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Oestrus Induction and Fertility Response in Post Partum Anoestrus Buffaloes using CIDR Alone or in Combination with Antioxidants

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ABSTRACT

The present study was carried out to study the effect of ControlledInternal Drug Releasing Device (CIDR-B) with and without antioxidants (vitamin E & selenium) on oestrus induction response and oxidative stress parameters in postpartum anoestrus buffaloes located in different villages of R. S. Pura areas of Jammu district. A total of 18 postpartum anoestrus buffaloes were equally divided into three groups with 6 buffaloes in each group. Group I animals were kept as control, Group II animals were treated with CIDR protocol and Group III animals were treated with CIDR protocol and were given two intramuscular injection of vit. E-Care-Se (50 mg α-tocopherol acetate and 1.5 mg selenium per ml, Vetcare, Banglore) at the dose rate of 1ml/50 kg BWT. on day 0 and day 7, respectively. Oestrus induction response was 100% in treated animals and onset of oestrus occured within 70.50±6.38 h and 68.33±3.12 h and mean duration of oestrus was 22.33±1.14 h and 20.75±1.30 h in Group II and III, respectively. The pregnancy rate was 0.00%, 50% and 83.33% in Group I, II and III respectively. Among the oxidative stress parameter, a significant decrease in lipid peroxidation (LPO) and superoxide dismutase (SOD) level along with significant increase in catalase (CAT) and glutathione peroxidase (GPx) level was observed by treating post partum anoestrus buffaloes with CIDR protocol either alone or in combination with vitamin E and Selenium.

Keywords: Catalase, glutathione peroxidase, lipid peroxidation, superoxide dismutase

Buffaloes are considered as an excellent converter of poorer quality roughages, well adapted to harsh environments and are more resistant to several bovine tropical diseases than cattle (Gordon, 1996). Despite these merits, buffaloes are blamed for slow reproduction, long calving interval, delayed puberty, poor estrus expression and seasonality in breeding and calving (Singh *et al.*, 2000). The reproductive performance is low in buffaloes because of post-partum anoestrus (Warriach *et al.*, 2008). During the last few years, several studies have been attempted to treat the prolonged postpartum anoestrum in cattles by using hormonal and non hormonal treatments such as gonadotropin releasing hormone (GnRH), estrogen, prostaglandin ($PGF_{2\alpha}$) and progesterone (Metwelly, 2001; Singh, 2003; Edwell *et al.*, 2004) Lugol's iodine, ovarian and uterine massage (Edwell *et al.*, 2004). Various fertility levels have been achieved through the use of progestogens such as PRID (Singh *et al.*, 1988) and CIDR (Murugavel *et al.*, 2010). Oxidative stress (OS) is caused by an imbalance between pro-oxidants and antioxidants. This ratio can be altered by increased levels of

reactive oxygen species (ROS) and/or reactive nitrogen species (RNS), or a decrease in antioxidant defense mechanisms (Ruder *et al.*, 2009). A certain amount of ROS is needed for the progression of normal cell functions, provided that upon oxidation, every molecule returns to its reduced state. Production of free radicals could represent a source of infertility, because ovarian steroidogenic tissue (Crowe, 2008), spermatozoa (Sanocka and Kurpisz, 2004) and preimplantation embryos (Fujitani *et al.*, 1997) are sensitive to free radicals damage.

Vitamin E and selenium are essential nutrients that are integral components of the antioxidant defense of tissues and cells. Vitamin E is a potent chain breaking antioxidant, which inhibits propagation of per-oxidation reactions by scavenging oxygen radicals and terminating free radical chain reaction (Ferrell and Robert, 1994).

Selenium, a component of enzyme Glutathionine Peroxidase (GPx), in combination with vitamin E serves as a biological antioxidant to maintain cellular integrity. The action of vitamin E and selenium appears to be synergistic (Papas *et al.*, 1990). In some studies, administration of vitamin E and selenium (Ahmed *et al.*, 2010) improved fertility of bovine, while in other studies, providing high amount of these antioxidants showed no beneficial effects on fertility (Stowe *et al.*, 1988). In the view of above information the present study was carried to evaluate the effectiveness of CIDR device alone or in combination with antioxidants (vitamin E and Selenium) in oestrus induction and fertility response in postpartum anoestrus buffaloes.

MATERIALS AND METHODS

Eperimental location

The present study was conducted at Division of Veterinary Gynaecology and Obstetrics, Faculty of Veterinary Science and Animal Husbandry, R.S. Pura, Jammu.

Selection of the Animals

The present study was carried out on 18 postpartum anestrus buffaloes aged between 5 to 8 years with parity 2-6, which had not exhibited any signs of estrus for 90 days or more postpartum. Selected buffaloes were per rectally explored twice, ten days apart to confirm ovarian activity and genital status. All animals were dewormed with 1.5 gm Fenbendazole bolus (Panacur) 15 days before start of treatment.

Grouping of animals

The selected buffaloes were divided into 3 groups consisting of 6 animals in each group. Group I animals (n=6) were injected with Normal Saline (2ml) as placebo and were kept as control. Group II animals (n=6) were treated with progesterone in the form of CIDR for 7 days and an injection of PGF 2α (clostenol) @ 500µg i.m was given one day before the removal of CIDR. Group III animals (n=6) were administered Vit. E and Se (E care Se) @ 1ml/50 kg body weight i/m. and CIDR implant on day 0, followed by day 6 $PGF_{2\alpha}$ (clostenol) @ 500µg i.m and Vit. E and Se (E care Se) @ 1ml/50 kg body weight i/m and on day 7 CIDR was removed. Estrus was detected by visual observation of estrus signs twice in a day and later confirmed by genitilia examination per rectum. The buffaloes were artificially inseminated using frozen thawed semen of acceptable quality, 10 to 12 hours after the onset of estrus. Pregnancy diagnosis was done by rectal examination after 60 days post service.

Estimation of oxidative stress indices

For estimation of oxidative stress indices, blood sample was collected on day 0 (pre treatment), day 7 and at the time of A.I by jugular vein puncture into a 30 ml stoppered heparinised mineral free glass vials (dipped overnight in 2 N HCl). The activity of lipid peroxidation in erythrocytes and tissues was determined according to method described by Shafiq-

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ur-Rehman (1984). Membrane peroxidative damage in erythrocytes was determined in terms of MDA (malonedialdehyde) production, using thiobarbituric acid reactive substance (TBA). The activity of SOD, GPx and Catalase in erythrocyte lysate was determined by method of Marklund and Marklund 1974, Hafeman *et al.,* (1974) and Aebi (1983).

Statistical analysis

For evaluating the effect of antioxidant supplement, data analysis was done by using two way ANOVA (using SPSS-16.0 Inc).

RESULTS AND DISCUSSION

None of the CIDR devices were lost during the experiment. In Group II and III buffaloes treated with CIDR and CIDR plus antioxidants respectively all 6 (100%) buffaloes responded to the treatment. However, none of the animals was induced to estrus in the control group. Onset of oestrus, duration of estrus, and pregnancy results are shown in Table 1 and the mean MDA, Glutathione peroxidase, SOD and Catalase level on day 0, 7 & at the time of A.I for Group I, II and III are presented in Table 2.

In Group II and III all 6 (100%) buffaloes responded to the treatment. Similarly, higher oestrus induction rate were reported by Ali *et al.* (2012) and Naseer *et al.* (2013). Anita *et al.* (2004) reported 100% oestrus response in postpartum anoestrus buffaloes supplemented with vitamin E & Se. Findings of the present study revealed that oestrus can be successfully induced using CIDR protocol alone or in combination with antioxidant (vitamin E & Se). The mean time required for the onset of oestrus in Group II and III was 70.50±6.38 h (50-96 h) & 68.33±3.12 h (56-79 h), respectively. There was no significant difference ($P<0.05$) in time required foronset of oestrus between the two treatment groups. These findings are in accordance with earlier reports of Singh (2003) and Caesar *et al.* (2011), in buffaloes. The average duration of oestrus in Group II was 22.33±1.14 h (19-26 h).These findings are in accordance with earlier reports of Alyas (2010) and Kalsotra (2011). However, In Group III, the average duration of oestrus was 20.75±1.30 h (17-26 h). Similar results were reported by Ezzo (1996) who reported shorter duration of first postpartum oestrus in buffaloes given vitamin E- Se supplement. In group II, out of 6 buffaloes, 3 (50.00%) conceived at first service and none (0.00%) out of 3 conceived at second oestrus. The overall conception rate was 3/6 (50.00%). Similar conception rates have been reported by Naikoo and Patel (2009) and Naikoo *et al.* (2010). In Group III, out of 6 buffaloes, 4 conceived at first oestrus, 1 (50%) out of 2 conceived at second oestrus. The overall conception rate was 5/6 (83.33%). Similar conception rates have been reported by Zanella *et al.* (2010) and Izquierdo (2010) in cows. On the contrary, Scales (1976) and Spears *et al.* (1986) found no beneficial effect of vitamin E and Se on the fertility of cows. The higher conception rate reported in present study may be due to incorporation of vitamin E and Se in CIDR protocol which might have altered immune status (Hogan *et al.*,

Table 1: Treatment response for estrus induction, onset of oestrus, duration of estrus and conception in anestrous buffaloes

Group	Oestrus Induction response	Onset of oestrus	Duration of oestrus	Conception rate		
				1st service conception rate	2nd service conception rate	Overall conception rate
II	100%	70.50 ±6.38h	22.33±1.14 h	3/6 (50.00%)	0/3 (0.00%)	3/6 (50.00%)
III	100%	68.33±3.12 h	20.750±1.30 h	4/6 (66.66%)	1/2 (50%)	5/6 (83.33%)

Table 2: Effect of CIDR alone or in combination with Vit. E and Se on mean Malonedialdehyde (nmol/gHb/hr), Glutathione peroxidase (U/mg Hb), Super oxide Dismutase (U/mg Hb) and Catalase (µmol/mghb/min) level

	Group	Sampling schedule		
		O Day	7th Day	At FTAI
LPO (nmol/gHb/hr)	I	19.52 ± 0.94^{Aa}	17.72±1.02^{Aa}	—
	II	18.40±1.04^{Aa}	16.71±0.71^{Aa}	13.39±1.15^{Ab}
	III	20.46±0.91^{Aa}	13.41±1.21^{Bb}	8.33±1.05^{Bc}
Glutathione peroxidase (U/mg Hb)	I	10.55 ± 0.58^{Aa}	10.28±0.40^{Aa}	—
	II	12.14±0.57^{Aa}	15.20±0.42^{Bb}	16.22±0.30^{Ab}
	III	11.03±0.37^{Aa}	18.18±1.09^{Cb}	22.92±1.55^{Bc}
SOD (U/mg Hb)	I	54.25±1.82^{Aa}	51.26±1.82^{Aa}	—
	II	49.95±1.10^{Aa}	47.14±0.74^{Aa}	44.92±0.81^{Ab}
	III	51.35±2.32^{Aa}	44.49±2.25^{Ba}	38.00±3.88^{Ab}
CAT (µmol/mghb/min)	I	52.39±2.93^{Aa}	48.76±1.64^{Aa}	—
	II	47.57±0.97^{Aa}	50.35±0.9^{Ab}	52.14±0.34^{Ab}
	III	48.05±1.37^{Aa}	53.50±2.64^{Ab}	55.20±0.66^{Ab}

The values having different capital superscripts within a coloum differ significantly (P<0.05).
The values having different small superscripts within a row differ significantly (P<0.05).

1990), increased sperm transport and uterine muscular function (Libby and Segerson, 1981) and protected the oocyte or developing embryo against ROS damage (Olson and Seidel, 2000).

Oxidative stress parameters

There was significant decrease (P<0.05) in the MDA level on day 7 and at the time of A.I. in Group III as compared to Group II animals. Our findings are collaborated by findings of Anita *et al.* (2004) who reported significant decrease in MDA level in anoestrus buffaloes supplemented with vitamin E and Se. However, in Group III animals the mean level of MDA decreased significantly both on day 7 and at the time of A.I as compared to day 0. Such decrease in MDA level have been reported by Anita *et al.* (2004) and Kahlon *et al.* (2006) in anoestrus buffaloes supplemented with vitamin E and Se. MDA is considered as the final product of lipid peroxidation and a marker of oxidative stress (Shafiq-ur-Rehman, 1984). The increased MDA level in anoestrus buffaloes indicate that they were in stress (Anita *et al.*, 2004). The increased lipid peroxidation in anoestrus buffaloes could be due to increased permeability and fluidity of erythrocytic membrane, which enhanced their osmotic fragility (Kahlon *et al.*, 2002) and hence oxidative stress. Therefore, consumption of NADPH by ROS reactions (Golden and Ramdath, 1987) and susceptibility of steroidogenic enzymes to lipid peroxidation (Staats *et al.*, 1988; Takayanagi *et al.*, 1986) can limit synthesis of steroid hormones under the stress of free radicals. Therefore, metabolic changes mediated by ROS and peroxidative inactivation of steroidogenic enzymes may contribute to anoestrus in buffaloes (Kahlon *et al.*, 2006).

\There was significant increase (P<0.05) in the mean glutathione peroxidase level on day 7 and at the time of A.I when compared to day 0 in Group II animals. Similar, findings were reported by Anita *et al.* (2004) who reported significant higher activity of GPx in normal cyclic than anoestrus buffaloes. On the contrary, Kahlon and Singh (2003) in anoestrus buffalo heifers reported increased GPx activity as compared to normal cyclic heifers. However, in Group III animals the mean level of GPx increase

significantly (P<0.05) both on day 7 and at the time of A.I as compared to day 0. Such increase in GPx level have been reported by Zanella *et al.* (2010) in cows. However, Kahlon and Singh (2003) and Anita *et al.* (2004) reported non-significant increase in GPx activity in anoestrus buffaloes supplemented with vitamin E and Se. Therefore increase in GPx activity in treated anoestrus buffaloes supplemented with vitamin E and selenium might had increased the level of α-tocopherol in erythrocytes, neutrophils and plasma, and enhanced the biochemical activity of glutathione peroxidase in these animals (Finch and Turner, 1996).

The mean SOD value decrease significant (P<0.05) in Group II and III animals at the time of A.I when compared to day 0 and day 7. Kahlon and Singh (2002) and Anita *et al.* (2004) also reported lower values of SOD in normal cycling than anoestrus buffaloes. On the contrary, SOD level was higher in cyclic buffaloes than anoestrus buffaloes as reported by Ahmed *et al.* (2010). However, the SOD activity decreased significantly after supplementation with vitamin E and selenium in Group III on day 7. Such decrease in SOD level have been reported by Kahlon and Singh (2002) and Anita *et al.* (2004) in anoestrus buffaloes supplemented with vitamin E and Se. Increased lipid peroxidation in anoestrus buffalo implied occurrence of oxidative stress and poor antioxidant status in these animals (Kahlon, 1999). Therefore, the increased activities of erythrocytic SOD in anoestrus buffaloes could be attributed to physiological upregulation of this enzyme in an attempt to mitigate superoxide radical damage. Supplementation of vitamin E and selenium might have relieved the oxidative stress in postpartum anoestrus buffaloes, thus lowering erythrocytic SOD activity (Kahlon and Singh, 2002).

The mean catalase level was significantly higher (P<0.05) in anoestrus buffaloes on day 7 and on the day of A.I as compared to day 0 in Group II and III. These values are in accordance with Ahmed *et al.* (2010) who reported significant higher concentration of catalase in cyclic than non-cyclic buffalo heifers. On the contrary, Lallawmkimi *et al.* (2013) reported decreased erythrocyte catalase activity in growing heifers and lactating buffaloes fed with vitamin E. Possible reason for decreased activity of catalase in anoestrus buffaloes could be because of increased activity of SOD which might have resulted into increased production of H_2O_2 and there by increased utilization of catalase for converting H_2O_2 into water (Beigh, 2010). Supplementation of vitamin E and Se to these animals decreased the SOD activity, which spared catalase from utilization against ROS. Also, Vitamin E plays an important role in the regulation of heme synthesis (Boyvin *et al.*, 2013) deficiency of which might be a reason for decreased catalase activity (Suga *et al.*, 1984). Thus, enhanced catalase activity in the present study might be due to the supplementation of vitamin E (Kokcam and Naziroglu, 2002) which could have a positive role in the biosynthesis of heme (Hanswirth *et al.*, 1975).

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