



ISSN (E): 2277- 7695  
ISSN (P): 2349-8242  
NAAS Rating: 5.03  
TPI 2018; 7(11): 202-204  
© 2018 TPI  
www.thepharmajournal.com  
Received: 13-09-2018  
Accepted: 14-10-2018

**Parveen Kumar**  
Veterinary Surgeon,  
Department of Animal  
Husbandry, Haryana, India

**Anand Kumar Pandey**  
Department of Veterinary  
Clinical Complex, LUVAS,  
Hisar, Haryana, India

**Ajit Verma**  
Department of Veterinary  
Gynaecology and Obstetrics,  
LUVAS, Hisar, Haryana, India

**Sandeep Kumar**  
Department of Veterinary  
Gynaecology and Obstetrics,  
LUVAS, Hisar, Haryana, India

**SK Phulia**  
ICAR- Central Institute for  
Research on Buffalo,  
Hisar, Haryana, India

**Sandeep Kumar**  
Department of Veterinary  
Physiology and Biochemistry,  
LUVAS, Hisar, Haryana, India

**RK Sharma**  
ICAR- Central Institute for  
Research on Buffalo, Hisar,  
Haryana, India

**Correspondence**  
**Parveen Kumar**  
Veterinary Surgeon,  
Department of Animal  
Husbandry, Haryana, India

## Evaluation of effect of different estrus synchronization protocol on oxidative stress during summer season in cyclic Murrah Buffaloes

**Parveen Kumar, Anand Kumar Pandey, Ajit Verma, Sandeep Kumar, SK Phulia, Sandeep Kumar and RK Sharma**

### Abstract

The present study was conducted with the objective to compare the estrus synchronization protocols on oxidative stress parameters in cyclic Murrah buffalo during summer season. Forty cyclic Murrah buffaloes were synchronized for estrus with standard Ovsynch (n=20) and Doublesynch protocol (n=20) and inseminated fixed time at 8 and 24hr of last GnRH injection. Blood samples were analyzed for the level of oxidative stress parameters. Overall, there was no significant difference ( $P<0.05$ ) found between Ovsynch and Doublesynch treated buffaloes on the day of start of protocol and day of AI (Artificial Insemination) in overall MDA, GSH-Px and SOD concentration.

**Keywords:** Summer, Murrah buffaloes, Oxidative stress

### Introduction

The physiological and environmental stressors arising from high milk production within intensive management systems impair reproductive performance in high yielding buffaloes. Oxidative stress occurs due to an imbalance between the systemic manifestations of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage [2]. ROS are also implicated in the reproductive functions such as ovarian steroidogenesis, oocyte maturation, corpus luteal function and luteolysis, thus being related to female fertility [3]. Due to harsh and dry weather during summer season enhance stress for the continuation of reproductive activity in buffalo, probably; an increase in summer-induced oxidative stress is one of the reasons and which estrus synchronization protocol is better in relation with oxidative stress. Therefore, the present study was planned to compare the oxidative stress in buffalo subjected to Ovsynch and Doublesynch estrus synchronization during summer season.

### Material and Method

The study was conducted on 40 cyclic Murrah buffaloes subjected to estrus synchronization via Ovsynch [5] and Doublesynch protocol for estrus synchronization [11]. Buffaloes were divided into two groups as listed 20 in each (having parity:- 1-5; body weight:- 400-600 kg and body condition score:- 3-5) maintained at two locations: (1) Dairy Farm of Central Institute for Research on Buffaloes (CIRB), Hisar and, (2) at Field, Fatehabad district. The blood samples were collected on day of beginning of the protocol 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 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°C until analysis. Lipid peroxidation was evaluated in terms of MDA formed by using thiobarbituric acid-reactive substances (TBARS; [13]). Also, the activity of GSH-Px [6] and SOD [9] in erythrocyte lysate was assayed. The student's T test was employed to compare the differences between the oxidative stress parameters between the day of start of study and day of AI in both groups.

**Result and discussion**

The impact of different estrus synchronization on oxidative stress in buffaloes was not evident in the present study during summer season (Table 1). In summer season, the overall lipid peroxidation in terms of MDA concentration was non-significant ( $P<0.05$ ) higher in Doublesynch protocol treated buffaloes as compared to Ovsynch treated buffaloes (Table 1). Moreover, in both protocol on the day of fixed time AI erythrocytic MDA concentration in buffaloes were invariably higher on the day of estrus/ A.I. as compared to day of start of protocol (Table 1) but there was no significant difference ( $P<0.05$ ) found between Ovsynch and Doublesynch treated buffaloes on the day of A.I. MDA concentration on day of start of protocol was higher in Ovsynch as compared to Doublesynch treated pregnant buffaloes ( $5.5\pm 0.8$  v/s  $4.9\pm 0.5$  nmol/ml of hemolysate; respectively) and similar trend was found on day of A.I. ( $7.6\pm 1.2$  v/s  $5.7\pm 0.4$  nmol/ml of hemolysate; respectively). This may be due to more number of pregnant animals in doublesynch group as compared to Ovsynch treatment group and in fact animal showing less oxidative stress has more chance to become pregnant. It is well known that oocyte and embryos of buffaloes are very susceptible to oxidative damages due to high content of lipid [4]. On the other hand, the dominant follicle at the time of AI on the ovary results in higher production of ROS leads to lipid peroxidation and ultimately results into high MDA level [7]. High level of lipid peroxidation during estrus phase was also reported in Egyptian buffalo [10]. Between Ovsynch and Doublesynch estrus synchronized buffaloes during summer season, the overall GSH-Px concentrations remained similar on day of start of protocol and day of A.I. (Table 1). However, the overall GSH-Px was higher on the day of estrus in summer in both treatment protocol (Table 1). This could be

due to increased aerobic metabolism on the day of estrus which results into more production of free radicals and  $H_2O_2$  [11]. Level of antioxidative enzyme GSH-Px was non significantly higher ( $P<0.05$ ) in Ovsynch as compared to Doublesynch treated pregnant buffaloes on day of start of protocol ( $19.8\pm 2.8$  v/s  $15.2\pm 2.7$  U/ml of hemolysate; respectively) and similar trend was found on day of A.I. ( $24.0\pm 1.6$  v/s  $21.0\pm 2.7$  U/ml of hemolysate; respectively). Moreover, in oviductal fluid of cow, mRNA expression for GSH-Px-1 was observed higher toward the end of the estrous cycle before ovulation [8]. However, GSH-Px levels were invariably similar between pregnant and non-pregnant buffalo in summer (Table 1). Also, between Ovsynch and Doublesynch protocol on day of start of protocol, the overall SOD concentration ( $189.9\pm 14.1$  v/s  $154.1\pm 13.2$  U/ml of hemolysate; respectively) was higher in Ovsynch treated buffaloes as compared to doublesynch treated buffaloes. However, the overall SOD concentration was lower on the day of estrus in summer in both treatment protocol as compared to start of protocol but buffaloes subjected to doublesynch protocol had lower SOD concentration on the day of A.I. as compared to Ovsynch treated buffaloes ( $129.1\pm 8.1$  v/s  $149.7\pm 8.1$  U/ml of hemolysate; respectively). Active CL on the day of start of protocol might be responsible for more SOD activity [12]. Lipid peroxidation and the antioxidant enzymes viz. GSH-Px and SOD remained invariably similar between buffaloes subjected to Ovsynch and Doublesynch estrus synchronization protocol during summer season. In brief, no significant effect on oxidative stress was observed in study between Ovsynch and Doublesynch estrus synchronization protocol on the day of start of synchronization and day of A.I. during summer season in buffaloes.

**Table 1:** Oxidative stress parameters (per ml of hemolysate; Mean  $\pm$  SE) in buffaloes at start and day of AI of Ovsynch (n=20; NP =16, P=4) and Doublesynch protocol (n=20; NP=12, P=8) in summer season (AI: Artificial insemination; d= Day; nmol: Nano mol.; NP: Non pregnant; P: Pregnant; U: Unit)

Season	Parameters	Pregnancy Status (P/NP)	Day of start of Ovsynch protocol	Day of start of Doublesynch protocol	dAI (Ovsynch)	dAI (Doublesynch)
Summer	MDA (nmol)	NP	4.9 $\pm$ 0.4	5.4 $\pm$ 0.5	7.2 $\pm$ 0.5	7.9 $\pm$ 0.7
		P	5.5 $\pm$ 0.8	4.9 $\pm$ 0.5	7.6 $\pm$ 1.2	5.7 $\pm$ 0.4
		Overall	5.1 $\pm$ 0.4	5.2 $\pm$ 0.3	7.3 $\pm$ 0.5	7.1 $\pm$ 0.5
	GSH-Px (U)	NP	16.5 $\pm$ 1.2	20.1 $\pm$ 2.5	22.8 $\pm$ 1.5	24.8 $\pm$ 1.9
		P	19.8 $\pm$ 2.8	15.2 $\pm$ 2.7	24.0 $\pm$ 1.6	21.0 $\pm$ 2.7
		Overall	17.2 $\pm$ 1.1	18.1 $\pm$ 1.9	23.0 $\pm$ 1.3	23.3 $\pm$ 1.6
	SOD (U)	NP	179.5 $\pm$ 13.4	142.7 $\pm$ 14.1	145.9 $\pm$ 9.0	122.9 $\pm$ 8.4
		P	231.6 $\pm$ 44.4	171.4 $\pm$ 25.4	165.1 $\pm$ 18.2	138.4 $\pm$ 16.2
		Overall	189.9 $\pm$ 14.1	154.1 $\pm$ 13.2	149.7 $\pm$ 8.1	129.1 $\pm$ 8.1

**References**

1. Agarwal A, Gupta S, Sikka S. The role of free radicals and antioxidants in reproduction. *urr. Opin. Obstet. Gynecol.* 2006; 18:325-332.
2. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in pathophysiology of human reproduction. *Fertil. Steril.* 2003; 79:824-843.
3. Behram HR, Kodaman PH, Preston SL. Oxidative stress and the ovary. *J Soc. GynecolInvesting.* 2001; 8(1):S40-2.
4. Boni R, Santella L, Dale B, Roviello S, Di Palo R, Barbieri V. An ultrastructural study of maturation in buffalo oocytes. *Acta Medica Veterinaria.* 1992; 38:153-161.
5. Cirit Ü, Ak K, Ileri IK. New strategies to improve the efficiency of the Ovsynch protocol in primiparous dairy cows. *Bull. Vet. Inst. Pulawy.* 2007; 51:47-51.
6. Hafeman DG, Sunde RA, Hoekstra WG. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in rat. *J Nutr.* 1974; 104:580-587.
7. Jan MH, Das GK, Khan FA, Singh J, Bashir ST, Khan S *et al.* Evaluation of follicular oxidant-antioxidant balance and oxidative damage during reproductive acyclicity in water buffalo (*Bubalus bubalis*). *Asian Pacific J Anim. Reprod.* 2014; 3(1):35-40.
8. Lapointe J, Bilodeau JF. Antioxidant defenses are modulated in the cow oviduct during the estrous cycle. *Biol. Reprod.* 2003; 68(4):1157-1164.
9. Madesh M, Balsubramanian KA. Microtitre plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian J Biochem. Biophys.* 1998; 35:184-188.
10. Megahed GA, Anwar MM, Wasfy SI, Hammad ME.

Influence of heat stress on the cortisol and oxidant-antioxidants balance during oestrous phase in buffalocows (*Bubalus bubalis*): thermo-protective role of antioxidant treatment. *Reprod. Domest. Anim.* 2008; 43:672-677.

11. Pursely JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF<sub>2α</sub> and GnRH. *Theriogenology.* 1995; 44:915-923.
12. Rapoport R, Sklan D, Wolfenson D, Shaham-Albalancy A, Hanukoglu I. Antioxidant capacity is correlated with steroidogenic status of the corpus luteum during the bovine estrous cycle. *Biochim. Biophys. Acta.* 1998; 1380:133-140.
13. Shafiq-U-Rehman. Lead-induced regional lipid peroxidation in brain. *Toxicology Letters.* 1984; 21:333-337.