International Journal of Veterinary Science

www.ijvets.com P-ISSN: 2304-3075 E-ISSN: 2305-4360 editor@ijvets.com

RESEARCH ARTICLE

Role of Oxidative Stress Biomarkers on Embryonic Mortality in Bovines

V Prabaharan[1], M Selvaraju[2], R Ezakial Napolean[3], KA Duraisamy[4] and A Natarajan[5]

[1 & 3]Department of Animal Reproduction, Gynaecology and Obstetrics; [2]Teaching Veterinary Clinical Complex, [4]Dean and [5]Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

ARTICLE INFO

Received: August 13, 2014
Revised: August 20, 2014
Accepted: November 10, 2014

Key words:
Embryonic mortality
GSH
MDA
Oxidative biomarkers
SOD

***Corresponding Author**
V Prabaharan
kullupraba@gmail.com

ABSTRACT

A total number of one hundred repeat breeder cows between second and fifth calving were selected, and randomly and equally divided into five groups as Group I to V. Cows of group I served as control and were inseminated during natural oestrus. Cows of group II were treated for 3 weeks with intramuscular injection of vitamin A, C and E. They were also supplemented with mineral mixture continuously for 20 days. At the end of treatment, these cows were observed for oestrus signs and inseminated at 16-18 hours after the onset of oestrum. Cows of group III were inseminated at 16-18 hours after the onset of natural oestrus and treated with an intramuscular injection of flunixin meglumine at 12, 13, 14 and 15 days of post-insemination. Cows of group IV were treated intravaginally with CIDR (Controlled internal drug release) for 9 days and following its removal, by observing oestrus signs, they were inseminated. Cows of group V were administered with two injections of $PGF_2\alpha$ at 11 days interval and they were observed for oestrus signs after second injection of $PGF_2\alpha$ and inseminated during induced oestrus. The serum oxidative biomarkers *viz*., reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) were estimated in pregnant cows of all the groups on days 0, 5, 10, 15, 20, 30, 45 and 60 and non-pregnant cows on days 0, 5, 10, 15 and 20. Among all the treatment groups, the serum oxidative biomarkers *viz*., GSH and SOD were higher in pregnant (day 0 to 60) and non pregnant (day 0 to 20) cows treated with antioxidant cows (group II). Comparing the pregnant and non pregnant cows in each group, GSH and SOD were higher in pregnant cows than non pregnant cows from day 0 to 20. The mean serum MDA was found to be lower in pregnant and non pregnant cows in group II than in other groups.

Cite This Article as: Prabaharan V, M Selvaraju, RE Napolean, KA Duraisamy and A Natarajan, 2015. Role of oxidative stress biomarkers on embryonic mortality in bovines. Inter J Vet Sci, 4(1): 6-9. www.ijvets.com

INTRODUCTION

Among the various forms of infertility in bovines, repeat breeding syndrome is considered as a common condition and is generally defined as any cow despite of normal estrus and health status of its genital tract fails to conceive after three or more artificial inseminations. The causes of repeat breeding syndrome can be divided into two major categories, fertilization failure and early embryonic mortality. But the exact etiology of the condition is complicated including a series of intrinsic and extrinsic factors, which can act independently or in combination (Amiridis *et al.,* 2009) to cause primarily embryonic mortality. It has been reported that one of the intrinsic causes of embryonic mortality leading to repeat breeding syndrome in cows is oxidative stress (Agarwal *et al.,* 2005). The present research investigation was made to establish the relationship between the oxidative stress and embryonic mortality.

MATERIALS AND METHODS

Cows of group II were treated for 3 weeks with intramuscular injection of Vitamin A, C and E and fed with 30 g of TANUVAS mineral mixture (Ca-23%; P-12%; Mg-6.5%; Fe-0.5%; I_2-0.026%; Cu-0.007%; Mn-0.12%; Co-0.012%; Zn-0.38%; S-0.5%; Fl-0.07% and Se-0.03%) continuously for 20 days. After the end of

Table 1: One hundred repeat breeder cows were equally divided into five groups.

Group	Treatment
I (control)	Artificial insemination during natural oestrus
II	I.M. injection of Vit. A, C and E @ 800 IU/kg/week/animal, 500 mg/day/animal and 8 mg/kg/week/animal, respectively and TANUVAS mineral mixture @ 30 g, through feed continuously for 20 days.
III	I.M. injection of flunixin meglumine @ 1.1 mg/kg on day 12, 13, 14 and 15 post-insemination
IV	CIDR intravaginally for 9 days
V	Double injections of $PGF_2\alpha$ at 11 days apart (at the total dose of 25 mg / injection)

Cows of group I (control) were artificially inseminated during natural oestrus.

aforementioned treatment these cows were observed for oestrus signs and inseminated at 16-18 hours after the onset of oestrum.

Cows of group III were inseminated at 16-18 hours after the onset of natural oestrus and administrated with an intramuscular injection of flunixin meglumine (COX_2-inhibitor which prevents the conversion of arachidonic acid to $PGF_2\alpha$) at the dose rate of 1.1 mg/kg on day 12, 13, 14 and 15 post-insemination.

Cows of group IV were treated with CIDR (Controlled internal drug release) intravaginally for 9 days and inseminated during induced oestrus following CIDR removal by observing estrus signs.

Cows in group V were administered with two injections of $PGF_2\alpha$ at 11 days interval (at the total dose of 25 mg / injection) and all the cows were observed for signs of oestrus following second $PGF_2\alpha$ injection and inseminated during induced oestrus.

The serum oxidative biomarkers *viz*., reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) were estimated in pregnant cows of all the groups on days 0, 5, 10, 15, 20, 30, 45 and 60 and on-pregnant cows on days 0, 5, 10, 15 and 20. SOD was estimated spectrophotometrically at 420 nm, based on the principle of degree of inhibition of auto-oxidation of pyrogallol at an alkaline pH, as explained by Marklund and Marklund (1974). GSH was measured as per the method of Ellman (1959), as described by Bulaj, *et al*. (1998), which was based on the reaction with Ellman's reagent. MDA was estimated according to method described by Yoshida *et al*. (2005).

RESULTS AND DISCUSSION

Reduced glutathione (GSH)

The mean (±SE) serum GSH levels (µmol/mg of protein) in repeat breeding pregnant and non pregnant cows during various phases of treatment are presented in Table 1(a) and 1(b), respectively.

In the present study, the mean serum GSH concentrations (µmol/mg) in pregnant cows were higher in all the groups than the non pregnant cows of respective group from day 0 to 20. Further, in pregnant cows from day 0 to 60 and in non pregnant cows from day 0 to 20, a gradual increase in the serum concentration of GSH was observed in this experiment. Among all the treatment groups, the mean GSH levels of pregnant and nonpregnant cows were higher in group II cows than other treatment and control groups. In the current experiment, the mean reduced glutathione (GSH) concentration ranged from 25.62±1.03 to 40.24±1.07 and 20.12±1.05 to 31.75±1.21 (µmol/mg of protein) in pregnant and non-pregnant cows respectively. Similar values were reported in normal and repeat breeder cows by Ahmed *et al.* (2010). GSH is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides (Sharma *et al*., 2011). Due to supplementation of antioxidants, there was an increase in concentration of GSH, from 0 to 60 days in pregnant cows and 0 to 20 days in non pregnant cows of group II compared to other groups. Brzeninka-Slebodzinsak *et al.* (1994) reported that the supplementation of vitamin E increased the level of GSH. However, supplementation of minerals plus vitamin E to the dry cows did not show any effect on GSH and SOD in red blood cells (Sharma *et al*., 2011). Kumar and Selvam (2003) reported that the administration of Se and vitamin E to the rats fed with caliculi producing diet, the level of lipid peroxidation was significantly reduced while the antioxidants GSH, vitamin C and E and the activities of the SOD were significantly increased. The increase in serum GSH in this study might have protected the embryonic cells from OS (oxidative stress) and it could be one of the reasons for increased conception rate in group II cows as demonstrated by Guerin *et al*. (2001).

Superoxide dismutase (SOD)

The mean (±SE) serum SOD levels (IU/ml) in repeat breeding pregnant and non pregnant cows during different phases of treatment in are presented in Table 2(a) and 2(b), respectively.

In the present study, the mean serum SOD concentrations (U/ml) in pregnant cows were higher in all the groups than the non pregnant cows of respective group from day 0 to 20. Further, in pregnant cows from day 0 to 60 and in non pregnant cows from day 0 to 20, a gradual increase in the serum concentration was observed in this experiment. Among all the treatment groups, the mean SOD levels of pregnant and non pregnant cows were higher in group II cows than other treatment and control groups. In the current experiment, the mean superoxide dismutase (SOD) concentration ranged from 28.91±1.45 to 46.41±1.75 and 26.10±1.43 to 42.51±1.45 (IU/ml) in pregnant and non pregnant cows respectively. Similar values were reported in normal and repeat breeder cows by Ahmed *et al.* (2010). However, lowest values of 6.99±0.45 and 6.37±0.72 IU/ml were reported in advanced pregnancy and early lactating cows, respectively (Sharma *et al.,* 2011). SOD is present in the ovarian tissue and there is a correlation between SOD and Ad4BP which is a steroidogenic transcription factor that induces transcription of the steroidogenic P450 enzyme. Thus, it controls steroidogenesis in the ovaries. The correlation between Ad4BP and SOD expression suggests an association between OS and ovarian steroidogenesis (Suzuki *et al.,* 1999). In group II cows, there was an

Table 2: Mean (±se) serum gsh in repeat breeder cows treated with antioxidants or cox_2 inhibitor or oestrus induction protocols

Treatment groups	1 (a). GSH (µmol/mg of protein)- Pregnant Animals							
	0^{th} day	5^{th} day	10^{th} day	15^{th} day	20^{th} day	30^{th} day	45^{th} day	60^{th} day
Group I	25.62^{aq}±1.03	26.12^{br}±1.04	27.45^{bq}±1.02	28.45^{bq}±1.25	29.23^{bq}±1.18	$31.56^{cq\pm}$1.04	32.12^{dq}±1.07	35.43^{dr}±1.12
Group II	28.78^{ap}±1.10	29.56^{ap}±1.12	31.72^{ap}±1.14	32.14^{bp}±1.01	32.38^{bp}±1.19	33.45^{cp}±1.19	35.23^{cp}±1.07	40.24^{dq}±1.07
Group III	26.54^{ap}±1.04	27.12^{ap}±1.03	28.78^{ap}±1.04	30.12^{ap}±1.16	34.43^{bp}±1.15	36.12^{bp}±1.01	36.56^{bp}±1.04	38.98^{cp}±1.02
Group IV	22.78^{ap}±1.10	24.56^{ap}±1.12	26.72^{ap}±1.14	27.14^{bp}±1.01	28.38^{bp}±1.19	30.45^{cp}±1.19	31.23^{cp}±1.07	33.24^{dq}±1.07
Group V	22.45^{ar}±1.12	25.12^{ar}±1.04	26.45^{aq}±1.25	28.43^{bq}±1.18	30.46^{cq}±1.07	31.54^{cq}±1.08	32.12^{cp}±1.12	$33.23^{dq\pm}$1.06
Treatment groups	1 (b). GSH (µmol/mg of protein)-Non pregnant Animals							
	0^{th} day	5^{th} day	10^{th} day	15^{th} day	20^{th} day			
Group I	24.34^{ar}±1.03	25.12^{bs}±1.12	26.48^{br}±1.21	26.32^{cs}±1.02	27.94^{cr}±1.09			
Group II	25.12^{ar}±1.10	29.48^{ar}±1.19	30.78^{aq}±1.15	31.12^{br}±1.11	31.75^{bq}±1.21			
Group III	24.45^{as}±1.12	25.43^{as}±1.02	26.75^{ar}±1.07	28.01^{bs}±1.13	29.43^{br}±1.15			
Group IV	20.12^{aq}±1.05	23.98^{aq}±1.05	24.12^{bq}±1.06	25.43^{bq}±1.07	26.65^{bp}±1.12			
Group V	20.78^{ap} ±1.04	23.92^{ap}±1.13	24.16^{bp}±1.07	26.78^{bp}±1.04	28.24^{cp}±1.08			

Mean values bearing superscripts between rows within a same column (a, b, c, d) and among columns within a row (p, q, r, s, t) differ significantly (P≤0.05). Group I – Control; Group II – Antioxidants; Group III - COX_2 inhibitor; Group IV – CIDR; Group V - $PGF_2\alpha$

Table 3: Mean (±se) serum sod levels in repeat breeder cows treated with antioxidants or cox_2 inhibitor or oestrus induction protocols

Treatment groups	2 (a). SOD (IU/ml)-Pregnant Animals							
	0^{th} day	5^{th} day	10^{th} day	15^{th} day	20^{th} day	30^{th} day	45^{th} day	60^{th} day
Group I	33.10^{ap}±1.50	33.31^{ap}±1.12	33.52^{ap}±1.10	33.91^{aq}±1.45	34.00^{ap}±1.45	34.12^{aq}±1.08	34.20^{ap}±1.01	34.32^{aq}±1.05
Group II	36.14^{ap}±1.52	38.10^{ap}±1.00	40.71^{aq}±1.24	44.00^{aq}±1.05	45.82^{ap}±1.12	46.21^{aq}±1.12	46.32^{ap}±1.12	46.41^{aq}±1.75
Group III	30.50^{ap}±1.35	31.61^{aq}±1.24	34.81^{ap}±1.00	35.31^{aq}±1.75	35.92^{aq}±1.23	36.00^{aq}±1.17	39.12^{aq}±1.23	39.21^{ar}±1.08
Group IV	29.01^{ap}±1.13	30.31^{ap}±1.13	30.52^{ap}±1.24	31.61^{ap}±1.10	32.71^{ap}±1.12	32.82^{ap}±1.42	33.90^{ap}±1.05	35.24^{br}±1.45
Group V	28.91^{ap}±1.45	30.12^{ap}±1.45	31.43^{ap}±1.10	31.31^{ap}±1.93	31.46^{ap}±1.78	31.65^{ap}±1.08	32.62^{ap}±1.75	33.00^{ap}±1.01
Treatment groups	2 (b). SOD (IU/ml)- Non pregnant Animals							
	0^{th} day	5^{th} day	10^{th} day	15^{th} day	20^{th} day			
Group I	32.21^{ap}±1.23	32.37^{ap}±1.01	32.11^{ap}±1.23	32.91^{aq}±1.98	33.01^{ap}±1.12			
Group II	35.82^{ap}±1.20	36.00^{ap} ±1.12	38.31^{ap}±1.14	40.42^{ap}±1.56	42.51^{ap}±1.45			
Group III	26.10^{ap}±1.43	29.11^{ap}±1.04	32.32^{ap}±1.45	34.43^{ap}±1.46	34.52^{ap}±1.12			
Group IV	27.53^{aq}±1.23	28.63^{aq} ±1.02	29.80^{aq}±1.11	30.90^{ar}±1.12	32.00^{aq}±1.10			
Group V	27.12^{ap}±1.45	30.31^{ap} ±1.45	30.52^{ap}±1.07	30.71^{ap}±1.09	30.20^{ap}±1.56			

Mean values bearing superscripts between rows within a same column (a, b, c, d) and among columns within a row (p, q, r, s, t) differ significantly (P≤0.05). Group I – Control; Group II – Antioxidants; Group III - COX_2 inhibitor; Group IV – CIDR; Group V - $PGF_2\alpha$

Table 4: Mean (±se) serum mda in repeat breeder cows treated with antioxidants or cox_2 inhibitor or oestrus induction protocols

Treatment groups	3 (a). MDA (mmol/ml) - Pregnant Animals							
	0^{th} day	5^{th} day	10^{th} day	15^{th} day	20^{th} day	30^{th} day	45^{th} day	60^{th} day
Group I	1.24^{ap}±0.31	1.38^{aq}±0.45	1.37^{aq}±0.22	1.35^{cs}±0.29	1.34^{cs}±0.29	1.31^{br}±0.22	1.33^{br}±0.30	1.35^{ap}±0.12
Group II	1.03^{dq}±0.12	1.28^{ap}±0.21	1.24^{ap}±0.22	1.28^{ap}±0.18	1.24^{bp}±0.32	1.22^{bp}±0.06	1.21^{cf}±0.04	1.23^{dp}±0.14
Group III	1.27^{dr}±0.18	1.31^{br}±0.31	1.33^{aq}±0.35	1.33^{bq}±0.07	1.31^{cq}±0.46	1.34^{aq}±0.18	1.27^{cq}±0.15	1.36^{dr}±0.44
Group IV	1.31^{aq}±0.01	1.21^{cs}±0.14	1.30^{cr}±0.23	1.38^{cr}±0.29	1.21^{cr}±0.12	1.33^{bq}±0.35	1.28^{dr}±0.42	1.92^{bq}±0.58
Group V	1.28^{br}±0.09	1.22^{cs}±0.22	1.22^{cr}±0.26	1.24^{bq}±0.07	1.32^{cr}±0.21	1.21^{cr}±0.39	1.27^{bq}±0.09	1.36^{ar}±0.07
Treatment groups	3 (b). MDA (mmol/ml) - Non pregnant Animals							
	0^{th} day	5^{th} day	10^{th} day	15^{th} day	20^{th} day			
Group I	1.29^{ap}±0.14	2.45^{aq}±0.34	2.71^{aq}±0.05	3.11^{br}±0.38	3.92^{bq}±0.58			
Group II	1.63^{cq}±0.12	2.14^{ap}±0.12	2.18^{bp}±0.04	2.41^{cp}±0.16	2.85^{dq}±0.06			
Group III	1.69^{br}±0.40	2.92^{ar}±0.26	2.39^{br}±0.21	3.07^{cr}±0.29	3.67^{cr}±0.10			
Group IV	1.92^{bs}±0.16	2.57^{bs}±0.45	2.72^{ar}±0.12	3.18^{cq}±0.08	3.66^{cp}±0.17			
Group V	1.88^{aq}±0.39	2.06^{bs}±0.26	2.52^{bs}±0.11	3.05^{cr}±0.02	3.90^{dr}±0.04			

Mean values bearing superscripts between rows within a same column (a, b, c, d) and among columns within a row (p, q, r, s, t) differ significantly (P≤0.05). Group I – Control; Group II – Antioxidants; Group III - COX_2 inhibitor; Group IV – CIDR; Group V - $PGF_2\alpha$

elevated levels of SOD from day 0 to 60 in pregnant and day 0 to 20 in non pregnant cows than other groups in this study**,** might be due to the administration of antioxidants. The increased levels of SOD in oxidative stress, protects cells against toxic oxygen radicals. Hydrogen peroxide, the by-product of SOD action is eliminated either by catalase or glutathione peroxidase (Guerin *et al.,* 2001). Hence, in group II cows, the elevated SOD might have favoured the highest conception rate.

Malondialdihyde (MDA)

The mean (±SE) serum MDA (mmol/ml) in repeat breeding cows during different phases of treatment in pregnant and non pregnant cows are given in Table 3(a) and 3(b), respectively.

In the present study, among all the groups, group II cows had decreased serum concentration of MDA in pregnant (day 0 to 60) and non pregnant cows (day 0 to 20) than other groups. Pregnant cows of all the groups showed fluctuations in serum MDA concentrations from day 0 to 60. Further, in each group, non pregnant cows had higher mean serum MDA levels than the pregnant cows. In the current study, the serum MDA (mmol/ml) levels ranged from 1.03 ±0.27 to 1.92±0.58 and 1.29±0.14 to 3.92±0.58 in pregnant and non pregnant cows respectively. Similar finding was made by Ahmed *et al.,*

(2010) in cows. MDA is a byproduct of lipid peroxidation and used as an index of the rate of tissue reaction chain. In addition, MDA is used as an indicator of OS in cells and tissues. Further, pregnant cows had lower serum MDA than non pregnant cows of corresponding group. These findings indicated that administration of antioxidants resulted in reduced production of MDA in group II as described by Amal *et al*. (2012) in mares. The reduction of mean serum MDA in pregnant cows than in non pregnant cows of this study was in concurrence with the findings of Amal *et al*. (2012) in mares. MDA has been reported to promote cross linking bonds in the cell membranes and leads to unfavorable effects such as changes in ion permeability and enzyme activity (Ahmed *et al*., 2010). The reduced production of MDA in pregnant cows might have caused protection of developing embryos and resulted successful conception in this experiment.

Conclusion

From day 0 to 60 in pregnant cows and day 0 to 20 in non pregnant repeat breeder cows, a gradual increase in oxidative biomarkers GSH and SOD in all the groups was observed. Among all the treatment groups, the serum GSH and SOD were higher in pregnant (day 0 to 60) and non pregnant (day 0 to 20) cows of group II (antioxidant treated cows). Comparing the pregnant and non pregnant cows in each group, these GSH and SOD were higher in pregnant cows than non pregnant cows from day 0 to 20. The mean serum MDA was found to be lower in pregnant and non pregnant cows in group II than in other groups. Hence, from this study, it is concluded that administration of antioxidants along with mineral mixture improved the conception rate in repeat breeder cows.

Acknowledgements

We are thankful to Tamilnadu Veterinary and Animal Sciences University for the facilities provided for permitting the first author to prosecute Ph.D.

REFERENCES

Agarwal AS, R Gupta and K Sharma, 2005. Role of oxidative stress in female reproduction. Reprod. Biol, Endocrin, 3, 28.

Ahmed WM, HH El-Khadrawy, AR Abd El Hameed and HA Amer, 2010. Applied investigations on ovarian inactivity in buffalo heifers. Inter J Acad Res, 2: 26-32.

Amal MAE, YHS Fawzia and IG Faten, 2012. Antioxidants markers, trace minerals and steroid hormones in preconceptional arab mares. Global Vet, 9: 311-318.

Amiridis GS, TH Tsiligianni, E Dovolou, C Rekkas, DV Ouzaras and I Menegatos, 2009. Combibed administration of gonadotropin releasing hormone, progesterone, and meloxicam is an effective treatment for the repeat breeder cow. Theriogenol, 72: 542-548.

Brzeninka-Slebodzinsak E, JK Miller, JD Quickery, JR Moore and FC Madsen, 1994. Antioxidant status of dairy cows supplemented prepartum with vitamin E and selenium. J Dairy Sci, 77: 3087-3095.

Bulaj G, T Kortemme and DP Goldenberg, 1998. Ionization: Reactivity relationships for cysteine thiols in polypeptides. Biochem, 37: 8965-8972.

Ellman GL, 1959. Determination of sulfhydryl group. Arch. Biochem. Biophys, 82: 70-74.

Guerin P, EIS Mouatassim and Y Menezo, 2001. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. Human Reprod, Update, 7: 175-189.

Kumar SM and R Selvam, 2003. Supplimentation of vitamin E and selenium prevents hyperoxaluria in experimental urolithic rats. J Nutr Biochem, 14: 306-313.

Marklund S and GA Marklund, 1974. Simple assay for super oxide dismutase using autooxidation of pyrogallol. European J Biochem, 47: 469-472.

Sharma N, NK Singh, OP Singh, V Pandey and PK Verma, 2011. Oxidative stress and antioxidant status during transition period in dairy cows. Asian-Aust J Anim Sci, 4: 479-484.

Suzuki T, N Sugino, T Fukaya, S Suguyama, T Uda, R Takaya, A Yajima and H Sasano, 1999. Superoxide dismutase in normal cyclicing human ovaries; Immunohistochemical localization and characterization. Fertil Steril, 72: 720-726.

Yoshida Y, N Itoh, M Hayakawa, R Piga, O Cynshi, K Jishage and E Niki, 2005. Lipid peroxidation induced by carbon tetrachloride and its inhibition by antioxidant as evaluated by an oxidative stress marker, HODE. Toxicol Appl Pharmacol, 208: 87-97.