EFFECT OF VITAMIN E AND MINERAL SUPPLEMENTATION ON BIOCHEMICAL PROFILE AND REPRODUCTIVE PERFORMANCE OF BUFFALOES

H.M. Khan¹, T.K. Mohanty², M. Bhakat², A.K. Gupta², A.K. Tyagi³ and G. Mondal³

ABSTRACT

The experiment was designed to provide higher plane of nutrition, vitamin E and mineral supplementation for augmenting the improvement in reproductive performance. In the present investigation, 10 Murrah buffaloes each in two groups, expected to calve in winter season were selected during prepartum period. None of the buffaloes during periparturient period suffered from any clinical metabolic disease or reproductive disorders. Plasma Ca and Plasma inorganic P concentration showed significant difference on day 15 prepartum (P<0.01); Zn on day 15 prepartum (P<0.05) and day 15 postpartum (P<0.01); Cu on day 30 prepartum (P<0.05) and Mn on day 30 prepartum (P<0.05), however, the differences in concentrations at all other stages were non-significant but the supplemented group had higher levels than the control group at all the stages. The concentration of the plasma glucose exhibited significant difference (P<0.01) at 45 days postpartum but the supplemented group had higher levels than the control group at all the stages. The plasma BUN showed significant difference (P<0.05) at days 30 and 15 prepartum, calving day and on day 30 postpartum (P<0.01) and Plasma NEFA and vitamin E values obtained were statistically not significant. However, the supplemented group had lower levels than the control group at all the stages but vitamin E values had higher levels than the control group at all the stages. Cervical and uterine involution was completed in lesser days, involutory changes took place at a faster pace and there were lesser percent of cows suffering from abnormal uterine changes in supplemented compared to control group. Supplemented group showed better reproductive performance considered in the study than control group. In total, around 12 days could be saved in days to first service if vitamin E and minerals were supplemented. Supplemented group showed early initiation of cyclicity (32 days postpartum) compared to control group (35 days postpartum). Cyclicity in most of the animals might have been initiated earlier than 30 days as was evident from progesterone concentration (>1 ng/ml). Short and long luteal phases were observed on appraisal of progesterone concentration in both the groups which delayed the days to first service in these animals. It can be concluded that mineral and vitamin E supplementation improved the reproductive performance of buffalo during periparturient period.

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INTRODUCTION

Nutritional management during the dry period is the main factor which may affect susceptibility of cows to metabolic and infectious diseases during the periparturient period (Dann et al., 2005; Campanile et al., 1997). Besides general nutritional status, deficiencies or imbalance with respect to specific nutrients (minerals and vitamins) have been found to have drastic effects on various determinants of reproductive performance leading to infertility. Vitamins and minerals (macro and microelements) in minute quantity play a decisive role in overall metabolism, normal growth, production and reproduction. Excess or even imbalance of some minerals and vitamins may have deleterious effect on health (Borghese, 2005). Further the impacts of such disturbances on general health including insidious sub-clinical diseases/disorders have been recognized as the most important but covert factors with deleterious consequences for reproductive performance.

Minerals and vitamins have direct or indirect relationship with productive and reproductive health of animals. Vitamin E is important for maintaining optimal immune function (Sikka et al., 2002; Sikka and Lal, 2006) and as anti-stress factors (Kahlon et al., 2006) and requirements are higher compared to production or reproduction requirements (Xin et al., 1991; Weiss, 1998). Deficiencies and imbalance of minerals during peri-parturient period are either solely incriminated for or associated with anestrous (Patil and Deshpande, 1979; Naidu and Rao, 1982; Agarwal et al., 1985; Singh and Vadhare, 1987), repeat breeding (Balakrishnan and Balagopal, 1994; Prajapati et al., 2005), metabolic disorders, retention of foetal membranes (Gupta et al., 2005), dystocia, abortion (McDowell 1992; Dutta et al., 2001; Sharma et al., 2005), weak calf syndrome (Logan et al., 1990), milk fever, vulval discharge (Husband, 2006) and poor conception rate (Khasatiya et al., 2005). Thus have negative impact on the subsequent fertility of the cow. Such disorders could probably be prevented by addressing to the basic etiology through balanced feeding and mineral supplementation during advanced pregnancy and early post-partum period, when the animals are highly prone to stress of heavy nutrient demand and drain (Mandali et al., 2002). Thus nutritional supplementations play important role to improve general, productive and reproductive health of animals (Kleczkowski et al., 2003). Further mineral (Sharma et al., 2003; Hussain et al., 2004; Borghese, 2005; Yildiz et al., 2006) and vitamin E supplementation (LeBlanc et al., 2004; Panda et al., 2006) improves reproductive performance because of their positive effect on steroid synthesis, release, follicular growth and symptoms of ovulatory oestrus (Srivastava, 2008). The impact of minerals and vitamins supplemented in the peripartum period in buffaloes on subsequent fertility is lacking and needs due importance for prevention of periparturient problems and improvement of fertility. In order to deal with above problems and to improve overall reproductive efficiency in buffaloes, the present investigation was undertaken to fill up the gaps in knowledge in Murrah buffaloes with the objective to investigate the role of prepartum mineral and vitamin E supplementation on postpartum reproductive performance.
MATERIALS AND METHODS

The present study was conducted on 20 pregnant dry Murrah buffaloes maintained at Cattle Yard of National Dairy Research Institute (NDRI), Karnal, India. Twenty Murrah buffaloes 60 days prepartum were selected and randomly assigned to two experimental groups with 10 animals in each group; group 1 (C) was provided 20% higher nutrients than Kearl’s Feeding Standard (Chauhan et al., 2000) and group 2 (T) was provided 20% higher nutrients than Kearl’s Feeding Standard (Chauhan et al., 2000) along with vitamin E (2000 IU from 60 days prepartum to 30 days postpartum and 1500 IU from 30 to 60 days postpartum) vitamin E 50% powder, Vet Chem supplementation (Panda et al., 2006) and 50 gm of commercial mineral mixture (Agrimin, Agrivet Farm Care Division) to meet the expected requirements of the minerals. The buffaloes used for the investigation were kept in conventional barns throughout the prepartum period and were shifted to calving pens 2 weeks prior to expected date of parturition for extra care and attention up to 5 days after parturition. After that they were shifted to loose housing and group management system where other lactating buffaloes were kept. The parameters (time of parturition, days required for cessation of lochia, days required for complete uterine involution, days at first heat, days at first service) were recorded.

All the experimental buffaloes were monitored regularly for estrus by visual observation and parading of vasectomised bull in the morning and evening hours. Animal were rectally confirmed for heat and inseminated with frozen semen by two inseminations at 12 h intervals. Buffaloes not returning to estrus after inseminations were examined per rectum on 45 for pregnancy confirmation.

Scoring of uterine discharges and uterine involution

The experimental animals under investigation were scored for uterine discharges and Uterine Involution on 7, 14, 21, 28 and 35 days postpartum as per Sheldon and Noakes (1998) for early diagnosis and treatment of uterine infections.

Plasma Biochemical Assay

The blood samples were collected from jugular vein into heparinized (20 IU heparin/ ml blood) tubes from all experimental animals at fortnightly interval from 60 days prepartum to 60 days postpartum. Immediately after sampling the blood was centrifuged at 3000 rpm for 15 to 20 minutes and the plasma was separated and stored frozen (-20°C) until analyzed. Following micronutrients and metabolites in control as well as in experimental groups were estimated:

Plasma Mineral and Metabolite Estimation

Plasma Ca, inorganic P, Zn, Cu and Mn were estimated at fortnightly intervals in both the groups. Plasma minerals (Ca, Zn, Mn and Cu) except phosphorous were estimated with the help of Atomic absorption Spectrophotometer (Model PU9100X Atomic absorption Spectrophotometer, Philips). The procedure described in AAS (1988) manual for preparation of stock and standard solutions and choice of instrumental conditions were followed. Plasma inorganic phosphorus was estimated following the method of Fiske and Subbarow (1925). An HPLC method for simultaneous estimation of α-tocopherol in plasma was adopted (Chawla and Kaur, 2001). The plasma urea was estimated according to Rahmatulla and Boyde in 1980. The copper soap extraction method modified by Shipe et al. (1980) was adopted for the determination of plasma NEFA and the standard
curve was prepared with palmitic acid as specified by Koops and Klomp (1977). Glucose in blood plasma was estimated by end-point o-Toluidine method.

Blood plasma analysis for progesterone quantification
The method of Kamboj and Prakash (1993) was followed through RIA for blood plasma progesterone estimation.

Statistical analysis
Effect of vitamin E and mineral supplementation on mineral and metabolic status and reproductive performance was calculated by t-test using Systat 6 software package.

RESULTS AND DISCUSSION
Feeding plays an important role in the performance of the animals. The experiment was designed to provide higher plane of nutrition (Chauhan et al., 2000) and vitamin E and mineral supplementation for augmenting the improvement in reproductive performance. In the present investigation, 10 buffaloes each expected to calve in winter season were selected during prepartum period for investigating the role of vitamin E and mineral supplementation supplemented through prepartum to postpartum period. None of the buffaloes during periparturient period suffered from any clinical metabolic disease or reproductive disorders. Two of the buffaloes, one each from both the groups due to chronic problem were removed from the experimental study.

Mineral profile of buffaloes
The results obtained are presented in the Table 1 for interpretation from 45 days prepartum to 45 days postpartum taking 0 day as the day of calving. The supplemented group had higher levels of plasma mineral (Ca, inorganic P, Zn, Cu and Mn), Plasma glucose, Plasma Vitamin E and lower level Plasma BUN and Plasma NEFA than the control group at all the stages reflecting improvement and beneficial effect due to vitamin E and mineral supplementation.

Plasma Ca
Plasma Ca concentration did not follow a specific pattern in both the groups. Plasma Ca decreased on days 30 and 15 prepartum in control and day 30 only in supplemented group during the prepartum period. These changes were attributed to growing needs of the fetus and changes in the available fodder. At parturition and 15 day postpartum there was a substantial increase, whereas days 30 and 45 showed a decrease in Ca level in both the groups. The changes reflected in both the groups were not significant except day 15 prepartum (P<0.01).

The increasing and decreasing trend of plasma Ca not following a specific pattern may be attributed to changes in available fodder. The drop in Ca concentration on day 30 and 45 might be due to its increased diversion for foetal growth and more secretion of Ca through colostrums and milk; however the concentrations were within the normal range. The normal values in cows vary between 8-12 mg/dl and hypocalcaemia occurs when value decreases to 3-7 mg/dl (Hidiroglou, 1979; McDowell, 1992; Shah et al., 2003). Buffalo calcium blood levels have been reported to show limited variability during lactation and dry milk period. Campanile et al. (1997) found constant values of about 10 mg/dl. Higher levels have been found in the last month of pregnancy and lower ones.
<table>
<thead>
<tr>
<th>Prepartum</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>-45d</td>
<td>0d</td>
</tr>
<tr>
<td>Control</td>
<td>Supplemented</td>
</tr>
<tr>
<td>Ca  A</td>
<td>9.97±0.26</td>
</tr>
<tr>
<td>P  A</td>
<td>5.42±0.18</td>
</tr>
<tr>
<td>Zn  B</td>
<td>2.46±0.27</td>
</tr>
<tr>
<td>Mn  B</td>
<td>0.96±0.08</td>
</tr>
<tr>
<td>Cu  B</td>
<td>1.40±0.10</td>
</tr>
</tbody>
</table>

A: mg%; B: ppm; * - Significant (P<0.05); ** - Significant (P<0.01)
at the end of the lactation period (Montemurro et al., 1997). A seasonal variation has been evidenced with higher values during the winter as leguminous fodder contains more calcium. Ca deficiency in cow causes reproductive disorders viz. prolapse of uterus, retained placenta, difficult delivery and delay in uterine involution. In buffalo, calcium excesses could alter the Ca/P ratio during the dry period, inducing parathyroid hypoactivity which would cause magnesium to increase and calcium to decrease at the beginning of the lactation due to a non immediate calcium mobilization by the bones. The altered Ca/Mg ratio has been incriminated for atony of uterus and eventually uterine prolapse (Campanile et al., 1997).

**Plasma inorganic P**

Plasma inorganic P showed a specific trend. It increased in the supplemented group upto days 15 prepartum and declined on calving day and showed an increasing trend thereafter, whereas in control group it declined upto day 15 prepartum and thereafter showed an increasing trend. The differences in concentration at all stages was non significant except day 15 prepartum (P<0.01).

The drop in concentration of plasma P during prepartum may be due to increasing demands of growing foetus and also due to changes in concentration of available fodder. Phosphorus levels in buffaloes have been found to be quite stable at 6 mg/dl (Campanile et al., 1997). An increasing trend has been evidenced starting from the pre-partum period (6.3 mg/dl) to 160 days of lactation (7.9 mg/dl) (Montemurro et al., 1997). Phosphorus deficiency during dry period has been recognized as the most frequent causes of vaginal and/or uterine prolapse. Dietary deficiency of P before calving has been found to cause decreased calcium levels at calving without altering serum P i.e. maintaining normal level (Campanile et al., 1997).

**Plasma Zn**

Plasma Zn concentration was lowest on the day of calving in both the groups. The Zn concentration dropped on day 30 prepartum and day of calving and showed an increasing trend thereafter in supplemented buffaloes, whereas it showed an increasing trend at all stages except day of calving and day 15 postpartum in control group. There was significant difference in plasma concentration of the Zn on day 15 prepartum (P<0.05) and day 15 postpartum (P<0.01). The differences in concentration at all other stages was non significant. The results regarding plasma Zn concentration in consonance with Panda (2003), who also reported decrease in plasma Zn concentration in buffaloes during late gestation and parturition.

**Plasma Cu and Mn**

Plasma Cu levels followed a particular trend in both the groups. It started declining upto calving and thereafter there was an increase in its concentration, except at day 30 in the supplemented group wherein it showed an increasing level. The extent of decrease in Cu concentration at parturition was more in comparison to other minerals. There was significant difference in plasma concentration of the Cu on day 30 prepartum (P<0.05), however, the differences in concentration at all other stages were non significant.

Plasma Mn levels followed a decreasing trend upto parturition and followed increasing trend following parturition except on day 30 in both the groups. The drop in the Mn concentration during prepartum might be due to the increasing demands of growing foetus and utilization for improving
antioxidant status. The drop following parturition on day 30 might be due to variation in available fodders. There was significant difference in plasma concentration of the Mn on day 30 prepartum (P<0.05), however, the differences in concentration at all other stages were non significant. Panda (2003) also found the similar trend in Cu and Mn concentration but reported higher levels than the present findings.

**Metabolic profile of buffaloes**

Metabolic profile reflects the nutritional and physiological status of the animal. In the present study, the metabolic profile of the animals in terms of blood glucose, BUN and NEFA were evaluated to delineate their effects on reproduction performance in control and supplemented groups. The metabolic profile in control and supplemented buffaloes is presented (Table 2) for ease of interpretation.

**Plasma glucose**

The concentration of the plasma glucose exhibited a highly significant difference (P<0.01) among the treatment groups at 45 days postpartum (Table 2). Plasma glucose followed an increasing trend throughout the experiment period except on day 30 when it showed slight decrease in both the groups which might be due to change or variation in the fodder supplied or other managemental and environmental effects. However, the differences in concentration at other periods was non significant.

**Plasma BUN**

The plasma BUN values obtained were lower in the prepartum than postpartum period. Plasma BUN decreased during prepartum period and increased during postpartum period. Plasma blood urea nitrogen showed significant difference (P<0.05) at days 30 and 15 prepartum, calving day and highly significant difference (P<0.01) on day 30 postpartum. The differences in concentration at other periods was non significant. Plasma blood urea concentrations were close to the normal ranges in buffaloes (Borghese, 2005). Urea concentration is an indicator of energy protein balance (Dhali, 2001; Campanile et al., 1998; Dhali et al., 2006) and is typically increased in cows deficient in energy.

**Plasma NEFA**

Plasma NEFA showed decreasing and increasing trend in both the groups and the values obtained were statistically non significant. During postpartum increasing trend was followed during 30 days signifying slight negative energy balance but the values are much lower than in cattle. Buffaloes have higher protein and energy utilizing efficiencies as compared to cattle at similar fat corrected milk production level, plane of energy and protein nutrition, body size and weight change (Paul et al., 2003) which could be the reason for less negative energy balance reflected in buffaloes during postpartum period.

**Plasma vitamin E**

The plasma vitamin E values obtained showed a decreasing trend in the control group during prepartum period and thereafter increased up to day 45 postpartum. In Supplemented group, there was slight decrease in the concentration on days 15 prepartum, calving day and 15 day postpartum. The difference in the concentration of two groups was statistically non significant. Campanile et al. (1997) reported that in buffaloes average value of vitamin E is 175 g/l and it increased with the distance from calving and reduced after 120 days of lactation. An increase in serum α-tocopherol to 1 μg/ml in the last week prepartum has been
Table 2. Metabolic status of vitamin E and mineral supplemented and control buffaloes (Mean ± S.E.).

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th></th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-45d</td>
<td>-30d</td>
<td>-15d</td>
</tr>
<tr>
<td>Glucose(^A)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplemented</td>
<td>57.53±2.73</td>
<td>62.06±1.72</td>
<td>65.46±3.44</td>
</tr>
<tr>
<td>Control</td>
<td>63.89±5.63</td>
<td>67.18±2.20</td>
<td>69.80±2.44</td>
</tr>
<tr>
<td>BUN(^A)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplemented</td>
<td>15.03±1.25</td>
<td>15.13±1.28*</td>
<td>12.19±1.38*</td>
</tr>
<tr>
<td>Control</td>
<td>24.95±5.64</td>
<td>24.71±4.11*</td>
<td>21.26±2.96*</td>
</tr>
<tr>
<td>NEFA(^B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplemented</td>
<td>305.89±10.98</td>
<td>275.64±19.31</td>
<td>298.81±10.76</td>
</tr>
<tr>
<td>Control</td>
<td>305.41±11.35</td>
<td>273.81±10.07</td>
<td>295.22±7.17</td>
</tr>
<tr>
<td>Vitamin E(^C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplemented</td>
<td>1.42±0.15</td>
<td>1.44±0.21</td>
<td>1.24±0.16</td>
</tr>
<tr>
<td>Control</td>
<td>1.40±0.19</td>
<td>1.25±0.27</td>
<td>1.23±0.17</td>
</tr>
</tbody>
</table>

\(^A\) – mg%; \(^B\) – μmol/l; \(^C\) – μg/ml; * – Significant (P<0.05); ** – Significant (P<0.01)

BUN – Blood urea nitrogen; NEFA – Non-esterified fatty acids
found to reduce the risk of retained placenta by 20% (LeBlanc et al., 2004). Peripartum decreases in serum concentrations of vitamins A and E have been incriminated for impaired immune function in dairy cows (LeBlanc et al., 2004). Oral administration of selenium along with vitamin E to anoestrus buffaloes is more beneficial in increasing the antioxidant status as revealed by the increase in the level of vitamin E, β-carotene and decrease in lipid per oxidation (Nayyar et al., 2002); higher levels glucose, cholesterol, triiodothyronine and thyroxine (Nayyar et al., 2003) and improvement of blood biochemical composition (Anita et al., 2004).

**Uterine and cervical involution**

During postpartum period, the reproductive organs were palpated transrectal on days 7, 14, 21, 28, 35 and 42 for observing cervical and uterine involutory changes, abnormal discharges if any as per the score card (Sheldon and Noakes, 1998) with slight modifications. The general goal for postpartum reproductive health is for the uterus to be completely involuted and free of infection, and for cows to be cyclic by the time they enter the breeding period (after 50 to 60 DIM) (LeBlanc et al., 2002). There was no significant difference in days to completion of cervical and uterine involution. In supplemented group, involuntary changes took place at a faster pace than the control group (Table 3). However, cervical and uterine involution was completed in shorter days (Table 3) and lesser percent of cows suffering from abnormal uterine changes in supplemented group than control group (Table 4) signifies the role of vitamin E and mineral supplementation which can further be substantiated by the mineral and metabolic profile of the supplemented group.

There were no cases of RFM and metritis in both the groups which may be attributed to better nutrition available to both groups. This is in agreement with findings of Chauhan et al. (2000). They also reported no case of RFM if buffaloes are fed at a higher rate than recommended by Kearl (1982). The buffaloes with abnormal uterine involutory changes particularly having foul smelling discharges or purulent discharge (puerperal metritis) and RFM cases were treated as soon as detected and were declared free of disease as per the score card after completion of uterine involution. The presence of mildly purulent uterine discharge in the first month postpartum likely reflects a successful immune response by the cow to a bacterial challenge. Uterine involution largely depends on the intrauterine contamination with pathogenic bacteria. However, presence of bacteria in the uterus of postpartum cows does not always indicate a disease condition. Bacterial presence in the uterus for the first 10-14th day postpartum has been considered usual and could be detected in more than 90% of the cows, regardless of disease signs (Sheldon and Dobson, 2004). The presence of bacteria has been found sporadic on 28-35 days after calving, and in normal healthy conditions the uterine cavity has to be sterile thereafter (Paisley et al., 1986; Hussain, 1989; Hussain and Daniel, 1991a,b). However the condition may be either clinical or sub-clinical as well as the overall effects vary depending upon the immune status of the host (Gilbert et al., 1998; LeBlanc et al., 2002; Kasimanickam et al., 2004). Although the role of host’s humoral immune response in disease remains poorly defined, in a physiological situation the self-defence mechanisms of the uterus are able to counteract the bacterial infection (Foldi et al., 2006). Sheldon (2004) propounded that 90% cows postpartum develop a mild, nonpathological form of endometritis. In majority of cases the local
Table 3. Effect of vitamin E and mineral supplementation on involutory changes.

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>Supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>4.89</td>
<td>5.55</td>
</tr>
<tr>
<td>14</td>
<td>3.56</td>
<td>3.22</td>
</tr>
<tr>
<td>21</td>
<td>2.33</td>
<td>1.33</td>
</tr>
<tr>
<td>28</td>
<td>1.33</td>
<td>0.22</td>
</tr>
<tr>
<td>35</td>
<td>0.33</td>
<td>0</td>
</tr>
<tr>
<td>42</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4. Effect of vitamin E and mineral supplementation on abnormal uterine involutory changes as per score card.

<table>
<thead>
<tr>
<th>Days</th>
<th>Control (%)</th>
<th>Supplemented (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>22.22</td>
<td>33.33</td>
</tr>
<tr>
<td>14</td>
<td>11.11</td>
<td>11.11</td>
</tr>
<tr>
<td>21</td>
<td>33.33</td>
<td>0</td>
</tr>
<tr>
<td>28</td>
<td>55.56</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>11.11</td>
<td>0</td>
</tr>
<tr>
<td>42</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 5. Effect of vitamin E and mineral supplementation on reproductive parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDD (h)</td>
<td>6.37±1.11</td>
<td>4.53±0.72</td>
</tr>
<tr>
<td>Uterine involution (Days)</td>
<td>34.22±2.17</td>
<td>28.78±1.40</td>
</tr>
<tr>
<td>DFS</td>
<td>73.25±10.82</td>
<td>61.88±6.92</td>
</tr>
<tr>
<td>Initiation of cyclicity P₄ &gt; 1 ng/ml (Days)</td>
<td>32</td>
<td>35</td>
</tr>
<tr>
<td>Risk to First service &lt;60 days (%)</td>
<td>44.44</td>
<td>44.44</td>
</tr>
<tr>
<td>Risk to First service &lt;90 days (%)</td>
<td>66.67</td>
<td>87.5</td>
</tr>
<tr>
<td>Risk to First service &gt;90 days (%)</td>
<td>33.33</td>
<td>12.5</td>
</tr>
</tbody>
</table>

* - Significant (P<0.05); ** - Significant (P<0.01)

PDD- Placental Delivery Duration; DFS- Days to First Service
antimicrobial defence mechanisms eliminates the pathogens and this mild non-pathological form of endometritis resolves within some days.

Supplementation of Vitamin E and selenium to Murrah buffaloes during prepartum period has been shown to shorten expulsion time of foetal membranes and early uterine involution (Qureshi et al., 1997; Mavi et al., 2006; Panda et al., 2006) and decrease metritis cases. Similar observations have been reported for Dairy cows supplemented with Vitamin E and Zinc (Campbell and Miller, 1998). However, LeBlanc et al. (2002) reported that supplementation caused only conditional benefit of treatment for reduction of the incidence of retained placenta and no significant effects could be observed in the incidence of retained placenta, clinical mastitis, metritis, endometritis, ketosis, displaced abomasum, or lameness. Metabolic disorders like hyperketonaemia and deficiency conditions such as selenium, vitamin E and vitamin A deficiency have been incriminated for altered competence of cellular self-defence mechanisms, which in turn increases the risk for developing metritis (Lewis, 1997; Reist et al., 2002; Sheldon and Dobson, 2004). Oral administration of selenium along with vitamin E in buffaloes is more beneficial as it increases the antioxidant status as revealed by the increase in the level of vitamin E (Nayyar et al., 2002); higher levels glucose (Nayyar et al., 2003) and improvement of blood biochemical composition (Anita et al., 2004). It has been opined that the effects of vitamin E and Se on neutrophils promote uterine modeling and involution.

Reproductive performance

Reproductive performance of the buffaloes in two different groups was evaluated on the basis of PDD, uterine involution, days to first service, risk to first service on days 60, 90 and >90 (Table 5). Risk to first service on days 60, 90 and >90 was calculated by the number of animals receiving first service by days 60, 90 and >90 divided by the total number of experimental animals.

Vitamin E and mineral supplemented group showed better reproductive performance in all the reproductive parameters considered in the study than control group. Mineral and metabolic status as already discussed substantiates the better performance of the supplemented group in the study regarding reproductive performance. In total around 12 days could be saved in days to first service if vitamin E and minerals are supplemented. The better performance in winter season of this experiment could be attributed mainly to high plane of nutrition provided in the prepartum period which is substantiated by the mineral and metabolic profile which was optimum required for better performance.

Progesterone estimation was done from 30 days postpartum to evaluate the effect of vitamin E and mineral supplementation on the initiation of cyclicity in the experimental buffaloes. Plasma progesterone concentration more than 1 ng/ml was used as criteria for assessing initiation of cyclicity. Supplemented group showed early initiation of cyclicity (32 days postpartum) compared to control group (35 days postpartum). Cyclicity in most of the animals might have been initiated earlier than 30 days as was evident from progesterone concentration. Short and long luteal phases were observed on appraisal of progesterone concentration in both the groups which delayed the days to first service in these animals.

Campbell and Miller (1998) also reported improvement of reproductive performance for dairy cows supplemented with vitamin E and Zn. Changes in immune function can contribute to improved reproductive efficiency. Immunopotentiation in
late gestation with vitamin E and Selenium has been shown to reduce the calving to first oestrus interval and the length of the postpartum service period (Qureshi et al., 1997; Panda et al., 2006). Supplementation of vitamin E and selenium to Murrah buffaloes during prepartum period has been shown to cause a significant increase in conception rate, decrease in service per conception, early initiation of postpartum ovarian activity and early exhibition of first postpartum heat (Mavi et al., 2006). The progesterone levels remained at basal levels from day 5 to day 30 postpartum and started rising thereafter (Bahga and Ganwar, 1988). Progesterone levels of 0.30, 1.43, 3.29 and 0.88 were reported by Qureshi et al. (2000) at estrus, developing, developed and regressing corpus luteum respectively. Progesterone analysis revealed that 23-70% of the postpartum buffaloes had one or more covert estruses before the first overt estrus (Batra and Pandey, 1983; Usmani et al., 1984; Sharma and Kaker, 1990). Also, duration of first progesterone rise of over 1 ng/ml of plasma was found to be significantly longer (Mavi et al., 2006) in supplemented buffaloes. Progesterone monitoring of postpartum buffaloes for detection of first ovulation offers an objective and accurate method for assessment of the reproductive potential of buffaloes (El-Wishy, 2007). Similar observations have been reported for Dairy cows supplemented with vitamin E and Zinc (Campbell and Miller, 1998). Shorter days to first service have been attributed to combined effect of mineral and vitamin supplementation because of their positive effect on steroid synthesis, release, follicular growth and symptoms of ovulatory oestrus (Srivastava, 2008).

Immunocompetence has been suggested as a useful tool for determining the requirements of some vitamins. Requirements that are based on measures of immune function have been reported to be higher than those that are based on production or reproduction (Weiss, 1998). It has been emphasised that the amount of micronutrients needed for optimal immune function may exceed that amount which will prevent more classical deficiency signs. In general, mineral deficiencies have been associated with altered metabolic profile leading to most periparturient disorders in buffaloes. Thus, such disorders could probably be prevented by addressing to the basic etiology through balanced feeding and mineral supplementation during advanced pregnancy and early post-partum period, when the animals are highly prone to stress of heavy nutrient demand and drain (Mandali et al., 2002).

**CONCLUSION**

Higher plane of nutrition needs to be followed during prepartum for meeting the requirements of buffaloes. Additionally vitamin E and mineral supplementation should be provided during the prepartum period to meet the requirements of critical elements for better reproductive performance.

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