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Effect of Seasonal Variation on Oxidative Stress Parameters in Cyclic Murrah Buffaloes Following Synchronized Estrus through Doublesynch Protocol

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ABSTRACT

The present study was conducted with the objective to see the effect of season on oxidative stress parameters in cyclic Murrah buffalo. Forty five cyclic Murrah buffaloes were synchronized for estrus with standard Doublesynch protocol and inseminated fixed time at 8 and 24hr of last GnRH injection in summer (n = 20) and winter season (n = 25). Blood samples were analyzed for the level of oxidative stress parameters. The overall MDA concentrations were higher ($P<0.05$) during summer compared to winter season. Furthermore, values were higher ($P<0.05$) on day of AI compared to days of start of protocol in both seasons. Glutathione peroxidase (GSH-Px) level were similar ($P>0.05$) between summer and winter season among days of protocol except on day 9, in which GSH-Px level was lower ($P<0.05$) in summer as compared to winter season (16.9 ± 1.8 *vs* 24.2 ± 2.0 U/ml of hemolysate). The Superoxide dismutase (SOD) concentrations were higher ($P<0.05$) in winter as compared to summer at the time of start of protocol however; on day 9, the values were lower ($P<0.05$) for winter than summer. In conclusion, MDA is more reliable parameter to measure oxidative stress in Murrah buffalo and it increase during summer season and hence reduce the conception rate.

Keywords: Buffalo, doublesynch, oxidative stress, season

The livestock are back bone of rural population of the country and Murrah buffalo is known as black gold of the dairy industry in India. High milk production along with intensive management system leads to increase in the physiological and environmental stressors which impair reproductive performance of buffaloes. Reactive oxygen species (ROS) or free radicals are two sides of a coin i.e. they have a physiological role in ovulation and embryo development (Desai *et al.*, 2009) and when their concentration increase beyond physiological level results in oxidative stress which leads to adverse effects (Goncalves *et al.*, 2010). Oxidative stress has negative correlation with reproduction in dairy animals. Adverse environment conditions increase the level of oxidative stress (Yarovan, 2008) and minerals play important role to enhance conception rate (Kumar *et al.*, 2015). Effect of oxidative stress in cyclic and repeat breeder cows have been established (Ali *et al.*, 2014) however, seasonal variation (summer and winter) of oxidative stress and its impact on pregnancy in cyclic Murrah buffaloes is lacking. Therefore the present study was aimed to investigate the impact of season on oxidative stress parameters in cyclic Murrah buffalo.

MATERIALS AND METHODS

Animals and Estrus synchronization

Total 45 cyclic Murrah buffaloes (in summer, n = 20 and winter, n = 25) were synchronized for estrus (Fig. 1) using Doublesynch protocol (Cirit *et al.*, 2007). The buffaloes were healthy (parity: 2 and 5; body weight: 400-600 Kg; body condition score: between 3 and 4 of 5 point scale), free from any apparent pathological disorders of reproductive organs and regular cycling. Transrectal sonography was done to assess the ovarian structure before the start of

Doublesynch estrus synchronization protocol. Blood samples were taken by jugular venipuncture on each day of treatment and on the day of artificial insemination (AI) to check the level of oxidative parameters i.e. Malondialehyde (MDA), Glutathione peroxidase (GSH-Px), Superoxide dismutase (SOD) in hemolysate and expressed as per ml of hemolysate.

Preparation of hemolysate, measurement of Oxidative stress parameters and statistical analysis

After separation of plasma from blood sample, erythrocytes were washed three times with normal saline solution and centrifuged at 1500Xg for 10 min. Thereafter supernatant was decanted and then chilled distilled water was added slowly to erythrocytes pellet with constant stirring up to the level of initial blood was taken. Hemolysate were stored at -20^{o}C until analysis. Lipid peroxidation was evaluated in terms of Malondialdehyde (MDA) formed by using thiobarbituric acid-reactive substances (TBARS) by method of Shafiq-u-Rehman (1984). The activity of Glutathione peroxidase and Superoxide dismutase (SOD) in erythrocyte lysate was assayed by the method of Hafeman *et al.* (1974) and Madesh and Balsubramaniam (1998), respectively. The data was analysed statistically by ANOVA and student's t-test using the SPSS (16.0) system for windows. The differences at 5% (P<0.05) level was sed as significant.

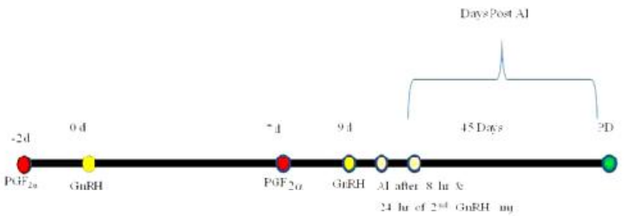

Fig. 1: Schematic diagramme of treatment schedule of Doublesynch protocol in buffalo; Inj. GnRH (Buserelin acetate, 10µg) i.m., $PGF_{2\alpha}$ (Cloprostenol, 500µg) i.m. (AI: Artificial insemination; d: Day; PD: Pregnancy diagnosis).

RESULTS AND DISCUSSION

Findings of the study are shown in Table 1. Buffaloes exhibited, MDA level was significantly higher (P<0.05) in summer as compared to winter season and the values were also found higher (P<0.05) on the day of AI as compared to the day of start of protocol in both seasons. Furthermore, in pregnant and non-pregnant buffaloes levels of MDA were significant higher (P<0.05) in summer as compared to winter season and, among days; MDA levels were maximum on the day of AI. The higher concentrations of MDA might be due to thermal stress during summer as compared to winter season (Jozwik *et al.*, 2012). Similar to present study Hozyen *et al.* (2014) observed higher level of serum MDA in buffaloes during summer as compared to winter season. The concentration of Hemoglobin increases on the day of estrus as compared to day of start of protocol to supply more oxygen (Kumar *et al.*, 2015). The considerable increase in oxygen requirements during time of increased metabolic demands (time of estrus) results in more production of reactive oxygen species (Agarwal *et al.*, 2006).

Similar findings in summer season were observed by Bernabucci *et al.* (2002) in cows. Findings of the present study suggest that Murrah buffaloes are also affected by a constant oxidative stress in summer similar to dairy cows (Bernabucci *et al.*, 2002; Tanaka *et al.*, 2007; Sakatani *et al.*, 2012). Due to high lipid content in oocyte and embryos of buffaloes leads to more oxidative damages (Boni *et al.*, 1992). Ovary contains dominant follicle on the day of estrus that results into production of more free radicals that results to more lipid peroxidation which could be responsible for increased level of MDA (Jan *et al.*, 2014). Similarly, Megahed *et al.* (2008) found higher lipid peroxidation during estrus phase of Egyptian buffaloes. Pregnant buffaloes, had higher (P<0.05) concentration of MDA on the day of AI than nonpregnant buffaloes in summer season. Moreover, MDA levels were similar in pregnant and nonpregnant buffaloes during winter season. It would suggest that non pregnant buffaloes produces more ROS and have higher lipid peroxidation as compared to pregnant ones in summer months. Higher level of ROS prevents preimplantation of embryo and cause death of embryo by changing the structure of mitochondrial enzymes (Takahasi *et al.*, 2000; Agarwal *et al.*, 2005). However, reason is not clear for similar values of pregnant and nonpregnant buffaloes during winter season.

Overall Glutathione peroxidase levels observed similar between summer and winter season among days of protocol as well as in pregnant and non-pregnant buffaloes. It would suggest that season has no effect

Table 1: Oxidative stress parameters (per ml of hemolysate; Mean ± SE) in buffaloes at different stage of Doublesynch protocol and on day of AI in summer and winter season (AI: Artificial insemination; d= Day; nmol: Nano mol.; NP: Non-pregnant; P: Pregnant; U: Unit)

Season		Summer (n = 20)			Winter (n = 25)		
Days	Parameters	Pregnancy status		Overall (n = 20)	Pregnancy status		Overall (n = 25)
		P (n = 8)	NP (n = 12)		P (n = 12)	NP (n = 13)	
-2d	MDA (nmol)	4.9 ± 0.5	5.4 ± 0.5^{B}	5.2 ± 0.3^{B}	4.8 ± 0.3	4.3 ± 0.3^{B}	4.5 ± 0.2^{B}
	GSH-PX (U)	15.2 ± 2.7	20.1 ± 2.5	18.1 ± 1.9	22.8 ± 2.4	22.8 ± 2.6	22.8 ± 1.7
	SOD (U)	171.4 ± 25.4	142.7 ± 14.1^{a}	154.1±13.2 ^{BC}	190.4 ± 12.1	184.1± 12.6$^{b, A}$	187.1 ± 8.6$^{\#,A}$
0d	MDA (nmol)	5.2 ± 0.7	6.1 ± 0.9	5.8 ± 0.6	5.8 ± 0.7^{A}	5.2 ± 0.4	5.5 ± 0.4^{A}
	GSH-PX (U)	19.9 ± 4.2	20.3 ± 2.4	20.1 ± 2.1	23.4 ± 4.0	25.7 ± 3.3	24.6 ± 2.5
	SOD (U)	175.4 ± 8.9	166.7±15.6^{A}	170.2± 9.0^{AB}	170.4 ± 11.5	196.8±15.7^{A}	184.1±10.0^{A}
7d	MDA (nmol)	5.4 ± 0.4^{c}	5.1 ± 0.4^{B}	5.3 ± 0.3$^{\#, B}$	4.2 ± 0.3$^{d, B}$	4.1 ± 0.3^{B}	4.2 ± 0.2^{B}
	GSH-PX (U)	21.3 ± 2.2	23.8 ± 1.7	22.8 ± 1.3^{A}	28.3 ± 3.3	25.2 ± 2.8	26.7 ± 2.1
	SOD (U)	180.6 ± 20.0	175.6± 17.1^{A}	177.6±12.7^{AB}	185.9 ± 34.5	153.3 ± 21.6	168.9 ± 19.8
9d	MDA (nmol)	5.3 ± 0.4^{c}	6.2 ± 0.6^{a}	5.8 ± 0.4$^{\#}$	4.1 ± 0.2$^{d, B}$	4.1 ± 0.3$^{b, B}$	4.1 ± 0.2^{B}
	GSH-PX (U)	16.1 ± 3.2	17.5 ± 2.2^{B}	16.9 ± 1.8 ^{B}	24.0 ± 2.8	24.4 ± 3.1	24.2 ± 2.0$^{\#}$
	SOD (U)	206.8 ± 17.9^{A}	180.4 ± 12.2$^{a, A}$	191.0 ± 10.3$^{\#, A}$	182.1 ± 15.9	129.0±15.0 $^{b, B}$	154.5 ± 12.0
d-AI	MDA (nmol)	5.7 ± 0.4	7.9±0.7$^{*, a, A}$	7.1 ± 0.5$^{\#, A}$	5.8 ± 0.5^{A}	5.5 ± 0.3$^{b, A}$	5.6 ± 0.3^{A}
	GSH-PX (U)	21.0 ± 2.7	24.8 ± 1.9^{A}	23.3 ± 1.6^{A}	27.4 ± 2.6	27.5 ± 2.8	27.4 ± 1.8
	SOD (U)	138.4 16.2^{B}	122.9± 8.4^{B}	129.1± 8.1^{C}	150.6± 11.6	129.5±12.6^{B}	139.6± 8.6^{B}

$^{a\ vs\ b,\ c\ vs\ d}$ (P<0.05): within row a parameter between summer and winter; $^{A\ vs\ B\ vs\ C}$(P<0.05) within column of a parameter; *(P<0.05): from non-pregnant of the respective group; $^{\#}$(P<0.05): within row a parameter between summer and winter.

on GSH-Px concentration. However, more studies are warranted at large scale to assess the effect of season on GSH-Px. Nevertheless, the level was significant lower (P<0.05) on day 9 of protocol in summer season. Similar finding was recorded by Sakatani *et al.* (2012) in Japanese black cows. Moreover, GSH-Px concentration was significant higher (P<0.05) on day of day of AI as compared to start of protocol in summer as well as in winter season. Concentration of Hemoglobin increases on the day of estrus (Kumar *et al.*, 2015) as compared to day of start of protocol to supply more oxygen for increased aerobic metabolism that results into more production of free radicals and H_2O_2 (Agarwal *et al.*, 2006). In another study, the highest levels of mRNA expression for GSH-Px-1(Glutathione peroxidase -1) were observed toward the end of the estrous cycle before ovulation in oviductal fluid of cow (Lapointe and Bilodeau, 2003).

The SOD concentrations were higher in winter group buffaloes as compared to summer ones at the time of start of treatment however, on day 9 it had lower level (P<0.05) in former as compared to later group. The reason for lower level of SOD on day 9 remains unclear. However, this might be due to effect of hormonal treatment for estrus synchronization. In summer season due to thermal stress, level of SOD decreased as compared to winter in Egyptian buffaloes and cattle (Megahed *et al.*, 2008; Sakatani *et al.*, 2012). Buffaloes of both season showed a lower concentrations (P<0.05) of SOD on the day of AI as compared to day of start of treatment. As the level of stress increase, it results into decrease concentration of SOD and active CL on the day of start of protocol might be responsible for more SOD activity (Rapoport *et al.*, 1998). In pregnant buffaloes, Superoxide dismutase concentrations were observed alike (P>0.05) between summer and winter season. Hozyen *et al.* (2014) observed similar SOD concentrations throughout year.

In conclusion, MDA is a more reliable parameter for estimation of oxidative stress parameters in Murrah buffalo. Concentrations of MDA increased during summer than winter season consequently affect the pregnancy rate in Murrah buffalo.

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