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ROLE OF ANTIOXIDANT VITAMINS ON OXIDATIVE STRESS AND EMBRYONIC MORTALITY IN BOVINES

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Abstract: A total number of one hundred repeat breeder cows between second and fifth parity were selected and randomly and equally divided into five groups as Group I to V. Cows of group I served as control and were artificially inseminated during natural oestrus. Cows of group II were treated for 3 weeks with intramuscular injection containing vitamin A, C and E. All the cows of this group were also administered orally with 30 g of mineral mixture through feed continuously for 20 days. After the end of treatment, these cows were observed for oestrus signs and AI was done at 16-18 hours after the onset of oestrum. Cows of group III were artificially inseminated at 16-18 hours after the onset of natural oestrus and treated with an intramuscular injection of flunixin meglumine at the dose rate of 1.1 mg/kg on days 12, 13, 14 and 15 post-insemination. Cows of group IV were treated with CIDR intravaginally for 9 days and AI was performed during induced oestrus following CIDR removal by the observation of oestrus signs. Cows of group V were administered with double injections of $PGF_2\alpha$ at 11 days apart and all the cows were observed for signs of oestrus following second $PGF_2\alpha$ injection and AI was performed during induced oestrus. The serum antioxidant vitamins *viz*., Vitamin A, C and E 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. Among all the treatment groups, the serum antioxidant vitamins *viz*., vitamin A, C and E were higher in pregnant (day 0 to 60) and nonpregnant (day 0 to 20) cows treated with antioxidant cows (group II). Comparing the pregnant and nonpregnant cows in each group, these vitamins were higher in pregnant cows than nonpregnant cows from day 0 to 20.

Keywords: Antioxidant; Oxidative stress; Vitamin A, C and E; Embryonic mortality.

Introduction

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 serious of intrinsic and extrinsic factors, which can act independently

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or in combination (Amiridis *et al.,* 2009) to cause primarily embryonic mortality. The relationship between nutrition and reproduction is complex and responses are often quite variable and inconsistent that affects reproductive functions in dairy cattle. Specific nutrient deficiencies or malnutrition can have a negative effect on the embryonic development. A severe deficiency of vitamins especially vitamin A that serve as regulators of metabolism can cause embryonic mortality. Vitamins A, C and E are essential in a wide range of physiological processes such as promotion of general growth, pregnancy, ameliorating increased OS during pregnancy and the provision of antioxidant defence to the embryo and fetus (Dejmek *et al.,* 2002). The present research investigation was made to establish the relationship between the oxidative stress, antioxidant vitamins and embryonic mortality.

Materials and methods

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.

Cows of group II were treated for 3 weeks with intramuscular injection of Vitamin A, C and E and were administered orally 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%) through feed continuously for 20 days. After the end of treatment with antioxidants, these cows were observed for oestrus signs and AI was done at 16-18 hours after the onset of oestrum.

Cows of group III were artificially inseminated at 16-18 hours after the onset of natural oestrus and treated 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 intravaginally for 9 days and AI was performed during induced oestrus following CIDR removal by the observation of oestrus signs.

Cows in group V were administered with double injections of $PGF_2\alpha$ at 11 days apart (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 AI was performed during induced oestrus.

The serum antioxidant vitamin A, C and E 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.

Vitamin A was estimated as per the method of Rutkowski *et al.* (2006). Vitamin C was estimated as per the method of Rutkowski and Grzegorczyk (1998). Vitamin E was estimated as per the method of Fabianek *et al.* (1968).

Results and discussion

Vitamin A

The mean vitamin A (µg/dl) concentrations in repeat breeding pregnant and nonpregnant cows during various phases of treatment are presented in Table 1(a) and 1(b), respectively.

In the current study, pregnant cows from day 0 to 60 in all the groups exhibited a gradual increase in the mean serum vitamin A concentration. In nonpregnant cows, from day 0 to 20 in all the groups, there was a gradual increase in serum vitamin A concentration. The pregnant and nonpregnant cows of group II had higher serum vitamin A concentrations than other treatment and control groups from day 0 to 60 and day 0 to 20, respectively.

The mean vitamin A (µg/dl) concentrations ranged from 28.41±0.66 to 42.10±0.15 in pregnant cows and in non-pregnant cows it was 26.12±0.16 to 36.98±0.41. Similar values were reported from day 0 to 21 in pregnant and nonpregnant cows by Ataman *et al*. (2010). However, higher values of serum vitamin A at 30 days (52.97±31) and 60 days (49.76±3.11, µg/dl) of gestation were reported (Kumar *et al.,* 2010). This finding supported our result of elevated levels of mean serum vitamin A concentrations in all the pregnant cows from day 0 to 60 in all the groups of this study. Ataman *et al.* (2010) reported that the mean vitamin A level was higher in pregnant cows than in nonpregnant cows 21 days after AI but there was no significant difference at the time of AI and 3 days and 12 days after AI. In nonpregnant cows, from day 0 to 15, mean serum vitamin A got increased in all the groups and thereafter on day 20, the levels reduced drastically in our study. In accordance with this finding, Ataman *et al.* (2010) reported that vitamin A levels were higher at the time of AI and 3 days after AI in nonpregnant cows. In group II cows, both in pregnant and nonpregnant cows, there was an elevated vitamin A concentrations when compared to other groups during all the

periods of blood collection. This could be the one of the reasons for the highest conception rate in this group as reported by Ataman *et al*. (2010) in cows. Vitamins are not directly involved in reproduction but may have an indirect role in proper functioning of reproductive system (Kumar *et al.,* 2010). Vitamin A regulates uterine growth and reproductive performances and affects the overall health of animals (Chew, 1984). Lack of vitamin A reduces the animal's resistance against invading pathogens and makes it more susceptible to infection. With special reference to reproduction, this micro nutrient affects the ovarian steroidogenesis and directly or indirectly through P_4 secretion which influences the uterine environment and early embryo and fetal development. Vitamin A deficiency produces degenerative changes in the mucus membrane of the uterus with the result that the nidation is prevented and death and resorption of the embryo follows (Kumar *et al.,* 2010).

Vitamin C

The mean serum vitamin C (µg/ml) levels in repeat breeding pregnant and nonpregnant cows during various phases of treatment are presented in Table 1(a) and 1(b), respectively.

In this study, pregnant cows from day 0 to 60 and nonpregnant cows from day 0 to 20 exhibited a gradual increase in the mean serum vitamin C concentrations in all the groups. In group II cows, the mean serum vitamin C concentrations were higher in pregnant cows (day 0 to 60) and nonpregnant cows (day 0 to 20) than other groups. In each experimental group, the serum vitamin C concentration was higher in pregnant cows than in nonpregnant cows.

The mean serum vitamin C (µg/ml) levels ranged from 2.15±0.17 to 8.72±0.12 in pregnant cows and in non-pregnant cows it was 2.13±0.16 to 3.75±0.12. Ataman *et al*. (2010) observed that there was no significant difference in vitamin C levels at the time of AI and 21 days after AI between pregnant and nonpregnant cows. In this study, in each group, the vitamin C levels in pregnant cows were higher in the nonpregnant cows and also in all the groups, the vitamin C concentration increased from day 0 to 60 in pregnant cows. Ascorbic acid protects against endogenous oxidative DNA damage (Fraga *et al.,* 1991). Furthermore, ascorbate, at physiological concentrations, induces the release of hypotaurine and taruine by oviduct epithelial cells (Guerin *et al.,* 2001). The liquid present in the ampullar section of the oviduct at the time of fertilization is a mixture of tubal and follicular fluids (Hansen *et al.,* 1991). It is possible that large amounts of ascorbate, present in follicular fluid (Paszkowski and Clarke, 1999) induce taurine and hypotaurine release in oviductal fluid at the time of ovulation. Among all the groups, pregnant and nonpregnant cows of group II had higher levels of serum ascorbic acid in this study. As an non-enzymatic antioxidant vitamin C might have favored

the embryonic development in group II cows and it could be the one of the reason for the highest conception rate in this group as described by Chen *et al*. (2001).

Vitamin E

The mean vitamin E (µg/dl) concentration in repeat breeding pregnant and nonpregnant cows during different phases of treatment in are presented in Table 2(a) and 2(b), respectively.

In this study, the serum vitamin E concentration showed increasing pattern from day 0 to 60 in pregnant cows and from day 0 to 20 in nonpregnant cows in all the groups. Similarly in all the groups, the mean serum concentration of vitamin E in pregnant cows was found to be higher than in non pregnant cows. In group II cows, the mean serum vitamin E concentrations were higher in pregnant (day 0 to 60) and nonpregnant cows (day 0 to 20) than other groups.

The mean vitamin E (µg/dl) concentration ranged from 1.10±0.03 to 2.84±0.07 in pregnant cows and in non-pregnant cows it was 1.05±0.04 to 2.30±0.04. Similar values were reported by Dobbelaar *et al.* (2010). From day 0 to 20, pregnant cows had higher serum vitamin E concentration than nonpregnant cows of respective group. Further, from day 0 to 60 in all the groups, there was a gradual increase in the serum vitamin E concentration in pregnant cows. The vitamin E, a cellular antioxidant, interacts with selenium-containing glutathione peroxidase to prevent oxidative breakdown of tissue membranes (Putnam and Comben, 1987) and lipid containing organelles by inhibition and destruction of endogenous peroxides, thus maintaining membrane integrity and reducing oxidative stress. Vitamin E and selenium showed synergistic effect in their antioxidant action and reproductive performance in dairy cows (Kim *et al.,* 1997). Administration of vitamin E before calving may be beneficial in improving the postpartum reproductive performance of dairy animals (Anita *et al.,* 2004). Vitamin E functions as a chain-breaking antioxidant, neutralizing free radicals and preventing peroxidation of lipids within membranes (McDowell, 2000). Vitamin E serves as the first line of defence against peroxidation of phospholipids. Selenium as part of GSH-Px is the second line of defence as the enzyme destroys peroxides and hydroperoxides. These might be the reasons for the influence of vitamin E and selenium indirectly to achieve highest conception rate in group II cows in this study.

Conclusion

The serum antioxidant vitamins *viz*., A, C and E were higher in pregnant (day 0 to 60) and nonpregnant (day 0 to 20) cows treated with antioxidant cows (group II). Comparing the pregnant and nonpregnant cows in each group, these vitamins were higher in pregnant cows than nonpregnant cows from day 0 to 20. Hence, from this study, it is concluded that

administration of antioxidants vitamins along with mineral mixture improved the conception rate in repeat breeder cows.

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MEAN (±SE) SERUM VITAMIN A LEVELS IN REPEAT BREEDER COWS TREATED WITH ANTIOXIDANTS OR COX, INHIBITOR OR OESTRUS INDUCTION PROTOCOLS

Table 1 (a) Vitamin A (µg/dl)- Pregnant Animals

S.No.	Treatment groups	0th day	5th day	10th day	15th day	20th day	30th day	45th day	60th day
1.	Group I	28.70^{ap}±0.39	29.58^{bp}±0.50	30.21^{bp}±0.33	31.60^{cp}±0.33	32.20^{cp}±0.33	34.10^{dp}±0.33	35.98^{ep}±0.33	36.13^{ep}±0.24
2.	Group II	34.35^{ar}±0.31	35.81^{bs}±0.15	36.19^{ct}±0.27	37.12^{dt}±0.16	38.37^{es}±0.15	39.23^{fs}±0.27	40.98^{gt}±0.39	42.10^{hs}±0.15
3.	Group III	29.58^{aq}±0.59	30.54^{bq}±0.16	31.69^{cs}±0.16	32.32^{dr}±0.16	34.19^{eq}±0.66	36.00^{fq}±0.20	37.89^{gs}±0.83	38.43^{gq}±0.33
4.	Group IV	28.41^{ap}±0.66	29.11^{ap}±0.50	30.98^{bq}±0.16	32.02^{cq}±0.16	34.86^{dq}±0.16	35.84^{eq}±0.16	37.01^{fq}±0.17	38.96^{gr}±0.50
5.	Group V	29.69^{aq}±0.33	30.81^{br}±0.39	31.12^{br}±0.48	34.0^{cs}±0.48	35.60^{dr}±0.33	36.32^{er}±0.32	37.21^{fr}±0.42	39.00^{gr}±0.34

Table 1 (b) Vitamin A (µg/dl)- Nonpregnant Animals

S.No.	Treatment groups	0th day	5th day	10th day	15th day	20th day
1.	Group I	26.12^{ar}±0.16	28.10^{bq}±0.33	29.36^{cq}±0.15	30.32^{dp}±0.16	30.70^{ep}±0.16
2.	Group II	31.51^{as}±0.15	33.91^{bs}±0.32	34.34^{ct}±0.27	35.89^{dt}±0.31	36.98^{et}±0.41
3.	Group III	24.78^{ap}±0.33	26.72^{bp}±0.16	27.98^{cp}±0.50	30.92^{dq}±0.16	33.73^{es}±0.17
4.	Group IV	25.98^{aq}±0.20	28.01^{bq}±0.16	30.18^{cr}±0.16	31.34^{dr}±1.66	32.61^{eq}±0.26
5	Group V	26.21^{ar}±0.16	29.14^{br}±0.30	30.45^{cs}±0.32	32.56^{ds}±0.30	33.08^{er}±0.15

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 - $PGF_2\alpha$

MEAN (±SE) SERUM VITAMIN C LEVELS IN REPEAT BREEDER COWS TREATED WITH ANTIOXIDANTS OR COX_2 INHIBITOR OR OESTRUS INDUCTION PROTOCOLS

Table 2 (a) Vitamin C (µg/ml)-Pregnant Animals

S.No.	Treatment groups	0^{th} day	5^{th} day	10^{th} day	15^{th} day	20^{th} day	30^{th} day	45^{th} day	60^{th} day
1.	Group I	2.15^{ap}±0.17	2.83^{bp}±0.86	2.96^{bp}±0.02	3.13^{bp}±0.06	3.66^{bp}±0.19	6.70^{cq}±0.08	7.23^{dr}±0.13	7.67^{eq}±0.18
2.	Group II	2.85^{ss}±0.05	3.93^{bq}±0.02	4.03^{cr}±0.61	4.61^{cs}±0.21	4.83^{ds}±0.12	7.60^{er}±0.04	7.72^{fs}±0.19	8.72^{gr}±0.12
3.	Group III	2.76^{ar}±0.06	2.82^{ap}±0.16	2.97^{bp}±0.07	4.16^{cr}±0.12	4.54^{dr}±0.03	6.67^{eq}±0.16	6.86^{eq}±0.15	7.50^{fp}±0.15
4.	Group IV	2.43^{aq}±0.09	2.82^{bp}±0.20	3.13c^{bq}±0.09	3.63^{dq}±0.19	4.22^{eq}±0.10	6.41^{fq}±0.90	6.51^{fp}±0.16	7.40^{gp}±0.18
5.	Group V	2.36^{ap}±0.12	2.91^{bp}±0.23	3.10^{bq}±0.11	3.83^{cq}±0.21	4.52^{dr}±0.13	6.00 ^{ep}±0.08	6.95^{fq}±0.13	7.79^{gq}±0.16

Table 2 (b) Vitamin C (µg/ml)-Nonpregnant Animals

S.No.	Treatment groups	0^{th} day	5^{th} day	10^{th} day	15^{th} day	20^{th} day
1.	Group I	2.13^{ap}±0.16	2.45^{bp}±0.23	2.47^{bp}±0.16	2.67^{bp}±0.44	2.82^{cp}±0.30
2.	Group II	2.62^{ar}±0.06	2.71^{bq}±0.06	3.13^{cr}±0.18	3.36^{dr}±0.20	3.75^{es}±0.12
3.	Group III	2.31^{aq}±0.06	2.40^{bp}±0.16	2.61^{cp}±0.13	3.16^{dq}±0.04	3.31^{er}±0.23
4.	Group IV	2.36^{aq}±0.09	2.53^{bp}±0.17	2.64^{bq}±0.19	2.79^{cp}±0.22	3.14^{dq}±0.09
5	Group V	2.31^{aq}±0.16	2.49^{bp}±0.12	2.65^{cq}±0.17	2.71^{cp}±0.15	3.16^{dq}±0.07

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 - $PGF_2\alpha$

MEAN (±SE) SERUM VITAMIN E LEVELS IN REPEAT BREEDER COWS TREATED WITH ANTIOXIDANTS OR COX, INHIBITOR OR OESTRUS INDUCTION PROTOCOLS

Table 3 (a)		**Vitamin E (µg/ml)-Pregnant Animals**							
S.No.	**Treatment groups**	0^{th} day	5^{th} day	10^{th} day	15^{th} day	20^{th} day	30^{th} day	45^{th} day	60^{th} day
1.	Group I	1.10^{ap}±0.03	1.22^{bp}±0.07	1.67^{cp}±0.01	1.98^{dr}±0.02	2.01^{dq}±0.01	2.12^{ep}±0.05	2.22^{fp}±0.03	2.49^{gp}±0.08
2.	Group II	1.47^{aq}±0.05	1.89^{bs}±0.08	2.10^{cr}±0.03	2.15^{cs}±0.08	2.37^{ds}±0.08	2.48^{dr}±0.09	2.53^{es}±0.05	2.84^{fs}±0.07
3.	Group III	1.38^{aq}±0.04	1.42^{br}±0.01	1.60^{cp}±0.04	2.07^{ds}±0.04	2.16^{er}±0.06	2.20^{eq}±0.04	2.27^{fq}±0.04	2.68^{gr}±0.09
4.	Group IV	1.18^{ap}±0.08	1.33^{bq}±0.04	1.57^{cp}±0.09	1.63^{cp}±0.09	1.72^{dp}±0.03	2.13^{ep}±0.05	2.27^{fq}±0.02	2.44^{gp}±0.05
5.	Group V	1.37^{aq}±0.09	1.44^{ar}±0.07	1.81^{bq}±0.08	1.92^{cq}±0.02	2.01^{dq}±0.04	2.20^{eq}±0.04	2.39^{fr}±0.09	2.54^{gq}±0.01

Table 3 (b)		**Vitamin E (µg/ml)-Nonpregnant Animals**				
S. No.	**Treatment groups**	0^{th} day	5^{th} day	10^{th} day	15^{th} day	20^{th} day
1.	Group I	1.05^{ap}±0.04	1.15^{bp}±0.02	1.52^{cq}±0.09	1.90^{dr}±0.01	1.95^{eq}±0.03
2.	Group II	1.37^{as}±0.05	1.42^{br}±0.01	$1.64^{cr\pm}$0.01	2.10^{ds}±0.05	2.30^{es}±0.04
3.	Group III	1.31^{ar}±0.03	1.39^{bq}±0.06	1.48^{bp}±0.07	1.80^{cq}±0.08	2.10^{dr}±0.08
4.	Group IV	1.16^{aq}±0.05	1.30^{bq}±0.08	1.42^{cp}±0.05	1.55^{dp}±0.03	1.67^{ep}±0.02
5	Group V	1.34^{ar}±0.02	1.38^{aq}±0.04	1.64^{bq}±0.03	1.72^{cq}±0.05	1.89^{dq}±0.03

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 - $PGF_2\alpha$