ABSENCE OF AN ASSOCIATION BETWEEN OXIDATIVE STRESS AND PREGNANCY OUTCOME FOLLOWING FTAI IN BUFFALO DURING SUMMER AND WINTER SEASON

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ABSTRACT

Thirty-eight cyclic Murrah buffalo in summer (n=20) and winter (n=18) season were subjected to a fixed time artificial insemination (FTAI) protocol. Oxidative stress parameters were assessed on different days of protocol. Season had no impact on pregnancy rate (summer, 25%; winter, 27.8%; p>0.05) following FTAI in buffalo. Lipid peroxidation in terms of malondialdehyde (MDA) concentration was higher during summer as well as on the day of AI in summer as well as in winter season (p<0.05). Glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activity was similar (p>0.05) between summer and winter season. In brief, oxidative stress parameters had no conclusive association with pregnancy outcome in buffalo during summer season.

Keywords: AI, Buffalo, Glutathione peroxidase, Malondialdehyde, Superoxide dismutase, Season

INTRODUCTION

Oxidative stress is the result of an imbalance between antioxidant system and reactive oxygen species (ROS). Increases in ROS beyond physiological levels lead to adverse impact on reproduction in dairy animals through DNA fragmentation, lipid peroxidation and denaturation of proteins (Litwinczuk *et al*., 2011). Summer season is especially stressful for the continuation of reproductive activity in buffalo, probably; an increase in summer-induced oxidative stress is one of the reasons. Therefore, the present study was planned to assess the oxidative stress in buffalo subjected to artificial insemination (AI) during summer and winter season.

MATERIALS AND METHODS

The regualr cyclic Murrah buffalo (n=38; Parity, 1-5; body weight, 400-600 kg; body condition score, 3-4) free from any apparent pathological disorders of reproductive organs were subjected to Fixed Time AI (FTAI) by ovsynch protocol in summer (n=20) and winter (n=18) season. The protocol involved administration (i.m.) of a synthetic prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogue (500µg, Cloprostenol sodium) on day 7, as well as two injections of a GnRH analogue (10µg, Buserelin acetate) on day 0 and day 9 followed by AI with frozen thawed semen at 8 h and 24 h after day 9 GnRH. Pregnancy diagnosis was carried out by ultrasonography on day 45 post AI. The blood samples were collected on each day of hormone administration and on the day of AI for assessing oxidative parameters viz. Malondialdehyde (MDA), Glutathione peroxidase (GSH-Px) and Superoxide dismutase (SOD) per ml of hemolysate. The procedure for hemolysate preparation involved separation of blood plasma, washing of erythrocytes three times with normal saline solution followed by centrifugation (1500g x 10 min). Thereafter, supernatant was decanted and chilled distilled water was added slowly to erythrocyte pellet with constant stirring up to the level of initial blood volume. The hemolysate were stored at -20^{0}C until analysis. Lipid peroxidation was evaluated in terms of MDA formed by using thiobarbituric acid-reactive substances (TBARS;

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Table 1: Oxidative stress parameters (per ml of hemolysate; Mean±SE) **in buffalo subjected to ovsynch protocol in summer and winter season.**

Group / Parameter			Days (d) of protocol			
			d0	d7	d9	dAI
Summer, NP=16, P=4	**MDA,** nmol	**NP**	4.9±0.4^{a,A}	5.4±0.2^{a,A}	6.4±0.5^{a}	7.2±0.5^{a,B}
		P	5.5±0.8	4.5±0.2^{A}	5.1±0.5	7.6±1.2^{B}
		Overall	5.1±0.4$^{\#,A}$	5.2±0.2$^{\#,A}$	6.1±0.4$^{\#}$	7.3±0.5$^{\#,B}$
	GSH-Px, U	**NP**	16.5±1.2^{A}	25.3±2.7^{C}	18.9±1.8^{BC}	22.8±1.5^{AB}
		P	19.8±2.8^{c}	17.6±2.4	21.4±2.8	24.0±1.6
		Overall	17.2±1.1^{A}	23.7±2.3^{B}	19.4±1.5	23.0±1.3^{B}
	SOD, U	**NP**	179.5±13.4^{A}	127.9±15.2^{a,A}	168.4±15.3^{B}	145.9±9.0
		P	231.6±44.4^{A}	122.5±17.6^{c,B}	185.5±32.9	165.1±18.2
		Overall	189.9±14.1^{A}	126.8±12.4$^{\#,C}$	171.9±13.5^{AB}	149.7±8.1^{BC}
Winter, NP=13, P=5	**MDA**	**NP**	3.7±0.2^{b,A}	3.8±0.2^{b,A}	3.9±0.2^{b,A}	4.8±0.2^{b,B}
		P	3.8±0.2^{A}	5.2±0.6	4.1±0.5	5.5±0.4^{B}
		Overall	3.7±0.1^{A}	4.2±0.2^{A}	4.0±0.2^{A}	5.0±0.2^{B}
	GSH-Px	**NP**	16.8±1.5^{A}	28.2±3.0^{B}	15.9±1.7^{A}	24.3±2.0^{B}
		P	12.5±1.0^{d}	24.2±4.6	13.5±4.0	23.6±3.8
		Overall	15.6±1.2^{A}	27.1±2.5^{B}	15.2±1.6^{A}	24.1±1.7^{B}
	SOD	**NP**	188.4±9.7^{A}	242.8±18.8^{b,B}	195.0±17.4^{A}	159.2±9.9^{A}
		P	175.4±20.9	225.5±15.0^{d,A}	159.9±20.7^{B}	140.6±16.3^{B}
		Overall	184.7±8.8^{A}	238.0±14.1^{B}	185.2±14.0^{A}	154.1±8.4^{A}

$^{A\ vs.\ B\ vs.\ C}$p<0.05, within a row; $^{a\ vs.\ b,\ c\ vs.\ d}$p<0.05, for a parameter within a column between season; $^{\#}$p<0.05, for overall value of a parameter within a column between season; AI Artificial insemination, NP Non pregnant, P Pregnant

Shafiq-u-Rehman, 1984). Also, the activity of GSH-Px (Hafeman *et al.,* 1974) and SOD (Madesh and Balsubramaniam, 1998) in erythrocyte lysate was assayed. The data was analyzed statistically by ANOVA and t-test using the SPSS (16.0) system for windows.

RESULTS AND DISCUSSION

The impact of seasonal stress on pregnancy rate in buffalo was not evident in the present study (summer, 25%; winter, 27.8%; p>0.05; Table 1). In summer season, the overall lipid peroxidation in terms of MDA concentration was higher on different days of FTAI protocol as compared to winter season (p<0.05, Table 1). Others also found higher serum MDA concentrations in buffalo during summer as compared to winter season (Hozyen *et al*., 2014). Moreover, in winter and summer season, as well as in pregnant and non-pregnant buffalo, erythrocytic MDA concentration in buffalo were invariably higher on the day of estrus/AI as compared to other days of FTAI protocol (p<0.05, Table 1). In fact, high lipid content in buffalo oocyte and embryos increases their sensitive to oxidative damages (Boni *et al.,* 1992). On the day of estrus, the dominant follicle in the ovary results in higher production of ROS and hence, lipid peroxidation and increased MDA level (Jan *et al*., 2014). Others also reported higher lipid peroxidation in estrus phase of Egyptian buffalo (Megahed *et al*., 2008).

Between winter and summer season, the overall GSH-Px concentrations remained similar on different days of observation (p<0.05, Table 1). However, the overall GSH-Px was higher on the day of estrus in

summer as well as in winter season ($p<0.05$, Table 1). This could be due to increased aerobic metabolism at estrus that results into production of free radicals and H_2O_2 (Agarwal *et al*., 2006). Moreover, the highest mRNA expression for GSH-Px-1 was observed toward the end of the estrous cycle before ovulation in oviductal fluid of cow (Lapointe and Bilodeau, 2003). However, GSH-Px levels were invariably similar between pregnant and non-pregnant buffalo in summer and winter season ($p>0.05$, Table 1).

Also, between winter and summer season as well as within a season on different days of observation, the overall SOD concentrations remained invariably similar ($p>0.05$, except day 7) and no consistent pattern was observed ($p<0.05$ - >0.05, Table 1). Previous studies suggested that in summer season due to thermal stress, the levels of SOD decrease as compared to winter (Megahed *et al*., 2008).

In brief, season had no impact on pregnancy rate following FTAI in buffalo. Lipid peroxidation was higher in summer season with elevated levels of MDA at the time of AI in summer as well as in winter season. However, the anti-oxidant enzymes viz. GSH-Px and SOD remained invariably similar between seasons as well as between pregnant and non-pregnant buffalo.

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