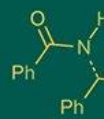


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Status and correlation of progesterone, 17 β -estradiol and minerals on treatment with conventional Ovsynch protocol and medicinal herbs in postpartum anestrus buffaloes

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Abstract

The present study was conducted on thirty two postpartum anestrus buffaloes. The animals were randomly divided into four groups comprising 8 buffaloes in each group. Group I served as control group and was subjected to standard Ovsynch protocol which consisted of administration of GnRH on day 0, PGF2 α on day 7 and GnRH on day 9. Group II was supplemented orally with 130 g per day of *Saraca asoca* leaves for 24 days. Group III received 200 g per day of *Trigonella foenum-graecum* seeds orally for 24 days. Group IV was administered a combination of 65 g of *Saraca asoca* leaves and 100 g of *Trigonella foenum-graecum* seeds orally per day for 24 days.

Blood samples were collected at four-day intervals on day 0, 4, 8, 12, 16 and 24 for estimation of serum hormonal profile, viz., progesterone and 17 β -estradiol. For mineral profile analysis, blood samples collected at eight-day intervals on day 0, 8, 16 and 24 were analyzed for calcium (Ca), phosphorus (P), zinc (Zn), selenium (Se), iron (Fe) and copper (Cu) using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). Estrous induction was observed in all experimental groups at varying time intervals.

The objective of the study was to assess the two-tailed correlation between serum reproductive hormones and mineral profile in postpartum anestrus buffaloes under different treatment regimens.

The correlation analysis between hormones and minerals revealed distinct patterns across treatment groups. In Group I, phosphorus, zinc and copper exhibited a positive correlation with progesterone, whereas calcium and phosphorus showed a negative correlation with 17 β -estradiol. In Groups II, III and IV, calcium, phosphorus, zinc, copper and selenium demonstrated a positive correlation with progesterone. However, a positive correlation with 17 β -estradiol was observed exclusively in Group IV, which received the combination treatment of *Saraca asoca* leaves and *Trigonella foenum-graecum* seeds.

Keywords: Progesterone, 17- β estradiol, minerals, Ovsynch, *Saraca asoca* leaves, *Trigonella foenum graecum* seeds

Introduction

Microminerals play a critical role in the reproductive performance of buffaloes. They significantly influence the synthesis, secretion and systemic regulation of progesterone and 17- β estradiol in buffaloes (Abhijit B *et al.*, 2015) [1]. For optimal reproductive performance there should essentially a specific balance between reproductive hormones and microminerals. Anestrus buffaloes have been reported to exhibit low concentrations of estradiol, thyroid hormones, inorganic phosphorus, iron and copper. (Ankita *et al.*, 2018; Sharma *et al.*, 2012) [3, 9]. Reproductive disorders such as anestrus and repeat-breeding in buffaloes consistently associated with suppressed phosphorus and progesterone levels compared to normal cycling animals. An optimum concentration of Calcium and Phosphorus is essential for reproductive performance. Calcium influences the ability of an animal to utilize other trace elements while inorganic phosphorus shows a positive correlation with cyclic ovarian activity. An imbalance in the Calcium to Phosphorus ratio can impair ovarian activity. Altered Ca:P ratio affects ovarian function through its blocking action on pituitary gland which results in prolongation of first oestrus, ovulation and prolonged uterine

involution. Ca:P ratio of under 1.5:1 or >3:1 could potentially lead to reproductive problems. (Pashudhan Praharee, 2024; Chaurasia *et al.*, 2010) [6, 4].

Trace minerals such as zinc (Zn), copper (Cu), manganese (Mn), selenium (Se) and cobalt (Co) serve as cofactors for critical enzymes that regulate steroidogenesis, corpus luteum (CL) lifespan and follicular development. Deficiency of these microminerals leads to altered enzymatic profiles, diminished circulating steroid levels, silent estrus and reproductive pathologies such as postpartum anestrus and repeat breeding (Wang *et al.*, 2026; Hafez *et al.*, 2019; Yatoo *et al.*, 2016) [12, 5, 13].

Feeding of certain herbal plants ameliorates anestrus and repeat breeding in postpartum buffaloes by providing phytochemicals along with mineral supplementation. *Saraca asoca* leaves contain high concentrations of minerals such as calcium, phosphorus, sodium, iodine, iron and manganese (Suresh *et al.*, 2024) [11]. *Trigonella foenum-graecum* seeds are rich in Ca, Mg, Na, K, Fe, Zn and Cu (Rabia Praveen *et al.*, 2024; Ahmad *et al.*, 2015) [8, 2]. These herbs provide mineral supplementation and also aid in exhibiting estrus in postpartum anestrus buffaloes by stimulating the production of progesterone and 17 β -estradiol. The concentration of minerals estimated in *Saraca asoca* leaves in recent ICP-OES studies showed total ash 7.28%, Calcium 1.364%, Phosphorus 0.328%, Iron 513 ppm, Zinc 224 ppm, Copper 11.12 ppm and Selenium 0.14 ppm. *Trigonella foenum-graecum* seeds are rich in minerals-total ash 3.75%, Calcium 0.437%, Phosphorus 0.439%, Iron 535 ppm, Zinc 250 ppm, Copper 20.32 ppm and Selenium 0.63 ppm, containing Ca, Mg, Na, K, Fe, Zn and Cu. These herbs provide mineral supplements and also aid in exhibiting estrus in postpartum anestrus buffaloes by stimulating production of progesterone and 17 β estradiol. (Priyadarshini. CH *et al.*, 2024) [7].

The present study was undertaken with the objective of feeding herbal plants *Saraca asoca* leaves and *Trigonella foenum-graecum* seeds, which are rich in microminerals, to postpartum anestrus buffaloes to induce estrus and to assess the correlation of sex hormones (progesterone and 17 β -estradiol) with trace minerals.

Materials and Methods

Postpartum anestrus buffaloes (>90 days postpartum) were identified and selected for the study. A total of thirty-two animals were randomly divided into four groups, with eight buffaloes in each group. Group I served as the control group and was treated with the Ovsynch protocol, consisting of GnRH on day 0, PGF2 α on day 7, and GnRH on day 9. Group II was supplemented orally with 130 g per day of *Saraca asoca* leaves powder for 24 days. Group III received 200 g per day of *Trigonella foenum-graecum* seed powder orally for 24 days. Group IV was administered a combination of 65 g of *Saraca asoca* leaves powder and 100 g of *Trigonella foenum-graecum* seed powder orally per day for 24 days. Blood samples were collected on days 0, 4, 8, 12, 16 and 24 for estimation of serum progesterone and 17 β -estradiol by UV-Vis spectrophotometry. For mineral estimation, blood samples collected on days 0, 8, 16 and 24 were analyzed for calcium, phosphorus, zinc, selenium, iron and copper by ICP-OES. Data obtained was subjected to statistical analysis by using Analysis of variance (ANOVA) as per the procedure of Snedecor and Cochran (1994) [10] by using statistical software IBM SPSS Ver.23.0 for windows.

Results

The overall mean value of serum progesterone and 17 β -estradiol were given in Table 1. The serum progesterone value in group II is higher compared to Group I, III, and Group IV.

Table 1: Mean ($\pm$ SE) values of serum hormones as affected by different experimental groups

Period (days)	Experimental groups			
	Group I (Synthetic hormone)	Group II (SAL)	Group III (FS)	Group IV (SAL and FS)
Progesterone (ng/ml)	1.141 $\pm$ 0.17	1.351 $\pm$ 0.20	0.768 $\pm$ 0.1	0.583 $\pm$ 0.03
17 β -estradiol (pg/ml)	17.29 $\pm$ 0.91	17.05 $\pm$ 0.88	19.90 $\pm$ 1.44	21.33 $\pm$ 1.37

Minerals

The serum calcium levels (Table 2 and Fig 1) were significantly ($p < 0.01$) were higher in order of group III followed by group II and group IV. Group I values were significant at $p < 0.05$ which showed the lower value in comparison with other groups. The serum phosphorus levels (Table 3 and Fig 2) were significantly different ($p < 0.01$) in group II, group IV and group III and significantly ($p < 0.05$) different in group I. Group II levels were higher than group

IV and group III. Group I showed the lower value comparatively with other groups.

The serum zinc values (Table 4 and Fig 3) were significantly higher ($p < 0.01$) in group II followed by group IV, group III and group I. Serum selenium values (Table 5 and Fig 4) were significantly ($p < 0.01$) higher in group II compared to group III then group IV. Iron levels (Table 6 and Fig 5) were non-significant in all groups. The serum values of copper (Table 7 and Fig 6) were highly significant in group II followed by group III, group IV and group I.

Table 2: Mean ($\pm$ SE) values of serum Calcium (mg %) as affected by different experimental groups

Period (days)	Experimental groups			
	Group I (Synthetic hormone)	Group II (SAL)	Group III (FS)	Group IV (SAL and FS)
0 th day	8.40 $\pm$ 0.32	8.43 $\pm$ 0.31	8.47 $\pm$ 0.31	8.39 $\pm$ 0.32
8 th day	9.18 $\pm$ 0.37	9.70 $\pm$ 0.40	9.52 $\pm$ 0.32	9.38 $\pm$ 0.36
16 th day	9.69 $\pm$ 0.37	10.03 $\pm$ 0.39	10.04 $\pm$ 0.38	10.07 $\pm$ 0.33
24 th day	10.19 $\pm$ 0.40	10.66 $\pm$ 0.39	11.38 $\pm$ 0.24	10.97 $\pm$ 0.32
Overall Mean $\pm$ S.E	9.36 $\pm$ 0.21	9.71 $\pm$ 0.23	9.86 $\pm$ 0.24	9.70 $\pm$ 0.23
F-value	4.278*	6.168**	14.245**	10.603**

** $p < 0.01$ ^{NS} $p < 0.05$

Means bearing same superscripts in small letters (row wise) do not differ significantly between groups

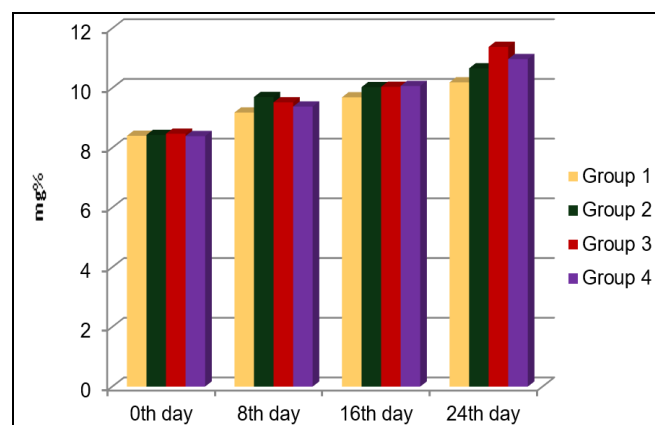


Fig 1: Mean values of serum Calcium (mg %) as affected by different experimental groups

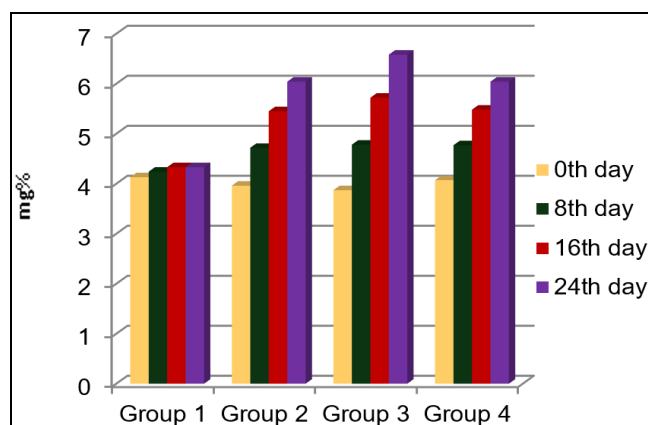


Fig 2: Mean values of serum Phosphorus (mg %) as affected by different experimental groups

Table 3: Mean ($\pm$ SE) values of serum Phosphorus (mg %) as affected by different experimental groups

Period (days)	Experimental groups			
	Group I (Synthetic hormone)	Group II (SAL)	Group III (FS)	Group IV (SAL and FS)
0 th day	4.13 $\pm$ 0.057	3.87 $\pm$ 0.161	3.96 $\pm$ 0.139	4.07 $\pm$ 0.163
8 th day	4.24 $\pm$ 0.049	4.78 $\pm$ 0.153	4.72 $\pm$ 0.161	4.77 $\pm$ 0.170
16 th day	4.33 ^a $\pm$ 0.032	5.72 ^b $\pm$ 0.170	5.45 ^b $\pm$ 0.163	5.48 ^b $\pm$ 0.166
24 th day	4.33 ^a $\pm$ 0.036	6.58 ^c $\pm$ 0.127	6.04 ^b $\pm$ 0.142	6.04 ^b $\pm$ 0.119
Overall Mean $\pm$ S.E	4.26 $\pm$ 0.026	5.24 $\pm$ 0.196	5.04 $\pm$ 0.158	5.09 $\pm$ 0.152
F-value	4.593*	58.050**	35.291**	30.145**

** $p < 0.01$ ^{NS} $p < 0.05$

Means bearing same superscripts in small letters (row wise) do not differ significantly between groups

Table 4: Mean ($\pm$ SE) values of serum Zinc (ppm) as affected by different experimental groups

Period (days)	Experimental groups			
	Group I (Synthetic hormone)	Group II (SAL)	Group III (FS)	Group IV (SAL and FS)
0 th day	0.80 $\pm$ 0.043	0.82 $\pm$ 0.030	0.84 $\pm$ 0.031	0.84 $\pm$ 0.032
8 th day	1.11 ^a $\pm$ 0.063	1.68 ^c $\pm$ 0.071	1.40 ^b $\pm$ 0.065	1.49 ^{bc} $\pm$ 0.085
16 th day	1.39 ^a $\pm$ 0.051	2.42 ^c $\pm$ 0.094	2.01 ^b $\pm$ 0.060	2.16 ^b $\pm$ 0.052
24 th day	1.63 ^a $\pm$ 0.050	3.08 ^c $\pm$ 0.136	2.66 ^b $\pm$ 0.060	2.82 ^b $\pm$ 0.064
Overall Mean $\pm$ S.E	1.23 $\pm$ 0.061	2.00 $\pm$ 0.157	1.73 $\pm$ 0.125	1.83 $\pm$ 0.136
F-value	46.372**	113.026**	198.511**	195.323**

** $p < 0.01$ ^{NS} $p < 0.05$

Means bearing same superscripts in small letters (row wise) do not differ significantly between groups

Table 5: Mean ($\pm$ SE) values of serum Selenium (ppm) as affected by different experimental groups

Period (days)	Experimental groups			
	Group I (Synthetic hormone)	Group II (SAL)	Group III (FS)	Group IV (SAL and FS)
0 th day	0.0692 ^a $\pm$ 0.001	0.0759 ^b $\pm$ 0.002	0.0689 ^a $\pm$ 0.000	0.0689 ^a $\pm$ 0.001
8 th day	0.0703 ^a $\pm$ 0.001	0.0852 ^b $\pm$ 0.002	0.0739 ^a $\pm$ 0.000	0.0720 ^a $\pm$ 0.000
16 th day	0.0711 ^a $\pm$ 0.002	0.0930 ^d $\pm$ 0.002	0.0788 ^c $\pm$ 0.000	0.0750 ^b $\pm$ 0.000
24 th day	0.0722 ^a $\pm$ 0.001	0.1042 ^d $\pm$ 0.003	0.0843 ^c $\pm$ 0.000	0.0784 ^b $\pm$ 0.001
Overall Mean $\pm$ S.E	0.0707 $\pm$ 0.000	0.0896 $\pm$ 0.002	0.0765 $\pm$ 0.001	0.0736 $\pm$ 0.001
F-value	3.091*	22.884**	242.665**	59.389**

** $p < 0.01$ ^{NS} $p < 0.05$

Means bearing same superscripts in small letters (row wise) do not differ significantly between groups

Table 6: Mean ($\pm$ SE) values of serum Iron (ppm) as affected by different experimental groups

Period (days)	Experimental groups			
	Group I (Synthetic hormone)	Group II (SAL)	Group III (FS)	Group IV (SAL and FS)
0 th day	4.05 $\pm$ 0.132	4.11 $\pm$ 0.153	4.14 $\pm$ 0.170	4.20 $\pm$ 0.168
8 th day	4.05 $\pm$ 0.132	4.25 $\pm$ 0.135	4.31 $\pm$ 0.165	4.40 $\pm$ 0.170
16 th day	4.06 $\pm$ 0.132	4.41 $\pm$ 0.146	4.53 $\pm$ 0.159	4.61 $\pm$ 0.167
24 th day	4.06 ^a $\pm$ 0.132	4.60 ^b $\pm$ 0.137	4.75 ^b $\pm$ 0.159	4.83 ^b $\pm$ 0.156
Overall Mean $\pm$ S.E	4.06 $\pm$ 0.063	4.34 $\pm$ 0.075	4.43 $\pm$ 0.088	4.51 $\pm$ 0.089
F-value	0.003 ^{NS}	2.173 ^{NS}	2.636 ^{NS}	2.648 ^{NS}

* $p < 0.01$ ^{NS} $p < 0.05$

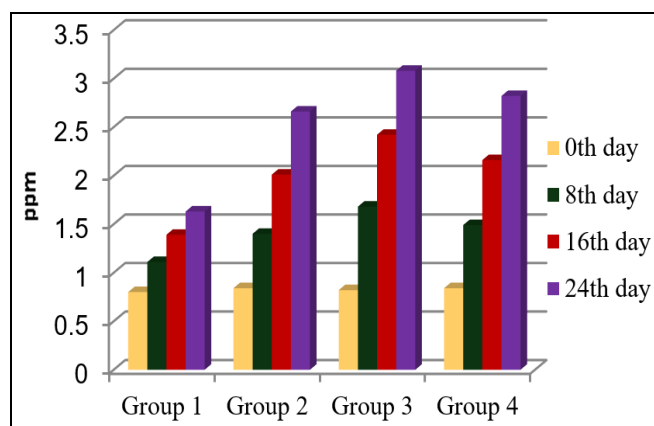
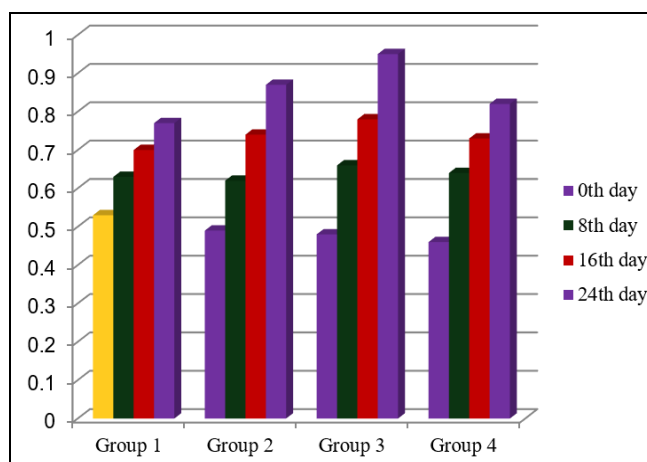
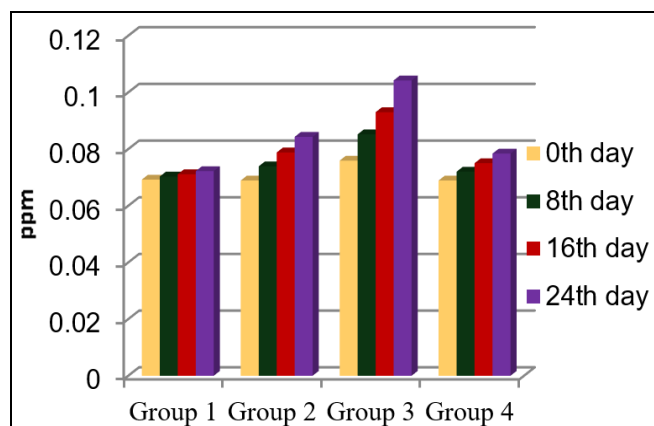
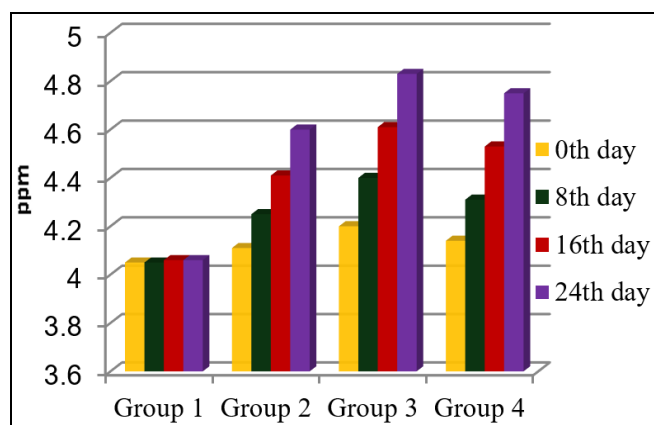
Means bearing same superscripts in small letters (row wise) do not differ significantly between groups

Table 7: Mean ($\pm$ SE) values of serum Copper (ppm) as affected by different experimental groups

Period (days)	Experimental groups			
	Group I (Synthetic hormone)	Group II (SAL)	Group III (FS)	Group IV (SAL and FS)
0 th day	0.53 ^c $\pm$ 0.010	0.48 ^{ab} $\pm$ 0.010	0.49 ^b $\pm$ 0.009	0.46 ^a $\pm$ 0.004
8 th day	0.63 $\pm$ 0.020	0.66 $\pm$ 0.008	0.62 $\pm$ 0.005	0.64 $\pm$ 0.006
16 th day	0.70 ^a $\pm$ 0.013	0.78 ^c $\pm$ 0.003	0.74 ^b $\pm$ 0.003	0.73 ^b $\pm$ 0.003
24 th day	0.77 ^a $\pm$ 0.008	0.95 ^d $\pm$ 0.004	0.87 ^c $\pm$ 0.008	0.82 ^b $\pm$ 0.005
Overall Mean $\pm$ S.E	0.66 $\pm$ 0.017	0.72 $\pm$ 0.031	0.68 $\pm$ 0.025	0.67 $\pm$ 0.024
F-value	56.038**	809.484**	647.031**	1110.625**

* $p < 0.01$ ^{NS} $p < 0.05$

Means bearing same superscripts in small letters (row wise) do not differ significantly between groups

**Fig 3:** Mean values of serum Zinc (ppm) as affected by different experimental groups**Fig 6:** Mean ($\pm$ SE) values of serum Copper (ppm) as affected by different experimental groups and days of collection**Fig 4:** Mean values of serum Selenium (ppm) as affected by different experimental groups**Fig 5:** Mean values of serum Iron (ppm) as affected by different experimental groups**Correlation between Hormones (Progesterone and 17 β -Estradiol) and minerals**

Correlation co-efficient between serum mineral levels and serum hormone (Progesterone and 17 β -estradiol) levels in four experimental groups is given in table 8.

Macrominerals

In Group I, serum calcium and phosphorus showed a positive correlation with progesterone concentration, with phosphorus exhibiting a positive and significant correlation ($p < 0.01$). In contrast, both calcium and phosphorus were negatively correlated with 17 β -estradiol. In Group II, serum calcium and phosphorus showed a positive and significant correlation ($p < 0.01$) with progesterone concentration. The correlation of phosphorus with progesterone was more significant in Group II compared to Group I. Calcium was negatively correlated with 17 β -estradiol, while phosphorus showed no significant correlation. In Group III, serum calcium and phosphorus exhibited a positive and significant correlation ($p < 0.01$) with progesterone concentration. The correlation of calcium with progesterone was higher in Group III compared to Groups II and I. Neither calcium nor phosphorus showed a significant correlation with 17 β -estradiol. In Group IV, serum calcium and phosphorus showed a positive and significant correlation ($p < 0.01$) with progesterone concentration. The correlation of calcium with progesterone was significantly higher ($p < 0.01$) in Group IV compared to Groups III, I, and II. Furthermore, calcium was positively and significantly correlated ($p < 0.05$), while phosphorus was positively and significantly correlated ($p < 0.01$) with 17 β -estradiol. Notably, both calcium and

phosphorus exhibited a significant correlation with 17 β -estradiol in Group IV compared to Groups I, II, and III.

Microminerals

In Group I, serum zinc and copper were found to be positively and significantly ($p < 0.01$) correlated with progesterone concentration, while selenium was positively correlated but non-significant. The correlation of zinc, copper, and selenium with 17 β -estradiol was positive but non-significant. In Group II, serum zinc and copper were positively and significantly ($p < 0.01$) correlated with progesterone, while selenium was positively and significantly ($p < 0.05$) correlated. The correlation of zinc, copper, and selenium with 17 β -estradiol remained positive but non-significant. In Group III, serum zinc, copper, and selenium were positively and significantly ($p < 0.01$) correlated with progesterone. The correlation of these minerals with 17 β -estradiol was positive. In Group IV, serum zinc, copper, and selenium were positively and significantly ($p < 0.01$) correlated with progesterone. The correlation of zinc and copper with 17 β -estradiol was positive and significant ($p < 0.01$), while selenium showed a positive and significant ($p < 0.05$) correlation with 17 β -estradiol. Regarding iron, in Group I it was negatively correlated with progesterone and positively correlated with 17 β -estradiol, but both correlations were non-significant. In Groups II and III, iron was positively correlated with both progesterone and 17 β -estradiol, but the correlations were non-significant. In Group IV, iron was positively correlated with progesterone and showed a positive and significant ($p < 0.05$) correlation with 17 β -estradiol. Overall, the correlation of minerals with progesterone was found to be positive and significant in all groups, except for iron. In contrast, the correlation of minerals with 17 β -estradiol was non-significant in all groups except Group IV, which showed significant correlations.

Table 8s: Correlation of minerals to hormones (Progesterone and 17 β -Estradiol)

Group	Mineral	Progesterone	17 β -estradiol
Group I	Ca	0.548	-0.277
	P	0.497**	-0.162
	Zn	0.522**	0.054
	Fe	-0.069	0.142
	Cu	0.545**	0.071
	Se	0.083	0.106
Group II	Ca	0.541**	-0.014
	P	0.645**	0.033
	Zn	0.594**	0.136
	Fe	0.091	0.274
	Cu	0.657**	0.138
	Se	0.396*	0.325
Group III	Ca	0.604**	0.253
	P	0.456**	0.147
	Zn	0.586**	0.201
	Fe	0.263	0.175
	Cu	0.599**	0.192
	Se	0.541**	0.171
Group IV	Ca	0.650**	.428*
	P	0.525**	.507**
	Zn	0.664**	.527**
	Fe	0.109	.384*
	Cu	0.559**	.552**
	Se	0.575**	.424*

** correlation is significant at the 0.01 level (2 tailed)

* correlation is significant at the 0.05 level (2 tailed)

Discussion

In the present study, serum macrominerals (Ca, P) and trace minerals (Zn, Cu, Se, Fe) showed a predominantly positive correlation with progesterone in all treatment groups, while their correlation with 17 β -estradiol was significant only in the combination group (Group IV). This differential relationship explains the reproductive physiology of buffaloes and the role of herbal mineral supplementation.

Calcium and Phosphorus: The positive and significant correlation of Ca and P with progesterone observed in all groups supports the findings of Chaurasia *et al.* (2010) [4] who reported that optimum Ca and P are essential for cyclic ovarian function. Calcium influences steroidogenesis as a second messenger in gonadotropin-mediated LH action on luteal cells, and regulates the utilization of other trace elements (Sharma *et al.*, 2012) [9]. The improved significance in Group II (*Saraca asoca* leaves) and Group III (*Trigonella foenum-graecum* seeds) compared to control is attributable to high mineral content reported in these herbs (Suresh *et al.*, 2024 [11]; Rabia Praveen *et al.*, 2024 [8]). *Saraca asoca* leaves are rich in Ca, P, Na, I, Fe and Mn, while fenugreek seeds are rich in Ca, Mg, Na, K, Fe, Zn and Cu (Ahmad *et al.*, 2015) [2]. The negative correlation of Ca and P with 17 β -estradiol in Group I (OvSynch) can be explained by hormonal antagonism during follicular phase. In contrast, the positive and significant correlation observed in Group IV indicates restoration of Ca:P ratio, which is critical. An imbalanced Ca:P ratio blocks pituitary function and prolongs ovulation (Chaurasia *et al.*, 2010) [4]. The combination of herbs at reduced doses (65g + 100g) provided a more balanced mineral supply, resulting in significant correlation of both Ca ($p < 0.05$) and P ($p < 0.01$) with estradiol, unlike individual herb groups.

Zinc, Copper and Selenium: Zn and Cu were positively and significantly correlated ($p < 0.01$) with progesterone in all groups, confirming their role as cofactors for enzymes governing steroidogenesis, corpus luteum lifespan and antioxidant defense (Wang B *et al.*, 2026 [12]; Hafez *et al.*, 2019 [5]). Copper is essential for cytochrome oxidase and lysyl oxidase involved in steroid synthesis, while zinc regulates progesterone synthesis via Zn-finger transcription factors. Selenium, as a component of glutathione peroxidase, protects luteal cells from oxidative damage, thereby maintaining progesterone secretion (Yatoo *et al.*, 2016) [13]. The non-significant correlation of these trace elements with 17 β -estradiol in Groups I, II and III and significant correlation in Group IV suggests that estradiol synthesis is more sensitive to combined mineral deficiency. Abhijit *et al.* (2015) [1] also emphasized that microminerals influence synthesis and systemic regulation of both progesterone and estradiol. The synergistic phytochemicals (β -sitosterol and flavonoids in *Saraca asoca* leaves and phytoestrogens, diosgenin in *Trigonella foenum graecum* seeds) along with Zn and Se supplementation in Group IV may have stimulated aromatase activity, enhancing conversion of androgens to estradiol in developing follicles.

Iron: Iron showed a negative to non-significant correlation with progesterone in Group I, which corroborates reports of low serum iron in anestrus buffaloes (Ankita *et al.*, 2018 [3]; Sharma *et al.*, 2012 [9]). Iron is required for heme-containing enzymes like cytochrome P₄₅₀, which catalyzes cholesterol

side-chain cleavage for steroidogenesis. The shift to positive correlation in Groups II, III and significant correlation with estradiol ($p < 0.05$) in Group IV indicates that herbal supplementation corrected the iron deficiency-induced anestrus.

In conclusion, the correlation pattern indicates that progesterone is highly mineral-dependent, while 17β -estradiol requires a synergistic balance of minerals. The combination of *Saraca asoca* leaves and *Trigonella foenum-graecum* seeds was superior in establishing significant correlations for both hormones, suggesting its potential as a cost-effective herbal strategy to ameliorate postpartum anestrus by improving mineral-hormonal cross-talk and exhibiting estrus in buffaloes.

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