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Research Article

INFLUENCE OF PARENTERAL ADMINISTRATION OF VITAMIN E AND SELENIUM DURING PERIPARTURIENT PERIOD ON THYROID (T_3& T_4) PROFILE IN SURTI BUFFALOES

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A study was conducted on twenty Surti buffaloes during their transient period maintained at the LRS, Navsari Agricultural University, Navsari, Gujarat. The animals were divided into two groups comprising of ten animals in each group as: Group-I: Treatment group of Surti buffaloes treated with inj. vitamin E and selenium (E-CARE Se) on 60th, 45th, 30th and 15th day before expected date of parturition and after parturition on 15th, 30th day intramuscular (IM) and Group-II: Control group of Surti buffaloes given inj. normal saline (IM) as placebo treatment. Blood samples were collected on same days before injection as well as on the day of parturition, 45 and 60 days postpartum in serum clotting vacutainer. The mean serum triiodothyronine (T_3) concentration was found to be significantly ($p<0.05$) higher on the day of parturition and significantly lower values at 30th day and 60th day postpartum in treatment than control group, while, non-significantly lower values at 15th day and higher at 45th day postpartum. The mean serum thyroxine (T_4) concentration was found non-significantly lower at 60th day prepartum in treatment than control group, but it was significantly higher at 45th day and non-significantly higher at 30th day and again significantly higher at 15th day before parturition and on the day of calving and thereafter fluctuated non-significantly higher at 15th day, lower at 30th day, higher at 45th day and non-significantly lower at 60th day after parturition. The mean serum T_3 and T_4 concentration did not differ significantly between pregnant and non-pregnant groups at any of the days studied.

Key words: Periparturient period, Selenium, Surti buffalo, Thyroid profile, Vitamin E

The transition or periparturient period, from 3 weeks before to 3 weeks after parturition, is a stressful time for dairy cows (Drackley, 1999). During the transition period,

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immunosuppression commonly occurs and cows exhibit great susceptibility to a number of diseases (Mallard *et al.*, 1998). 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). Moreover, vitamin E is involved in the formation of leukotrienes, prostaglandin and prostacyclin and has got a role in the duration of postpartum interval. 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).The hormonal activity of thyroid gland has an important role in the transitional period for determining cell metabolism of lipid and carbohydrates, and the lactation course itself by its thyroid hormones (Nikolic *et al.*, 1997). Thyroid hormones, triiodthyronine (T_3) and thyroxin (T_4) have a major role in differentiation, growth and development of animals (Bani Ismail *et al.*, 2009) and its estimation is a well-accepted indicator and it gives clear-cut idea by throwing the light on its level during two month before to two month after the parturition.

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) and 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 intramuscular on 60^{th}, 45^{th}, 30^{th} and 15^{th} day before expected date of parturition (prepartum) 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 intramuscular on 60^{th}, 45^{th}, 30^{th} and 15^{th} day before expected date of parturition (prepartum) 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 samples were collected from all those selected animals before the treatment as per the groups 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 vacutainer and serum was separated immediately after its collection and stored at –20°C in deep freezer until analysis. Serum concentrations of triiodothyroxine (T_3) and thyroxine (T_4) were measured by standard Enzyme Linked Immuno Sorbent Assay (ELISA) technique using assay kits and procedures described by Products No. DNOV053 & DNOV054, NovaTec Immundiagnostica GmbH Technologie &Waldpark, Germany, respectively.

The tests of significance for treatment vs. control and pregnant vs. non-pregnant groups were made by Standard Paired Student '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 (DMRT) at 1 per cent and 5 per cent level of significance.

RESULTS

The mean serum T_3 concentration was found to be significantly ($p<0.05$) higher on the day of parturition and significantly ($p<0.05$ and $p<0.01$) lower values at 30th day and 60th day postpartum in treatment than control group. While, non-significantly ($p>0.05$) lower values of 3.014 ± 0.027 ng/mL vs. 3.043 ± 0.016 ng/mL at 15th day and higher 3.273 ± 0.014 ng/mL vs. 3.257 ± 0.012 ng/mL were found at 45th day postpartum in treatment group as compared to control group (Table 1).

Overall mean serum T_3 concentrations were found non-significantly ($p>0.05$) and significantly ($p<0.05$) lower before parturition as compared to after parturition in both treatment (3.149 ± 0.017 ng/mL vs. 3.170 ± 0.019 ng/mL) and control (3.141 ± 0.021 ng/mL vs. 3.201 ± 0.019 ng/mL) groups with overall pooled mean of 3.145 ± 0.013 ng/mL vs. 3.185 ± 0.013 ng/mL ($P<0.05$) (Table 1). Whereas, overall prepartum mean serum T_3 concentration in the treatment group was found non-significantly ($p>0.05$) higher as compared to control group (3.149 ± 0.017 ng/mL vs. 3.141 ± 0.021 ng/mL), while overall postpartum mean was found non-significantly ($p>0.05$) lower (3.170 ± 0.019 ng/mL vs. 3.201 ± 0.019 ng/mL), and thereby finally the overall pooled mean serum T_3 concentration was non-significantly ($p>0.05$) higher in treatment group as compared to control group (3.106 ± 0.021 ng/mL vs. 3.099 ± 0.026 ng/mL)(Table 1).

Moreover, the prepartum mean serum triiodothyronine levels in the treatment group were found non-significantly ($p>0.05$) lower when compared between prepartum and postpartum phases at respective interval, viz. at 60th day prepartum and postpartum; 45th day prepartum and postpartum; 30th day prepartum and postpartum and 15th day prepartum and postpartum. Whereas, in control group it was significantly ($p<0.05$) lower at 60th day prepartum than that of 60th day postpartum; 30th day prepartum than that of 30th day postpartum and 15th day prepartum than that of 15th day postpartum (Table 1).

The mean serum triiodothyronine concentration was also found non-significantly ($p>0.05$) different between pregnant and non-pregnant groups at different intervals peripartum. On the day of calving non-significantly lower values was observed in pregnant group as compared to non-pregnant group (2.580 ± 0.052 ng/mL vs. 2.631 ± 0.052 ng/mL) (Table 1).

The mean serum triiodothyronine concentration was gradually decreased in trend from 60th day (3.233 ± 0.004 ng/mL; 3.245 ± 0.009 ng/mL) prepartum to significantly ($p<0.05$) on the day of calving (2.580 ± 0.052 ng/mL; 2.631 ± 0.052 ng/mL) and it was gradually increased in trend from day of parturition to significantly ($p<0.05$) on 60th day (3.285 ± 0.012 ng/mL; 3.329 ± 0.025 ng/mL) postpartum in pregnant and non-pregnant groups of Surti buffaloes, respectively (Table 1).

The mean serum thyroxine concentration was found non-significantly ($p>0.05$) lower at 60th day prepartum in treatment than control group, but it was significantly ($p<0.05$) higher at 45th day and non-significantly ($p>0.05$) higher at 30th day and again significantly ($p<0.01$) higher at 15th day before parturition and on the day of calving and thereafter fluctuated non-significantly($p>0.05$) higher at 15th day, lower at 30th day, higher at 45th day and non-significantly ($p>0.05$) lower at 60th day after parturition (Table 2).

The overall prepartum and postpartum mean serum thyroxine concentrations in the treatment group were found non-significantly ($p>0.05$) higher as compared to control group as 59.075 ± 0.984 ng/mL vs. 56.494 ± 1.309 ng/mL and 58.675 ± 0.987 ng/mL vs. 57.475 ± 1.156 ng/mL, and thereby overall pooled mean 57.267 ± 0.794 ng/mL vs. 54.953 ± 0.986 ng/mL (Table 2).

In addition to this, the prepartum mean serum thyroxine level was non-significantly differ when compared between prepartum and postpartum at various intervals (Table 2).

In the pregnant and non-pregnant groups, the mean serum thyroxine concentration was found non-significantly ($p>0.05$) higher during different phases/days in the pregnant group as compared to non-pregnant groups (Table 2).

Table 1. Mean serum triiodothyronine (T_3) 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	Triiodothyronine (T_3) ng/mL: Treatment (n=10)	Control (n=10)	't' Value	Pooled (n=20)	Pregnant (n=13)	Non-pregnant (n=7)	't' value
Prepartum	60	3.228_{y}^{d} ±0.003	3.246_{x}^{e} ±0.007	-2.583^{*}	3.237^{e} ±0.004	3.233^{d} ±0.004	3.245^{d} ±0.009	1.485
	45	3.261^{d} ±0.006	3.275^{e} ±0.006	-1.658	3.268^{ef} ±0.004	3.265^{d} ±0.006	3.275^{de} ±0.007	1.089
	30	3.096^{c} ±0.011	3.068^{c} ±0.018	1.294	3.082^{c} ±0.011	3.094^{c} ±0.011	3.060^{b} ±0.022	1.527
	15	3.013^{b} ±0.013	2.974^{b} ±0.022	1.513	2.994^{b} ±0.013	2.988^{b} ±0.019	3.005^{b} ±0.017	0.599
	Overall	**3.149 ±0.017**	**3.141 ±0.021***	**0.315**	**3.145 ±0.013***	**3.145 ±0.016**	**3.146 ±0.023**	**0.052**
Day of parturition	0	2.674_{x}^{a} ±0.057	2.521_{y}^{a} ±0.039	2.229^{*}	2.598^{a} ±0.038	2.580^{a} ±0.052	2.631^{a} ±0.052	0.631
Postpartum	15	3.014^{b} ±0.027	3.043^{c} ±0.016	-0.902	3.028^{b} ±0.016	3.021^{b} ±0.022	3.042^{b} ±0.018	0.642
	30	3.126_{y}^{c} ±0.005	3.169_{x}^{d} ±0.018	-2.282^{*}	3.148^{d} ±0.011	3.139^{c} ±0.010	3.163^{c} ±0.024	1.094
	45	3.273^{d} ±0.014	3.257^{e} ±0.012	0.891	3.265^{ef} ±0.009	3.274^{d} ±0.011	3.248^{d} ±0.016	1.353
	60	3.265_{y}^{d} ±0.009	3.336_{x}^{f} ±0.016	-3.795^{**}	3.300^{f} ±0.012	3.285^{d} ±0.012	3.329^{e} ±0.025	1.799
	Overall	**3.170 ±0.019**	**3.201 ±0.019**	**-1.180**	**3.185 ±0.013**	**3.180 ±0.017**	**3.196 ±0.023**	**0.563**
Overall	't'- Value	-0.800	-2.113^{*}	—	-2.117^{*}	-1.493	-1.521	—
	P- Value	0.426	0.038	—	0.036	0.139	0.134	—
	Pooled	**3.106 ±0.021**	**3.099 ±0.026**	**0.208**	**3.102 ±0.016**	**3.098 ±0.021**	**3.111 ±0.027**	**0.389**

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); *p<0.05 between prepartum and postpartum phase

Table 2. Mean serum thyroxine (T_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	Thyroxine (T_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	63.899^{d} ±2.099	66.874^{e} ±1.459	1.164	65.387^{e} ± 1.290	66.349^{f} ±1.488	63.599^{e} ±2.452	1.018
	45	62.200_{x}^{d} ±1.263	58.300_{y}^{d} ±1.001	2.420^{*}	60.250^{d} ± 0.903	60.385^{d} ±1.141	60.000^{de} ±1.589	0.198
	30	57.200^{c} ±1.200	53.900^{c} ±1.090	2.036	55.550^{c} ± 0.875	56.308^{c} ±1.190	54.143^{c} ±1.079	1.193
	15	53.000_{x}^{b} ±1.085	46.900_{y}^{b} ±1.433	3.393^{**}	49.950^{b} ± 1.120	50.615^{b} ±1.269	48.714^{b} ±2.233	0.802
	Overall	**59.075 ±0.984**	**56.494 ±1.309**	**1.577**	**57.784 ±0.826**	**58.414 ±1.016**	**56.614 ±1.417**	**1.040**
Day of Parturition	0	44.400_{x}^{a} ±1.310	38.700_{y}^{a} ±0.716	3.819^{**}	41.550^{a} ± 0.977	41.692^{a} ±1.195	41.286^{a} ±1.822	0.193
Postpartum	15	51.000^{b} ±1.193	47.000^{b} ±1.619	1.989	49.000^{b} ±1.081	50.154^{b} ±1.372	46.857^{b} ±1.550	1.502
	30	56.800^{c} ±0.975	57.700^{d} ±0.700	0.750	57.250^{c} ±0.593	57.077^{c} ±0.796	57.571^{d} ±0.896	0.389
	45	62.400^{d} ±1.166	60.400^{d} ±0.686	1.478	61.400^{d} ±0.697	61.923^{de} ±0.895	60.429^{de} ±1.088	1.023
	60	64.500^{d} ±0.957	64.800^{e} ±0.680	0.255	64.650^{e} ± 0.573	64.846^{ef} ±0.775	64.286^{e} ±0.837	0.457
	Overall	**58.675 ±0.987**	**57.475 ±1.156**	**0.789**	**58.075 ±0.758**	**58.500 ±0.915**	**57.286 ±1.355**	**0.762**
Overall	't'- value	0.287	0.562	—	0.259	0.063	0.343	—
	P-Value	0.775	0.576	—	0.796	0.950	0.733	—
	Pooled	**57.267 ±0.794**	**54.953 ±0.986**	**1.827**	**56.110 ±0.637**	**56.594 ±0.788**	**55.209 ±1.083**	**1.037**

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<0.0)

Further, the overall mean serum thyroxine level in the present study were found non-significantly ($p>0.05$) lower before parturition as compared to after parturition as 58.414 ± 1.016 ng/mL vs. 58.500 ± 0.915 ng/mL and 56.614 ± 1.417 ng/mL vs. 57.286 ± 1.355 ng/mL in the pregnant and non-pregnant groups, respectively (Table 2).

Overall mean serum thyroxine concentration before and after parturition in the pregnant group were found non-significantly ($p>0.05$) higher as compared to non-pregnant group as 58.414 ± 1.016 ng/mL vs. 56.614 ± 1.417 ng/mL and 58.500 ± 0.915 ng/mL vs. 57.286 ± 1.355 ng/mL, respectively and the overall pooled mean serum thyroxine concentration was also found non-significantly ($p>0.05$) higher 56.594 ± 0.788 ng/mL vs. 55.209 ± 1.083 ng/mL in pregnant group as compared to non-pregnant group, respectively (Table 2).

The mean serum thyroxine level was significantly ($p<0.05$) and non-significantly ($p>0.05$) decreased in trend from 60th day (66.349 ± 1.488 ng/mL; 63.599 ± 2.452 ng/mL) prepartum to significantly ($p<0.05$) on the day of calving (41.692 ± 1.195 ng/mL; 41.286 ± 1.822 ng/mL) and it was again found increased significantly ($p<0.05$) and non-significantly ($p>0.05$) in trend from day of parturition to significantly ($p<0.05$) higher concentration were observed at 60th day (64.846 ± 0.775 ng/mL; 64.286 ± 0.837 ng/mL) postpartum in pregnant and non-pregnant groups of Surti buffaloes, respectively (Table 2).

DISCUSSION

In the present study, the overall pooled mean values of T_3 and T_4 were observed as 3.102 ± 0.016 ng/mL and 56.110 ± 0.637 ng/mL, respectively, which were higher as compared to average plasma T_3 (1.83 ± 0.04 ng/mL) and T_4 (48.25 ± 0.54 ng/mL) reported by Aggarwal and Singh (2010) in Murrah buffaloes in hot-humid season; 27.43 ng/mL and 24.45 ng/mL mean value of T_4 on the day of calving and 21st day postpartum, respectively, reported by Lohan *et al.* (1989) and 0.75 to 1.25 ng/mL and 21.08 to 25.75 ng/mL for T_3 and T_4 concentrations before and after parturition reported by Cernescu *et al.* (2010) in cows.

Moreover, the present study also showed that thyroid activity could be modified in buffaloes injected with vitamin E plus selenium, as those buffaloes received injection of vitamin E plus selenium had significantly ($p<0.05$ & $p<0.01$) higher plasma levels of T_3 (2.674 ± 0.057 ng/mL vs. 2.521 ± 0.039 ng/mL) and T_4 (44.400 ± 1.310 ng/mL vs. 38.700 ± 0.716 ng/mL) at calving than that of untreated buffaloes. Similar response was also reported by Soliman *et al.* (2012) in ewes, and Pavlata *et al.* (2004) in dairy cows. The cows administered parentally with vitamin E and Se at 4th week prior to parturition had significantly ($p<0.05$) increased T_3 and non-significantly increased T_4 concentration (3.05 ± 0.42 nmol/L vs. 1.88 ± 0.71 nmol/L and 56.10 ± 13.18 nmol/L vs. 48.64 ± 15.98 nmol/L), respectively at parturition (Pavlata *et al.,* 2004). Further, such significant increases in T_3 level could probably be explained by an increased activity of iodothyronine deiodinase, which involves in the conversion of T_4 into active T_3 as its activity is influenced by Selenium (Kohrle, 2000).

Similarly, positive effect of Se on T_3 concentrations has been documented by Wichtel *et al.* (1996) and Awadeh *et al.* (1998), who noticed increased T_3 concentrations in cows after higher Se intake, as selenium is essential for normal thyroid hormone metabolism, and selenoperoxidases protect the thyroid gland from peroxides produced during the synthesis of hormones, a matter that play an important role in regulation of thyroid status in animal tissues in various physiological and pathological situations (Arthur *et al.*, 1992). It can therefore be concluded from our observations that, after a repeated administration of a supplement containing the combination of selenium and vitamin E during the period prior to parturition a positive effect of increased selenium intake resulted on higher T_3 concentrations.

The present trend of serum triiodothyronine (T_3) and thyroxine (T_4) level were in agreement with Alkalby and Mohammad (2013), who found that high level of thyroid hormones were recorded in late pregnancy which was followed by a significant ($p<0.05$) decrease in the periparturition period and earliest day of lactation in cows. Moreover, various research workers indicated that cows in postpartum negative energy balance responded to decrease the level of T_3 and T_4 and increase the level of rT_3 (Yambayamba *et al.*,1996) but in the present study, significant ($p<0.05$ and $p<0.01$) increase in mean serum T_3 and T_4 level was observed at 15^{th} day postpartum and thereafter significant ($p<0.01$) increased in T_3 at 60^{th} day postpartum, when it compared with the day of parturition, in both treatment and control groups; suggestive of the fact that such negative energy balance might not be there, thereafter in the buffaloes.

The significant decrease in free T_3 and T_4 levels noted in the study during late pregnancy may be due to high estrogen level during advanced pregnancy which stimulate production of thyroid hormone-binding globulin, leading to a rise in level of bound T_3 & T_4 and simultaneous drop in level of free T_3 & T_4, with the drop in thyroid binding globulin following delivery, level of free T_3 & T_4 increase.

Moreover, in the present study, T_3 and T_4 levels were found significantly ($p<0.05$) decreased from advanced pregnancy (two month before parturition) as parturition approached and lowest on the day of calving as well as, at one month postpartum T_3 and T_4 levels were found significantly ($p<0.05$) increased when compared with the levels at day of calving. These results were in agreement with those found by Pichaicharnarong *et al.* (1982) who demonstrated that the total serum T_3 and T_4 of late pregnant buffaloes (9-10 month) markedly decreased in comparison with that of 8–9 month of pregnancy and at 1 month postpartum, T_3 and T_4 rose to 143.4 ± 33.0 µg/100 mL and 5.1 ± 3.4 µg/100 mL, respectively. Lohan *et al.* (1989) also reported rising in T_4 level trend after 21[st] day of parturition. This could be regarded as a part of a complex multi-hormonal regulation of follicular steroidogenesis in bovine.

Djokovic *et al.* (2014) in Holstein cows reported T_3 and T_4 concentrations started to decrease before parturition and minimum levels were found soon after parturition. This might be due to increase in metabolic rate after calving as an anabolic process to rebuild destructive tissues and to compensate the deficiency of blood metabolites, which could occur during pregnancy.

In the present study, no significant ($p>0.05$) differeces were observed in T_4 concentration at 15th, 30th, 45th and 60th day before and after parturition as well as no significant ($p>0.05$) differences were observed between prepartum and postpartum T_3 and T_4 levels in the treated group and T_4 in the control group. However, in the control group T_3 values were found significantly ($p<0.05$) lower as compared to treatment group. Moreover, significantly ($p<0.05$) lower T_3 and T_4 concentration was found at two week before parturition than that of one month after parturition in Surti buffaloes. In agreement with the present study, Chaiyabutr *et al.* (2000) also found significantly higher T_3 and T_4 levels at 30 days postpartum, compared to late pregnant i.e. 20-23 days before parturition.

On the contrary, Teama and Gad (2014) reported significantly ($p<0.05$) higher T_4 concentration in one, two, three and four week before parturition as compared to one, two, three and four week postpartum in crossbred cows. Mostafa *et al.* (2014) reported non-significantly ($p>0.05$) lower T_3 and T_4 concentration at two week before parturition than that of one month after parturition in crossbred cows.

In general, the lower and/or higher level of thyroid hormones T_3 and T_4 observed by some authors as compared to present findings might be due to breed, species and nutritional status apart from analytical variation. Though, lower concentrations of thyroid hormone have been suggested to be associated with low reproductive performance in the postpartum cow (Huszenicza *et al.*, 2002).

The study reported that after a repeated administration of a combination of selenium and vitamin E during the peripartum period, a positive effect of increased selenium intake results on higher T_3 concentrations. Further, the decreased level of T_4 might support the mammary gland in partitioning of nutrients between mammary and non-mammary tissue at the onset of lactation.

Conflict of Interest: Authors declare that there is no conflict of interest regarding the present research work.

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