B-4938
[1-10]

RESEARCH ARTICLE Indian Journal of Animal Research

Peripartum Supplementation of Rumen Protected Choline and Fat and Se+Vit E in Gir Cows Economically Improves Endocrine Profile and Reproductive Performance

A.J. Dhami, K.B. Vala[1], M.R. Chavda[1], D.N. Patel, S.S. Parikh[2], B.B. Javia[1], N.P. Sarvaiya **10.18805/IJAR.B-4938**

ABSTRACT

Background: The negative energy balance around calving and in early lactation is the major cause of increased incidence of postpartum metabolic disorders, reduced milk production and impaired reproductive performance. This study was aimed to evaluate the effect of rumen protected choline, fat supplementation and Vit-E and selenium injections on plasma endocrine profile, postpartum productivity, fertility and economics in transitional Gir cows.

Methods: Forty advanced pregnant multiprous Gir cows of an organized farm were randomly distributed into five equal groups (T1 to T5; n=8 each) and were fed and managed individually from 30 days prepartum till 60 days postpartum. Cows in T1 (control) group were fed basal diet, while cows in treatment groups received basal diet + supplementation of rumen protected choline-RPC @ 45 g/d/cow (T2), rumen protected fat-RPF @ 80 g/d/cow from -30 to +15 days peripartum and then @ 10 g/kg milk yield (T3), RPC and RPF in combination @ as above (T4) and Inj. E-Care Se(R) 10 ml i/m (Vit-E 500 mg and Se 15 mg) on days -30, -15, 0, +15, +30 and +45 peripartum (T5) along with blood samples for estimation of plasma levels of insulin, IGF1, PGFM, estradiol (E_2), progesterone (P_4) and cortisol. The puerperal events and fertility parameters were recorded till 10 months postpartum.

Result: The fortnightly plasma P_4 concentration decreased, while E_2, PGFM and cortisol increased gradually and significantly (P<0.01) from 30 days prepartum to day of calving and thereafter P_4, E_2 and cortisol remained basal or very low, while PGFM decreased till 45-60 days postpartum, overall and in all groups. The treatment effect was significant (P<0.01) only for E_2 and PGFM, with higher values of E_2 in T3 and T4 groups and of PGFM in T1 and T2 than in T5 group. The insulin and IGF1 concentrations fluctuated insignificantly during prepartum phase with significantly lowest values till 15 and 30 days postpartum and thereafter only IGF1 increased significantly by day 60 postpartum. The treatment effect was also significant at some intervals with overall highest values of both the metabolic hormones in T1 followed by T5 and T2 than T3 and T4 groups. The uterine involution was significantly earlier in all treatment groups than in control. The first heat postpartum (days) was significantly earlier in group T5 (33.6±3.4), delayed in group T1 (63.5±9.5) and intermediate in Group T2 to T4. The conception rates postpartum were 50.0, 75.0, 62.5, 75.0 and 87.5% in groups T1 to T5, respectively, with significantly reduced service period (days) in T5 (96.3±14.6), followed by T2 (137.3±11.2), than T1, T3 and T4 groups (187 to 214 days). The overall net returns over feed cost of milk yield and reduced service period in T2, T3, T4 and T5 groups over control T1 were Rs. +15898, +6150, +7165 and +24060, respectively. The findings reflected the beneficial and economic role of RPF alone or in combination with RPC and of Se + Vit E supplementation on peripartum endocrine profile and improved postpartum fertility in Gir cows.

Key words: Economics, Endocrine profile, Gir cows, Peripartum supplementation, Postpartum fertility, Rumen protected choline and fat, Vit-E plus Selenium.

INTRODUCTION

The postpartum health, production and reproduction of dairy animals largely depend on the nutritional and energy status peripartum. The negative energy balance (NEB) around calving and in early lactation is the major cause of increased incidence of postpartum metabolic diseases, reduced milk production and impaired reproductive performance. NEB is a normal adaptive mechanism in high yielding transition dairy animals by mobilizing adipose tissue reserve (Wankhade *et al.,* 2017), as a result more non-esterified fatty acids (NEFAs) are drained towards liver (Drackley *et al.,* 2014). Though the resumption of ovarian activity is initiated in early lactation, NEB has adverse effects on subsequent productivity and fertility due to poor conception rates (Wathes *et al.,* 2007). At the time of parturition, progesterone, estradiol concentrations cascade to basal level and cortisol to peak level facilitating parturition within 3-5 days (Dhami *et al.,* 2017; Vala *et al.,* 2019).

Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand-388 001, Gujarat, India.
[1]College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh-362 001, Gujarat, India.
[2]Cattle Breeding Farm, Junagdh Agricultural University, Junagadh-362 001, Gujarat, India.

Corresponding Author: A.J. Dhami, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand-388 001, Gujarat, India. Email: ajdhami59@gmail.com

How to cite this article: Dhami, A.J., Vala, K.B., Chavda, M.R., Patel, D.N., Parikh, S.S., Javia, B.B. and Sarvaiya, N.P. (2022). Peripartum Supplementation of Rumen Protected Choline and Fat and Se+Vit E in Gir Cows Economically Improves Endocrine Profile and Reproductive Performance. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4938.

Submitted: 19-05-2022 **Accepted:** 08-12-2022 **Online:** 14-12-2022

Choline is involved in the metabolism of fatty acids in the liver and plays an important role in very low density lipoprotein synthesis and thereby contributes to fat export from the liver (Acharya *et al.,* 2019a). The bioavailability of free choline and phosphatidyl choline present in most of feedstuffs and diet is very low in ruminants (<30%) as they are rapidly degraded by the rumen micro-organisms, hence these must be supplemented in the protected form (Elek *et al.,* 2008). Supplementing rumen protected fat (RPF) to high producing lactating cows can enhance energy density of ration and energy intake in early lactation without compromising rumen cellulolytic bacterial activity and reduces the deleterious effect of NEB during early lactation (Ganjkhanlou *et al.,* 2009). Supplementation of bypass fat before parturition could improve lactation performance as well as lower the metabolic problems (Duske *et al.,* 2009). Vit-E and selenium are known antioxidants with established positive effects in body metabolism and fertility (Mavi *et al.,* 2006; Jovanovic *et al*., 2015; Hosnedlova *et al*., 2017). Considering the role of rumen protected choline and fat and vit-E and selenium in the diet of transitional dairy cows towards economically improving postpartum productivity and fertility (Singh *et al*., 2014; Sankhpal *et al.,* 2016, Anonymous, 2020), this study was planned to evaluate the effect of peripartum supplementation of rumen protected choline (RPC), rumen protected fat (RPF) alone and in combination and Vit-E + Selenium on metabolic and reproductive hormones, postpartum fertility and its economics in Gir cows.

MATERIALS AND METHODS

Selection and management of animals

This investigation was carried out during the period from August, 2020 to June, 2021 on 40 selected advanced pregnant Gir cows of Cattle Breeding Farm, JAU, Junagadh, following ethical approval of the Institutional Animal Ethics Committee of the College. The cows were equally and randomly divided into five groups comprising of 8 animals in each, *viz.,* T1 (control), T2 (RPC), T3 (RPF), T4 (RPC + RPF) and T5 (Vit-E + Se). In T1 group, animals were fed with basal diet of 10 kg green sorghum, mature pasture grass/hay *ad libitum* and compound cattle feed with 250 g maize bhardo and cotton seed cake to meet their nutrient requirement as per ICAR (2013) feeding standards followed on the farm. The cows in groups T2 to T5 received additional supplements, *viz.*, RPC @ 45 g/day (T2, Kemin Industries South Asia Pvt. Ltd), RPF @ 80 g/d from -30 to +15 days peripartum and then @ 10 g/kg milk yield (T3, Kemin Industries South Asia Pvt. Ltd), RPC @ 45 g/day + RPF @ 80 g/d from -30 to +15 days peripartum and then @ 10 g/kg milk yield, orally (T4) and Vit-E 500 mg + Se 15 mg, i/m (T5, Inj. E-Care Se(R), 10 ml fortnightly, Cadila-Zydus Pharma) along with basal diet, starting from 30 days before expected date of calving to 60 days postpartum. All the animals were housed in well ventilated hygienic sheds with access to wholesome drinking water *ad libitum.*

Blood analysis and postpartum reproductive parameters

Jugular blood samples were collected in heparinised vacutainers from all the cows on days -30, 15, 0, 15, 30, 45 and 60 peripartum. The plasma samples separated out immediately after blood sampling by centrifugation were stored at -80°C with a drop of merthiolate until analyzed. Plasma progesterone, estradiol-17β and cortisol levels were estimated by standard RIA techniques using procedures and kits of Beckman and Coulter procured from Immunotech, France and the concentrations of PGFM were determined by 13,14-dihydro-15-keto prostaglandin $F_2\alpha$, ELISA kits and procedure of Cayman Chemicals, USA. Plasma Insulin and IGF-1 concentrations were determined by standard ELISA technique using Bovine Insulin and Bovine IGF-1, ELISA kits of MybioSource, Inc., USA as per the manufacturer's instructions.

Uterine involution was monitored at weekly interval using transrectal ultrasonography as well as per rectal palpation. The occurrence of first estrus and fertile estrus postpartum, as well as conception rates and service period with numbers of services per conception in each group were recorded for cows conceived within 10 months postpartum. Cows showing estrus 60 days postpartum were only inseminated and pregnancy was confirmed by palpation per rectum 45 days after last AI.

Economics of supplementation and statistical analyses

The economics of nutrients supplementation peripartium for 90 days was calculated based on prevailing market price of feeds, fodder and milk and actual market price of supplements. The data on different hormonal profiles were analyzed by two-way analysis of variance for treatment and period effects and those on reproductive performance postpartum by one-way ANOVA or Chi-square test for treatment effect using online SAS program (Snedecor and Cochran, 1994). Duncan's New Multiple Range test was used to check mean differences for significance at p<0.05.

RESULTS AND DISCUSSION

The fortnightly mean (±SE) values of various hormonal parameters obtained in different groups of parturient Gir cows are presented in Table 1, 2, 3, Fig 1, the observations on postpartum reproductive performance are shown in Table 4 and the comparative economics of various supplements in Table 5.

Plasma insulin and insulin-like growth factor-1 (IGF-1)

The mean plasma insulin levels across the groups revealed significant (P<0.01) effect of treatments, the values being lower in all treatment groups than in control (Table 1, Fig 1). Moreover, the insulin levels were found to be reduced significantly (p<0.05) on day 15 postpartum, overall and in T1, T3 and T5 groups and non-significantly in T2 and T4 groups. It showed decline in all groups from day 30 prepartum to day of calving and then had rising trend till day 45/60 postpartum. Comparatively more decline in insulin

Table 1: Effect of RPC, RPF and Se + Vit-E supplementation on fortnightly plasma insulin and IGF-1 profile in periparturient Gir cows (Mean±SE).

Days	Dietary treatment groups					
peripartum	T1	T2	T3	T4	T5	Overall***
Insulin (IU/L)						
-30	6.22^{bC}±0.80	4.76^{ab}±0.56	4.50^{aB}±0.56	4.62^{a}±0.42	5.17^{abB}±0.45	5.06^{D}±0.25
-15	5.92^{bC}±0.82	4.65^{ab} ±0.71	3.42^{aAB}±0.54	4.57^{ab}±0.43	4.69^{abB}±0.30	4.65^{DC}±0.37
0	5.64^{bBC}±0.65	4.30^{ab}±0.58	3.14^{aAB}±0.27	3.14^{a}±0.36	3.58^{aAB}±0.30	3.96^{B}±0.37
15	3.82^{A}±0.64	3.14±0.66	2.58^{A}±0.38	2.38±0.23	3.71^{A}±0.59	3.13^{A}±0.23
30	4.24^{ab}±1.10	3.34±0.58	2.65^{A}±0.44	3.01±0.19	3.68^{AB}±0.48	3.38^{AB}±0.22
45	5.50^{bBC}±0.76	3.67^{a}±0.37	3.62^{aAB}±0.39	3.98^{a}±0.33	3.55^{aAB}±0.27	4.06^{BC}±0.29
60	3.93^{A}±0.69	3.18±0.43	3.00^{AB}±0.30	3.27±0.39	3.64^{AB}±0.26	3.40^{AB}±0.13
Overall***	5.04^{c}±0.36	3.86^{a}±0.25	3.27^{a}±0.23	3.57^{ab}±0.30	4.00^{b}±0.23	
IGF-1 (ng/ml)						
-30	16.50^{bC}±0.79	13.61^{abAB} ±0.72	10.65^{a}±1.08	13.08^{abBC}±1.22	16.05^{bBC}±1.13	13.98^{C}±0.84
-15	14.31^{abBC}±0.80	11.95^{abAB}±1.05	11.16^{ab}±1.66	14.30^{aC}±1.24	15.63^{bBC}±1.39	13.47^{C}± 0.65
0	16.00^{cC}±0.63	14.47^{bcB}±1.14	10.37^{a}±0.55	11.58^{abABC}±0.84	17.08^{cC}±1.22	13.90^{C}±1.01
15	13.21^{BC}±2.04	9.76^{A}±1.43	12.63±0.73	10.12^{AB}±0.84	12.46^{AB} ±0.83	11.64^{B}±0.56
30	8.70^{A}±0.47	9.38^{A}±1.34	9.03±0.29	8.11^{A}±0.581	10.26^{A}±0.71	9.09^{A}±0.28
45	11.11^{AB}±1.83	11.77^{AB}±1.26	10.16±1.04	8.03^{A}±0.48	10.31^{A}±0.89	10.28^{AB}±0.50
60	14.46^{cBC}±1.16	11.30^{abcAB}±0.96	12.00^{bc}±1.04	7.86^{aA}±0.68	9.88^{abA}±0.93	11.10^{B}±0.87
Overall***	13.47^{c}±0.98	11.75^{ab}±0.66	10.86^{a}±0.42	10.44^{a}±0.93	13.09^{bc}±1.09	

T1 Control; T2 RPC; T3 RPF, T4 RPC + RPF, T5 Se + Vit-E, ***P<0.001. Means bearing different small superscripts (a, b, c) within the row and capital superscripts (A, B, C, D) within the column differ significantly (P<0.05) for a parameter.

Table 2: Effect of RPC, RPF and Se + Vit-E supplementation on fortnightly plasma progesterone and estradiol profile in periparturient Gir cows (Mean±SE).

Days	Dietary treatment groups					
peripartum	T1	T2	T3	T4	T5	Overall***
Plasma progesterone (ng/ml)						
-30	5.90^{C}±0.85	6.53^{C}±0.93	5.41^{B}±0.66	6.72^{D}±0.49	6.18^{C}±0.48	6.15^{D}±0.18
-15	4.53^{B}±0.53	4.34^{B}±0.67	4.30^{B}±0.56	4.94^{C}±0.49	5.21^{C}±0.47	4.66^{C}±0.14
0	0.53^{A}±0.13	1.19^{A}±0.39	0.71^{A}±0.19	0.61^{A}±0.17	0.38^{A}±0.06	0.68^{A}±0.11
15	0.65^{A}±0.49	0.21^{A}±0.06	0.27^{A}±0.06	0.22^{A}±0.03	0.41^{A}±0.20	0.35^{A}±0.07
30	0.23^{A}±0.06	0.27^{A}±0.13	0.72^{A}±0.29	0.56^{A}±0.26	1.27^{A}±0.83	0.61^{A}±0.15
45	1.39^{A}±0.43	0.69^{A}±0.30	0.46^{A}±0.14	0.65^{A}±0.37	0.54^{A}±0.17	0.75^{A}±0.13
60	1.06^{A}±0.29	0.47^{A}±0.08	1.40^{A}±0.61	2.08^{B}±0.83	3.00^{B}±1.06	1.60^{B}±0.34
Overall	2.04±0.79	1.96±0.88	1.90±0.73	2.25±0.91	2.43±0.86	
Plasma estradiol-17β (pg/ml)						
-30	129.13^{B}±16.09	160.19^{B}±18.16	173.06^{B}±14.91	175.88^{B}±18.82	139.94^{B}±20.58	155.64^{B}±7.25
-15	196.31^{C}±29.33	203.75^{BC}±22.11	221.25^{C} ±15.85	218.75^{B} ±18.32	175.00^{B}±24.55	203.01^{C}±6.64
0	235.63^{abC}±31.29	231.25^{abC}±25.09	275.13^{bD}±17.63	266.13^{abC}±23.49	220.19^{aC}±29.38	245.66^{D}±8.37
15	62.00^{A}±11.02	75.69^{A}±8.79	82.63^{A}±7.37	60.63^{A}±11.21	47.13^{A}±9.71	65.61^{A}±4.90
30	68.38^{A}±10.95	63.13^{A}±8.68	62.75^{A}±11.37	47.50^{A}±8.61	57.75^{A}±9.92	59.90^{A}±2.79
45	59.8^{A}8 ±9.44	47.38^{A}±6.14	58.44^{A}±11.69	70.94^{A}±9.24	62.81^{A}±10.26	59.89^{A}±3.01
60	64.75^{A}±10.18	66.50^{A}±9.44	69.25^{A}±7.32	57.94^{A}±8.74	55.50^{A}±7.18	62.79^{A}±2.06
Overall*	116.58^{ab}±25.76	121.13^{ab}±26.73	134.64^{b}±31.16	128.25^{b}±31.86	108.33^{a}±24.62	

T1 Control; T2 RPC; T3 RPF, T4 RPC + RPF, T5 Se + Vit-E, *P<0.05; Means bearing different small superscripts (a, b, c) within the row and capital superscripts (A, B, C, D) within the column differ significantly (P<0.05) for a parameter.

levels observed in T3 and T4 groups than in T1, T2 and T5 groups might be due to beneficial effect of RPF supplement on this hormone. Overall plasma insulin levels were significantly higher in T1 and T5 groups as compared to others. Supplementation of both RPC and RPF alone or in combination reduced the plasma insulin concentration in periparturient Gir cows. Garnsworthy *et al.* (2008) also reported significantly decreased plasma insulin concentration in dairy cows supplemented with bypass fat, while Leiva *et al.* (2015) and Acharya *et al.* (2019[a]) reported significantly higher blood insulin level in the dairy cows supplemented with RPC during peripartum periods. However, a non-significant effect on plasma insulin concentration with RPC supplementation (Chung *et al.,* 2009) in lactating Holstein cows and bypass fat or prill fat from 40 days prepartum till 90 days postpartum (Tyagi *et al.,* 2010; Singh *et al.,* 2014) has been reported earlier.

Like insulin, the overall mean values of plasma IGF-1 were found to be significantly ($p<0.001$) lower in groups T3 and T4 and non-significantly lower in T2, as compared to T5 (Se + Vit-E) and T1 (control). The significant differences were also observed between peripartum periods in all groups (Table 1, Fig 1). Plasma IGF-1 levels were significantly ($p<0.001$) reduced on day 30th postpartum overall and in all groups, except T3, as compared to values of prepartum and postpartum periods. Further, the IGF-1 levels declined gradually and significantly from day 30 prepartum till day 30 postpartum and then further rose till day 45/60 postpartum in all supplemented and control groups. Acharya *et al.* (2019a) reported significantly higher plasma IGF-1 level in cows supplemented with RPC, while Leiva *et al.* (2015) reported non-significant effect. We observed significant decrease in plasma IGF-1 level in the cows supplemented with RPF and RPF+RPC as compared to control or Se + Vit-E supplemented group. However, Garnsworthy *et al.* (2008) did not observe significant effect of various levels of bypass fat on plasma IGF-1 concentration in lactating dairy cows.

Effect on plasma progesterone and estradiol profile

The mean plasma progesterone (P_4) concentrations were maximum on day 30 prepartum in all the groups, which declined significantly ($p<0.01$) by day 15 preparturm and then dropped abruptly to the basal levels (<1 ng/ml) on the day of parturition. Subsequently these values fluctuated non-significantly around the basal levels from day 15 with rising trend around day 45/60 postpartum (Table 2, Fig, 1), suggestive of presence of luteinized follicles or corpora lutea preceded by silent ovulations in some of the animals, particularly in groups T5, T4 and T3. The mean plasma estradiol-17β concentrations (E_2) recorded in cows of different groups at day 30 prepartum increased significantly or apparently at day 15 prepartum with highly significant rise ($P<0.01$) on the day of calving. Thereafter, there was a sudden and significant ($p<0.01$) drop in the mean plasma estradiol levels to the basal values by day 15 postpartum and then it fluctuated insignificantly between days 15 and 60 postpartum in all groups (Table 2). The basal value of

Table 3: Effect of RPC, RPF and Se + Vit-E supplementation on fortnightly plasma PGFM and cortisol profile in periparturient Gir cows (Mean±SE).

Days	Dietary treatment groups					
peripartum	T1	T2	T3	T4	T5	Overall***
Plasma PGF metabolites (pg/ml)						
-30	103.61^{abA}±2.10	106.09^{bA}±2.05	101.91^{abA}±2.15	97.94^{aA}±1.69	97.79^{aA}±1.94	101.47^{A}±1.28
-15	145.48^{cD}±1.87	142.28^{cC}±2.20	136.15^{bD}±2.50	129.10^{aD}±1.71	126.61^{aC}±1.58	135.92^{D}±2.88
0	155.99^{bE}±0.91	154.04^{bD}±2.05	140.84^{aD}±2.45	138.55^{aE}±0.93	136.83^{aD}±1.09	145.25^{E}±3.20
15	156.61^{cE}±1.03	159.06^{cD}±4.01	147.34^{bE}±2.68	139.81^{aE}±1.83	136.58^{aD}±1.35	147.88^{F}±3.51
30	134.83^{cC}±1.18	128.79^{bB}±0.84	127.48^{bC}±2.24	119.66^{aC}±2.31	115.83^{aB}±0.93	125.32^{C}±2.67
45	130.51^{bC} ±5.17	130.06^{bB}±1.16	128.68^{bC}±2.81	120.33^{aC}±2.11	118.89^{aB}±1.85	125.69^{C}±1.99
60	116.15^{cB}±0.76	109.48^{bA}±2.00	109.25^{bB}±2.05	104.58^{abB}±2.35	100.75^{aA}±1.37	108.04^{B}±2.05
Overall***	134.74^{d}±7.03	132.83^{d}±7.23	127.38^{c}±5.83	121.42^{b} ±5.64	119.04^{a} ±5.55	
Plasma cortisol concentrations (ng/ml)						
-30	22.44^{A}±3.18	21.65^{A}±2.68	23.80^{A}±1.46	27.44^{AB}±1.80	25.86^{AB}±1.76	24.24^{A} ±0.85
-15	26.03^{AB}±3.19	24.86^{A}±2.79	28.42^{A}±1.21	32.38^{B}±2.02	31.36^{BC}±2.30	28.61^{C}±1.15
0	31.91^{B}±3.23	38.14^{B}±4.18	37.15^{B}±3.52	41.18^{C}±2.46	36.37^{C}±2.83	36.95^{D}±1.19
15	19.81 ^{A}±1.68	23.69^{A}±2.26	25.38^{A}±4.04	23.25^{A}±2.56	19.14^{A}±2.49	22.25^{AB}±0.94
30*	23.73^{A}±2.03	23.16^{A}±1.95	22.28^{A}±3.52	21.16^{A}±2.06	22.60^{A}±1.42	22.58^{AB}±0.34
45	22.08^{A}±2.40	18.22^{A}±2.00	21.99^{A}±3.22	21.09^{A}±1.79	19.42^{A}±1.85	20.56^{A}±0.60
60	18.79^{A}±2.77	20.89^{A}±2.16	22.34^{A}±1.79	21.14^{A}±1.68	20.75^{A}±2.78	20.78^{A}±0.45
Overall	23.54±1.55	24.37±2.28	25.91±1.93	26.81±2.69	25.07±2.33	

T1 Control; T2 RPC; T3 RPF, T4 RPC + RPF, T5 Se + Vit-E, ***$P<0.001$; Means bearing different small superscripts (a, b, c) within the row and capital superscripts (A, B, C, D) within the column differ significantly ($P<0.05$) for a parameter.

progesterone found on the day of calving was suggestive of complete luteolysis at parturition and corroborated with the findings of Dhami *et al.* (2017) and Kalasariya *et al.* (2017). The overall trend of peripartum E_2 profile found in animals under study coincided well with that reported by many of the earlier researchers in dairy animals (Dugwekar *et al.*, 2008; Dhami *et al.*, 2017; Kalasariya *et al.*, 2017).

The period wise or overall mean values of plasma P_4 however did not vary significantly between groups, though it was apparently much higher on day 60 postpartum in groups T5, T4 and T3. The E_2 levels varied significantly overall and at most peripartum intervals between groups, the values being lowest in T5 and higher in T3 and T4 (Table 2). Dietary supplementation of fat increases the circulating concentrations of cholesterol, which serves as a precursor for the synthesis of steroid hormones by ovarian theca and luteal cells and also increases the lifespan of induced CL (Rahbar *et al.,* 2014). Tyagi *et al.* (2010) and Kalasariya *et al.* (2017) also found that the fatty acids and protein supplemented cows had non-significantly higher progesterone values as compared to control group. The observed higher level of plasma P_4 might be due to the substantial production by the functional CL. The prolonged period of inhibition during pregnancy from continuous negative-feedback effect of P_4 secreted by the CL and placenta causes the pituitary to become refractory to GnRH

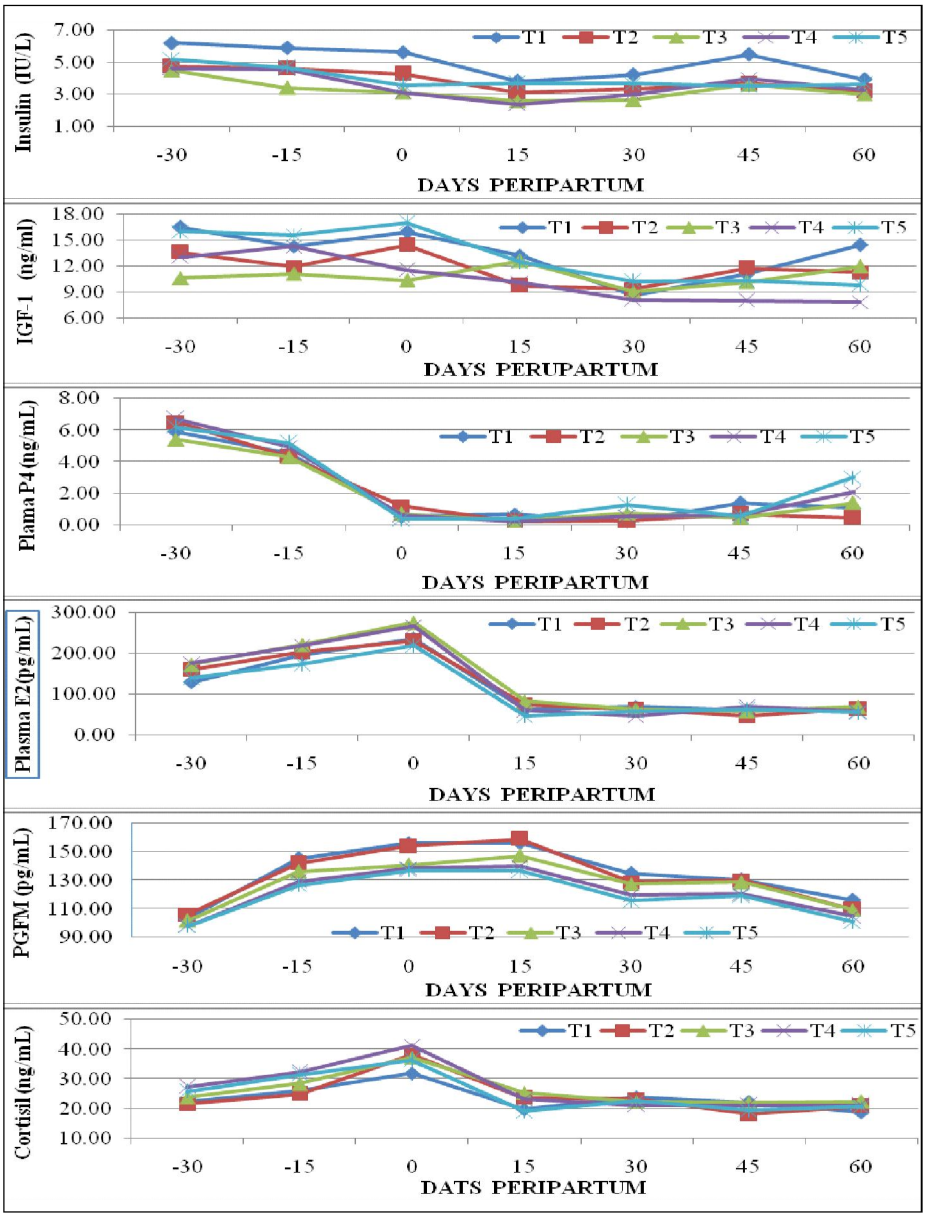

Fig 1: Impact of peripartum supplementation of RPC (T2), RPF (T3), RPC+RPF (T4) and Se+Vit-E (T5) over control (T1) on plasma endocrine profile in Gir cows.

postpartum (Lammoglia *et al.,* 1997). This eventually recovers with time. As a result of the absence or low output of gonadotrophins in early postpartum phase the ovaries remain relatively quiescent and the cow shows anestrum. The trend of plasma P_4 concentration observed in peripartum periods of Gir cows corroborated well with the findings of Chaiyabutr *et al.* (2000), Ishikawa *et al.* (2004) and Dhami *et al.* (2017). The overall trend of E_2 profile found coincided well with observations of earlier researchers (Kindahl *et al.,* 2004; Shah *et al.*, 2006, Kalasariya *et al.,* 2017). It is fact that after parturition the levels of estradiol-17β markedly decrease and fluctuate at basal levels until the initiation of postpartum follicular activity in dairy bovines (Dhami *et al.,* 2017; Vala *et al.,* 2019).

Effect on plasma PGFM and cortisol profile

The plasma concentration of PGFM (13, 14-dihydro, 15-keto-$PGF_2\alpha$) was found to be lowest or basal on day 30 prepartum, which increased gradually and significantly ($p<0.01$) in all groups reaching peak values of 1.5-fold greater on the day of calving, which then further increased non-significantly by day 15 postpartum and then declined gradually and significantly till day 60 postpartum, attaining the basal values at par with those of 30 days prepartum. The rise was significantly ($p<0.01$) lower in nutrients supplemented groups T3, T4 and T5 than in treatment T2 and control group T1 (Table 3, Fig 1). In our earlier studies almost similar trend, but relatively much higher values (8-12 fold) of peripartum PGFM profile in bypass fat and minerals supplemented crossbred cows (Dhami *et al.,* 2017) and Jaffarabadi buffaloes (Vala *et al.,* 2019) were observed. The trend of PGFM recorded peripartum is a reflection of PGF2α production around parturition for inducing luteolysis and for uterine involution. There is an intense production of PGF2α by inter-caruncular region of endometrial epithelial surface during early puerperium, which correlates to uterine involution process (Skarzynski *et al.,* 2000; Dhami *et al.,* 2017). High concentration of PGFM beyond day 20 postpartum may suggest the presence of uterine inflammation and/or infection and thereby delaying involution of uterus (Kindahl *et al.,* 2004). Archbald *et al.* (1998) opined that high plasma PGFM in cows between days 24 and 29 postpartum are indicative of uterine infection, that results in infertility. The PGFM in the early postpartum period was found (P=0.10) to be higher in cows fed fatty acids and affected the uterine health and reproductive efficiency through alteration of PGFM concentration (Lammoglia *et al.,* 1997; Rodriguez-Sallaberry *et al.,* 2007). The present findings are in close agreement with earlier reports (Sheldon and Dobson, 2000; Heppelmann *et al.,* 2013).

The mean plasma cortisol concentrations in cows of different groups varied non-significantly between 21.65±2.68 and 27.44±1.80 ng/ml at day 30 prepartum, which increased significantly or apparently by day 15 prepartum, but increased rapidly to nearly 1.5-fold ($p<0.01$) on the day of parturition reaching a peak, the rise was more in all treatment groups compared to control. Thereafter, the levels decreased significantly on day 15 postpartum and then fluctuated non-significantly (Table 3, Fig 1). The nutrients supplemented cows tended to have non-significantly ($p<0.01$) higher cortisol values than the control cows. Further, it was found that the occurrence of the postpartum estrus was earlier in cows of all the treatment groups than the control. Very similar trend but with relatively much lower values of peripartum cortisol profile in bypass fat supplemented cows (Dang *et al.,* 2013; Dhami *et al.,* 2017) and comparable values in Jaffarabadi buffaloes (Vala *et al.,* 2019) have been reported earlier. It is suggested that cortisol may play a role in the CL as an anti-apoptotic factor in the bovine luteal cells (Rueda *et al.,* 2000) and that the CLs have the potential to respond to a locally generated cortisol (Michael *et al.,* 2003). The observed levels and trend of changes in cortisol values were found to be similar to the findings of Chaiyabutr *et al.* (2000), who reported that the cortisol levels in the prepartum days were initially low, but increased as parturition approached.

Effect on postpartum fertility

Uterine involution observed by ultrasonography was found to be earlier in all supplemented groups over control with or without significant differences. Moreover in all the nutrients supplemented groups of Gir cows, the commencement of cyclicity was significantly earlier ($p<0.001$) as compared to control (Table 4) and the values of T2, T3 and T4 groups were found to be statistically similar with either T1 or T5. A positive effect of supplementation of bypass fat and choline (McNamara *et al.,* 2003; Tyagi *et al.,* 2010; Khalil *et al.,* 2012) and of Vit E and Se (Mavi *et al.,* 2006; Jovanovic *et al.,* 2015; Hosnedlova *et al.,* 2017) on uterine involution and occurrence of first estrus postpartum with improved conception rate, as observed in present study, has also been reported by earlier researchers. Onset of postpartum cyclicity is related with the process of involution and health of the uterus. Uterine involution was reported to be enhanced significantly by Pirestani and Aghakhani (2018) and non-significantly by Acharya *et al.* (2019b) in cows fed with RPC. Further, in contrast to present findings, supplementation of RPC during peripartum period did not influence cyclicity in earlier studies (Amrutkar *et al.,* 2015; Anonymous, 2020). The significant effect of RPF supplementation peripartum on commencement of cylicity observed in present study was in agreement with findings of Dhami *et al.* (2017) and Vala *et al.* (2019), who observed significantly reduced time for commencement of cyclicity in peripartum RPF supplemented crossbred cows and Jaffarabadi buffaloes as compared to control. In contrast, Nirwan *et al.* (2019) observed non-significant difference for this trait in peripartum RPF supplemented cows.

Service period was significantly shortened with numerically reduced number of services per conception (Table 4) in T5 group supplemented with Se + Vit-E than that of control and the values for RPC, RPF and its combined supplementation were statistically similar with either T1 or T5 group, suggesting beneficial effect of all four supplementation tested in transition cows and T5 in particular. Our results were in agreement with Tyagi *et al.* (2010), who reported shorter service period in bypass fat supplemented groups of cows than in control and Pirestani and Aghakhani (2018) observed improvement in service period with reduced AI per conception in the cows receiving RPC in peripartum periods. However, Khalil *et al.* (2012) did not find such effect of nutrient supplements on service period, although the conception rate was improved. In some other studies, the service period and AI/conception were reported to be improved significantly in dairy cows and buffaloes due to supplementation of RPC or protein (Amrutkar *et al.*, 2015; Acharya *et al.*, 2019b; Anonymous, 2020) and RPF (Dhami *et al.*, 2017; Nirwan *et al.*, 2019; Vala *et al.,* 2019) during peripartum period. Fats and choline in the diet influence reproduction positively by altering both ovarian follicle and CL function via improved energy status and by increasing precursors insulin and IGF-I for the synthesis of reproductive hormones such as steroids and prostaglandins. The increase in insulin plays a role in mediating increased follicular growth, either directly through its own receptor or indirectly by modulating granulosa cell IGF-I production which is required for follicle development (Rahbar *et al.,* 2014; Anonymous, 2020).

In the present study, postpartum fertility was better with Vit-E and Selenium injections than with RPC and RPF supplementation in diet, perhaps due to their potent

Table 4: Effect of peripartum RPC, RPF and Se + Vit E supplementation on postpartum reproductive performance in Gir cows (Mean±SE).

Particulars	Dietary treatment groups				
	T1	T2	T3	T4	T5
Uterine involution (days)*	31.38^{b}±0.60	29.63^{ab} ±0.60	29.38^{ab}±0.37	29.25^{a}±0.53	29.38^{ab}±0.35
FOPP (days)*	63.50^{b}±9.52	49.63^{ab}±3.09	51.75^{ab}±5.43	50.88^{ab}±5.72	33.63^{a}±3.44
Service period*	214.25^{b}±60.17	137.17^{ab}±11.26	193.40^{ab}±40.69	186.83^{ab}±29.36	96.29^{a}±14.78
Conception rate (%)**	50.00 (4/8)	75.00 (6/8)	62.50 (5/8)	75.00 (6/8)	87.50(7/8)
No. of AIs/CR for Pregnant cows	2.50±1.11	1.67±0.33	2.20±0.65	2.83±0.70	1.29±0.28

T1 Control; T2 RPC; T3 RPF, T4 RPC + RPF, T5 Se+Vit-E, FOPP First oestrus postpartum,*p<0.05, **p<0.01.

Means bearing uncommon superscripts (a, b) within the row differ significantly (p<0.05).

Table 5: Comparative economics of peripartum supplementation of RPC, RPF and Se + Vit-E in Gir cows based on market price.

Particulars	Dietary treatment groups				
	T1	T2	T3	T4	T5
Cost of feeding/supplement (Rs./head) in 90 days					
Green fodder @ Rs.5/kg	3924.00	4077.00	3811.50	3694.50	3627.00
Dry fodder @ Rs.15/kg	3510.00	3942.00	3375.00	3645.00	3429.00
Compound cattle feed @Rs. 28/kg	3906.00	4284.00	4233.60	3704.40	3528.00
CS cake @ Rs. 30/kg	5697.00	6156.00	6318.00	5508.00	5049.00
Maize bhardo @ Rs. 22/kg	495.00	495.00	495.00	495.00	495.00
RPC @ Rs.390/kg	0	1579.50	0	1579.50	0
RPF @ Rs.100/kg	0	0	720.00	720.00	0
Se + Vitamin E @ Rs. 115 per 10 ml for seven dose	0	0	0	0	805.00
Total	17532.0	20533.5	18953.1	19346.4	16818.0
Average cost of feeding and supple (Rs. /head/d)	194.80	228.15	210.59	214.96	186.87
Av. milk yield per animal (kg/d)	7.32	8.62	8.48	8.56	7.44
Cost of milk production (Rs/kg)	26.61	26.47	24.83	25.11	25.12
Service period-SP (days)	214.00	137.00	193.00	187.00.	96.00
SP shortened by days over control	-	77.00	21.00	27.00	118.00
Receipt from sale of milk (Rs./head) @ Rs. 50/kg milk during experimental period	21960.0	25860.0	25440.0	25680.0	22320.0
Net return over feed cost(Rs./head)	4428.00	5326.50	6486.90	6333.60	5502.00
Difference (Rs/head) over control	-	+898.50	+2058.90	+1905.60	+1074.00
Saving in feed cost due to improved reproductive performance (Rs./head)	-	+14999.60	+4090.80	+5259.60	+22986.40
Net saving (Rs/head) over control	-	+15898.10	+6149.70	+7165.20	+24060.40

T1 Control; T2 RPC; T3 RPF, T4 RPC + RPF, T5 Se+Vit.E.

antioxidant potential alleviating oxidative stress and thereby better feed intake preventing NEB. In earlier studies also, Vit-E and Selenium injections or oral supplements in diet prepartum and/or peripartum have been reported to reduce significantly the oxidative stress (Dimri *et al.,* 2010; Modi *et al.,* 2016; Khatti *et al.,* 2017), incidence of retained placenta (Arechiga *et al.,* 1994; Gupta *et al.,* 2005; Moeini *et al.,* 2009; Damarany, 2021) and to improve postpartum reproductive performance (Sattar *et al.,* 2007; Dimri *et al.,* 2010; Qureshi *et al.,* 2010; Pontes *et al.,* 2015; Khatti *et al.,* 2017) in dairy cows and buffaloes.

Economics of peripartum supplementation in Gir cows

Average daily cost of feeding (Rs./head) for T1, T2, T3 T4 and T5 group was calculated to be 194.80, 228.15, 210.59, 214.96 and 186.87, respectively. The corresponding net return over feed cost of milk yield (Rs./head) was 4428.00, 5326.50, 6486.90, 6333.60 and 5502.00, respectively and that due to reduced service period over control T1 was +14999.60, +4090.80, +5259.60 and +22986.40 for T2, T3 T4 and T5, respectively. The overall net returns over feed cost of milk yield and reduced service period (Rs./head) in T2, T3, T4 and T5 groups over control T1 were +15898.10, +6149.70, +7165.20 and +24060.40, respectively, indicating that it was highest in T5 followed by T2 and in T3 group (Table 5). The data proved that both production and reproduction efficiencies and thereby economic of Gir cows supplemented with Se+Vit-E, followed by RPC and RPF alone for 90 days peripartum were markedly improved. Very similar economic benefits of these supplementations either during peripartum or postpartum period in dairy cows have been reported by several earlier researchers (Mohsen *et al.,* 2011; Singh *et al.*, 2014; Sankhpal *et al.,* 2016, Anonymous, 2020) from different parts of country. Hence farmers should be advised to incorporate Se+Vit-E, followed by RPC and RPF alone as per economic merit in dairy animals for harvesting its benefits.

CONCLUSION

Based on the results, it can be concluded that peripartum supplementation of RPC and RPF alone or in combination and Vit E + Se have significant effect on concentration of plasma insulin and IGF1 in Gir cows, both being declined in supplemented groups as compared to control. All these supplementations also improve plasma steroid hormones, particularly E_2 and PGFM profile and thereby postpartum reproductive performance in terms of shorter period of uterine involution with earlier onset of first estrus postpartum ($p<0.05$) and reduced service period with higher pregnancy rate and lesser number of services per conception, particularly with Vit E + Se injections, than the control cows. These changes in plasma profile reflected the normal puerperal physiology and suggest that nutrient supplementation peripartum, particularly with Vit E + Se as antioxidants definitely has advantage to combat parturient and oxidative stress and economically enhance postpartum fertility in Gir cows.

ACKNOWLEDGEMENT

Authors thank the authorities of AAU, Anand and JAU, Junagadh and the Research Scientist, Cattle Breeding Farm, JAU, Junagadh for the facilities and cooperation extended for this collaborative research work under AICR of AAU, Anand. The financial assistance by ICAR-NIANP, Bangalore to Dr. A.J. Dhami, PI at AAU, Anand, through "AICRP on nutritional and physiological interventions for enhancing reproductive performance of animals" is also gratefully acknowledged.

Conflict of interest: None.

REFERENCES

Acharya, P., Lathwal, S.S., Baithalu, R., Patnaik, N., Thul, M.R., Moharana, B. (2019b). Supplementing rumen-protected choline with green tea extract improves reproductive performances in transition Karan Fries cows. Indian Journal of Animal Research. 54(4): 452-455.

Acharya, P., Lathwal, S.S., Patnaik, N.M., Moharana, B. (2019a). Rumen protected choline along with green tea extract maintain glucose homeostasis in transition Karan Fries cows. Indian Journal of Animal Nutrition. 36(3): 276-280.

Amrutkar, S.A., Pawar, S.P., Thakur, S.S., Kewalramani, N.J., Mahesh, M.S. (2015). Dietary supplementation of rumen-protected methionine, lysine and choline improves lactation performance and blood metabolic profile of Karan-Fries cows. Agricultural Research. 4(4): 396-404.

Anonymous. (2020). The effect of feeding protected choline on milk and production efficiency in dairy cows. In: Research Report (2019-20), 18th Meeting of Agricultural Research Subcommittee on Animal Production and Fisheries Subcommittee, College of Veterinary Science, Anand Agricultural University, Anand, Gujarat. pp. 298-303.

Archbald, L.F., Tsai, I.F., Thatcher, W.W., Tran, T., Wolfsdorf, K., Risco, C. (1998). Use of plasma concentrations of 13, 14-dihydro, 15-keto-PGF2 alpha (PGFM) in the diagnosis of sub-clinical endometritis and its relationship to fertility in the postpartum dairy cows. Theriogenology. 49: 1425-1436.

Arechiga, C.F., Ortiz, O., Hansen, P.J. (1994). Effect of prepartum injection of vitamin E and selenium on postpartum reproductive function of dairy cattle. Therigenology. 41: 1251-1258.

Chaiyabutr, N., Prueksagorn, S., Komolvanich, S., Chanpongsang, S. (2000). Plasma levels of hormones and metabolites as affected by the forages type in two different types of crossbred Holstein cattle. Asian-Australasian Journal of Animal Science. 13(10): 1359-1366.

Chung, Y.H., Brown, N.E., Martinez, C.M., Cassidy, T.W., Varga, G.A. (2009). Effects of rumen-protected choline and dry propylene glycol on feed intake and blood parameters for Holstein dairy cows in early lactation. Journal of Dairy Science. 92(6): 2729-2736.

Damarany, A.I. (2021). Effect of treatment with vitamin E and selenium during late gestation period on mastitis, retained placenta and postpartum reproductive parameters in Egyptian Baladi cows. Egyptian Journal of Animal Production. 58(2): 47-56.

Dang, A.K., Prasad, S., De, K., Pal, S., Mukherjee, J. (2013). Effect of supplementation of vitamin E, copper and zinc on the *in vitro* phagocytic activity and lymphocyte proliferation index of peripartum Sahiwal cows. Journal of Animal Physiology and Animal Nutrition. 97: 315-321.

Dhami, A.J., Theodore, V.K., Panchal, M.T., Hadiya, K.K., Lunagariya, P.M., Sarvaiya, N.P. (2017). Effects of peripartum nutritional supplementation on postpartum fertility and blood biochemical and steroid hormone profile in crossbred cows. Indian Journal of Animal Research. 51(5): 821-826.

Dimri, U., Ranjan, R., Sharma, M.C., Varshney, V.P. (2010). Effect of vitamin E and selenium supplementation on oxidative stress indices and cortisol level in blood in water buffaloes during pregnancy and early postpartum period. Tropical Animal Health and Production. 42: 405-410.

Drackley, J.K., Wallace, R.L., Graugnard, D., Vasquez, J., Richards, B.F., Loor, J.J. (2014). Visceral adipose tissue mass in non-lactating dairy cows fed diets differing in energy density. Journal of Dairy Science. 97(6): 3420-3430.

Dugwekar, Y.G., Sarvaiya, N.P., Patel, M.D., Tajne, K.R., Shah, R.R. (2008). Serum progesterone and estradiol levels in Jafarabadi buffaloes. Indian Journal of Animal Reproduction. 29: 177-180.

Duske, K., Hammon, H.M., Langhof, A.K., Bellmann, O., Losand, B., Nürnberg, K., Nurnberg, G., Sauerwein, H., Seyfert, H.M., Metges, C.C. (2009). Metabolism and lactation performance in dairy cows fed a diet containing rumen-protected fat during the last twelve weeks of gestation. Journal of Dairy Science. 92(4): 1670-1684.

Elek, P., Newbold, J.R., Gall, T., Wagner, L., Husventh, F. (2008). Effects of rumen protected choline supplementation on milk production and choline supply of peri-parturient dairy cows. Animal. 2(11): 1595-1601.

Ganjkhanlou, M., Rezayazdi, K., Ghorbani, G.R., Banadaky, M.D., Morraveg, H., Yang, W.Z. (2009). Effects of protected fat supplements on production of early lactation Holstein cows. Animal Feed Science and Technology. 154(3-4): 276-283.

Garnsworthy, P.C., Lock, A., Mann, G.E., Sinclair, K.D., Webb, R. (2008). Nutrition, metabolism and fertility in dairy cows: 2. Dietary fatty acids and ovarian function. Journal of Dairy Science. 91(10): 3824-3833.

Gupta, S., Gupta, H.K., Soni, J. (2005). Effect of vitamin E and selenium supplementation on concentrations of plasma cortisol and erythrocyte lipid peroxides and the incidence of retained fetal membranes in crossbred dairy cattle. Theriogenology. 64: 1273-1286.

Heppelmann, M., Weinert, M., Brommling, A., Piechotta, M., Hoedemaker, M., Bollwein, H. (2013). The effect of puerperal uterine disease on uterine involution in cows assessed by Doppler sonography of the uterine arteries. Animal Reproduction Science. 143: 1-7.

Hosnedlova, B., Kepinska, M., Skalickova, S., Fernandez, C., Rittkay -Nedecky, B., Malevu, T.D., Sochor, J., Baron, M., Melcova, M. Zidkova, J., Kizek, R. (2017). A summary of new findings on the biological effects of selenium in selected animal species - A critical review. International Journal of Molecular Sciences. 18(2209): 1-47.

ICAR (2013). Nutrient Requirements of Cattle and Buffaloes. Indian Council of Agricultural Research, New Delhi, India.

Ishikawa. Y., Nakada, K., Hagiwara, K., Kirisawa, R., Iwai, H., Moriyoshi, M., Sawamukai. (2004). Changes in Interleukin-6 concentration in peripheral blood of pre- and postpartum dairy cattle and its relationship to postpartum reproductive diseases. Journal of Veterinary Medical Science. 66: 1403-1408.

Jovanovic, B., Velickovic, M., Milanovic, S., Valcic, O., Gvozdic, D., Vranjes-Duric S. (2015). Supplemental selenium reduces the levels of biomarkers of oxidative and general stress in peripartum dairy cows. Acta Veterinaria-Beograd. 65(2): 191-201.

Kalasariya, R.M., Dhami, A.J., Hadiya, K.K., Borkhatariya, D.N., Patel, J.A. (2017). Effect of peripartum nutritional management on plasma profile of steroid hormones, metabolites and postpartum fertility in buffaloes, Veterinary World, 10: 302-310.

Khalil, W.A., El-Harairy, M.A., Abul-Atta, A.A. (2012). Impact of dietary protected fat (Magnapac) on productive and reproductive performances of lactating Holstein cows. Journal of Animal and Poultry Production, Mansoura Univ. 3(10): 437-50.

Khatti, A., Mehrotra, S., Patel, P.K., Singh, G., Maurya, V.P., Mahla, A.S., Chaudhari, R.K., Narayanan, K., Das, G.K., Singh, M., Sarkar, M., Gupta, H.K. (2017). Supplementation of vitamin E, selenium and increased energy allowance mitigates transition stress and improves postpartum reproductive performance in crossbred cow. Theriogenology. 88: 1225-1231.

Kindahl, H., Kornmatitsuk, B., Gustafsson, H. (2004). The cow in endocrine focus before and after calving. Reproduction in Domestic Animal. 39: 217-221.

Lammoglia, M.A., Willard, S.T., Oldham, J.R., Randel, R.D. (1997). Effects of dietary fat and season on steroid hormonal profiles before parturition and on hormonal, cholesterol, triglycerides, follicular patterns and postpartum reproduction in Brahman cows. Journal of Animal Science. 74: 2253-62.

Leiva, T., Cooke, R.F., Brandao, A.P., Marques, R.S., Vasconcelos, J.L.M. (2015). Effects of rumen-protected choline supplementation on metabolic and performance responses of transition dairy cows. Journal of Animal Science. 93(4): 1896-1904.

Mavi, P.S., Pangaonkar, G.R., Sharmn, R.K. (2006). Effect of vitamin E and selenium on postpartum reproductive performance of buffaloes. Indian Journal of Animal Sciences. 76(4): 308-310.

McNamara, S., Butler, T., Ryan, D.P., Mee, J.F., O’Mara, F.P., Butler, S.T., Anglesey, D., Rath, M., Dillon, P., Murphy, J.J. (2003). Effect of offering rumen-protected fat supplements on fertility and performance in spring-calving HF cows. Animal Reproduction Science. 79: 45-56.

Michael, A.E., Thuston, L.M., Rae, M.T. (2003). Glucocorticoid metabolism and reproduction: A tale of two enzymes. Reproduction. 126: 425-441.

Modi, L.C., Khasatiya, C.T., Patel, M.D., Modi, F. (2016). Impact of vitamin E and Selenium administration during periparturient period on reproductive performance of Surti buffaloes. Indian Journal of Animal Reproduction. 37(1): 30-31.

Moieni, M.M., Karami, H., Mikaeili, E. (2009). Effect of selenium and vitamin E supplementation during the late pregnancy on reproductive indices and milk production in heifers. Animal Reproduction Science. 114: 109-114.

Mohsen, M.K., Gaafar, H.M.A., Khalafalla, M.M., Shitta, A.A., Yousif, A.M. (2011). Effect of rumen protected choline supplementation on digestibility, rumen activity and milk yield in lactating Friesian cows. Slovak Journal of Animal Science. 44(1): 13-20.

Nirwan, S.S., Mehta, J.S., Kumar, A., Kumar, P., Kumar, A., Singh, V. (2019). Effects of bypass fat on postpartum reproductive performance in dairy cattle. Indian Journal of Dairy Science. 72(2): 194-200.

Pirestani, A. and Aghakhani, M. (2018). The effects of rumen-protected choline and L-carnitine supplementation in the transition period on reproduction, production and some metabolic diseases of dairy cattle. Journal of Applied Animal Research. 46(1): 435-440.

Pontes, G.C.S., Monteiro Jr., P.L.J. Prata, A.B., Guardieiro, M.M., Pinto, D.A.M., Fernandes, G.O., Wiltbank, M.C., Santos, J.E.P., Sartori, R. (2015). Effect of injectable vitamin E on incidence of retained fetal membranes and reproductive performance of dairy cows. Journal of Dairy Science. 98: 2437-2449.

Qureshi, Z.I., Siddiq, M., Lodhi, L.A., Muhammad, G., Jamil, H. (2010). Effect of vitamin E-selenium administration during late gestation on productive and reproductive performance in dairy buffaloes and on growth performance of their calves. Pakistan Veterinary Journal. 30(2): 83-86.

Rahbar, B., Safdar, A.H.A., Kor, N.M. (2014). Mechanisms through which fat supplementation could enhance reproduction in farm animal. European Journal of Experimental Biology. 4(1): 340-348.

Rodriguez-Sallaberry, C., Caldari-Torres, C., Callante, W., Staples, C.R., Badinga, L. (2007). Plasma prostaglandin and cytokine concentrations in periparturient Holstein cows fed diet enriched in saturated trans-fatty acids. Journal of Dairy Science. 90: 5446-5452.

Rueda, B.R., Hendry, I.R., Hendry, I.W., Stormshak, F., Slayden, O.D., Davis, J.S. (2000). Decreased progesterone levels and progesterone receptor antagonists promote apoptotic cell death in bovine luteal cells. Biology of Reproduction. 62: 269-276.

Sattar, A., Mirza, R.H., Hussain, M.I. (2007). Effect of prepartum treatment of vitamin E-selenium on postpartum reproductive and productive performance of exotic cows and their calves under subtropical conditions. Pakistan Veterinary Journal. 27(3): 105-108.

Shah, K.D., Nakao, T., Kubota, H. (2006). Plasma estrone sulphate and estradiol-17â profiles during pregnancy and their relationship with the relaxation of sacrosciatic ligament and prediction of calving time in Holstein Friesian cattle. Animal Reproduction Science. 95: 38-53.

Shankhpal, S., Gupta, R.S., Parnerkar, S., Dhami, A.J. (2016). Effect of feeding bypass fat and unprotected oil on feed intake, digestibility and production performance in lactating crossbred cows. Indian Journal of Animal Nutrition. 33(3): 266-272.

Sheldon, I.M. and Dobson, H. (2000). Effect of administration of eCG to postpartum cows on folliculogenesis in the ovary ipsilateral to the previously gravid uterine horn and uterine involution. Journal of Reproduction and Fertility. 119: 157-163.

Singh, M., Sehgal, J.P., Roy, A.K., Pandita, S., Rajesh, G. (2014). Effect of prill fat supplementation on hormones, milk production and energy metabolites during mid lactation in crossbred cows. Veterinary World. 7(6): 384-388.

Skarzynski, D.J., Miyamoto, Y., Okuda, K. (2000). Production of prostaglandin F2α by cultured bovine endometrial cells in response to tumor necrosis factor α: Cell type specificity and intracellular mechanisms. Biology of Reproduction. 62: 1116-1120.

Snedecor, G.W. and Cochran, W.G. (1994). Statistical Methods. 8th edn. Oxford and IBH Publishing House, New Delhi, India, pp. 312-317.

Tyagi, N., Thakur, S.S. and Shelke, S.K. (2010). Effect of bypass fat supplementation on productive and reproductive performance in crossbred cows. Tropical Animal Health and Production. 42(8): 1749-1755.

Vala, K.B., Dhami, A.J., Kavani, F.S., Parmar, S.C., Raval, R.J., Sarvaiya, N.P., Gajbhiye, P.U. (2019). Impact of bypass fat and mineral supplementation peripartum on plasma profile of steroid hormones, PGFM and postpartum fertility in Jaffarabadi buffaloes. Indian Journal of Anima; Research. 53(8): 1042-1048.

Wankhade, P.R., Manimaran, A., Kumaresan, A., Jeyakumar, S., Ramesha, K.P., Sejian, V., Rajendran, D., Varghese, M.R. (2017). Metabolic and immunological changes in transition dairy cows: A review. Veterinary World. 10(11): 1367-1377.