mL), and RP (6.63 ± 0.80 ng/mL). Similarly, plasma insulin level was significantly higher ($P<0.05$) in buffaloes infected with endometritis (1.53 ± 0.21 ng/mL), mastitis (1.32 ± 0.26 ng/mL), and RP (1.24 ± 0.15 ng/mL). But, the plasma IGF-1 was significantly lower in buffaloes exhibiting symptoms of metritis (128.43 ± 5.38 ng/mL), endometritis (112.65 ± 4.38 ng/mL), and mastitis (123.61 ± 3.99 ng/mL) except in RP (122.21 ± 5.10 ng/mL) than normally calved buffaloes (123.80 ± 4.96 ng/mL). From the experiment, it could be inferred that the levels plasma growth hormone, insulin, and IGF-1 differed significantly among buffaloes with and without postpartum infections and could be used for the risk assessment of postpartum infection in buffaloes.

Keywords: Buffaloes, Growth Hormone, Insulin, Insulin like Growth Factor-1, Postpartum Infections

The period around parturition in dairy cattle is characterized by negative energy balance (NEB), insulin resistance, reduced feed intake, hypocalcemia, and impaired immune functions (Mili et al. 2014, Sundrum, 2015, Mili et al. 2015a). The changes of hormones of the somatotrophic axis (i.e., GH-insulin-IGF-1-glucose signaling pathway) help in energy homeostasis during the transition period in dairy cattle. However, the inability of dairy cows to cope with metabolic demands, hormonal milieu coupled with impaired immune functions make them susceptible to infectious diseases (metritis, endometritis, mastitis, RP, etc.), and thereby production performance of the dairy animal is seriously affected in terms of economic loss to farmers. Deviation of hormones of the somatotrophic axis could be helpful as risk predictors for diagnosing metabolic and postpartum infections/ disorders in dairy cows. Therefore, the present study was aimed to find out the association of crucial hormones of the somatotrophic axis with postpartum infections/ disorders in buffaloes.

The present experiment was conducted between September 2011 till May 2012 at ICAR-National Dairy Research Institute (NDRI), Karnal. The institute is located at an altitude of 250 m above mean sea level, latitude and longitude position $29^{\circ}42'N$ and $79^{\circ}54'E$, respectively. The maximum ambient temperature in summer goes up to $45^{\circ}C$, and the minimum temperature in winter comes down to $0^{\circ}C$ with a diurnal variation in the order of $15-20^{\circ}C$. The average annual rainfall is 700 mm from early July to mid-September.

A total of six (n=6) numbers of healthy pregnant Murrah buffaloes and 23 symptomatic buffaloes with symptoms indicative of metritis (n=5), endometritis (n=6), mastitis (n=8), and retained placenta (n=4) were selected from the institute cattle herd. Metritis, endometritis, and mastitis infections were diagnosed by the institute's herd veterinary officer based on symptoms described by Sheldon et al. (2006). Metritis was diagnosed by the presence of systemic signs of sickness, including fever, red-brown watery, foul-smelling uterine discharge, dullness, elevated heart rate, and low production. In contrast, clinical endometritis was diagnosed

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Table 1 Plasma hormone concentration in buffaloes exhibiting postpartum infections / disorders

Parameters	Healthy	Metritis	Postpartum infections Endometritis	Mastitis	Day 0 (Normally calved)	RP (After 12h of parturition)
IGF-1(ng/mL)	134.34±8.20 ^a	128.43±5.38 ^b	112.65±4.38 ^b	123.61±3.99 ^b	123.80±4.96	122.21±5.10
Insulin(ng/mL)	1.07±0.06 ^a	0.86±0.12 ^b	1.53±0.21 ^b	1.32±0.26 ^b	0.63±0.14 ^a	1.24±0.15 ^b
GH(ng/mL)	1.48±0.15 ^a	7.40±0.96 ^b	1.66±0.18	9.23±1.60 ^b	2.47±0.55 ^a	6.63±0.80 ^b

Bearing different superscripts (a, b) in the mean value for the metritis, endometritis, mastitis indicated significant differences at $P < 0.05$ with that of healthy buffaloes, whereas RP compared to normally calved buffaloes (Day 0).

by the presence of purulent (>50% pus) or mucopurulent (approximately 50% pus, 50% mucus) uterine exudates in the vagina, 21 days or more postpartum. Clinical mastitis was diagnosed by an elevated somatic cell count in milk and visual signs of inflammation such as clumpy, watery, bloody, or yellowish milk. The buffaloes that did not shed the fetal membrane within 12 hours of parturition were considered as cases of RP. All these buffaloes were maintained under general managerial practices as followed at the institute.

A blood sample (15ml) was drawn in sterile heparinized vacutainer tubes by jugular venipuncture from each healthy buffalo on days -21, -14, -7, 0, +7, +14, +21 relative to calving. Blood was collected on the day of diagnosis of the infections and on the alternate day in the infected group. The heparinized samples were centrifuged at 3000 rpm for 15 minutes, plasma aliquoted, and stored at -20°C for further analysis.

Growth hormone and Insulin was measured in plasma using a bovine Growth hormone ELISA test kit purchased from endocrine technologies, inc. USA and Endocrine Technologies, 35325 Fircrest Street, USA, respectively. IGF-I activity was quantified by Bovine IGF-1 ELISA test kit[®] obtained from Life Science Inc, Wuhan 430056, and P.R. China, with detection limit range between 2.4 ng/ml-60 ng/ml.

The data for healthy buffaloes were analyzed by one-way analysis of variance through graph prism version 5 to quantify postpartum variations for peripheral concentrations of growth hormone, Insulin, and IGF-1. Since prepartum and postpartum values were not statistically significant between days except IGF-1 (already published Mili et al. 2015b), the data from day 7 to 21 postpartum values were served as the reference value for healthy buffaloes, whereas the day of calving (day 0) values of healthy buffaloes was taken as a control for RP. Similarly for unhealthy buffaloes, all the values were expressed as mean ± standard error (SEM). The unpaired student “t” test using GraphPad prism version 5 was applied to compare the data of healthy and infected buffaloes.

The changes in plasma growth hormone, insulin, and IGF-1 levels in buffaloes infected with metritis, endometritis, mastitis, and RP compared to healthy ones is presented in Table 1. Plasma IGF-1

concentrations was significantly lower in buffaloes with metritis (128.43±5.38 ng/mL), endometritis (112.65±4.38 ng/mL), and mastitis (123.61±3.99 ng/mL) as against the healthy (134.34±8.20 ng/mL) ones expect RP, whereas plasma IGF-1 concentration was not statistically different among the two groups. These results are in agreement with previous studies (Kikukawa et al. 2002; Nikolic et al. 2003; Kasimanickam et al. 2013; Giuliadori et al. 2013; Beltman et al. 2020). Earlier, a low IGF-1 concentration was registered in mastitis cows (Nikolic et al. 2003; Huszenicza et al. 2004), and cows with uterine infections (53.8 ± 4.4 ng/mL) compared to healthy (66.9 ± 3.2 ng/mL) cows (Beltman et al. 2020). Kasimanickam et al. (2013) revealed that the IGF-1 concentrations remained high in cows infected with subclinical endometritis whereas these hormone levels were low in cows with clinical endometritis and metritis.

Plasma insulin concentration was significantly higher ($P < .05$) in buffaloes infected with endometritis (1.53±0.21 ng/mL), mastitis (1.32±0.26 ng/mL) and RP (1.24±0.15 ng/mL) when compared to healthy (1.07±0.06 ng/mL) and normally calved buffaloes (0.63 ±0.14 ng/ mL), respectively, except for buffaloes with metritis, where it was significantly lower than the healthy animals. Our findings are in agreement with earlier reports on mastitis (Nikolic et al. 2003; Huszenicza et al. 2004), metritis, and endometritis (Kasimanickam et al. 2013). Earlier, higher insulin levels were recorded in cows infected with mastitis (Nikolic et al. 2003; Huszenicza et al. 2004). Likewise, significantly higher insulin levels were recorded in subclinical endometritis cows compared to clinical endometritis, and metritis (Kasimanickam et al. 2013).

The plasma growth hormone was significantly elevated in metritis (7.40±0.96 ng/mL), mastitis (9.23±1.60 ng/mL), RP (6.63±0.80 ng/mL) when compared to healthy (1.48±0.15 ng/ mL) and normally calved buffaloes (2.47 ±0.55 ng/mL), respectively except endometritis buffaloes, where it was no significant changes than the healthy animals. Our findings are in agreement with earlier reports on mastitis (Gubbiotti et al. 2007). They revealed 2.5 times higher levels of growth hormones in Mastitis cows compared to healthy cows. An elevated level of growth hormones is expected for energy homeostasis during the transition period in buffaloes due to NEB. Earlier reports have indicated that the prolonged periods of NEB are associated with a decrease in insulin secretion

by the pancreas, which results in a lesser concentration of growth hormone receptors during the transition period (Pell and Bates, 1990). Therefore, an increased circulatory level of growth hormones with a lower IGF-1 level is expected due to uncoupled growth hormone-insulin-IGF-1-glucose signaling pathway for energy homeostasis (Mili et al. 2015b). These hormonal changes combined with alteration in humoral and cellular immune responses coupled with low antioxidant defense system during the transition period (Mili et al. 2015a), might explain the deviations of metabolic hormones are the predisposing risk indicators of postpartum infections and RP in buffaloes.

Conclusions

Hence, it can be inferred that plasma growth hormone, insulin, and IGF-1 differed significantly among buffaloes with or without postpartum infections and hence could be used for the risk assessment of postpartum infection. However, large-scale studies are required to determine the crucial threshold levels of these attributes to trace out the onset/ early identification of postpartum reproductive infections.

Acknowledgments

The authors thankfully acknowledge the Director, Indian Council of Agricultural Research – National Dairy Research Institute, Karnal, Haryana, India, for the research grant to conduct this study.

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