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Effect of Antioxidant Administration during Transient Period on Progesterone Profile in Surti Buffaloes

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A B S T R A C T

The study was carried out on 20 Surti buffaloes during their transient period maintained at the Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat. These animals were divided into two groups of ten each. Buffaloes of group-I were treated with Inj. Vitamin E and Selenium (E-CARE Se) on 60th, 45th, 30thand 15thday before expected date of parturition and after parturition on 15th, 30thday, while buffaloes of group-II were treated with Inj. Normal Saline as placebo treatment IM. Blood samples were collected on similar days before injection as well as on the day of parturition, 45th and 60th days postpartum. The mean serum progesterone concentration did not differ significantly ($p>0.05$) at different days/phases interval in between treatment and control groups. The overall (before parturition) mean serum progesterone in the treatment group were non-significantly ($p>0.05$) higher as compared to control group but overall (after parturition) it was found non-significantly ($p>0.05$) lower in treatment group as compared to control group, respectively. Similarly, the mean serum progesterone did not differ significantly ($p>0.05$) in between pregnant &non-pregnant groups at different days interval except at 15th day after parturition it was significantly ($p<0.05$) lower in pregnant group as compared to non-pregnant group of Surti buffaloes, respectively.

Introduction

The transition or periparturient period, from 3 weeks before to 3 weeks after parturition, is a stressful time for dairy cows (Drackley, 1999). The production of free radicals leads to infertility because of their effect on sterioidogenic enzymes (Miller *et al.*, 1993), ovarian steriodogenic tissue (Margolin *et al.*, 1990) all are sensitive to free radicles damage. During gestation oxidative stress plays a role in the initiation of pre-term labor (Pressman *et al.*, 2003) and during normal parturition (Fainaru *et al.*, 2002) assuring ovulation, ovarian steroidogenesis, oocyte maturation,

blastocyst formation, luteolysis and luteal maintenance in pregnancy (Sugino *et al.*, 2000). Vitamin E is an important antioxidant that has been shown to play an important role in immuno responsiveness and health in dairy cows (Weiss and Spears, 2006).In Vitamin E and Selenium deficiency condition, free radicals accumulate and not only damage cell membranes, but also disrupt several processes linked to the synthesis of steroids (Seagerson and Libby, 1982) and prostaglandins (Harrison and Conrad, 1984). It is not surprising therefore that negative impacts of Vitamin E and Selenium deficiencies have been observed on various components of the reproductive events, including post-partum activities (Jie *et al.,* 2004). Steroid hormone like, progesterone is necessary for maintenance of pregnancy throughout gestation period in cattle and values are found varied during pregnancy, parturition and lactation and its estimation provides important clue about reproductive status of animals.

Materials and Methods

The present research work was undertaken on twenty (20) Surti buffaloes during their transient period i.e. two month before their expected date of parturition to two month after parturition, dividing into treatment (n=10) & control (n=10)) groups, at Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat, over a period from May, 2014 to April, 2015. The animals were fed green fodder, hay and compounded concentrate, as per the standard feeding schedule followed on the farm. The animals had free access to drinking water. The animals were also washed and sprinkled with water twice daily or were allowed to wallow in the pond during hot noon hours of summer season to reduce heat stress and to improve oestrus expression in them. In control group of 10 animals to which 10 ml normal saline injected IM on 60^{th}, 45^{th}, 30^{th} and 15^{th}day before expected date of parturition and after parturition on 15^{th}, 30^{th} day. Treatment Group of 10 animals to which the injectable product E-CARE Se (DL-α Tocopheryl Acetate I.P. equivalent to Tocopherol (Vitamin E) Base - 50mg, Sodium Selenite U.S.P. equivalent to Selenium Base -1.5mg in each ml) was administered IM on 60^{th}, 45^{th}, 30^{th} and 15^{th}day before expected date of parturition and after parturition on 15^{th}, 30^{th} day at the dose rate of 10 ml (500 mg vit. E and 15 mg Se.). Pregnancy diagnosis was carried out per rectally at 90 days post breeding. Again the group was made from all 20 animals irrespective of treatment and control group on the base of its conception in pregnant (n=13) and non-pregnant (n=7) groups.

Blood collection and laboratory examination

Blood samples were collected from all those selected animals on approximate day 60, 45, 30, 15 before the expected date of parturition, on the day of parturition and 15, 30, 45 and 60 day after parturition in serum clotting vaccutainer for serum. The serum was separated from vaccutainers containing 5 ml blood samples immediately after its collection and stored at –20°C in deep freezer until analysis. Serum progesterone (P_4) concentration was measured by standard Enzyme Linked Immuno Sorbent Assay (ELISA) technique using assay kits and procedure described by Product No. DNOV006, Nova Tec Immundiagnostica GmbH Technologie & Waldpark, Germany.

Statistical analysis

The tests of significance for pregnant vs. non-pregnant in treatment vs. control groups were made by Standard Student's paired 't' test. The fortnight-wise variation within the group was tested for each trait by using completely randomized design as well as the mean

differences between and within the groups were tested using Duncan's New Multiple Range Test (DNMRT) at 1 per cent and 5 per cent level of significance.

Results and Discussion

The mean serum progesterone concentration was observed to differ non-significantly between treatment and control groups at different peripartum intervals. The mean serum progesterone concentration was found non-significantly ($p>0.05$) higher at 60th day (3.660 ± 0.080 vs. 3.547 ± 0.094 ng/ml) but non-significantly ($p>0.05$) lower at 45th day (2.901 ± 0.115 vs. 3.062 ± 0.151 ng/ml) and again non-significantly ($p>0.05$) higher at 30th day (2.529 ± 0.157 vs. 2.393 ± 0.131 ng/ml) and 15th day (2.266 ± 0.149 vs. 2.170 ± 0.142 ng/ml)before parturition in treatment group as compared to control group. Whereas, non-significantly ($p>0.05$) lower (0.456 ± 0.076 vs. 0.515 ± 0.110 ng/ml) on day of calving and thereafter at 15th day (0.857 ± 0.080 vs. 1.128 ± 0.138 ng/ml) postpartum, after that non-significantly ($p>0.05$) higher (1.189 ± 0.181 vs. 1.008 ± 0.130 ng/ml), lower (1.170 ± 0.139 vs. 1.262 ± 0.228 ng/ml) and higher (2.083 ± 0.177 vs. 1.984 ± 0.175 ng/ml) at 30th, 45th and 60th day postpartum, respectively in treatment group as compared to control group (Table 1).

The prepartum progesterone concentrations were significantly ($p<0.05$) higher when compared between prepartum and postpartum phases at various interval in treatment and control groups, viz. at 60th day prepartum (3.660 ± 0.080 ng/ml; 3.547 ± 0.094 ng/ml) than that of 60th day postpartum (2.083 ± 0.177 ng/ml; 1.984 ± 0.175 ng/ml); 45th day prepartum (2.901 ± 0.115 ng/ml; 3.062 ± 0.151 ng/ml) than that of 45th day postpartum (1.170 ± 0.139 ng/ml; 1.262 ± 0.228 ng/ml); 30th day prepartum (2.529 ± 0.157 ng/ml; 2.393 ± 0.131 ng/ml) than that of 30th day postpartum (1.189 ± 0.181 ng/ml; 1.008 ± 0.130 ng/ml) and 15th day prepartum (2.266 ± 0.149 ng/ml; 2.170 ± 0.142 ng/ml) than that of 15th day postpartum (0.857 ± 0.080 ng/ml; 1.128 ± 0.138 ng/ml) in both treatment and control groups of Surti buffaloes, respectively (Table 1). The pooled mean serum progesterone concentration was found to decrease significantly ($p<0.05$) trend from 60th day (3.603 ± 0.061 ng/ml) to 45th day (2.981 ± 0.094 ng/ml) and 30th day (2.461 ± 0.101 ng/ml) and thereafter non-significantly ($p>0.05$) at 15th day prepartum (2.218 ± 0.101 ng/ml) and significantly ($p<0.05$) decreased on the day of calving (0.486 ± 0.065 ng/ml) and increased in trend significantly ($p<0.05$) at 15th day (0.992 ± 0.084 ng/ml) postpartum and thereafter increased non-significantly ($p>0.05$) at 30th day (1.099 ± 0.110 ng/ml), 45th day (1.216 ± 0.130 ng/ml) and significantly ($p<0.05$) at 60th day (2.034 ± 0.122 ng/ml) postpartum. Almost similar trend was observed in both treatment and control groups also (Table 1).

The mean serum progesterone concentration was also found non-significantly ($p>0.05$) different between overall pregnant and non-pregnant groups at different peripartum intervals, except at 15th day postpartum, where it was significantly ($p<0.05$) lower in pregnant group (0.875 ± 0.074 vs. 1.211 ± 0.176 ng/ml) as compared to non-pregnant group. In the pregnant and non-pregnant group, the mean serum progesterone concentration was non-significantly ($p>0.05$) higher at 60th and 30th day prepartum as well as, at 30th days postpartum, while rest of the days in prepartum and postpartum phase including the day of calving mean serum progesterone concentration was non-significantly ($p>0.05$) lower in pregnant as compared to non-pregnant groups (Table 1). The mean serum progesterone concentration gradually decreased from 60th day (3.628 ± 0.067 ng/ml; 3.558 ± 0.129 ng/ml) prepartum to

significantly ($p<0.05$) on the day of calving (0.408 ± 0.075 ng/ml; 0.629 ± 0.110 ng/ml) and it was gradually increased in trend from day of parturition to significantly ($p<0.05$) at 15th day (0.875 ± 0.074 vs. 1.211 ± 0.176 ng/ml) thereafter non-significantly ($p>0.05$) at 30th day (1.124 ± 0.161 vs. 1.052 ± 0.114 ng/ml), 45th day (1.165 ± 0.163 ng/ml; 1.313 ± 0.229 ng/ml) and increased significantly ($p<0.05$) at 60th day (1.961 ± 0.176 ng/ml; 2.169 ± 0.118 ng/ml) postpartum in pregnant and non-pregnant groups of buffaloes, respectively (Table 1).

The pooled mean serum progesterone concentration in the present study, was found significantly ($p<0.05$) decreased from 60th day (3.603± 0.061 ng/ml) prepartum to lowest (0.486 ± 0.065 ng/ml) on the day of calving and significantly ($p<0.05$) increased from 15th day (0.992 ± 0.084 ng/ml) till 60th day (2.034 ± 0.122 ng/ml) postpartum. The declined trend in plasma progesterone level during late gestation and lowest on the day of calving was in agreement with Smith *et al.,* (1973) in Holstein heifers, Arora and Pandey (1982) in Murrah buffaloes, Sawada *et al.,* (1988) in cows, El-Massry *et al.,* (1997) in buffaloes, Habeeb *et al.,* (1999) in Friesian cows, Chaiyabutr *et al.,* (2000) in crossbred cattle, Ishikawa *et al.,* (2004) in HF cows, Cernescu *et al.,* (2010) in cows, Mostafa *et al.,* (2014) in crossbred cows and Patel (2014) in HF crossbred cows. Moreover, Estergreen *et al.,* (1967) suggested that corpus luteum was the main source of progesterone during pregnancy in cows. Huffmann *et al.,* (1976) reported that uterine vein of cow did not make net contribution of circulating level of progesterone and there was no significant secretion from placenta into the foetal compartment. Therefore, it appears that decline in progesterone concentration before parturition may well have been resulted due to the regression of corpus luteum, which is probably brought about by $PGF_{2\alpha}$ at last stage in buffaloes as demonstrated in cows. In addition to this, vitamin E and selenium affect reproductive tissue through their antioxidant role as well as involvement in prostaglandin synthesis. Vitamin E has been implicated in the control of phospholipase A_2 activity, which is responsible for cleaving arachidonic acid from membranes phospholipids for synthesis of prostaglandins (Robinson *et al.,* 2006). Selenium preferentially accumulates in ovary, pituitary, placenta and adrenal glands suggesting specific requirements for selenium in these tissues for proper function (Hefnawy and Tortora, 2010). These aforesaid findings were in accordance with the concept of progesterone "block" being removed from the myometrium before or during the progress of parturition (Yannone *et al.,* 1968) and these findings may be attributed to present study in which higher (3.660 ± 0.080 vs. 3.547 ± 0.094 ng/ml) level of progesterone was observed at 60th day in vitamin E and Selenium treated and control groups that was declined to the lower level (0.456 ± 0.076 vs. 0.515 ± 0.110 ng/ml) at parturition as compared to control group.

Moreover, the overall trend of prepartum and postpartum progesterone profile found in buffaloes coincided well with that reported by Smith *et al.,* (1973), who observed progesterone concentration remained high until 2 day prepartum (7.6 ± 0.9 ng/ml) that fell down to 3.0 ± 0.7 ng/ml at one day prepartum and to 0.6 ± 0.1 ng/ml at parturition there after remained near this low level for 9 days postpartum in Holstein heifers. Similarly, the progesterone (P_4) concentration gradually decreased from 60 days (4.0 ± 0.2 ng/ml) to 5 days (2.0 ± 0.2 ng/ml) before parturition, thereafter rapidly decreased from 3 days (1.6 ± 0.1 ng/ml) to the day of parturition (0.11 ± 0.01 ng/ml) and remained at low level of less than 1 ng/ml between the day of parturition and 24 days postpartum in Holstein-Friesian cows (Ishikawa *et al.,* 2004).

Table.1 Mean serum Progesterone (P_4) levels (ng/ml) at different fortnightly intervals peripartum in antioxidant treated and control groups as well as pregnant and non-pregnant groups of Surti buffaloes (Mean±SE)

Peripartum Phases	Days	Progesterone (P_4) ng/ml						
		Treatment (n=10)	**Control (n=10)**	**'t' - Value**	**Pooled (n=20)**	**Pregnant (n=13)**	**Non-pregnant (n=7)**	**'t'-value**
Prepartum	60	3.660 ± 0.080^{f}	3.547 ± 0.094^{e}	0.914	3.603 ± 0.061^{f}	3.628 ± 0.067^{f}	3.558 ± 0.129^{d}	0.538
	45	2.901 ± 0.115^{e}	3.062 ± 0.151^{d}	0.848	2.981 ± 0.094^{e}	2.895 ± 0.096^{e}	3.141 ± 0.198^{d}	1.263
	30	2.529 ± 0.157^{de}	2.393 ± 0.131^{c}	0.666	2.461 ± 0.101^{d}	2.490 ± 0.140^{d}	2.408 ± 0.133^{c}	0.380
	15	2.266 ± 0.149^{cd}	2.170 ± 0.142^{c}	0.467	2.218 ± 0.101^{cd}	2.181 ± 0.148^{cd}	2.287 ± 0.094^{c}	0.492
	Overall	**$2.839\pm0.104^{**}$**	**$2.793\pm0.108^{**}$**	**0.305**	**$2.816\pm0.075^{**}$**	**$2.798\pm0.095^{**}$**	**$2.848\pm0.121^{**}$**	**0.317**
Day of Parturition	0	0.456 ± 0.076^{a}	0.515 ± 0.110^{a}	0.443	0.486 ± 0.065^{a}	0.408 ± 0.075^{a}	0.629 ± 0.110^{a}	1.693
Postpartum	15	0.857 ± 0.080^{b}	1.008 ± 0.130^{b}	1.701	0.992 ± 0.084^{b}	$0.875\pm0.074_{y}^{b}$	$1.211\pm 0.176_{x}^{b}$	2.075^{*}
	30	1.189 ± 0.181^{b}	1.128 ± 0.138^{b}	0.811	1.099 ± 0.110^{b}	1.124 ± 0.161^{b}	1.052 ± 0.114^{ab}	0.305
	45	1.170 ± 0.139^{b}	1.262 ± 0.228^{b}	0.345	1.216 ± 0.130^{b}	1.165 ± 0.163^{b}	1.313 ± 0.229^{b}	0.532
	60	2.083 ± 0.177^{c}	1.984 ± 0.175^{c}	0.398	2.034 ± 0.122^{c}	1.961 ± 0.176^{c}	2.169 ± 0.118^{c}	0.811
	Overall	**1.325±0.103**	**1.346±0.103**	**0.144**	**1.335±0.072**	**1.281±0.092**	**1.436±0.114**	**1.026**
Overall	**'t' -Value**	10.344^{**}	9.729^{**}	--	14.275^{**}	11.464^{**}	8.465^{**}	--
	P-Value	0.000	0.000	--	0.000	0.000	0.000	--
	Pooled	**$1.901\pm0.114_{x}$**	**$1.897\pm0.111_{x}$**	**0.029**	**1.899±0.079**	**$1.858\pm0.101_{x}$**	**$1.974\pm0.128_{x}$**	**0.695**

Means bearing different superscripts (a,b,c) within a column (between phase intervals) differ significantly ($p<0.05$). Means bearing different subscripts (x,y,z) within a row (between groups) differ significantly ($p<0.05$); $^{**}p<0.01$ between pre- and post-partum phase.

Pargaonkar and Kaikini (1989) reported the level of progesterone in non-descript cows during 280 days of pregnancy and 5th day postpartum as 5.90 ng/ml and 0.23 ng/ml, respectively corroborated well with the present study.

In addition to this, the findings of linear increased in plasma progesterone concentration from parturition to postpartum period in the present study were also in close agreement with the reports of Edgerton and Hafs (1973) and Echternkamp and Hansel (1973) in non-suckled dairy cows; Pahwa and Pande (1983) in Murrah buffaloes, who all reported decreased progesterone level on the day of calving that increased linearly after parturition to rise with appearance of ovarian follicular activity.

Significant increase in progesterone occurred by 15th day postpartum in the present study. These deviations from low level after parturition probably resulted from follicular luteinization or adrenal contribution (Wagner *et al.,* 1969), since the postpartum level of progesterone remained low until the initiation of CL formation following ovulation (Echternkamp and Hansel, 1973). Short (1962) suggested that ovarian follicles could be a significant source of progesterone at 5 to 7 day after calving, but no such evidence exists in buffalo so far. Similarly, Edgerton and Hafs (1973) reported a linear increase in plasma progesterone concentration at one week to eight weeks postpartum in non-suckled dairy cows, whereas it remained at a low level (<0.3 ng/ml) from parturition until first oestrus postpartum in non-suckled cows (Echternkamp and Hansel, 1973). Arora and Pandey (1982) recorded the lowest overall mean level of progesterone on the day of parturition, followed by a significant ($p<0.05$) increase by day 16th postpartum in Murrah buffaloes. Similarly, Pahwa and Pandey (1983) and Khasatiya *et al.,* (2006) in buffaloes, and Patel *et al.,* (2013), and Patel (2014) in cows also reported increased progesterone level around 15th day postpartum. While, Smolders *et al.,* (1996) reported progesterone concentration first time increased on 28th day after calving and the level was found increased around 30th day postpartum by others (Cernescu *et al.,* 2010) and very much earlier transient increased trend of mean plasma progesterone concentration as 0.74 ± 0.09 ng/ml and 1.01 ± 0.12 ng/ml at 6 hrs and 24 hrs postpartum from the day of parturition, respectively as compared to present study has been reported by Patel *et al.,* (2013) in HF cows.

Further, the explanation for abrupt increasing progesterone trend at 15th to 30th day postpartum might be attributed to early onset of follicular activity and ovulation contra lateral to the gravid horn or might be due to gradual rise in progesterone prior to the first postpartum estrus (Pope *et al.,* 1969). Moreover, the source of this progesterone had not been elucidated, since there were apparently no functional corpora lutea present, it was conceivable that either luteinized follicles or adrenal glands might be responsible for the pre-estrual progesterone secretion. While, Callahan *et al.,* (1971) reported that from day 8 to 20 postpartum size of ovaries increased in normal dairy cows. The sudden increasing serum progesterone concentration trend observed at 15th to 30th day after calving might give some clue on hidden follicular phenomenon that usually take place postpartum. Similarly, Smolders *et al.,* (1996) also reported first increase in progesterone by about 28 days after calving and in 28 per cent of the cows, first oestrus cycle was normal with luteal phase of 12-17 days, but at the second cycle, 56 per cent of cows had a normal luteal phase.

In addition to this, mean serum progesterone concentration increased (>1 ng/ml) from the

day 30th postpartum to the day 45th postpartum in the present study, might be due to establishment of normal pattern of progesterone secretion in cyclic animals. These findings were supported by Tegegne *et al.,* (1993), who observed the African Zebu cows that cycled earlier expressed irregular and short-lived progesterone rises (>1 ng/ml). In the present study, buffaloes supplemented with injectable vitamin E and Selenium at 60th day, 45th day, 30th day, 15th day before parturition and 15th and 30th day after parturition had higher pregnancy rate as compared to control group of Surti buffaloes (80 vs. 50 %), suggesting little bit earlier onset of follicular activity with higher conception rate in treated group.

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Conflict of interest statement

Authors declare that they have no conflict of interest.

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