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Effect of Ovsynch Estrus Synchronization Protocol on Certain Mineral Profile of Cyclic Murrah Buffaloes in Summer and Winter Season

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ABSTRACT

The present study aimed to investigate the effect of Ovsynch estrus synchronization protocol on plasma Zn, Cu, Ca and P levels in cyclic Murrah buffaloes during summer and winter season. The buffaloes were categorized in to two groups: 1) summer (n = 20) and, 2) winter (n = 18). Estrus cycle of buffaloes were synchronized with Ovsynch protocol (Pursley *et al.* 1995) as per standard procedure followed by artificial insemination (AI) at 8 and 24hrs after second GnRH treatment. The blood samples were collected on the day of start of protocol (d0) and day of AI (dAI) for the estimation of minerals concentration. The pregnancy was confirmed through sonography on day 45 post-AI. Levels of Zn were lower (P<0.05) in summer as compared to winter season (1.0 ± 0.1 *vs.* 1.5 ± 0.1 and 1.3 ± 0.1 *vs.* 1.9 ± 0.1 ppm, respectively) whereas Cu, Ca and P levels showed no seasonal variation. Pregnant and Non pregnant buffaloes had significant lower (P<0.05) concentration of Zn on d0 and AI in summer as compared to winter season (0.9 ± 0.1 *vs* 1.6 ± 0.2 and 1.2 ± 0.2 *vs.* 1.7 ± 0.1ppm, respectively and1.1 ± 0.1 *vs.* 1.5 ± 0.1 ; 1.3 ± 0.1 *vs.* 1.6 ± 0.1 ppm, respectively). The pregnant and non-pregnant buffaloes exhibited similar mineral profiles in both seasons. In conclusion, plasma mineral concentrations remain lower during summer as compared to winter season in buffaloes that could be responsible for lower fertility in summer.

Keywords: Buffalo, estrus, minerals, ovsynch, season

The livestock resources play a key role in the upliftment of the socio-economic status of the rural population of the India. The buffalo population of the world is about 199.78 million (FAOSTAT, 2013). India alone has 115.42 million buffalo population (FAOSTAT, 2013). Haryana is the home land of Murrah buffalo which is called as "Black Gold". Minerals play an incredible role in production via increased reproduction by acting as co-factors for action of hormones and enzymes at sub cellular levels in an integrated fashion (Amle *et al.* 2014). Deficiency of minerals causes various reproductive failures such as infertility, repeat breeding, embryonic loss, poor conception rate, anestrus condition etc. The influence of micro-minerals on reproductive efficiency, ovulatory mechanism and maintenance of pregnancies and regulation of endocrine functions in the animals is important (Islam *et al.* 2012). Copper and Zinc plays key role in progesterone production by luteal cells and prevents ovaries from free radical damage and help in resumption of ovarian activity (Ahmed *et al.* 2009).

Phosphorus is important component of cellular energy transfer via the ADP, ATP system (Chaudhary and Singh, 2004). Literatures in dairy cattle suggest that nutrient deficiency in marginal level may be manifested as reduced fertility before other clinical symptoms are apparent (Hadiya *et al.* 2010 and Panda *et al.* 2015). Therefore, extensive interest is being focused to identify specific reproductive and nutritional problems and thereby to augment bovine fertility. Although, much work has so far been carried out to see the effect of mineral profile in cattle on reproduction but literatures are lacking about the plasma mineral profile following synchronized estrus cycle with Ovsynch protocol in summer and winter season. Therefore, the present study was designed to investigate the effect of season on mineral profiles following ovsynch protocol in buffalo.

MATERIALS AND METHODS

Animals

The present study was conducted on 38 cyclic Murrah buffaloes (Parity: 1st to 5th; body weight: between 400-600 Kg; body condition score: between 3 to 4) in summer (n = 20) and winter (n = 18) season. All buffaloes were healthy, free from any apparent pathological disorders of reproductive organs and cycling at regular intervals. Transrectal sonography was carried out before the start of estrus synchronization protocol to detect the corpus luteum on either of ovaries. Estrus cycle of buffaloes were synchronized by administration (i.m.) of two injections of a GnRH analogue (10µg, Buserelin acetate, Gynarich, Intas pharmaceutical ltd., India) on days 0 and 9, and an injection of a synthetic $PGF_{2\alpha}$ analogue (500µg, Cloprostenol Sodium, Vetmate™, Vetcare, Banglore, India) on day 7 (Fig. 1) followed by artificial insemination (AI) with frozen thawed semen at 8 and 24hrs of second GnRH injection.

Blood sampling and mineral estimation

Blood samples were taken on the day of start of protocol (d0) and day of AI (dAI) in heparinised vials. After centrifugation at 1500 X g for 10 minutes, plasma was harvested and stored at -20°C until analysis. Plasma Ca and P concentrations were estimated by fully automated Random Access Clinical Chemistry Analyzer (EM 200™ Erba Mannheim – Germany) by using kits procured from Transasia Biomedical Limited, Germany (Arsenazo and UV Phosphomolybdate method, respectively) while plasma concentrations of Zn and Cu were estimated with the help of atomic absorption flame photometer (PinAAcle 900T, Perkin Elmer) by wet digestion of the plasma samples. For wet digestion, 1 ml plasma sample was taken in 50 ml conical flask and added 10ml digestion mixtures of acids (Nitric acid and Perchloric acid in 4:1 ratio). Thereafter, it was left overnight and the next morning, sample was heated over hot plate til colour of fumes changed. Then after cooling, 15 ml final volume was made by adding distilled water slowly into conical flask, mixed properly and stored into 30ml capped vials until analysis.

Statistical analysis

Student's t test was used to find the statistical significance of plasma mineral profiles between summer and winter season and between pregnant and non-pregnant buffaloes. The differences at 5% (P<0.05) level was used as significant. Statistical analysis were done by SPPS version 16 using Student's T test.

Ovsynch

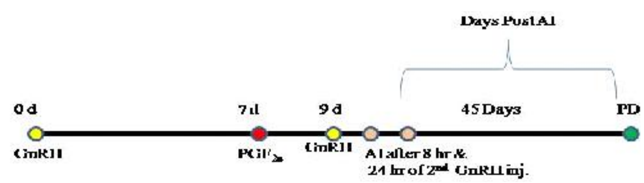

Fig. 1: Schematic diagram of treatment schedule of Ovsynch protocol in buffalo; Inj. GnRH (Buserelin acetate, 10µg) i.m., $PGF_{2\alpha}$ (Cloprostenol, 500µg) i.m. (d: Day; AI: Artificial Insemination; PD: Pregnancy Diagnosis).

RESULTS AND DISCUSSION

Results of the mineral profiles (Mean ± SE) are shown in table 1. In the present study, level of Zn was lower (P<0.05) in buffaloes during summer as compared to winter season group (1.0 ± 0.1 *vs.* 1.5 ± 0.1 and 1.3 ± 0.1 *vs.* 1.9 ± 0.1 ppm, respectively) on d0 and dAI. Furthermore, pregnant and non-pregnant buffaloes also had significant lower (P<0.05) concentration of Zn on days 0 and AI in summer as compared to winter season. Nevertheless, Cu, Ca and P levels were similar in both seasons. In pregnant and non-pregnant buffaloes also, Cu and Ca levels were similar on days 0 and AI between seasons. Buffaloes meet out their mineral requirements through feed and fodder (Suttle, 2010). In winter season fodder contains high amount of minerals as compared to summer season and also the feed intake is low during summer as compared to winter (Chhabra *et al.* 2015). In the present study, during summer season, Phosphorus level was found significantly lower (P<0.05) on the day 0 as compared to winter season (4.2 ± 0.3 *vs* 5.3 ± 0.2mg/dl) but levels were found to be non significant on the day of AI in pregnant buffaloes. In non-pregnant buffaloes, plasma P levels were observed non significant on days 0 and AI between summer and winter season (Table 1). In past, studies showed no seasonal (winter *vs* fall) differences in serum Ca and P concentrations in cattle (Kiatoko *et al.* 1982 and Merkel *et al.* 1990). The Zn levels were higher (P<0.05) on the day of AI as compared to start of protocol in summer as well as in winter season (1.0 ± 0.1 *vs* 1.3 ± 0.1 and 1.5 ± 0.1 *vs* 1.9 ± 0.1 ppm, respectively) however, Cu concentrations were higher significantly (P<0.05) on the day of AI as compared to start of protocol in summer season group and marginal increase in winter season. The higher concentrations of Zn might be due to role of Zn in secretion of GnRH from hypothalamus which ultimately leads to increase levels of FSH and LH and help in ovulation (Kaswan and Bedwal, 1995). During follicular phase of the estrous cycle ovarian tissues requires more Zn (Brem *et al.* 2003) and, Copper plays a lead role in steroid hormone synthesis and it prevents ovaries from free radical damage (Ahmed *et al.* 2009) and it improves the plasma biochemical profile also (Godara *et al.* 2015). During the follicular phase, estrogen hormone concentration increases and earlier study has suggested that Cu level increase under the influence of estrogen hormone (Sato and Henkin, 1973). Kulkarni (1993) reported higher serum copper levels in follicular phase (112.2 ± 0.1 μg/ml) than luteal phase (95.7 ± 0.5 μg/ml) in buffaloes.

Calcium and P level was observed non significantly higher on the day of AI as compared to day of start of protocol in summer as well as in winter season in pregnant and non pregnant buffaloes (Table 1). Earlier studies have also reported marginal increase of Ca and P level on the day of estrus than the day of start of protocol in buffaloes and cattle (Khasatiya *et al.* 2004; Virmani *et al.* 2011; Parmar *et al.* 2012; Nakrani *et al.* 2014; Savalia *et al.* 2014 and Kumar *et al.* 2015). Calcium controls the membrane potential of oocyte and participates in regulation of gap junctions between cumulus cells resulting in disruption of cohesiveness of cumulus cells (Peracchia *et al.* 1978). The higher level of P in the present study might be due to its role in cAMP production and hence necessary for steroid synthesis and regulation of pitutary-hypophysial axis activity (Bhaskaran and Patil, 1982).

The present study found similar mineral profiles between pregnant and non pregnant animals following synchronized estrus in both seasons. No other literature

Table 1: Mineral profile of buffaloes (Mean ± SE) on d0 and dAI following synchronized estrus with Ovsynch protocol in summer and winter season (AI: Artificial insemination; d= Day; ppm: parts per million; NP: Non pregnant; P: Pregnant)

Season	Parameters	Pregnancy status (P/NP)	Days (d)	
			d0	dAI
Summer (n= 20)	Zn (ppm)	NP (n=16)	1.1 ± 0.1^{bB}	1.3 ± 0.1^{bA}
		P (n=4)	0.9 ± 0.1^{c}	1.2 ± 0.2^{c}
		Overall	1.0 ± 0.1^{B}	1.3 ± 0.1^{A}
	Cu (ppm)	NP (n=16)	1.1 ± 0.1^{B}	1.83 ± 0.1^{A}
		P (n=4)	1.2 ± 0.1^{B}	2.1 ± 0.1^{A}
		Overall	1.2 ± 0.1^{B}	1.9 ± 0.1^{A}
	Ca (mg/dl)	NP (n=16)	8.4 ± 0.3	9.1 ± 0.2
		P (n=4)	8.0 ± 0.3	9.1 ± 0.4
		Overall	8.2 ± 0.3^{B}	9.2 ± 0.3^{A}
	P (mg/dl)	NP (n=16)	5.1± 0.2	5.3 ± 0.2
		P (n=4)	4.2 ± 0.3^{c}	5.0 ± 0.3
		Overall	4.6 ± 0.3^{B}	5.2 ± 0.3^{A}
Winter (n= 18)	Zn (ppm)	NP (n=13)	1.5 ± 0.1^{a}	1.6 ± 0.1^{a}
		P (n=5)	1.6 ± 0.2^{d}	1.7 ± 0.1^{d}
		Overall	$1.5 \pm 0.1^{\#, B}$	$1.9 \pm 0.1^{\#, A}$
	Cu (ppm)	NP (n=13)	1.5 ± 0.2	1.6 ± 0.2
		P (n=5)	1.5 ± 0.2	1.8 ± 0.3
		Overall	1.5 ± 0.1	1.7 ± 0.2
	Ca (mg/dl)	NP (n=13)	9.1 ± 0.6	9.4 ± 0.5
		P (n=5)	9.6 ± 0.6	9.8 ± 0.3
		Overall	9.3 ± 0.6	9.6 ± 0.4
	P (mg/dl)	NP (n=13)	5.3±0.3	5.7 ± 0.3
		P (n=5)	5.3 ± 0.2^{d}	5.7 ± 0.1
		Overall	$5.3 \pm 0.3^{\#}$	5.7 ± 0.2

$^{a\ vs\ b,\ c\ vs\ d}$(P<0.05): within column a parameter between summer and winter; $^{A\ vs\ B}$(P<0.05) within a row; $^{\#}$(P<0.05): within column a parameter between summer and winter season (overall).

is available to compare these data so, the findings of the study would suggest that if, the concentration of minerals are within normal physiological range than they could not influence pregnancy rate in buffaloes.

In conclusion, the mineral profiles increase more or less at the time of estrus irrespective of season indicating that minerals play some role in follicular phase of estrous cycle. During summer season, plasma mineral concentration remains low as compared to winter season that could be one of the reasons for lower fertility in summer season.

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