Indian J. Anim. Res., 53(8) 2019: 1042-1048
Print ISSN:0367-6722 / Online ISSN:0976-0555

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com

Impact of bypass fat and mineral supplementation peripartum on plasma profile of steroid hormones, PGFM and postpartum fertility in Jaffarabadi buffaloes*

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Received: 17-05-2018 Accepted: 01-08-2018 DOI: 10.18805/ijar.B-3631

ABSTRACT

Advanced pregnant Jaffarabadi buffaloes (n=40) of 2-4 parity selected on an organized farm were divided equally into control (routine farm feeding-RFF) and treatment/nutrients supplementation (RFF + bypass fat @ 150-200 g/h/d and ASMM @ 50 g/h/d) groups and were studied from 6 wks prepartum to 8 wks postpartum for plasma profile of steroid hormones and PGF2α metabolites on days -45, -30, -7, 0, +7, +15, +30, +45 and +60 peripartum as well as for puerperal events and postpartum fertility. Half of the buffaloes in both the groups also received parenteral microminerals (Inj. Stimvet 5 ml i/m) twice, 45 days before and on the day of calving. Again half of them were treated with ecbolic (Exapar) 2 boli bid for first 4 days postpartum. The mean plasma progesterone values were maximum (>4 ng/ml) on day 45 prepartum, which declined significantly ($p<0.01$) on day 7 prepartum reached to the basal levels (<1 ng/ml) on the day of calving, remained basal till day 15, and thereafter showed a rising trend on days 30, 45 and 60 postpartum. The oestradiol-17β values were at its peak on the day of calving ($p<0.01$), showed a rapid fall by day 7 postpartum and remained low till recrudesce of follicular activity around day 45 and 60 postpartum. The levels of cortisol were significantly higher on the day of parturition as compared to values on day 7 pre- and post-partum. The plasma concentration of PGFM was low on day 45 prepartum, which increased gradually and significantly by almost 10-folds to reach peak values on the day of calving in both control and treatment groups and then declined gradually and significantly till day 45 postpartum. The rise was little more in nutrients supplemented group with higher mean values at most intervals peripartum than in control group. The periods for uterine involution, first postpartum estrus and days open were significantly shorter with higher conception rate in nutrient supplemented group. It is concluded that the peripartum nutrient supplementation in Jaffarabadi buffaloes is beneficial and has positive effect on the postpartum fertility and plasma progesterone and PGFM profile.

Key words: Jaffarabadi buffalo, Nutrient supplementation, PGFM, Postpartum fertility, Steroid hormones profile, Transitional period.

INTRODUCTION

About 50 per cent of the rural poor in India are dependent on livestock for their livelihood (Thorton *et al*., 2002). Buffaloes are preferred over cattle in India because they are well adapted to hot and hot-humid climate, have better feed conversion efficiency, greater resistance to diseases and higher milk fat percentage. The periparturient events are crucial in dairy animals. The foetal hypothalamo-hypophyseal-adrenal (HPA) axis serves to initiate parturition by secreting adreno-corticotrophic hormone (ACTH) from the anterior pituitary, which stimulates production of cortisol and other glucocorticoids from the adrenal cortex (Kindahl *et al*., 2004). Rueda *et al.* (2000) suggested that cortisol may play a role in the corpus luteum as an anti-apoptotic factor in bovines. Furthermore, the corpus luteum was thought to have the potential to respond to a locally generated cortisol (Michael *et al*., 2003). At the time of parturition, progesterone and oestradiol concentrations cascade to basal and peak levels, respectively, facilitating the almost immediate resumption of recurrent transient increase in FSH within 3-5 days of parturition that produces a dominant follicle by days 7-10 postpartum (Crowe *et al*., 1993).

Fats and minerals in the diet can influence reproduction positively by altering both ovarian follicle and corpus luteum function via improved energy status and by increasing precursors and catalysts for the synthesis of reproductive hormones such as steroids and prostaglandins (Rahbar *et al.*, 2014; Mane *et al.*, 2016). Uterine ecbolics are also shown to enhance expulsion of fetal membranes, uterine involution and onset of postpartum ovarian activity in dairy animals (Gautam *et al.*, 2005). Duration of postpartum anestrus has an important influence on reproductive performance. Factors such as limited energy intake, lower body reserves, and postpartum diseases can delay the uterine involution and thereby return to cyclicity. Therefore, the event of parturition and the time thereafter play a key role in

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resuming the reproductive life and come back to cycle. A trouble-free calving predisposes to prompt resumption of postpartum ovarian activity. Ideally, this should be followed by a minimal period of negative energy balance (NEB). The nutritional, managemental and environmental factors have impact on fertility. Transitional period and early postpartum phase in particular exerts biological and physiological stress on the dam (Setia *et al.*, 1992). Such studies on Jaffarabadi, the world famous largest buffalo breed, are scarce. Hence, this investigation was planned to evaluate whether the nutrient management of transition period influence the peripartum plasma profile of steroid hormones, PGFM and postpartum fertility in Jaffarabadi buffaloes under farm conditions.

MATERIALS AND METHODS

Experimental animals and supplements: The prior approval from the Institutional Animal Ethics Committee was obtained for the use of farm animals in this study. In all 40 advanced pregnant Jaffarabadi buffaloes of 2-4 parity were selected from Cattle Breeding Farm of JAU, Junagadh. All buffaloes were maintained in well ventilated hygienic sheds and were fed green fodder, hay and compounded concentrate, as per standard feeding schedule followed on the farm. The buffaloes were divided into two equal groups, *viz.*, control and treatment groups (n=20 each). The control animals were maintained on standard routine farm feeding schedule and the animals of treatment group were given additional oral supplements daily with 50 g of chelated mineral mixture and 150 g of bypass fat along with concentrates for 6 weeks prepartum and 2 weeks postpartum, and then bypass fat was given @ 15 g/lit of milk limiting to 200 g/day/head till 60 days postpartum. Both the groups were again equally divided into two subgroups each of 10 animals to evaluate the effect of i/m Injection of Stimvet 5 ml (containing Se, Zn, Cu, Mn; 25, 200, 75 and 50 mg, respectively, Wellcon Animal Health Pvt Ltd, Mumbai) around day 45 prepartum and on day of calving, keeping rests as Stimvet controls. They were further subgrouped and one of them (n=5) received Exapar boli (Indian Herbs) 2/day for 4 consecutive days postpartum. Uterine involution was monitored by per rectal palpation and trans-rectal ultrasonography at weekly intervals from day 7 to day 42 postpartum. The puerperal events and periods for uterine involution, first estrus postpartum, conception and service period were recorded for each animal.

Blood sampling and assay techniques: Blood samples (7 ml) were collected from all animals by jugular vein puncture in heparinized vacutainers on days -45, -30, -7, 0, 7, 15, 30, 45 and 60 peripartum (day 0 is day of parturition). The plasma was separated immediately after the collection of blood by centrifugation of samples at 3000 rpm for 10 minutes. The plasma was stored at -80°C with a drop of merthiolate until analyzed. The levels of plasma progesterone, estradiol-17β and cortisol were determined by employing standard RIA techniques of Kubasic *et al.* (1984), Robertson (1979) and Brock *et al.* (1978), respectively. Labelled antigen (with I^{125}), antibody coated tubes and standards were procured from Immunotech-SA, Marsielle Cedex, France. The sensitivity of the progesterone, estradiol-17β and cortisol assays were 0.1, 9.58 and 0.1 ng/ml, respectively. The intra- and inter-assay coefficients of variation were 5.4 and 9.1 per cent for progesterone; 14.4 and 14.5 per cent for estradiol and 5.8 and 9.2 per cent for cortisol, respectively. The levels of 13, 14-dihydro-15-keto PGF2α metabolites (PGFM) were determined in blood plasma using standard procedure and EIA diagnostic kits (Cayman Chemical Co., Ann Arbor, USA) as described by Mishra *et al.* (2003).

Statistical analyses: The puerperal events were compared between groups by 't' test. The data on hormonal profile and PGFM within group were analyzed using ANOVA and DMRT and between groups by 't' test for each trait employing SPSS software version 20.00.

RESULTS AND DISCUSSION

Plasma progesterone: The mean plasma progesterone concentrations in buffaloes are presented in Table 1. The prepartum mean plasma progesterone concentrations in both the groups declined significantly ($p<0.01$) on the day of calving, and reached to the basal/lowest levels (<0.5 ng/ml) by day 15 postpartum. Subsequently, these levels showed a significant rising trend on days 30 to 45 postpartum with still higher values on day 60, suggestive of contributory progesterone production by luteinized follicles that emerged out on the ovaries during early postpartum period followed by follicular atresia and/or formation of corpora lutea preceded by silent ovulations. The day-wise pooled mean plasma progesterone concentrations in control group tended to be lower than the respective values in treatment group, with statistically significant ($p<0.05$) difference in overall pooled means.

The present significant decreasing trend prepartum and increasing trend postpartum of plasma progesterone concentrations observed in buffaloes during transitional period corroborated with the earlier findings of Ullah *et al.* (2010), Ashmawy (2015) and Kalasariya *et al.* (2017) in buffaloes and Gowda *et al.* (2015) and Dhami *et al.* (2017) in cattle. In the present study, the basal value of progesterone found on the day of calving was suggestive of complete luteolysis at parturition and corroborated with the findings of Momongan *et al.* (1990) and Kalasariya *et al.* (2017). These findings are also in agreement with the Pahwa and Pandey (1983) and El-Belely *et al.* (1988), who reported that the concentration of progesterone tended to decline from the day of parturition and then decreased linearly until complete regression of the residual corpus luteum of pregnancy by day 15 postpartum in non-suckled Murrah and Egyptian buffaloes, respectively.

Cerri *et al.* (2009) and Tyagi *et al.* (2010) found that the nutrients supplemented cows had significantly higher

Table 1: Plasma progesterone concentrations (ng/ml) in Jaffarabadi buffaloes given peripartum nutrients supplementation and treatment with Stimvet and Exapar alone or in combination.

Peripartum Period	Overall nutrient groups			Treatment subgroups			
	Control (N=20)	Supplement (N=20)	Pooled (N=40)	Stimvet (N=10)	Exapar (N=10)	S+E (N=10)	None (N=10)
-45 day	4.43±0.15^{d}	4.83±0.29^{d}	4.63±0.16^{e}	5.07±0.36	4.19±0.11	4.13±0.13	5.15±0.45
-30 day	4.08±0.17^{d}	4.66±0.33^{d}	4.37±0.19^{e}	4.10±0.22	3.79±0.15	4.30±0.35	5.28±0.54
-7 day	3.98±0.35^{d}	4.41±0.36^{d}	4.20±0.25^{e}	4.06±0.51	3.61±0.20	4.22±0.52	4.97±0.65
0 day	0.89±0.10^{a}	1.13±0.18^{b}	1.01±0.10^{bc}	0.94±0.17	0.84±0.17	0.83±0.13	1.41±0.30
7 day	0.40±0.05^{a}	0.58±0.08^{a}	0.49±0.05^{ab}	0.46±0.09	0.54±0.13	0.43±0.08	0.53±0.10
15 day	0.35±0.04^{a}	0.48±0.05^{a}	0.41±0.04^{a}	0.41±0.07	0.42±0.08	0.37±0.06	0.47±0.08
30 day	1.07±0.27^{ab}	1.45±0.35^{b}	1.26±0.22^{c}	0.70±0.15	2.12±0.60	1.09±0.28	1.13±0.51
45 day	1.68±0.46^{bc}	1.24±0.24^{b}	1.46±0.26^{c}	2.34±0.78	1.02±0.15	1.06±0.29	1.41±0.55
60 day	2.23±0.35^{c}	2.88±0.43^{c}	2.55±0.28^{d}	2.23±0.42	2.87±0.52	1.84±0.52	3.27±0.71
Overall	**2.12±0.14**	****2.41±0.16**	**2.26±0.11**	**2.25±0.21**	**2.16±0.18**	**2.03±0.20**	**2.62±0.25**

N= Number of animals; **P<0.01 between control and supplemented groups.
Means bearing uncommon superscripts within the column differ significantly between periods (p<0.05).

progesterone values as compared to control group. In the present study, the progesterone levels of nutrient supplemented group were however non-significantly higher as compared to control buffaloes. The observed higher level of plasma progesterone might be due to the substantial production of progesterone by the functional CL. Dietary supplementation of fat increases the circulating concentrations of cholesterol, which serves as a precursor for the synthesis of progesterone by ovarian luteal cells and also increases the lifespan of induced CL (Rahbar *et al.*, 2014). The gradual rise in mean plasma progesterone level on day 45 and 60 postpartum in the present study is supported by the findings of previous workers (Dhami *et al.*, 2017; Kalasariya *et al.*, 2017), who also observed gradual rise in progesterone prior to first postpartum estrus in cattle and buffalo.

The injection Stimvet and oral Exapar alone or in combination did not influence the plasma progesterone concentrations, irrespective of oral nutrient supplements, when compared with non-treated subcontrol group at any of the peripartum intervals, except at day 30 and 45 postpartum, where the groups treated with Stimvet and Exapar, respectively, had significant rise in plasma progesterone as compared to other subgroups and by day 60 postpartum all the subgroups revealed significantly elevated plasma progesterone concentrations showing resumption of ovarian cyclicity and presence of luteal activity (Table 1, Fig 1). Almost similar patterns of plasma progesterone were noted with these treatments under major control and oral nutrient supplemented/treatment groups.

Plasma estradiol-17β: The mean plasma estradiol-17β concentrations at day 45 prepartum in buffaloes of both control and treatment groups increased gradually and significantly (p<0.01) as parturition approached reaching the highest on the day of calving. Thereafter, there was a sudden and significant (p<0.01) drop in the levels by day 7 (29.46±2.06 and 30.35±2.06 pg/ml) postpartum, which then fluctuated non-significantly at the same level till day 45-60 postpartum (Table 2).

The injection Stimvet and oral Exapar alone or in combination did not influence the plasma estradiol concentrations, irrespective of oral nutrients supplements, when compared with non-treated control subgroup at any of the peripartum intervals, except at day 45 postpartum, where the subgroups treated with Exapar and Stimvet + Exapar had significant rise in plasma estradiol as compared to other subgroups and by day 60 postpartum all the subgroups revealed significantly elevated plasma estradiol concentrations over day 15 postpartum, showing resumption of ovarian follicular activity, which was also substantiated with presence of luteal activity and increased progesterone levels (Table 2). Almost similar trends / patterns of plasma estradiol were noted with these treatments under major control and oral nutrients supplemented/treatment groups. The overall trend of prepartum and postpartum estradiol-17β profile found

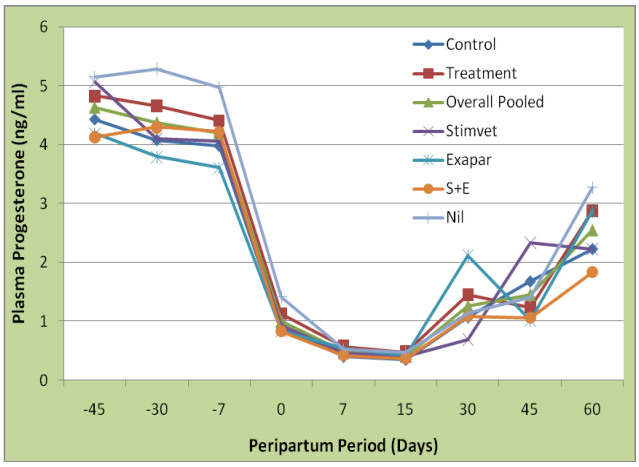

Fig 1: Influence of Farm feeding Vs Nutrients supplementation peripartum and treatment with Stimvet and Exapar alone or in combination postpartum on plasma profile of progesterone in Jaffarabadi buffaloes.

Table 2: Plasma estradiol concentrations (pg/ml) in Jaffarabadi buffaloes given peripartum nutrients supplementation and treatment with Stimvet and Exapar alone or in combination.

Peripartum Period	Overall nutrient groups				Treatment subgroups		
	Control (N=20)	Supplement (N=20)	Pooled (N=40)	Stimvet (N=10)	Exapar (N=10)	S+E (N=10)	None (N=10)
-45 day	39.24±2.12^{c}	40.14±2.19^{c}	39.69±1.51^{d}	40.58±3.42	37.00±1.89	39.23±2.22	41.96±4.21
-30 day	45.83±2.29^{d}	46.83±2.86^{d}	46.33±1.74^{e}	47.00±3.64	44.15±2.59	45.75±3.37	48.40±4.45
-7 day	59.36±2.25^{e}	56.53±2.65^{e}	57.94±1.73^{f}	56.30±3.36	57.63±2.84	58.05±3.72	59.80±4.22
0 day	85.21±2.48^{f}	82.74±2.12^{f}	83.98±1.62^{g}	81.25±3.62	83.79±3.25	88.33±2.70	82.54±3.40
7 day	29.46±2.06^{b}	30.35±2.06^{b}	29.90±1.44^{c}	34.05±4.13	28.05±1.87	25.82±1.35	31.70±3.00
15 day	22.56±1.41^{a}	22.36±1.62^{a}	22.46±1.06^{a}	22.35±2.70	22.58±1.75	20.75±1.32	24.18±2.59
30 day	24.25±1.33^{ab}	22.04±1.45^{a}	23.15±0.99^{ab}	23.73±2.39	23.96±0.83	22.67±1.83	22.23±2.62
45 day	27.36±1.95^{ab}	25.08±1.38^{ab}	26.22±1.19^{abc}	22.73±1.71	27.47±2.91	27.98±3.00	26.71±1.52
60 day	28.20±2.43^{ab}	26.396±1.82^{ab}	27.28±1.51^{bc}	26.75±1.86	28.87±3.19	30.65±4.23	22.85±2.02
Overall	**40.16±1.60**	**39.16±1.57**	**39.66±1.12**	**39.41±2.20**	**39.28±2.16**	**39.91±2.35**	**40.04±2.30**

N= Number of animals.

Means bearing uncommon superscripts within the column differ significantly between periods ($p<0.05$).

in animals under study coincided well with that reported by many of the earlier researchers in buffaloes (Arya and Madan, 2001; Dugwekar *et al.*, 2008; Ashmawy, 2015 and Kalasariya *et al.*, 2017) and in cattle (Dhami *et al.*, 2017).

It is well established fact that after parturition the levels of circulating plasma progesterone and estradiol-17β markedly decrease and fluctuate at basal levels until the initiation of postpartum ovarian activity. However in the present study, no significant effect of oral nutrient supplementation was noted on these hormones profiles as compared to control group. In addition to this, plasma estradiol concentrations were variable but minimal during the early postpartum period similar to those during the luteal phase of the estrous cycle and concurred with the reports of Dugwekar *et al.* (2008), Dhami *et al.* (2017) and Kalasariya *et al.* (2017) in bovines.

Plasma cortisol: The mean plasma cortisol concentrations were initially at the lowest level (18-22 ng/ml) between day 30 and 7 prepartum, but increased abruptly and significantly on the day of parturition in both control and treatment groups (25.17±2.32 and 28.55±1.80 ng/ml, respectively). Thereafter, the levels decreased significantly to those of prepartum phase by day 7 postpartum and fluctuated non-significantly in both the groups for all the subsequent days postpartum (Table 3).

The maternal glucocorticoids concentrations show a sharp peak around 2 days before parturition, after which the concentrations return to basal levels by 2 days after parturition (Smith *et al.*, 1973). High maternal cortisol levels during parturition could be attributed to the stress of labour; however, cortisol is also necessary for other physiological processes around the time of parturition, particularly for gluconeogenesis (Smith *et al.*, 1973; Dhami *et al.*, 2017). Further, the observed trend of changes in cortisol values was found to be similar to the findings of Dang *et al.* (2013), Ashmawy (2015) and Dhami *et al.* (2017), who reported that the cortisol levels in the prepartum days were initially low, but increased as parturition approached. These levels came back to normal levels after calving.

The oral nutrients supplementation did not influence the plasma cortisol levels in transitional buffaloes. The injection Stimvet and oral Exapar alone or in combination also did not influence the plasma cortisol concentrations, irrespective of oral nutrients supplementation, when compared with non-treated control subgroup at any of the peripartum intervals, yet the levels in Exapar and Stimvet + Exapar subgroups were apparently lower than other two groups at most peripartum intervals (Table 3). However, the occurrence of the postpartum first estrus was earlier in the treatment than control group.

Plasma PGFM: The plasma concentrations of 13, 14-dihydro-15-keto PGF2α metabolites (PGFM) were found to be low or basal on day 45 prepartum, which increased gradually and significantly ($p<0.01$) by almost 9 to 10-fold to reach peak values on the day of calving in both control and treatment groups and then declined gradually and significantly till day 45 postpartum, which then further decreased marginally by day 60 postpartum (Table 4, Fig 2). The rise was little more in nutrients supplemented group with higher values at most intervals peripartum than in control group.

The observed trend of changes in PGFM values was found to be similar to the findings of Thompson *et al.* (1987), Toribio *et al.* (1994) and Dhami *et al.* (2017), who all reported that the PGFM levels in the prepartum days were low, but increased many fold around parturition. The levels thereafter came back to normal within 20-30 days postpartum. The trend of PGFM recorded peripartum is a reflection of prostaglandin F_2 alpha production around parturition for inducing luteolysis and for uterine involution. There is an intense production of prostaglandin F2α (PGF2α) by inter-caruncular region of endometrial epithelial surface during early puerperium

Table 3: Plasma cortisol concentrations (ng/ml) in Jaffarabadi buffaloes given peripartum nutrients supplementation and treatment with Stimvet and Exapar alone or in combination.

Peripartum Period	Overall nutrient groups				Treatment subgroups		
	Control (N=20)	Supplement (N=20)	Pooled (N=40)	Stimvet (N=10)	Exapar (N=10)	S+E (N=10)	None (N=10)
-45 day	19.20±2.11^{a}	19.09±2.33^{a}	19.14±1.55^{a}	22.01±3.42	15.53±2.57	17.48±2.82	21.56±3.47
-30 day	19.46±2.28^{a}	18.33±1.93^{a}	18.89±1.48^{a}	20.78±3.40	15.36±2.05	20.03±2.95	19.41±3.37
-7 day	21.59±2.29^{ab}	21.36±2.33^{a}	22.47±1.61^{a}	22.53±3.36	17.43±2.81	21.70±2.93	24.23±3.81
0 day	25.17±2.32^{b}	28.55±1.80^{b}	26.86±1.45^{b}	29.62±3.38	23.40±1.99	21.33±3.00	28.11±3.10
7 day	18.35±2.16^{a}	17.71±1.72^{a}	18.03±1.63^{a}	29.64±2.90	16.50±2.14	16.14±2.45	19.84±3.43
15 day	16.86±1.64^{a}	20.23±1.72^{a}	18.54±1.20^{a}	19.91±2.48	16.78±2.29	18.01±1.80	19.47±3.13
30 day	17.39±1.79^{a}	20.75±1.98^{a}	19.07±1.34^{a}	17.98±2.25	17.19±2.38	21.67±3.44	19.45±2.74
45 day	16.23±1.86^{a}	20.17±1.68^{a}	18.20±1.28^{a}	19.65±2.29	14.99±2.73	18.92±2.29	19.24±2.97
60 day	16.99±1.92^{a}	18.57±1.45^{a}	17.78±1.20^{a}	18.18±2.24	17.34±2.83	18.37±1.76	17.22±2.92
Overall	**19.03±0.70**	***20.53±0.66**	**19.78±0.48**	**21.14±0.99**	**17.17±0.82**	**19.87±0.90**	**20.95±1.08**

N= Number of animals; *P<0.05 between control and supplemented groups.
Period means within the column did not differ significantly (p>0.05).

Table 4: Plasma PGFM concentrations (ng/ml) in Jaffarabadi buffaloes given peripartum nutrients supplementation and treatment with Stimvet and Exapar alone or in combination.

Peripartum Period	Overall nutrient groups				Treatment subgroups		
	Control (N=20)	Supplement (N=20)	Pooled (N=40)	Stimvet (N=10)	Exapar (N=10)	S+E (N=10)	None (N=10)
-45 day	224.41±16.68^{b}	275.01±14.89^{ab}	249.71±11.76^{b}	242.99±19.54	254.93±32.37	244.75±27.69	256.18±13.55
-30 day	295.08±13.13^{c}	348.71±31.10^{b}	321.89±17.20^{c}	328.07±10.62	343.02±66.45	308.46±20.31	308.03±11.70
-7 day	855.37±22.37^{e}	891.41±20.16^{d}	773.39±15.14^{e}	876.17±21.70	887.12±40.33	872.43±35.03	857.85±24.50
0 day	2209.7±37.71^{g}	2266.3±72.78^{f}	2238.0±40.71^{g}	2314.5±55.77	2110.0±99.07	2317.4±68.98	2210.1±49.52
7 day	1309.8±35.04^{f}	1397.8±42.68^{e}	1353.8±28.15^{f}	1373.4±28.85	1265.1±65.64	1398.0±67.95	1378.8±52.65
15 day	771.40±21.67^{d}	796.64±20.83^{c}	784.02±14.97^{d}	796.05±25.43	774.55±40.88	798.78±37.53	766.71±18.63
30 day	325.63±18.75^{c}	357.13±14.33^{b}	341.38±11.92^{c}	335.68±22.74	328.90±22.16	365.68±28.55	335.26±23.39
45 day	170.56±7.91^{ab}	209.91±10.22^{a}	190.24±7.11^{a}	204.36±10.13	197.00±15.72	191.35±17.04	168.23±12.49
60 day	142.34±4.93^{a}	182.79±9.53^{a}	162.57±6.21^{a}	171.77±8.51	162.24±16.37	152.94±14.45	163.31±9.95
Overall	**700.49±49.42**	**747.30±50.30**	**723.90±35.13**	**738.11±71.96**	**702.54±67.19**	**738.87±73.18**	**716.06±69.65**

N= Number of animals.
Period means within the column did not differ significantly (p>0.05).

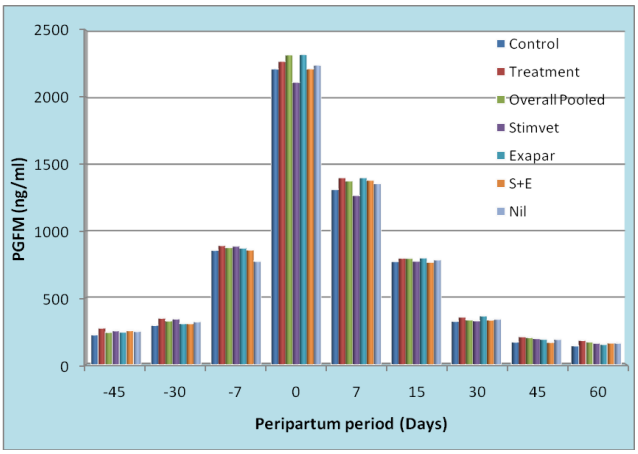

Fig.2: Influence of Farm feeding Vs Nutrients supplementation peripartum and treatment with Stimvet and Exapar alone or in combination postpartum on plasma profile of PGFM in Jaffarabadi buffaloes.

(Asselin *et al.*, 1997; Skarzynski *et al.*, 2000), which correlates to uterine involution process (Kindahl *et al.*, 1999; Dhami *et al.*, 2017). In the present study, somewhat higher PGFM concentrations recorded throughout the study period in nutrients supplemented group were correlated with rapid uterine involution and early onset of ovarian activity with better fertility as compared to control group.

The injection Stimvet and oral Exapar alone or in combination did not influence the plasma PGFM concentrations, irrespective of oral nutrients supplements. Almost similar inconsistent trends / patterns of plasma PGFM were noted with these treatments under major control and oral nutrients supplemented / treatment groups. However, no such study of Stimvet or Exapar on PGFM was available to support or contradict the present findings in buffaloes.

Postpartum fertility: The feeding of bypass fat and chelated minerals peripartum had significant effect on the time required for expulsion of placenta (3.93±0.24 vs 7.18±0.72 hrs; p<0.01), uterine involution (32.75±0.57 vs 37.00±0.56 days;

p<0.05), interval for first estrus postpartum (79.05±3.82 vs 100.55±3.47 days p<0.05) and service period (107.10±4.43 vs 133.65±6.04 days, p<0.05), being significantly earlier/ shorter as compared to control group, with higher conception rate by day 120 postpartum (85 vs 50%). These findings corroborated well with the reports of Mavi *et al.* (2006) and Modi *et al*. (2016) for vitamin E and Se supplemented buffaloes. Similarly, Khan *et al.* (2015), Mane *et al.* (2016), Dhami *et al.* (2017) and Kalasariya *et al.* (2017) found beneficial effect of peripartum nutritional supplementation on uterine involution and postpartum fertility in cattle and buffaloes. These observations clearly indicated that there was a positive effect of peripartum nutrient supplementation in buffaloes so far as uterine involution and onset of postpartum ovarian activity and fertility are concerned. The earlier resumption of cyclicity in the buffaloes under treatment group could be attributed to the effect of fat and additional minerals supplementation in their diet.

However, there was no significant effect of Stimvet injection and Exapar boli either alone or in combination over control group on the traits studied. Although the conception rate was apparently higher in Stimvet plus Exapar treated group as compared to individual treatment groups. Dhakal (1999) recorded expulsion of placenta in 100 per cent animals with conception rate of 72 vs. 40 % in buffaloes and 55 vs. 25 % in cows following use of Exapar. Similar significant positive effect was also seen on expulsion of placenta, cessation of lochial discharge and uterine involution in dairy animals following use of Exapar by others (Gautam *et al.*, 2005; Thakur *et al.*, 2013). However, in all these studies no other nutrients supplements were provided to the animals. In the present study, the non-significant influence of Stimvet injection and Exapar bolus alone or in combination could be attributed to the oral supplementation of chelated minerals and bypass fat to all the animals on the farm, which might have optimized the circulatory nutrients requirements of animals, not requiring extra parenteral or oral supplement/ treatment.

CONCLUSION

The results showed that there was sudden drop in plasma progesterone and increase in estradiol-17β concentrations at calving with subsequent drop in both during early postpartum and an increase during 30-60 days postpartum, till recrudescence of ovarian follicles and ovulation/CL formation. Significant increase in plasma cortisol level on the day of calving compared to 7 days before and after calving indicated parturition stress and levels were significantly higher in fat supplemented group. PGFM levels were higher in fat supplemented group throughout the study period and it was the highest on the day of calving in all buffaloes. In short, peripartum nutrient supplementation (bypass fat & ASMM) in Jaffarabadi buffaloes yielded significant beneficial effect on puerperal events and postpartum fertility as revealed by significantly shorter period of placental expulsion and uterine involution, early onset of first postpartum estrus with apparently shorter service period and enhanced pregnancy rate in comparison to control group, hence farmers could be advised to follow this practice.

ACKNOWLEDGEMENT

We thank the Officer in Charge of CBF, JAU, Junagadh and their staff for permitting us to use their animals and kind cooperation as well as ICAR for financial support through sanction of project "AICRP on nutritional & physiological interventions for enhancing reproductive performance in animals" at AAU, Anand with Dr. A.J. Dhami as principal investigator.

REFERENCES

Arya, J.S. and Madan, M.L. (2001). Postpartum reproductive cyclicity based on ovarian steroids in suckled and weaned buffaloes. *Buffalo Journal*, **17**: 361-369.

Ashmawy, N.A. (2015). Changes in peripheral plasma hormone concentrations and metabolites during the last trimester of pregnancy and around parturition in the Egyptian buffalo and Baladi cows. *International Journal of Advanced Research*. **3**: 1377-1390.

Asselin, E., Droplet, P. and Fortier, M.A. (1997). Cellular mechanisms involved during oxytocin induced prostaglandin F2α production in endometrial cells *in vitro*: role of cycloxygenase 2. *Endocrinology.* **138**: 4798-4805.

Brock, P., Eldred, E.W., Woiswillo, J.E., Doran, M. And Schoemaker, H.J. (1978). Direct solid phase I^{25I} radioimmunoassay of serum cortisol. *Clinical Chemistry*. **24**: 1595-98.

Cerri, R.L.A., Juchem, S.O., Chebel, R.C., Rutigliano, H.M., Bruno, R.G.S., Galvão, K.N., Thatcher, W.W. and Santos, J.E.P. (2009). Effect of fat source differing in fatty acid profile on metabolic parameters, fertilization, and embryo quality in high-producing dairy cows. *Journal of Dairy Science*. **92**: 1520-1531.

Crowe, M.A., Goulding, D., Baguisi, A., Boland, M.P., Roche, J.F. (1993). Induced ovulation of the ûrst postpartum dominant follicle in beef suckler cows using a GnRH analogue. *Journal of Reproduction and Fertility.* **99**: 551-555.

Dang, A.K., Prasad, S., De, K., Pal, S., Mukherjee, J. *et al.* (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.

Dhakal, I.P. (1999). Efficacy of "Exapar" for the expulsion of placenta and as uterine tonic in cows and buffaloes. *Indian Journal of Animal Reproduction*. **20**: 33-34.

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

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

El-Belely, M.S., Zaki, K. and Grunert, E. (1988). Plasma profiles of progesterone and total estrogens in buffaloes (*Bubalus bubalis*). *Journal of Agricultural Sciences Cambridge,*.**111**: 519-524.

Gautam, R.P., Tiwari, R.P., Koley, K.M. and Hore, S.K. (2005). The Exapar in induction of uterine contraction *in vitro* and expulsion of fetal membranes in buffaloes. *Indian Journal of Animal Reproduction*. **26**(2): 126-128.

Gowda, A.J.S., Devaraj, M., Krishnaswamy, A., Ranganath, L., Ravindra, J.P. and Gupta, P.S.P. (2015). The impact of feeding propylene glycol, bypass fat and bypass protein on progesterone concentration in postpartum dairy cattle. *Journal of Cell and Tissue Research*. **15**: 5079-5084.

Kalasariya, R.M., Dhami, A.J., Hadiya, K.K., Borkhatariya, D.N. and 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.

Khan, H.M., Mohanty, T.K., Bhakat, M., Gupta, A.K., Tyagi, A.K. and Mondal, G. (2015). Effect of vitamin E and mineral supplementation on biochemical profile and reproductive performance of buffaloes. *Buffalo Bulletin.* **34**: 63-72.

Kindahl, H., Bekana, M., Kask, K., Konigsson, K., Gustafsson, H. and Odensvik, K. (1999). Endocrine aspects of uterine involution in the cow. *Reproduction in Domestic Animals.* **34**: 261-268.

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

Kubasic, N.P., Hallauer, G.D. and Brodows, R.G. (1984). Evaluation of direct solid phase RIA for progesterone, useful for monitoring luteal function. *Clinical Chemistry.* **30**: 284-286.

Mane, P.M., Gaikwad, S.M., Dhoble, R.L., Chaudhari, R.J., Sawale, A.G., Suryawanshi, P.R. and Dawane, S.C. (2016). Effect of mineral supplementation on involution, postpartum ovarian activity and conception rate in Marathwadi buffaloes. *Buffalo Bulletin.* **35**: 247-257.

Mavi, P.S., Pangaonkar, G.R. and 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.

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

Mishra, D.P., Meyer, H.H.D., Prakash, B.S. (2003). Validation of a sensitive enzymeimmunoassay for 13, 14-dihydro-15-keto-PGF2 in buffalo plasma and its application for reproductive health status monitoring. *Animal Reproduction Science.* **78**: 33-46.

Modi, L.C., Khasatiya, C.T., Patel, M.D. and 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.

Momongan, V.G., Sarabia, A.S., Roxas, N.P., Palad, O.A., Obsioma, A.R., Nava, Z.M., Del Barrio, A.N. (1990). Increasing the productive efficiency of Caraboas under small holder farming systems. *In: Domestic Buffalo Production in Asia*. IAEA, Vienna, p. 167-178.

Pahwa, G.S. and Pandey, R.S. (1983). Hormonal changes in postpartum blood plasma and milk of buffaloes (*Bubalus bubalis*). *Animal Production*. **37**: 237-246.

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

Robertson, R.D. (1979). Assessment of ovulation by ultrasound and plasma estradiol determination. *Obstetrics and Gynaecology*. **54**: 686-690.

Rueda, B.R., Hendry, I.R., Hendry, I.W., Stormshak, F., Slayden, O.D. and 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.

Setia, M.S., Duggal, R.S. and Singh, R. (1992). Biochemical constituents of blood in buffaloes and cows during late pregnancy and different stages of lactation - A longitudinal study. *Buffalo Journal*. **8**(2): 123-129.

Skarzynski, D.J., Miyamoto, Y. and 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.

Smith, V.G., Edgerton, L.A., Hafs, H.D., Convey, E.M. (1973). Bovine serum estrogens, progestins and glucocorticoids during late pregnancy, parturition and early lactation. *Journal of Animal Science.* **36**: 391-396.

Thakur, A., Ravikanth, K., Maini, S., Patil, A.D., Deshmukh, A.A. and Patil, A.D. (2013). Management of postparturient reproductive disorders in dairy animals with herbal uterine cleanser Exapar-n. *Advanced Research in Pharmacology and Biology.* **3**: 517-519.

Thompson, F.N., Page, R.D., Cook, C.B. and Caudle, A.B. (1987). Prostaglandin F2á metabolite levels in normal and uterine-infected postpartum cows. *Veterinary Research Communication*. **11**(6): 503-507.

Thorton, P.K., Kruska, R.L., Henninglr, N., Kristijanan, P.M., Reid, R.S., Atieno, F., Odero, A.N. and Ndegwa. T. (2002). *Mapping poverty and livestock in the developing world*. *Nairobi: International Livestock Research Institute.*

Toribio, R.E., Molina, J.R., Bolanos, J.M. and Kindahl, H. (1994). Blood levels of the prostaglandin F2α metabolite during the postpartum period in *Bos indicus* cows in the humid tropics. *Journal of Veterinary Medicine-A.* **41**: 630-639.

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**:1749-1755.

Ullah, N., Anwar, M., Andrabi, S.M.H., Murtaza, S., Ali, Q. and Asif, M. (2010). Effect of mineral supplementation on postpartum ovarian activity in Nili-Ravi buffaloes. *Pakistan Journal of Zoology.* **43**(2): 195-200.