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Differential Fertility in Dairy Buffalo: Role of Thyroid and Blood Plasma Biochemical Milieu

Research Article

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ABSTRACT

The present study was conducted on buffaloes that were non-cycling (heifers, n=32; lactating, n=13) or cycling (heifers, n=11; lactating, n=27) and in the buffaloes that conceived (heifers; n=11; lactating, n=23) or failed to conceive (heifers, n=32; lactating, n=17) subsequent to artificial insemination. Plasma samples were analyzed for thyroid stimulating hormone, thyroid hormones, glucose, total proteins, creatinine, blood urea nitrogen, total cholesterol and triglycerides. None of the plasma biochemicals were significantly different (P>0.05) between non-cycling or cycling and pregnant or non-pregnant buffaloes. However, non-cycling and non-pregnant lactating buffaloes had lower (P<0.05) plasma concentrations of thyroid stimulating hormone and thyroid hormones, respectively, compared to their counterparts. Thus, blood metabolite status is not the cause of differential fertility in buffaloes. However, a subtle thyroid activity in lactating buffaloes may have some impact on their fertility status.

KEY WORDS blood biochemicals, buffalo, fertility, thyroid.

INTRODUCTION

Poor breeding efficiency remains one of the frustrating problems confronting the buffalo dairy industry (Ghuman *et al.* 2008). Initiation of ovarian cyclicity in post-pubertal and post-partum dairy animals and subsequently their ability to conceive are affected by nutrition status. Attainment of a critical level of body fat is important for the onset of puberty. This strongly suggests that there are links between the reproductive endocrine system and metabolic status of the animal (Schillo *et al.* 1992). During immediate post-partum period, metabolic changes induced by the difference in energy uptake and energy requirements may lead to negative energy balance, thus having repercussions on subsequent fertility (Jorritsma *et al.* 2003). Intriguingly, repercussions on ovarian cyclicity are not exhibited by all the buffaloes of comparable age, body condition, parity and lactation status (Dadarwal *et al.* 2009). It was suggested that the metabolic profile of dairy animals can reveal the reasons behind differential fertility (Jorritsma *et al.* 2003). Epidemiological studies have suggested the role of thyroid hormones in the onset of ovarian activity as thyroid hormones are part of the complex hormonal mechanism that regulates steroidogenesis in the ovary (Suriyasathaporn, 2000).

The persistent interest in the information about the relation between metabolic status and reproductive performance from both farmers and veterinarians emphasizes upon the importance of the present study. Hence, the present work was planned to examine whether differences exist in the plasma concentrations of thyroid stimulating hormones, thyroid hormones and various biochemicals in buffaloes (heifers and lactating) showing disparity in ovarian cyclicity (non-cycling or cycling) and their ability to conceive.

MATERIALS AND METHODS

Animals and location of work

This study was conducted on buffalo heifers (mean age: 3.37±0.11 years, mean body weight: 247.4±6.28 kg, mean body condition score: 2.90±0.06) and lactating buffaloes (mean age: 6.66±0.38 years, mean body weight: 358.9±8.3 kg, mean body condition score: 3.01±0.07) housed at private dairy farms of Punjab state (latitude: 30º56´N, longitude: 75º52´E), India, during the hot-humid month from June to August (referred to as the 'summer season' with maximum ambient temperatures and relative humidity ranging from 36 to 45ºC and 30 to 80%, respectively). Using an ultrasound scanner, all the buffaloes were subjected to gynecological examination before inclusion in the study, and the buffaloes diagnosed with any pathological condition of the reproductive tract were excluded. Cardinal clinical signs of iodine deficiency were absent in the animals of the present study. All the buffaloes were offered ad-libitum feeds and fodders twice a day with fresh drinking water throughout the day and night.

Groups

Estrus detection was carried out regularly through close observation of estrus behavior. Buffaloes were declared non-cycling (heifers, n=32; lactating, n=13) or cycling (heifers, n=11; lactating, n=27) based upon the history during the period of at least three months before the start of the study and based upon the observations of transrectal ovarian ultrasonography. Also, study was continued in buffaloes that became pregnant (heifers, n=11; lactating, n=23) or were diagnosed non-pregnant (heifers, n=32; lactating, n=17) by rectal examination about 90 days after the artificial insemination. Inseminations were done by one person after properly checking the microscopic picture of frozen thawed semen.

Blood sampling

Jugular vein heparinized blood samples were collected randomly from non-cycling and cycling buffaloes. Blood sample were also collected at the time of artificial insemination. Plasma was separated (1500×*g* for 10 min) and was stored at −20 ºC.

Plasma analysis

Plasma concentrations of hypophysial hormone (Thyrotropin/Thyroid stimulating hormone) and thyroid hormones (Total triiodothyronine and Total thyroxine) were determined using commercially available ELISA kits (Accu-Bind, Monobind Inc., Lake forest, CA, USA). Plasma concentrations of glucose, total proteins, creatinine, blood urea nitrogen-BUN, total cholesterol and triglycerides, were determined colorimetrically with auto analyzer using commercially available kits (Siemens Healthcare Diagnostics Ltd., New Delhi).

Statistical analysis

Differences between data (mean ±SEM) of non-cycling or cycling buffaloes and non-pregnant and pregnant buffaloes were examined *post-hoc* using Student's paired t-test (two tails; Dytham, 1999). Probabilities of P<0.05 were considered significant. Statistical procedures were performed in Minitab release 14.2 statistical software (Dytham, 1999).

RESULTS AND DISCUSSION

Similar body weight and body condition score of non-cycling or cycling and non-pregnant or pregnant heifers as well as lactating buffaloes (P>0.05, Tables 1 and 2) suggested that estrus expression or ability to conceive of the animals used in the present study was not dependent upon their body weight. Moreover, the lactation status of buffaloes was similar between various sub-groups (p>0.05, Tables 1 and 2). In a previous study, dairy cattle in low body condition score had decreased follicular development compared with cattle in adequate body condition score (Formigoni *et al*. 2003). The observation that body condition score was similar between groups can be explained by the fact that body condition score may truly detect only a small proportion of bovines in negative energy balance (Jorritsma *et al*. 2003), as well as by a lack of a sensitive method for measuring body condition score in buffaloes.

Thyroid hormones are part of the complex hormonal mechanism that regulates steroidogenesis in the ovary (Jorritsma *et al*. 2003). Deranged thyroid activity is a potential cause for reproductive abnormalities in the females of farm animals (Aggarwal and Singh, 2010). In a previous study, the mean prevalence of iodine deficiency in crossbred cattle (Taurus×Indicus) was 35.9% and showed considerable geographical variation from 0 to 86% within Punjab (Randhawa and Randhawa, 2001). In the present study, compared to cycling, acycling lactating buffaloes had lower concentrations of thyroid stimulating hormone (p<0.05) as well as total triiodothyronine and total thyroxine (p>0.05, Table 1). Infertility and a wide variety of estrous cycle irregularities, from complete cessation of cycles to abnormally frequent cycles have been attributed to hypothyroidism (Johnson, 1994).

In fact, thyroid hormones have direct stimulatory effects on ovarian function in cattle, acting at the level of granulosa and thecal cells (Mutinati *et al.* 2010). In the current study, low thyroid activity might have suppressed steroidogenesis of ovarian cells thus leading to anestrus and reduced expression of estrus. Low triiodothyronine concentrations were associated with lower concentrations of estradiol and diminished estrus expression (Jorritsma *et al.* 2003).

(Ashkar *et al.* 2010). Increasing iodine concentrations via intramuscular injection of iodine (1 mL of 78% ethiodised oil) could provide a method for preventing iodine deficiency for more than 70 days (Randhawa and Randhawa, 2001). Actually, subsequent to maintenance of adequate concentrations of thyroid hormones, ovulatory dysfunctions attributed to hypothyroidism were corrected leading to the establishment of pregnancy (Lincoln *et al.* 1999).

Table 1 Body condition, lactation status, thyroid hormone status and plasma biochemical profile of non-cycling or cycling buffalo heifers and lactating buffaloes*

Parameters		Buffalo heifers		Lactating buffaloes	
		Acycling (n=32)	Cycling (n=11)	Acycling (n=13)	Cycling (n=27)
Body condition	Age (yr)	3.3±0.1	3.6±0.2	6.5±0.7	6.72±0.4
	Body weight (kg)	244.8±8.1	255.0±7.3	331.4±18.6	373.9±9.8
	Body condition score	2.8±0.1	3.1±0.1	2.9±0.1	3.1±0.1
Lactation status	Parity	-	-	2.4±0.3	2.8±0.3
	Days from last calving (days)	-	-	279.5±32.2	271.5±26.4
	Milk yield (L/day)	-	-	5.96±0.5	4.70±0.7
	Suckled animals (%)	-	-	52.0	48.0
Hypophysial hormone	Thyroid stimulating hormone (µIU/mL)	2.7±1.6	1.9±0.6	0.3±0.2[a]	1.7±0.4[b]
Thyroid hormones	Total triiodothyronine (ng/mL)	4.7±0.1	4.8±0.3	3.8±0.2	4.2±0.3
	Total thyroxine (µg/dL)	10.3±0.4	9.7±0.9	7.6±0.5	9.2±0.6
Plasma biochemical profile	Glucose (mg/dL)	58.8±2.0	59.9±1.8	53.6±3.7	54.1±2.2
	Total protein (g/dL)	7.4±0.2	7.6±0.2	7.6±0.2	7.57±0.19
	Creatinine (mg/dL)	1.33±0.03	1.44±0.1	1.29±0.05	1.39±0.06
	Blood urea nitrogen (mg/dL)	11.2±0.6	11.5±1.4	13.1±1.4	13.5±0.7
	Cholesterol (mg/dL)	52.9±2.0	51.7±2.9	57.6±4.6	67.23±4.0
	Triglyceride (mg/dL)	37.1±2.5	45.5±4.6	27.0±2.7	30.3±1.3

[*] The means that have common letter, do not have significant difference (P>0.05).

Furthermore, thyroid hormones play an important role in the pre- and post-implantation embryo development in cattle (Ashkar *et al.* 2010). Induced hypothyroidism was known to reduce the fertilization rate in dairy cattle (Bernal *et al.* 1999). In the present study, plasma concentrations of thyroid hormones were less in heifers (P>0.05) as well as lactating buffaloes (P<0.05) that failed to conceive (Table 2).

In an *in vitro* study, embryo quality was significantly improved following thyroid hormone supplementation

Under nutrition of dairy cattle is communicated to the hypothalamic-pituitary-ovarian axis via blood metabolites (Westwood *et al.* 2002). Optimum concentrations of blood glucose and proteins induce estrus cyclicity via hypothalamo-hypophyseal system (Tandle *et al.* 1998). Detrimental effects of protein metabolites (blood urea nitrogen) may occur not only at different stages of oocyte development, but also during fertilization and blastocyst formation (Jorritsma *et al.* 2003).

High plasma glucose and proteins were recently reported in cycling dairy cattle compared to non-cycling (Kumar *et al.* 2010). On the contrary, the present investigation revealed similar concentrations (P>0.05) of plasma glucose, total proteins, creatinine and blood urea nitrogen in non-cycling or cycling and non-pregnant or pregnant heifers and lactating buffaloes (Tables 1 and 2). Similarly, no alterations were observed in total cholesterol and triglyceride profile of non-cycling and non-pregnant buffalo heifers and lactating buffaloes compared to their respective counterparts (p>0.05, Tables 1 and 2). In an earlier study, the majority of the parameters of lipid profile were also not different between non-cycling or cycling buffaloes (nulliparous and pluriparous, Dadarwal *et al.* 2009). In contrast, dairy animals with higher plasma cholesterol are more likely to express estrus as lipids are the precursors of gonadal steroid hormones (Jorritsma *et al.* 2003). Moreover, conception at first service was positively related to energy balance at first service and plasma progesterone 10-13 days after first service (Reist *et al.* 2003). Nevertheless, the present study advocates that blood plasma biochemicals were not the factors responsible for the exhibition of differential fertility by the buffaloes during peak summer season.

On the other hand, summer stress is known to particularly alter the biochemical concentrations in the follicular fluid of the dominant follicle which may result in inferior oocyte and granulosa cell quality and hence, poorer fertility in dairy cattle (Shehab-El-Deen *et al.* 2010). Whether a subgroup of buffaloes exhibiting differential fertility during peak summer season is prone to heat stress-induced alterations of thyroid and metabolic profile in the follicular fluid remains to be investigated. Furthermore, it is possible that buffaloes with the same body condition score and the same blood biochemical profile may face a different level of actual metabolic deficiencies, because a sub-group of buffaloes may use less energy for processes like fertility (Jorritsma *et al.* 2003). Whether this re-allocation process is present in buffaloes and plays a role in the fertility status is unclear.

In summary, additional research is needed to determine how blood plasma concentrations of thyroid hormones, and blood biochemicals may be used to enhance reproductive

Table 2 Body condition, lactation status, thyroid hormone status and plasma biochemical profile at breeding in buffaloes that subsequently conceived or failed to conceive*

	Parameters	Buffalo heifers		Lactating buffaloes	
		Pregnant (n=11)	Non-pregnant (n=32)	Pregnant (n=23)	Non-pregnant (n=17)
Body condition	Age (yr)	3.4±0.2	3.33±0.12	6.7±0.5	6.1±0.7
	Body weight (kg)	188.1±21.5	244.0±8.0	346.4±8.5	259.0±26.2
	Body condition score	2.9±0.1	2.9±0.1	3.0±0.1	2.9±0.2
Lactation status	Parity	-	-	2.3±0.3	2.7±0.4
	Days from last calving (days)	-	-	295.6±30.1	255.3±29.7
	Milk yield (L/day)	-	-	4.4±0.6	5.4±0.6
	Suckled animals (%)	-	-	48.0	52.0
Hypophysial hormone	Thyroid stimulating hormone (µIU/mL)	4.2±1.9	2.5±0.7	1.2±0.3	1.2±0.4
Thyroid hormones	Total triiodothyronine (ng/mL)	4.9±0.1	4.4±0.2	4.5±0.2[a]	3.4±0.3[b]
	Total thyroxine (µg/dL)	10.8±0.4	9.7±0.8	9.7±0.5[a]	7.5±0.6[b]
Plasma biochemical profile	Glucose (mg/dL)	59.4±4.9	55.3±2.1	65.6±3.9	68.0±3.8
	Total protein (g/dL)	7.5±0.2	7.4±0.2	7.5±0.2	7.6±0.2
	Creatinine (mg/dL)	37.2±2.6	37.1±2.0	28.3±1.5	27.2±1.9
	Blood urea nitrogen (mg/dL)	12.7±1.3	11.2±0.7	12.2±0.8	13.0±1.2
	Cholesterol (mg/dL)	1.2±0.1	1.3±0.0	1.3±0.1	1.2±0.0
	Triglyceride (mg/dL)	54.4±3.3	56.6±2.2	52.2±2.5	44.7±1.7

*The means that have one common letter, do not have significant difference (P>0.05).

efficiency in non-cycling and (or) non-conceiving dairy buffalo.

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