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Summary and Conclusions

The present study “Gynaeco-clinical ultrasonographic studies with reference to nutritional management of transition period on uterine involution and postpartum fertility in Jaffarabadi buffaloes” was undertaken during June 2015 to December 2017 at Cattle Breeding Farm, JAU, Junagadh, with the support of “AICRP on Nutritional and Physiological Interventions for Enhancement of Reproductive Performance of Animals” of AAU, Anand, on a total of 40 healthy advanced pregnant Jaffarabadi buffaloes of 2 to 4 parity and of nearly identical body size. The major objectives were (i) to study the effect of peripartum nutritional (multi-minerals and bypass fat) supplementation on uterine involution and postpartum ovarian activities, (ii) to evaluate the effect of herbal ecbolic (Exapar) and injectable micro-minerals (Stimvet) formulations on uterine involution and postpartum fertility, and (iii) to study alterations in metabolic, mineral and hormonal attributes with the process of parturition and uterine involution in treated and control animals, including influence of various factors affecting the uterine involution and postpartum fertility in Jaffarabadi buffaloes.

The animals were randomly divided into two equal groups, viz., Control and Treatment (supplement) groups, each of 20 buffaloes. The Control animals were maintained on standard routine farm feeding schedule (20 kg seasonal green + ad lib dry fodder + concentrate mixture + 50 g mineral mixture to meet the DCP & TDN requirements) during last two months of pregnancy. After calving, the level of compounded concentrate fed was @ 50 per cent of milk produced. These 20 animals were equally divided into 2 subgroups (C1 & C2). In C1 group (n=10), multi-minerals Inj. Stimvet 5 ml (Wellcon Animal Health Pvt Ltd) (containing Se, Zn, Cu, Mn; 25, 200, 75 and 50 mg, respectively) was given i/m twice, at 45 days before parturition and on the day of parturition. Moreover, herbal ecbolic boli (Exapar bolus, Ayurved Ltd) were given orally 2 boli bid for first 4 consecutive days postpartum to 5 buffaloes and the rest 5 were kept as ecbolic control. While buffaloes of C2 group (n=10) were kept as control without Inj. Stimvet, but 5 of them received herbal ecbolic 2 boli orally bid as above and the rest 5 served as control.
The buffaloes under Treatment group (n=20) were subjected to additional nutritional supplements, over the routine farm feeding schedule, orally daily with 50 g of chelated multi-minerals (AAU, Anand) and 150 g of by-pass fat (Sunegry, Polchem) with concentrates for 6 weeks prepartum and 2 weeks postpartum. Then the bypass fat was increased as per the milk production @ 15 g/litre of milk produced maximum up to 200 g/head/day till 60 days postpartum. These animals were also equally divided into 2 subgroups (T1 & T2), and were managed like C1 and C2 subgroups with and without Stimvet injection and oral ecbolic Exapar boli. Blood samples were collected from jugular vein in heparinized vacutainers at days -45, -30, -7, 0, 7, 15, 30, 45 and 60 (day 0 is day of parturition) in both the groups. The plasma separated out was stored in sterilized plastic vials at -80°C with a drop of merthiolate until analyzed for various hormonal, metabolic and mineral profile.

The important puerperal events, viz., calf birth weight & sex, placental weight, placental expulsion time, side of pregnancy were recorded. Per-rectal (precalibrated operators’ hand) as well as ultrasonographic (5.0-7.5 MHz transrectal transducer) evaluation of uterine and cervical involution as well as ovarian follicular activities were carried out at day 7, 15, 30, 45 and 60 postpartum in both the control and treatment groups. The animals were then followed at least up to 120 days and beyond postpartum for first estrus postpartum, fertile estrus, service period and conception rate. The findings are summarized below.

5.1 Findings of Trans-Rectal Palpation and Ultrasonography

On per rectal palpation by day 7 postpartum, gravid and non-gravid uterine horns were lying cranial and ventral to pelvic brim in abdominal cavity as large, soft, flabby water bag like without any tonicity and elasticity in all the animals. The gravid uterine horn was not palpable up to its full length but the non-gravid horn could be palpated to its full length with little difficulties, in both the groups of animals. By day 15, in comparison to the location of gravid horn, the non-gravid horn and the cervix were found to be nearer to the pelvic brim or in pelvic cavity in buffaloes of both the groups, and the uterine horns appeared to resemble a water-hose having soft consistency with a mild increase in tone and elasticity. The gravid horn, however, was either touching the pelvic brim or suspended in the abdominal cavity. The decrease in size of uterine horns was observed to be faster up to day 15 postpartum and thereafter it decreased very marginally. The increasing trend in the tonicity, elasticity and curling of horns was observed from day 15 postpartum with firm consistency on day
21 and 28 postpartum for all the animals, being more pronounced in Treatment group. On day 35 postpartum, the horns evinced decreased tonicity and elasticity (slight doughy consistency), with lesser curling of horns. The gross involution of the genitalia was observed to be completed by day 37.00±0.56 and 32.75±0.57 (p<0.05) in buffaloes under Control and Treatment groups with significant effect of nutrients supplemented.

On ultrasonography, the wall of the cervix appeared as bright hyperechoic structure while its lumen was found to be hypoechoic with bright hyperechoic spots of cervical folds. By day 7, the cervix was found to be reduced in diameter considerably with constant reformation of the cervical folds. The echogenicity of the cervix became even more pronounced in subsequent days postpartum. The walls of the gravid and non-gravid uterine horns on the 7th day postpartum showed hyperechoic structures and the lumen was anechoic with some hyperechoic spots of lochia. On the later stages, the gravid uterine horns were seen as hyperechoic wall and hypoechoic lumen. The lochia was observed to be snowy. The scanned uterine caruncles on day 7 postpartum revealed bright hyperechoic structures resembling mushroom, protruding in the anechoic uterine lumen, encircled with bright visible hyperechoic line. The caruncular texture became more hyperechoic in the later days postpartum.

The mean diameter of the cervix in animals on day 7 postpartum was recorded as 6.51±0.02 cm, which subsequently reduced gradually and significantly (p<0.05) to 5.25±0.03 and 3.80±0.02 cm on days 15 and 30 postpartum, respectively. Thereafter, it reduced non-significantly to 3.32±0.03 and 3.30±0.03 cm on days 45 and 60, respectively. The mean thickness of the cervical wall on days 7, 15, 30, 45 and 60 postpartum were recorded to be 1.86±0.02, 1.26±0.01, 1.18±0.01, 1.13±0.01 and 1.11±0.01 cm, respectively, with progressive significant reduction (p<0.05) till day 15 postpartum, and thereafter no further reduction was noted between day 30 and 60 postpartum. The cervical involution was completed by day 30 postpartum.

The mean diameters of past gravid and non-gravid uterine horns in buffaloes on day 7 postpartum were 7.78±0.03 and 6.31±0.02 cm, which reduced significantly (p<0.01) and progressively on day 15 (5.18±0.02 and 5.02±0.01 cm) and 30 (4.85±0.03 and 4.37±0.03 cm, respectively) postpartum. Thereafter, it decreased non-significantly on days 45 and 60 postpartum. The mean thickness of wall of the gravid and non-gravid horns on day 7, 15, 30 and 45 postpartum were recorded as 2.48±0.02 & 1.34±0.02, 1.32±0.02 & 0.120±0.03, 0.70±0.02 & 0.89±0.01, and 0.67±0.01 &
0.67±0.01 cm, respectively, with significant (p<0.05) reduction from day 7 to day 30 postpartum in both the horns. The mean caruncular lengths in animals on day 7 and 15 postpartum were found to be 4.59±0.04 and 2.37±0.03 cm (p<0.05), respectively.

5.2 Effect of Nutritional Supplementation on Uterine Involution and Postpartum Fertility

The feeding of bypass fat and minerals had significant effect on the time required for expulsion of placenta (3.93±0.24 vs 7.18±0.72 hrs; p<0.01); however, the calf birth weight (33.43±1.13 vs 30.99±1.13 kg), though apparently higher, did not differ from the Control group, and weight of placenta was almost same in both Control and Treatment groups. The mean period for uterus involution was found to be 37.00±0.56 and 32.75±0.57 days (p<0.05) in the buffaloes of Control and Treatment groups, respectively, with a pooled mean of 34.88±0.52 days. The mean intervals for first estrus postpartum and service period observed in buffaloes under Control group were 100.55±3.47 and 133.65±6.04 days, whereas these day were significantly (p<0.05) lesser 79.05±3.82 and 107.10±4.43 days in Treatment group. The overall conception rates in Control and Treatment groups by day 120 postpartum were 50 and 85% (p<0.05), respectively. Moreover, the first 90 days milk yield was also higher in Treated than Control group.

5.3 Effect of Stimvet and Herbal Ecbolic Exapar alone or in Combination on Uterine Involution and Postpartum Fertility

The overall calf birth weight, placental weight, placental expulsion time, uterine involution, first estrus postpartum, service period, conception within 120 days postpartum and first 90 days milk yield in Jaffarabadi buffaloes, irrespective of nutrients treatment and control group, were observed to be 32.21 ±0.81 kg, 6.46 ±0.18 kg, 5.55 ±0.45 hr, 34.88 ±0.52 days, 89.80 ±3.07 days, 120.38 ±4.27 days, 69.50 per cent and 679.65 ±39.32 kg, respectively. There was no significant effect of Stimvet injection and Exapar boli either alone or in combination over non-treated control group on these traits. However, the conception rate was apparently higher in Stimvet plus Exapar treated group as compared to other groups, perhaps due to optimized nutrient requirements from oral supplements.

5.4 Factors Affecting Uterine Involution

The sex of calf born to Jaffarabadi buffaloes had no significant effect on puerperal events and postpartum fertility. However, the male calves were little heavier (32.77±1.22 vs 30.49±1.76. kg) and their dams had slightly longer gestation
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(310.08±1.61 vs 308.14±0.94 days) and took little longer time to expel somewhat heavier fetal membranes (6.00±0.78 vs 5.43±0.70 hrs). The birth weight of calf (</> 32 kg) had significant (p<0.05) effect on fetal membranes weight and gestation length, both being more in heavier calves. However, it had no significant effect on fetal membranes expulsion time, uterine involution, first postpartum estrus or service period.

The values for calf birth weight, fetal membranes weight, gestation length, uterine involution, first postpartum estrus and service period were found to be non-significantly higher in animals, which took more than 5 hrs to expel their fetal membranes. Weight of placenta (</> 6 kg) had significant effect on calf birth weight and gestation length, being higher for births with heavier placenta. Other traits were however not influenced adversely by the placental weight. The gestation length had significant effect on birth weight, heavier calves being born to buffaloes having gestation length beyond 309 days. However, there was no effect of gestation length on other traits studied. There was no effect of side of previous pregnancy on puerperal events and postpartum fertility.

5.5 Hormonal Profiles

The pooled mean plasma progesterone concentrations of buffaloes, irrespective of subgroups, under Control group at days 45, 30 and 7 prepartum, day of calving and on days 7, 15, 30, 45 and 60 postpartum were recorded as 4.43±0.15, 4.08±0.17, 3.98±0.35, 0.89±0.10, 0.40±0.05, 0.35±0.04, 1.07±0.27, 1.68±0.46, and 2.23±0.35 ng/ml, respectively. The corresponding values in the buffaloes under nutrient supplemented / Treatment group were 4.83±0.29, 4.66±0.33, 4.41±0.36, 1.13±0.18, 0.58±0.08, 0.48±0.05, 1.45±0.35, 1.24±0.24, and 2.88±0.43 ng/ml. 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.

The injection Stimvet and oral Exapar alone or in combination did not influence the plasma progesterone concentrations, irrespective of oral nutrients supplement groups, when compared with non-treated control subgroup at any of the peripartum intervals, except at day 30 and 45 postpartum, where the subgroups treated with Stimvet and Exapar, respectively, had significant rise in plasma progesterone as compared to other subgroups, and by 60 postpartum all the subgroups revealed
significantly elevated plasma progesterone concentrations showing resumption of ovarian cyclicity and presence of luteal activity.

The pooled mean plasma estradiol-17β concentrations in buffaloes of Control group, irrespective of subgroups, increased gradually and significantly (p<0.01) from day 45, 30 and 7 prepartum to day of calving with values of 39.24±2.12, 45.83±2.29, 59.36±2.25 and 85.21±2.48 pg/ml, respectively. Thereafter, there was a sudden and significant (p<0.01) drop in the mean plasma estradiol level on day 15 postpartum (22.56±1.41 pg/ml). The mean values of plasma estradiol on days 30, 45 and 60 postpartum were recorded to be 24.25±1.33, 27.36±1.95 and 28.20±2.43 pg/ml, respectively. The mean values in buffaloes of Treatment group followed a similar significant increasing trend for days 45, 30 and 7 prepartum to day of parturition with values of 40.14±2.19, 46.83±2.86, 56.53±2.65 and 82.74±2.12 pg/ml, respectively. Thereafter, there was a rapid and significant (p<0.01) drop in the mean plasma estradiol levels by day 7 to day 15 postpartum. The values for days 15, 30, 45 and 60 postpartum were 22.36±1.62, 22.04±1.45, 25.08±1.38, and 26.396±1.82 pg/ml, respectively, showing initiation of follicular activity from day 45 postpartum.

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 groups, and by 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. Almost similar trend / patterns of plasma estradiol were noted with these treatments under major Control and Treatment groups.

The pooled mean plasma cortisol concentrations in buffaloes of Control group, irrespective of subgroups, on days 45, 30 and 7 prepartum, day of calving and on days 7, 15, 30, 45 and 60 postpartum were 19.20±2.11, 19.46±2.28, 21.59±2.29, 25.17±2.325, 18.35±2.16, 16.86±1.64, 17.39±1.79, 16.23±1.86 and 16.99±1.92 ng/ml, respectively. The corresponding values for buffaloes under Treatment group were 19.09±2.33, 18.33±1.93, 21.36±2.33, 28.55±1.80, 17.71±1.72, 20.23±1.72, 20.75±1.98, 20.17±1.68 and 18.57±1.45 ng/ml. The mean plasma values in both the groups were initially at the lowest level (18 to 22 ng/ml) between day 30 and 7
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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), reflecting stress of calving. 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.

The oral nutrients supplementation did not influence the plasma cortisol levels in transitional buffaloes. Similarly, the injection Stimvet and oral Exapar alone or in combination also did not influence the plasma cortisol concentrations, when compared with non-treated control group at any of the peripartum intervals, yet the levels in Exapar and Stimvet + Exapar subgroups were apparently lower than other two sub-groups at most peripartum intervals.

The pooled mean plasma concentrations of 13, 14-dihydro, 15-keto-PGF₂α (PGFM) in buffaloes of Control group, irrespective of subgroups, on days 45, 30 and 7 prepartum, day of calving and on days 7, 15, 30, 45 and 60 postpartum were 224.41±16.68, 295.08±13.13, 855.37±22.37, 2209.75±37.71, 1309.85±35.04, 771.40±21.67, 325.63±18.75, 170.56±7.91 and 142.34±4.93 ng/ml, respectively. The corresponding values for buffaloes of Treatment group were 275.01±14.89, 348.71±31.10, 891.41±20.16, 2266.29±72.78, 1397.85±42.68, 796.64±20.83, 357.13±14.33, 209.91±10.22 and 182.79±9.53 ng/ml. The plasma concentration of PGFM was 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. The rise was little more in nutrients supplemented group with higher values at most intervals peripartum than in Control group.

Injection Stimvet and oral Exapar alone or in combination did not influence the plasma PGFM concentrations, irrespective of oral nutrients supplements, when compared with non-treated control subgroup at any of the peripartum intervals, except at early postpartum, where the subgroups treated with Stimvet and Stimvet + Exapar had little higher plasma levels of PGFM as compared to other subgroups, indicating some sort of beneficial role of these therapies in enhancing uterine involution and postpartum fertility, though no significant differences were noted among these groups. Almost similar inconsistent patterns of plasma PGFM were noted with these treatments under major Control and oral nutrients supplemented / Treatment groups.

5.6 Blood Biochemical Profiles
The pooled mean \textit{blood glucose} levels in animals of Control group, irrespective of subgroups, for days 45, 30 and 7 prepartum, the day of calving and on days 7, 15, 30, 45 and 60 postpartum were 67.50±0.96, 66.85±1.15, 65.95±0.79, 69.20±1.27, 65.50±0.89, 66.95±1.36, 65.40±1.04, 65.95±0.77 and 67.15±1.03 mg/dl, respectively, with an overall pooled mean of 66.72±0.35 mg/dl. The corresponding values in Treatment group were 68.65±0.63, 71.25±1.04, 72.00±0.92, 76.60±1.39, 73.35±1.40, 73.95±1.42, 76.85±0.97, 75.15±0.99 and 77.35±0.74 mg/dl, with an overall pooled mean of 73.91±0.41 mg/dl. The mean blood glucose values of prepartum period spiked up (p<0.01) to reach the highest on the day of calving and reduced back to normal levels within next 7 day postpartum, with same trend in both the groups. The values thereafter were found to be fluctuating non-significantly till 45-60 days postpartum. The buffaloes supplemented with peripartum nutrients were having significantly (p<0.01) higher blood glucose levels than the Control buffaloes from day 30 prepartum till day 60 postpartum with an overall mean of 73.91±0.41 vs. 66.72±0.35 mg/dl. This can be attributed to the effect of bypass fat supplementation in the diet of treatment group of buffaloes.

Injection Stimvet and oral Exapar alone or in combination did not influence significantly the blood glucose levels within Control and oral nutrients Treatment groups, or, among themselves irrespective of major Control and Treatment groups, when compared with non-treated control subgroup, yet the postpartum values for Stimvet and Stimvet + Exapar subgroups were apparently higher than other two subgroups during early postpartum period.

The pooled mean plasma \textbf{total protein} levels in animals of Control group, irrespective of subgroups, for days 45, 30 and 7 prepartum, the day of calving and on days 7, 15, 30, 45 and 60 postpartum were 8.12±0.11, 7.99±0.14, 7.77±0.11, 7.81±0.08, 7.89±0.11, 7.93±0.10, 8.10±0.10, 7.98±0.13 and 8.07±0.14 g/dl, respectively, with an overall mean of 7.16±0.04 g/dl. The corresponding values in nutrients supplemented / Treatment group were 8.09±0.13, 8.14±0.147, 7.89±0.15, 7.89±0.11, 7.81±0.12, 7.78±0.13, 7.88±0.13, 7.97±0.12 and 7.97±0.14 g/dl, with an overall mean of 7.92±0.04 g/dl. The levels varied non-significantly between days within the group as well as between the groups. The mean plasma total protein levels in Control and Treatment groups of animals fluctuated non-significantly yet the level in close peripartum period were little lower than 30 days before and after calving in all the groups. Bypass fat and mineral supplementation did not evince any beneficial
effect on the total protein values in buffaloes. Moreover, the injection Stimvet and oral Exapar alone or in combination also did not influence the plasma protein concentrations when compared with non-treated control subgroup at any of the peripartum intervals.

The pooled mean plasma cholesterol concentrations of animals in Control group, irrespective of subgroups, for days 45, 30 and 7 prepartum, the day of calving and on days 7, 15, 30, 45 and 60 postpartum were 106.47±2.08, 101.19±1.75, 97.05±2.29, 92.85±1.88, 101.34±1.74, 109.53±2.38, 122.17±3.30, 131.69±4.79 and 136.58±4.88 mg/dl respectively, with an overall mean of 110.99±1.48 mg/dl. The corresponding values in nutrients Treatment group were 105.43±1.98, 106.50±2.61, 103.66±2.78, 95.24±2.46, 102.22±1.89, 106.83±2.62, 124.29±3.30, 134.34±4.22, and 134.62±3.17 mg/dl, with an overall mean of 112.57±1.39 mg/dl. The prepartum mean plasma total cholesterol concentrations in animals of both the groups decreased gradually as parturition approached with the lowest value at calving. Thereafter, the values again increased in the subsequent days parturition to reach the highest value (p<0.01) at day 45 postpartum in both the groups concurrent with ovarian follicular activity. Injection Stimvet and oral Exapar alone or in combination, irrespective of oral nutrients supplementation, did not influence the cholesterol profile, yet the concentrations for Stimvet subgroup were lower than the Exapar and untreated control subgroups at most of the peripartum intervals, and similar was the trend/pattern of cholesterol for various subgroups under major Control and Treatment groups.

The pooled mean plasma levels of non-esterified fatty acids (NEFA) levels in animals of Control group, irrespective of subgroups, for days 45, 30 and 7 prepartum, the day of calving and on days 7, 15, 30, 45 and 60 postpartum were 162.80±7.24, 178.57±12.59, 176.88±10.33, 197.60±12.85, 230.32±11.29, 212.66±16.36, 191.85±14.69, 205.22±16.65 and 197.34±11.77 µmol/l, respectively, with an overall mean of 194.80±4.26 µmol/l. The corresponding values for nutrient Treatment group were 159.12±09.49, 163.88±07.77, 179.42±07.04, 187.99±11.03, 216.16±14.54, 199.23±12.07, 198.52±14.55, 194.24±12.82 and 165.37±11.02 µmol/l, respectively, with an overall pooled mean of 184.88±03.97 µmol/l. The mean plasma NEFA concentrations evinced an increasing trend from day 45 prepartum to the highest on day 7 postpartum, and thereafter the values declined with significant variation till day 30 parturition. The mean plasma NEFA concentrations were little higher in buffaloes of Control group than Treatment group throughout the study period. Injection Stimvet
and oral Exapar alone or in combination did not influence the plasma NEFA concentrations, yet the values till day 30 postpartum were apparently higher in Exapar and Stimvet + Exapar subgroups.

The pooled mean plasma levels of beta-hydroxybutyrate (BHBA) in animals of Control group, irrespective of subgroups, for days 45, 30 and 7 prepartum, the day of calving and on days 7, 15, 30, 45 and 60 postpartum were 747.54±20.76, 780.77±20.91, 986.25±26.52, 1113.75±40.48, 1073.86±30.51, 1020.07±17.73, 940.98±25.77, 856.33±20.90, and 837.57±14.87 µmol/l, respectively, with an overall mean of 928.57±12.35 µmol/l. The corresponding values in Treatment group were 726.13±18.47, 768.69±23.96, 878.74±22.84, 1013.51±46.12, 1024.72±27.40, 950.68±15.42, 913.49±20.16, 828.84±21.94, and 814.98±14.14 µmol/l, with an overall mean of 879.98±11.00 µmol/l. The peripartum pooled mean plasma BHBA values, irrespective of the subgroups, increased (p<0.01) as parturition approached with the peak levels on the day of calving. Thereafter the values declined in subsequent days postpartum at par with 30 days prepartum values. The BHBA levels tended to be higher in Control than Treatment group at most of the peripartum intervals. Injection Stimvet and oral Exapar alone or in combination within the major Control and Treatment groups, or irrespective of these groups, did not influence the plasma BHBA levels at any of the peripartum intervals, and the values followed the same trend among all subgroups over peripartum period studied.

5.7 Plasma Macro-Mineral Profiles

The pooled mean plasma calcium concentrations in buffaloes of Control group, irrespective of subgroups, recorded on day 45, 30, 7 prepartum, on the day of calving, and on day 7, 15, 30, 45 and 60 postpartum were found to be 11.96±0.30, 12.48±0.27, 12.25±0.28, 11.41±0.25, 11.71±0.21, 11.98±0.20, 12.42±0.21, 12.03±0.17 and 12.24±0.16 mg/dl, respectively, with an overall mean of 12.05±0.08 mg/dl. The corresponding levels in nutrients Treatment group were 12.83±0.26, 13.15±0.24, 12.89±0.23, 11.76±0.21, 12.27±0.23, 12.29±0.27, 12.38±0.23, 12.59±0.13 and 12.80±0.19 mg/dl, respectively, with an overall mean of 12.55±0.08 mg/dl. The prepartum mean plasma calcium levels in the buffaloes under both the groups decreased significantly (p<0.01) on the day of calving. These values then gradually increased in the subsequent days postpartum again reaching at par with prepartum levels at around day 15-30 postpartum. Injection Stimvet and oral Exapar alone or in
combination did not influence significantly the plasma calcium concentrations at any of the peripartum intervals, overall or among Control and nutrients Treatment groups.

The pooled mean plasma phosphorus concentrations in buffaloes of Control group, irrespective of subgroups, found on day 45, 30, 07 prepartum, on the day of calving, and on day 7, 15, 30, 45 and 60 postpartum were found to be 5.26±0.20, 5.68±0.15, 5.57±0.11, 4.82±0.14, 5.19±0.12, 5.16±0.18, 5.37±0.14, 5.26±0.16 and 5.28±0.14 mg/dl, respectively, with an overall mean of 5.29±0.05 mg/dl. The corresponding phosphorus levels (mg/dl) in nutrients Treatment group were 5.72±0.21, 6.06±0.19, 5.85±0.20, 5.33±0.18, 5.43±0.15, 5.36±0.17, 5.20±0.15, 5.11±0.14 and 5.46±0.17 mg/dl, respectively, with an overall mean of 5.50±0.06 mg/dl. The mean phosphorus levels followed the trend of plasma calcium, with significantly lower values on the day of calving in both Control and Treatment groups including pooled means. However the levels fluctuated non-significantly between different time intervals, except 7 days before and after parturition, where they were apparently higher in untreated subgroup as compared to Stimvet and Exapar alone or its combination subgroups.

The pooled mean plasma magnesium levels in animals of Control group, irrespective of subgroups, for days 45, 30 and 7 prepartum, day of calving and days 7, 15, 30, 45 and 60 postpartum were 2.64±0.12, 2.51±0.07, 2.52±0.10, 2.45±0.08, 2.58±0.10, 2.65±0.10, 2.59±0.09, 2.47±0.11 and 2.53±0.09 mg/dl, respectively, with an overall mean of 2.55±0.03 mg/dl. The corresponding mean magnesium levels in animals of Treatment group were 2.84±0.10, 2.87±0.12, 3.00±0.10, 2.80±0.09, 2.87±0.09, 3.00±0.10, 2.82±0.09, 2.72±0.09 and 2.91±0.11 mg/dl, respectively, with an overall mean of 2.87±0.03 mg/dl. The mean plasma magnesium concentrations were found to be almost consistent during entire peripartum period studied in both the groups with apparently higher values in Treatment than Control group with significant difference in overall pooled means (2.87±0.03 vs. 2.55±0.03 mg/dl). Very similar trend was also found in Stimvet and oral Exapar alone or in combination groups, being little higher in Stimvet injected subgroup. There was a non-significant decline in plasma level of magnesium at calving in all subgroups.

5.6 Plasma Micro-Minerals Profile

The pooled mean plasma zinc (ppm) levels in buffaloes of Control group, irrespective of subgroups, for days 45, 30 and 7 prepartum, day of calving and days 7, 15, 30, 45 and 60 postpartum were 1.085±0.019, 1.091±0.018, 1.102±0.017,
Summary & Conclusions

1.085±0.010, 1.099±0.017, 1.070±0.005, 1.090±0.017, 1.098±0.018 and 1.120±0.025, respectively, with an overall mean of 1.093±0.006. The corresponding mean zinc levels in buffaloes of Treatment group were 1.123±0.009, 1.177±0.019, 1.168±0.010, 1.208±0.023, 1.178±0.013, 1.198±0.012, 1.169±0.010, 1.194±0.020 and 1.178±0.011 ppm, respectively, with an overall mean of 1.184±0.005 ppm. The levels were high in Treatment group as compared to Control group throughout the peripartum period with significant (p<0.01) difference in overall pooled means (1.184±0.005 vs. 1.093±0.006 ppm). Further, the plasma zinc concentrations fluctuated inconsistently between Stimvet, Exapar, Stimvet+Exapar and non-treated control subgroup within major Control and Treatment groups. The values were higher in Stimvet and Stimvet + Exapar treated subgroups particularly at 30 days prepartum and 7-15 days postpartum.

The pooled mean plasma iron (ppm) levels in buffaloes of Control group, irrespective of subgroups, for days 45, 30 and 7 prepartum, day of calving and days 7, 15, 30, 45 and 60 postpartum were 2.376±0.025, 2.369±0.025, 2.368±0.023, 2.379±0.032, 2.363±0.024, 2.352±0.027, 2.342±0.026, 2.385±0.024 and 2.439±0.057, respectively, with an overall mean of 2.375±0.010. The corresponding values in Treatment group were 2.379±0.029, 2.450±0.032, 2.528±0.043, 2.611±0.044, 2.573±0.038, 2.563±0.030, 2.550±0.059, 2.556±0.042 and 2.609±0.058, respectively, with an overall mean of 2.571±0.014. The mean plasma iron concentration showed a gradual increasing trend, irrespective of groups, from day 30 to 60 postpartum, but the differences between periods were not significant. The highest concentrations of plasma iron were observed on day 60 postpartum, with apparently higher values in the buffaloes of Treatment group compared to Control group at most of all the peripartum periods with significant difference in the overall pooled means (2.571±0.014 vs. 2.375±0.010 ppm). Moreover, the plasma iron concentrations were nearly the same at all intervals peripartum among Exapar, Stimvet + Exapar and non-treated control subgroups, with slight higher values postpartum in Stimvet injected subgroup, irrespective of oral nutrient supplementation or even within major Control and Treatment groups.

The pooled mean plasma copper (ppm) levels in buffaloes of Control group, irrespective of subgroups, for days 45, 30 and 7 prepartum, day of calving and days 7, 15, 30, 45 and 60 postpartum were 1.023±0.013, 1.031±0.014, 1.033±0.013, 1.027±0.11, 1.038±0.022, 1.007±0.010, 1.024±0.015, 1.032±0.020 and 1.040±0.019, respectively, with an overall mean of 1.028±0.005. The corresponding values in
buffaloes of Treatment group were 1.103±0.021, 1.195±0.018, 1.198±0.025, 1.268±0.028, 1.200±0.023, 1.248±0.020, 1.205±0.027, 1.219±0.023 and 1.178±0.020, respectively, with an overall mean of 1.217±0.08. The plasma levels of copper were consistently higher (p<0.05) in oral nutrients supplemented / Treatment group over Control group throughout the peripartum period with highly significant difference in overall pooled means (1.217±0.08 vs. 1.028±0.005 ppm).

The pooled mean plasma cobalt levels in buffaloes of Control group, irrespective of subgroups, for days 45, 30 and 7 prepartum, day of calving and days 7, 15, 30, 45 and 60 postpartum recorded were 0.494±0.045, 0.425±0.021, 0.469±0.043, 0.436±0.027, 0.427±0.029, 0.497±0.034, 0.450±0.0230, 0.413±0.041 and 0.407 ±0.029 ppm, respectively, with an overall mean of 0.447±0.011 ppm. The corresponding values in buffaloes of Treatment Group were 0.530±0.038, 0.540 ±0.032, 0.572±0.024, 0.503±0.027, 0.525±0.027, 0.559±0.034, 0.573±0.037, 0.487±0.029 and 0.596±0.032 ppm, respectively, with an overall mean of 0.543 ±0.011 ppm. The levels were significantly higher in Treatment group as compared to Control group at most peripartum intervals; however the period effect was non-significant among both the groups.

The overall pooled mean plasma manganese levels in animals of Control and Treatment groups were 0.049±0.001 and 0.055±0.001 ppm (p<0.05), respectively. The differences between periods within the group or between groups at different time intervals were however statistically insignificant. Further, the effect of Stimvet and Exapar alone or in combination when analysed irrespective of oral nutrients supplementation did not reveal any significant influence on plasma copper, cobalt and manganese.

From the above results, following conclusions could be drawn.

CONCLUSIONS

1. Assessing the uterine involution by rectal palpation coupled with trans-rectal ultrasonography was found to be complementary to each other. Location, size and tonicity of the uterus could be assessed grossly well by rectal palpation, while the dimensions were well apprehended by trans-rectal ultrasonography.

2. The wall of the cervix and uterus, and uterine caruncles appeared as bright hyperechoic structures, while the cervical and uterine lumens were found to be hypoechoic with bright hyperechoic spots. The process of uterine involution...
seemed to be time dependent, regressive changes being faster in non-gravid horn than gravid horn.

3. 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.

4. Injection Stimvet and oral Exapar alone or in combination did not show any effect on uterine involution and postpartum fertility or blood profile studied, except shortened placental expulsion time by Exapar, perhaps due to optimized nutritional status of animals achieved with chelated ASMM and bypass fat supplementation.

5. The sudden drop in plasma progesterone at calving with subsequent increase during 30-60 days postpartum indicated initiation of postpartum ovarian activity, while plasma oestradiol-17β levels showed an inverse trend with peak value at calving, which later reduced to basal levels till the recrudescence of ovarian activity. But these hormones were not influenced significantly by nutrients supplementation or other treatments used.

6. 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.

7. PGFM levels were higher in fat supplemented group throughout the study period and it was the highest on the days of calving in all buffaloes.

8. The mean blood glucose level was the highest on the day of calving, with significantly higher contents in nutrient supplemented than control buffaloes. The plasma total cholesterol level was observed to be low at parturition and seven days postpartum, with a gradual increase with advancing lactation.

9. Significantly higher mean plasma NEFA and BHBA levels were found at 7 days postpartum, and in control than treated group, reflecting energy status of animals.

10. The plasma calcium, inorganic phosphorus zinc and copper levels were found to be significantly higher in nutrient supplemented group as compared to control, with drop in the levels on the day of calving, however, plasma magnesium and
other micro-minerals (Co, Fe, Mn) levels did not reveal significant variation between groups.

11. Additional nutrients supplementation over routine feeding to their animals during transitional period from 45 days prepartum till 60 days postpartum (50 g chelated ASMM and 150-200 g bypass fat daily) to significantly improve the uterine health and postpartum fertility with reduced calving interval for better economic return.