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Comparative study of oxidative stress between ovsynch and doublesynch estrus synchronization protocols on the day of AI in cyclic murrah buffaloes during winter

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Abstract
The aim of present study was to compare the effect on oxidative stress parameters on the day of AI after synchronizing estrous of cyclic Murrah Buffalo with Ovsynch and Doublesynch protocols during winter. Forty three cyclic Murrah buffaloes were synchronized for estrus with standard Ovsynch (n=18) and Doublesynch protocol (n=25) 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 (P<0.05) difference found in blood plasma concentrations of MDA, GSH-Px and SOD day of AI. In conclusion, the synchronization protocols do not impact on oxidative stress parameters in cyclic Murrah buffaloes.

Keywords: Ovsynch, doublesynch, murrah buffalo, day of AI, oxidative stress, winter

Introduction
The free radical and endogenous antioxidant balance is essential for normal cell activity. The balance gets altered when there is reduction in the endogenous antioxidant status of the cell. Such a situation has been observed following deficiencies of trace minerals [7]. Inorganic phosphorus [11]. Vitamins A, C, E. Reactive oxygen species (ROS) are double edge sword which at low level have a physiological roles in oocyte maturation and acquisition of developmental competence [4]. While higher level cause DNA fragmentation, lipid peroxidation, denaturation of protein result into embryonic death [9, 14, 20]. ROS level which overcome antioxidant defense system result into suppression of subsequent growth to ovulation of follicles, decreased LH receptor level, estradiol synthesis in the follicles and affect luteolysis [6, 13, 17, 19]. Therefore, the present study was planned to compare the effect on oxidative stress parameters after synchronizing estrous of cyclic Murrah Buffalo with Ovsynch and Doublesynch protocols during winter.

Material and method
The study was conducted on 43 cyclic Murrah buffaloes subjected to estrus synchronization via Ovsynch [15.] And Doublesynch protocol [3]. Buffaloes were divided into two groups: 1) Ovsynch group (n=18) and, 2) Doublesynch group (n=25). All the buffaloes were belonging to parity between 2 and 5, body weight ranged from 400 to 600 kg, body condition score between 3 and 5, and were 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 day of AI for assessing oxidative stress parameters viz. Malondialdehyde (MDA), Glutathione peroxidase (GSH-Px) and Superoxide dismutase (SOD) per ml of hemolysate. The hemolysate prepared after separation of blood plasma and erythrocytes washed three times with normal saline solution followed by centrifugation (1500g x 10 min). Thereafter, supernatant decanted and erythrocyte pellet was dissolved with chilled distilled water (added slowly to 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; [18]). Also, the activity of GSH-Px [5]. And SOD [10]. In erythrocyte lysate was assayed. The student's T test employed to compare the significant differences between days of AI in both protocol groups. The value less than 5% was considered as significant (P<0.05).

Result and discussion

The Mean ± SE of oxidative stress parameters are presented here in Table 1. There was marginal difference ($P>0.05$) found between Ovsynch and Doublesynch treated buffaloes on the day of AI (5.0 ± 0.2 v/s 5.6 ± 0.3 nmol/ml of hemolysis, respectively) but in pregnant buffaloes there was no significant difference found in the study ($P<0.05$). In fact, due to high content of lipid in oocyte and embryos of buffaloes makes them susceptible to oxidative damages [2]. On the day of estrus ovary contain dominant follicle which results into higher production of ROS (Reactive oxygen species) and leads to lipid peroxidation and ultimately result into high MDA level [6]. And in Egyptian buffalo level of lipid peroxidation during estrus phase was also reported higher [12]. The overall GSH-Px was non significantly higher ($P<0.05$) on the day of AI in doublesynch group as compared to ovsynch treatment group (Table 1) but in pregnant buffaloes there was no significant difference (23.6 ± 3.8 v/s 27.4 ± 1.8 U/ml of hemolysate) found in the study ($P<0.05$). It may be due to increased aerobic metabolism on the day of estrus which results into more production of free radicals and H_2O_2 [1]. Moreover, mRNA expression for GSH-Px-1 in oviductal fluid of cow was found higher before ovulation [8]. Also, between Ovsynch and Doublesynch protocol on day of AI, the overall SOD concentration (154.1 ± 8.4 v/s 139.6± 8.6 U/ml of hemolysate; respectively) was non-significant ($P<0.05$) and similar trend also found on the day of AI between pregnant animals. However, the SOD concentration was lower on the day of AI in Doublesynch protocols as compared to Ovsynch protocol in non-pregnant animals. It may be due to slight more ROS (Reactive oxygen species) in non-pregnant animals due to more no handing in Doublesynch group as compared to Ovsynch group. Active CL on the day of start (cyclic animal) of protocol might be responsible for more SOD activity in cyclic buffaloes [16]. Lipid peroxidation and the anti-oxidant enzymes viz. GSH-Px and SOD remained invariably similar between buffaloes subjected to Ovsynch and Doublesynch estrus synchronization protocol during winter season on the day of AI. Overall MDA, GSH-Px and SOD level in Doublesynch group indicate non-significant more ROS as compared to Ovsynch treated buffaloes. It may be due to extra handling and hormonal treatment in Doublesynch as compared to Ovsynch. In brief, no effect on oxidative stress was observed in study between Ovsynch and Doublesynch estrus synchronization protocol on day of AI during winter season in buffaloes, it means application of different estrus synchronization protocols does not differ much regarding oxidative stress in winter season in buffaloes.

Table 1: Oxidative stress parameters (per ml of hemolysate; Mean ± SE) in Murrah Buffaloes on day of AI following Ovsynch (n=18; NP =13, P=5) and Doublesynch protocol (n=25; NP=13, P=12) in winter season (AI: Artificial insemination; d0= Day of start of protocol; dAI= day of artificial insemination; nmol: Nano mol.; NP: Non pregnant; P: Pregnant; U: Unit)

Parameters	Pregnancy Status (P/NP)	dAI (Ovsynch)	dAI (Doublesynch)
MDA (nmol)	NP	4.8 ± 0.2	5.5 ± 0.3
	P	5.5 ± 0.4	5.8 ± 0.5
	Overall	5.0 ± 0.2	5.6 ± 0.3
GSH-Px (U)	NP	24.3 ± 2.0	27.5 ± 2.8
	P	23.6 ± 3.8	27.4 ± 2.6
	Overall	24.1 ± 1.7	27.4 ± 1.8
SOD (U)	NP	159.2 ± 9.9[a]	129.5±12.6[b]
	P	140.6 ± 16.3	150.6± 11.6
	Overall	154.1 ± 8.4	139.6± 8.6

[a vs b] Differ significantly in a row ($P<0.05$).

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