RESEARCH ARTICLE Indian Journal of Animal Research, Volume 57 Issue 12: 1606-1612 (December 2023)

Changes in Mineral Profiles in Progesterone-based Estrus Induction Protocols and their Relationship with the Phases of Estrous Cycle in Kangayam Cows

U. Lakshmikantan[1], M. Selvaraju[1], M. Palanisamy[1],
P. Selvaraj[2], P. Vasanthakumar[3], R. Mathivanan[4]

10.18805/IJAR.B-5191

ABSTRACT

Background: For the successful reproduction in Kangayam cows, adequate nutrition containing sufficient quantum of minerals is necessary. Minerals are important cofactors in majority of the metabolic and immune reactions and hormone synthesis that could affect reproduction. Present study was planned to analyse whether mineral levels change in different treatment days following estrus induction and corresponding changes in stages of the estrous cycle.

Methods: Non pregnant, pluriparous Kangayam cow (n= 70) were allotted to control and different treatment protocols (each consist of 10 cows) based on the transdermal (PNC based groups) and transvaginal application of progesterone (CIDR based group) with a shot of $PGF_2\alpha$ one day preceding the day of progesterone removal. Ovulation was induced either using estradiol benzoate or buserelin acetate. Mineral profiles were analysed on the day of progesterone application (APP), removal (REM), AI and 10 days post insemination (10 DPAI).

Result: It indicated that changes in the mineral levels between the treatment days especially on the day of AI and 10DAPAI could be correlated with the corresponding follicle and luteal phases and it could be concluded that there were significant changes especially in selenium, manganese, zinc and copper levels between the treatment days in each group which could be extrapolated to have specific roles in stages of estrous cycle. Among these minerals copper levels could be correlated to affect the steroid concentration by altering its metabolism thereby its clearance in *Bos indicus* breed ie Kangayam breed of Tamil Nadu.

Key words: *Bos indicus*, Estrus induction, Kangayam cow, Mineral profile, Steroid metabolism.

INTRODUCTION

A successful reproductive life in cow is dependent on many factors, among which minerals play a major role. Impact of estrus induction protocols are observed on the pregnancy rate achieved and in majority of the estrus induction programmes minerals are supplemented invariably. In some reports it was stated that all the minerals could play a major role in activating the specific genes in the dominant follicle (Gagnon *et al*., 2015) and this could influence the size of the follicle. Size of the follicle determines the steroid production and further ovulation. Phillippo *et al*. (1987) stated that deficiency in copper led to reduced first service conception, impaired embryonic survival, poor display of estrus signs, impaired LH secretion and finally reduced pregnancy rates. Hence Copper has long been described to impact fertility in cows.

Trace minerals are components of metalloenzymes, hormones and cofactors of functional proteins, or enzymes that are involved in many aspects of energy and protein metabolism, hormones synthesis and immune function (McClure, 1994). Some of the minerals are frequently related to anestrus, subestrus, irregular estrous cycle in cows (Parkinson, 2019). Phosphorus is considered a very important mineral in maintaining the fertility in cows. Manganese is involved in cholesterol synthesis and cholesterol is the precursor for any steroid production. Hence deficiency of manganese could affect the steroid synthesis.

[1]Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal-637 002, Tamil Nadu, India.
[2]Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal-637 002, Tamil Nadu, India.
[3]Department of Animal Nutrition, Veterinary College and Research Institute, Namakkal-637 002, Tamil Nadu, India.
[4]Veterinary University Training and Research Centre, Tirupur-641 604, Tamil Nadu, India.

Corresponding Author: U. Lakshmikantan, Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal-637 002, Tamil Nadu, India.
Email: vetlakshmi@gmail.com

How to cite this article: Lakshmikantan, U., Selvaraju, M., Palanisamy, M., Selvaraj, P., Vasanthakumar, P. and Mathivanan, R. (2023). Changes in Mineral Profiles in Progesterone-based Estrus Induction Protocols and their Relationship with the Phases of Estrous Cycle in Kangayam Cows. Indian Journal of Animal Research. 57(12): 1606-1619. doi: 10.18805/IJAR.B-5191.

Submitted: 04-07-2023 **Accepted:** 27-09-2023 **Online:** 05-10-2023

Expression of estrus signs are sometimes difficult to observe in Bos indicus breeds (Sartori *et al*., 2019). Hence unobserved estrus and silent estrus are more common in

these breeds. This issue necessitated the use of estrus induction protocols (Naikoo *et al*., 2016; Devipriya *et al*., 2020; Periyannan *et al*., 2021; Manokaran *et al*., 2023) to achieve a favourable conception rate in indigenous breeds of cattle. Allan *et al*. (1993) stated that pregnancy rates were increased in cows that were supplemented with trace elements with Vitamin bolus. Ahola *et al*. (2004) observed that increased pregnancy rates after synchronised AI in cows after 2 years of supplementation with copper, zinc and manganese.

This study was primarily conducted to evaluate the mineral profile during the progesterone based estrus induction protocols especially on the day of Artificial Insemination (AI) and 10 days post insemination. Moreover, changes found in the mineral levels could be related to any of the reproductive status and/or phases of estrous cycle in Kangayam cows.

MATERIALS AND METHODS

Estrus induction protocols were performed in the non-pregnant, pluriparous Kangayam cows which are reared (n = 70) in and around Tirupur district. The work was carried out in Veterinary University Training and Research Centre (VUTRC), Tirupur during the period of April 2021 to June 2023. All the cows were supplemented with mineral mixture 50 g/day at least for 20 days prior to the estrus induction procedure. For control animals AI was performed twice at observed estrus after mineral mixture supplementation for 20 days. Estrus induction was carried out using the progesterone hormone device either Controlled Internal Drug Releasing Device (CIDR based group) through transvaginal application or ProSync-NC (purchased from TRPVB a constituent laboratory under TANUVAS [PNC based group]) through transdermal application (Lakshmikantan *et al*., 2021) for seven and five days respectively. On the preceding date of progesterone removal 500 microgram of Cloprostenol sodium was injected in the treated cows. For the induction of ovulation either estradiol benzoate 1 milligram (CIDR-EB and PNC-EB) 24 hours before AI or buserelin acetate 10 micrograms (CIDR-G and PNC-G) at AI was used. Blood samples were collected at progesterone application (APP), removal (REM), AI and 10 days post AI (10 DPAI) in clot activator vials (5 ml) and submitted to a private laboratory on the same day to estimate the mineral profile. Results were analysed using one way ANOVA and if the ALPHA level estimated was ($P \leq 0.05$) then bonferroni correction was carried out and student t test (post-hoc analysis) was conducted to identify the significant difference between the treatment days in each group using Microsoft Excel 365.

RESULTS AND DISCUSSION

In the present study, following minerals such as chromium (µg/L), cobalt (µg/L), molybdenum (µg/L), selenium (µg/L), manganese (µg/L), phosphorus (mg/dl), magnesium (mg/dl), zinc (µg/L), calcium (mg/dl) and copper (µg/L) were estimated and analysed with corresponding reproductive status or stage of the estrous cycle in different treatment groups. Analysis was planned to mimic either the follicular or luteal phase in different treatment days, hence the following days of treatment were selected *viz*., at the initiation progesterone treatment (in which the cows selected for the study could be at different reproductive status because treatment protocols were initiated irrespective of the stage of estrous cycle), at the time of progesterone removal *i.e* one day after the administration of luteolytic agent, Cloprostenol sodium, on the day of AI and then the 10th day post AI (10 DPAI). In which at the time of progesterone application some cows were in anestrus so the mineral levels obtained may or may not reflect either follicular or luteal phase. Even though progesterone was supplemented through external source on the day of removal and on the day of AI, both could be considered for the follicular phase of the estrous cycle because impact of the corpus luteum is minimal or nil in these days respectively. 10 DPAI could be considered as the luteal phase. Levels of mineral in different groups-control and treatment groups were provided in Table 1 to 7. Table 8 shows the values of minerals in all the cows selected for the study irrespective of the treatment protocol adopted.

Chromium

In Kangayam cows, there were variations in the levels of chromium on the day of AI when compared with other treatment days. Otherwise, it could be stated that during the follicular phase the levels were slightly higher than the luteal phase even though levels did not differ statistically between days of treatment in different groups. Hence it could be assumed that chromium was less utilized during the follicle development and might had a role in the luteal phase. In other words, it could be hypothesized that this mineral has important role in the maintenance of corpus luteum or progesterone production because during this period levels were low indicating its utilization.

Cobalt

Levels of cobalt in different treatment groups did not differ significantly. However, there was a pattern observed in this mineral profile between the sources of progesterone (transdermal and transvaginal route). In PNC treated groups (PNC, PNC-EB and PNC-G) on the day of AI the levels were slightly higher. But on the CIDR treated groups (CIDR, CIDR-EB and CIDR-G) the levels on the day of AI were slightly lower when compared to 10 DPAI. However, when the data was analysed, irrespective of the treatment groups the levels of Cobalt were very much less at around the period of corpus luteum lysis. It was evident that the levels were very much low at the time of progesterone removal *i.e* one day after the cloprostenol sodium administration as a luteolytic agent.

Molybdenum

Treated Kangayam cows did not show any significant differences in the levels of molybdenum. But control cows showed significant difference between the day of AI and

Table 1: Mineral levels in control group on the day of AI and 10 DPAI.

CON	Chromium µg/L	Cobalt µg/L	Molybdenum µg/L	Selenium µg/L	Manganese µg/L	Phosphorus mg/dl	Magnesium mg/dl	Zinc µg/L	Calcium mg/dl	Copper µg/L
AI	3.03±0.08	0.77±0.03	4.71±0.53[a]	179.15±1.83[a]	19.43±1.27[a]	4.08±0.16	2.89±0.05	99.98±3.22[a]	9.58±0.16	90.53±5.87[a]
10D PAI	2.76±0.12	1.00±0.14	0.93±0.08[a]	154.31±3.40[a]	12.40±0.68[a]	4.58±0.59	2.38±0.33	88.17±3.23[a]	9.28±0.18	187.00±3.74[a]

Same superscripts differ significantly within the column (≤0.01).

Table 2: Mineral levels in PNC group during the progesterone-based estrus induction protocol.

PNC	Chromium µg/L	Cobalt µg/L	Molybdenum µg/L	Selenium µg/L	Manganese µg/L	Phosphorus mg/dl	Magnesium mg/dl	Zinc µg/L	Calcium mg/dl	Copper µg/L
APP	2.76±0.35	0.96±0.17	2.69±0.61	102.62±6.99	14.61±1.42	4.97±0.63	2.66±0.29	164.91±6.61[a]	9.48±0.18	59.34±2.72[a]
REM	3.00±0.50	0.57±0.10	2.57±0.60	110.87±9.99	15.48±1.41	3.97±0.35[a]	2.97±0.18	171.14±4.74[b,c]	9.67±0.16	66.15±4.80[c,d]
AI	3.13±0.13	1.17±0.23	2.21±0.56	86.32±3.32	20.19±1.92	4.92±0.49	2.95±0.35	122.10±4.84[a,b]	9.40±0.22	93.46±4.99[a,c,e]
10 DPAI	2.57±0.25	1.04±0.27	2.72±0.60	93.27±5.66	15.60±1.27	6.60±0.56[a]	2.37±0.30	118.77±4.06[a,c]	9.37±0.30	196.56±7.13[a,b,d,e]

Same superscripts differ significantly within the column (≤0.01).

Table 3: Mineral levels in CIDR group during the progesterone-based estrus induction protocol.

CIDR	Chromium µg/L	Cobalt µg/L	Molybdenum µg/L	Selenium µg/L	Manganese µg/L	Phosphorus mg/dl	Magnesium mg/dl	Zinc µg/L	Calcium mg/dl	Copper µg/L
APP	2.50±0.22	0.82±0.08	2.07±0.32	99.55±5.76	14.58±0.55	5.05±0.65	2.39±0.19	110.08±8.06[a,b,c]	9.03±0.61	116.98±2.32[a,b,c]
REM	2.60±0.14	0.64±0.05	1.49±0.12[a]	93.10±4.32	14.37±0.87	4.34±0.11	2.86±0.32	168.20±4.82[a,d]	9.36±0.13	70.70±4.91[a,d]
AI	2.97±0.21	0.88±0.07	2.29±0.27	105.90±3.70	16.53±1.49	4.48±0.18	2.22±0.18	253.98±8.90[b,d,e]	9.45±0.20	75.28±4.96[b,e]
10 DPAI	2.58±0.15	0.81±0.07	3.07±0.40[a]	103.80±3.23	14.53±0.88	7.46±1.23	2.16±0.16	175.68±6.24[c,e]	8.86±0.13	95.69±3.93[c,d,e]

Same superscripts differ significantly within the column (≤0.01).

Table 4: Mineral levels in PNC-EB group during the progesterone-based estrus induction protocols.

PNC-EB	Chromium µg/L	Cobalt µg/L	Molybdenum µg/L	Selenium µg/L	Manganese µg/L	Phosphorus mg/dl	Magnesium mg/dl	Zinc µg/L	Calcium mg/dl	Copper µg/L
APP	3.69±0.31	0.88±0.14	2.74±0.52	103.97±4.90^{a}	15.09±1.57	4.99±0.30	2.60±0.31	185.00±4.88^{a,b,c}	9.43±0.22	71.23±4.59^{a,b}
REM	3.65±0.57	0.66±0.08	2.20±0.60	129.45±6.62^{a}	19.44±2.01	5.11±0.28	2.74±0.19	123.33±4.83^{a}	9.87±0.24	66.55±2.93^{c,d}
AI	2.82±0.27	0.96±0.14	3.05±0.74	116.02±5.42	17.71±1.22	4.83±0.35	2.21±0.24	119.60±4.24^{b}	10.01±0.37	103.59±3.51^{a,c,e}
10 DPAI	3.22±0.21	0.97±0.17	2.08±0.41	105.86±5.76	16.33±1.11	5.51±0.20	2.24±0.09	117.43±5.48^{c}	9.26±0.24	204.37±6.50^{b,d,e}

Same superscripts differ significantly within the column (≤0.01).

Table 5: Mineral levels in CIDR-EB group during the progesterone-based estrus induction protocols.

CIDR-EB	Chromium µg/L	Cobalt µg/L	Molybdenum µg/L	Selenium µg/L	Manganese µg/L	Phosphorus mg/dl	Magnesium mg/dl	Zinc µg/L	Calcium mg/dl	Copper µg/L
APP	3.32±0.43	0.97±0.21	4.80±1.82	102.42±5.71^{a}	20.43±1.41	5.93±0.47	2.92±0.23	153.20±4.80^{a,b,c}	9.23±0.41	110.88±5.09^{a}
REM	1.57±0.54	0.65±0.06	2.62±0.70	104.38±8.78^{b}	18.18±2.31	4.98±0.67	3.12±0.13	99.52±4.53^{a,d}	10.33±0.58	84.98±8.26^{b}
AI	2.84±0.39	0.82±0.13	4.06±1.05	170.95±4.71^{a,b,c}	17.68±1.12	4.77±0.33	2.98±0.26	104.72±7.46^{b}	9.62±0.26	49.74±3.91^{a,b,c}
10 DPAI	2.68±0.36	1.30±0.35	1.88±0.44	119.81±8.52^{c}	18.51±1.94	5.88±0.58	2.80±0.31	117.67±3.46^{c,d}	9.37±0.28	94.44±5.32^{c}

Same superscripts differ significantly within the column (≤0.01).

Table 6: Mineral levels in PNC-G group during the progesterone-based estrus induction protocols.

PNC-G	Chromium µg/L	Cobalt µg/L	Molybdenum µg/L	Selenium µg/L	Manganese µg/L	Phosphorus mg/dl	Magnesium mg/dl	Zinc µg/L	Calcium mg/dl	Copper µg/L
APP	3.18±0.18	0.95±0.20	1.81±0.66	104.59±7.22	16.66±2.02	5.85±0.54	2.97±0.26	147.67±4.05^{a,b}	9.40±0.20	63.59±5.04^{a,b}
REM	3.70±0.57	0.86±0.20	3.11±1.12	125.77±5.85	17.83±2.41	5.28±0.58	3.20±0.07	111.72±6.47^{a,c}	9.80±0.20	59.65±4.92^{c,d}
AI	2.80±0.34	1.15±0.31	1.72±0.32	109.54±6.04	18.30±2.66	4.21±0.23^{a}	2.66±0.32	124.38±4.37^{b,e}	9.83±0.52	114.65±4.21^{a,c,e}
10 DPAI	3.27±0.27	0.96±0.22	2.07±0.70	131.99±5.63	16.02±1.92	6.35±0.44^{a}	2.98±0.23	162.44±5.81^{c,e}	9.45±0.22	147.55±6.06^{b,d,e}

Same superscripts differ significantly within the column (≤0.01).

the day of 10 DPAI. However, such differences were not found in the progesterone-based treatments provided in other groups.

Selenium

Mehdi and Dufrasne, (2016) stated that selenium is involved in increasing fertility which could be attributed to the reduction in the embryonic death during the first month of gestation. This statement indicates that the utilization of selenium could be more on 10 DPAI and hence the level might be comparatively low during these days as it is getting utilized. Ceko *et al*. (2015) found GPx-1 (seleno protein gene) significantly increased in granulosa cells of large healthy follicles. So, antioxidant role of selenoproteins is necessary for the development of healthy follicles. Wilde, (2006) stated that selenium is required in reducing the occurrence of ovarian cysts in cows. In the treatments, PNC based treatment groups even though the levels were not statistically significant between days of treatment except PNC-EB in which the levels were slightly high on the day of AI. In this group it creates the suspicion that injection of estradiol benzoate might have increased the levels of Selenium at AI. In contrast CIDR treated groups on the day of AI the mineral level where high in CIDR-G and also in control group and the levels were significantly different from the levels of other treatment days. In majority of the treatment groups, 10 DPAI levels were low compared to the day of AI indicating its utilization as stated by Mehdi and Dufrasne, (2016).

Manganese

Levels of manganese were high on the day of AI than the other treatment days. This difference was not statistically significant among different treatment and control groups but when considered irrespective of the treatment groups there was a significant difference between the day of AI and the day of 10 DPAI in which the levels were low on the 10 DPAI. It could be assumed that this mineral is involved in cholesterol synthesis. Melovanate kinase, Geranyl pyrophosphate synthetase and Farnesyl pyrophosphate synthetase these enzymes are involved in cholesterol synthesis that require Manganese for its function (Studer *et al*., 2022).

Phosphorus

In PNC group phosphorous levels were significantly different between the day of progesterone removal and on the day of 10 DPAI. This pattern of reduced levels of phosphorus on the day of AI was observed in all treatment groups even though there was no significant difference except PNC-G and in combined irrespective of the treatment group.

Magnesium

As for as the magnesium concentration in the serum of treated and control Kangayam cows were concerned there were no considerable change in the levels noticed in the different days of treatment in any group. However, when considered irrespective of the treatment groups there were mild changes that were statistically significant between the day of progesterone removal, day of AI and on the day of 10 DPAI.

Table 7: Mineral levels in CIDR-G group during the progesterone-based estrus induction protocols.

CIDR-G	Chromium µg/L	Cobalt µg/L	Molybdenum µg/L	Selenium µg/L	Manganese µg/L	Phosphorus mg/dl	Magnesium mg/dl	Zinc µg/L	Calcium mg/dl	Copper µg/L
APP	2.43±0.30	1.39±0.41	4.18±1.02	97.67±7.48^{a}	19.33±1.63	5.95±0.21	3.13±0.20	147.17±4.31^{a,b,c}	8.88±0.34^{a}	104.17±3.49^{a,b}
REM	2.35±0.62	0.55±0.09	2.61±0.67	99.76±5.14^{b}	18.93±1.65	5.15±0.39	2.98±0.16	105.85±4.42^{a}	10.25±0.30^{a}	71.50±4.77^{a,c,d}
AI	2.69±0.07	1.11±0.23	2.40±0.60	151.23±4.76^{a,b,c}	18.07±1.48	4.95±0.44	2.67±0.11	86.17±4.97^{b,d}	9.45±0.31	49.22±3.24^{b,c,e}
10 DPAI	1.99±0.20	1.73±0.41	2.41±0.88	104.60±7.19^{c}	15.85±1.03	5.32±0.16	2.92±0.11	114.28±4.85^{c,d}	9.30±0.27	93.60±4.23^{d,e}

Same superscripts differ significantly within the column (≤0.01).

Zinc

Zinc levels are comparatively high on the day of AI in the following groups: Control, PNC, CIDR groups and less in CIDR-EB, PNC-G, CIDR-G. Zinc is involved in the regulation of expression of steroidogenic enzymes (StAR, Cyp11a1, Hsd3b1, Cyp17a1) and in turn could regulate steroidogenesis (Li *et al*., 2015). Zinc levels on 10 DPAI were significantly less than the day of AI in Control, PNC, CIDR and the levels were significantly high in PNC-G and CIDR-G indicating that GnRH treatment could alter the profile of zinc and might influence steroidogenesis.

Calcium

Calcium levels in the treated and control group cows were not found with significant difference between the days of treatment. However, levels were significantly different when analysed irrespective of the treatment groups concerning all the cows under study on the day of progesterone application, removal, on the day of AI and 10 DPAI.

Copper

There was certain pattern that could be observed in the Copper levels in treated and control groups in Kangayam cows especially on the day of AI and 10 DPAI. In all the treated groups copper levels were less on AI day when compared to 10 DPAI. Copper ions are reducing the metabolism of steroids in liver by manipulating the cytochrome P450 and NADPH-P450 reductase enzymes (Kim *et al*., 2002). In *Bos indicus* cows it was found that the concentration of circulating reproductive steroid hormone was high when compared to the *Bos taurus* breeds (Wiltbank *et al*., 2014) and stated that in *Bos indicus* cows there is a change in blood supply to the liver that alters the steroid metabolism. Hence steroid clearance is based on increased or decreased blood supply to the liver. Wiltbank *et al*. (2014) also, stated that increased feed intake in high producing or lactating or suckled cows had increased blood supply to liver when compared to non-productive cows. In this present study copper levels are comparatively low on the day of AI. Hence it could be hypothesized that when steroid hormone concentration is getting high at AI, copper concentration is getting low. it might be due to utilization of copper, or the copper ions in the liver to regulate the metabolism of steroid hormone. The concentration of copper was getting increased at luteal phase as the long-standing requirement of copper is necessary to control or to manipulate the metabolism of steroid hormone in the liver until the corpus luteum get lysed. So, it could be interpreted that Copper levels initially at AI was low because of utilisation of this mineral in the steroid metabolism and slowly getting increased when the liver reserves are getting released to match the requirement of metabolism and hence the serum concentration is getting increased. So, in addition to the changes in the blood flow to the liver as stated by Wiltbank *et al*. (2014) changes in the Copper levels may also be responsible for the reduced steroid metabolism.

Table 8: Mineral levels in all the cows selected for the study irrespective of the treatment protocol adopted.

All groups	Chromium µg/L	Cobalt µg/L	Molybdenum µg/L	Selenium µg/L	Manganese µg/L	Phosphorus mg/dl	Magnesium mg/dl	Zinc µg/L	Calcium mg/dl	Copper µg/L
APP	2.92±0.13	0.98±0.08^{a}	2.98±0.38	101.76±2.45^{a,b}	16.57±0.66	5.41±0.21^{a}	2.75±0.10	150.45±4.47	9.24±0.15^{a}	88.03± 4.10^{a,b}
REM	2.83±0.21	0.65±0.04^{a,b,c}	2.39±0.26	110.16±3.44^{c}	17.22±0.74	4.77±0.17^{b,c}	2.98±0.07^{a,b}	132.98±5.14	9.85±0.12^{a,b}	69.60±2.32^{a,c,d}
AI	2.90±0.09	0.98±0.07^{b}	2.88±0.26	128.89±5.11^{a,c}	18.23±0.61^{a}	4.61±0.13^{a}	2.63±0.10^{a}	135.11±8.61	9.62±0.11^{c}	82.75±3.76^{c,e}
10 DPAI	2.73±0.10	1.10±0.09^{c}	2.22±0.21	114.97±3.48^{b}	15.57±0.52^{a}	6.03±0.28^{b,c}	2.51±0.09^{b}	129.44±4.63	9.25±0.09^{b,c}	145.82±7.36^{b,d,e}

Same superscripts differ significantly within the column (≤0.01).

CONCLUSION

The levels of chromium, selenium, manganese, zinc (except GnRH treated animals) were comparatively or significantly less in 10 DPAI than the day of AI and majority of these minerals such as selenium, manganese, zinc were utilized for the luteal function by involving themselves as antioxidants, in cholesterol synthesis and steroidogenesis respectively. Copper levels were high on 10 DPAI compared to the day of AI in all the groups leading to the assumption that as described by Kim *et al*. (2002) Copper ions were responsible for the reduced activity of enzymes that are involved in the metabolism of steroid hormones. In *Bos indicus* breeds it was proved that the levels of steroid hormones were comparatively high than the *Bos taurus*. Hence it could be concluded that there were significant changes especially in Selenium, Manganese, Zinc and Copper levels between the treatment days in each group which could be extrapolated to have specific roles in estrous cycle by manipulating steroidogenesis and its metabolism, especially changes in copper levels could be correlated to have a role in altering steroid metabolism that could be attributed to the steroid concentration in *Bos indicus* breeds.

ACKNOWLEDGEMENT

The author wish to thank the Dean, Veterinary College and Research Institute, Namakkal and Director of clinics, Tamil Nadu Veterinary and Animal Sciences University for the guidance and technical support and National Bank for Agriculture and Rural Development (NABARD), Chennai for their financial support.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

Ahola, J.K., Baker, D.S., Burns, P.D., Mortimer, R.G., Enns, R.M., Whittier, J.C., Geary, T.W. and Engle, T.E. (2004). Effect of copper, zinc and manganese supplementation and source on reproduction, mineral status and performance in grazing beef cattle over a two-year period. Journal of Animal Science. 82(8): 2375-2383.

Allan, C.L., Hemingway, R.G. and Parkins, J.J. (1993). Improved reproductive performance in cattle dosed with trace element/ vitamin boluses. The Veterinary Record. 132(18): 463-464.

Ceko, M.J., Hummitzsch, K., Hatzirodos, N., Bonner, W.M., Aitken, J.B., Russell, D.L., Lane, M., Rodgers, R.J. and Harris, H.H. (2015). X-Ray fluorescence imaging and other analyses identify selenium and GPX1 as important in female reproductive function. Metallomics. 7(1): 71-82.

Devipriya, K., Selvaraj, P., Jayachandran, S., Balasundaram, K., Sivakumar, K. and Manokaran, S. (2020). Efficacy of intravaginal progesterone sponge in estrus induction and fertility rate in indigenous kangayam cows under field conditions. Int. J. Curr. Microbiol. App. Sci. 9(8): 2051-2056.

Gagnon, A., Khan, D.R., Sirard, M.A., Girard, C.L., Laforest, J.P. and Richard, F.J. (2015). Effects of intramuscular administration of folic acid and vitamin B12 on granulosa cells gene expression in postpartum dairy cows. Journal of Dairy Science. 98(11): 7797-7809.

Kim, J.S., Ahn, T., Yim, S.K. and Yun, C.H. (2002). Differential effect of copper (II) on the cytochrome P450 enzymes and NADPH" cytochrome P450 Reductase: Inhibition of Cytochrome P450-Catalyzed Reactions by Copper (II) Ion. Biochemistry. 41(30): 9438-9447.

Lakshmikantan, U., Vadivoo, V.S., Geetha, T. and Mathivanan, R. (2021). Effect of progesterone coated nano fibre dermal patch (ProSync-NF) on cattle diagnosed with Anestrus and silent or unobserved estrus. Indian Journal of Animal Sciences. 91(1): 5-8.

Li, Y., He, X., Yang, X., Huang, K., Luo, Y., Zhu, L., Li, Y. and Xu, W. (2015). Zinc inhibits the reproductive toxicity of Zearalenone in immortalized murine ovarian granular KK-1 cells. Scientific Reports. 5(1): 14277. doi: 10.1038/srep14277.

Manokaran, S., Selvaraju, M., Geetha, T., Palanisamy, M., Devipriya, K. and Periyannan, M. (2023). Comparative study on effect of different estrus synchronization protocols on the pattern of estrus, conception rate and serum hormonal profile in indigenous Kangayam cows. Indian Journal of Animal Research. 57(2): 172-177.

McCLURE, T.J. (1994). Nutritional and Metabolic Infertility in the Cow. Willunga, Cassilis. New South Wales, Australia: CAB International.

Mehdi, Y. and Dufrasne, I. (2016). Selenium in cattle: A review. Molecules. 21(4): 545. https://doi.org/10.3390/molecules 21040545.

Naikoo, M., Dhami, A.J. and Ramakrishnan, A. (2016). Effect of estrus synchronization on plasma progesterone profile and fertility response in postpartum suckled anestrous Kankrej cows. Indian Journal of Animal Research. 50(4): 460-465.

Parkinson, T.J. (2019). Infertility in the Cow Due to Functional and Management Deficiencies. In: Veterinary Reproduction and Obstetrics. [Noakes, D.E., Parkinson, T.J. and England, G.C.W. (eds.)], Elsevier. Pp: 394-398.

Periyannan, M., Selvaraju, M., Ravikumar, K. and Raja, A. (2021). Pattern of induced estrus during superovulatory programme in Kangayam donor cows. The Pharma Innovation. 10(4): 5-8.

Phillippo, M., Humphries, W.R., Atkinson, T., Henderson, G.D. and Garthwaite, P.H. (1987). The effect of dietary molybdenum and iron on copper status, puberty, fertility and oestrous cycles in cattle. The Journal of Agricultural Science. 109(2): 321-336.

Studer, J.M., Schweer, W.P., Gabler, N.K. and Ross, J.W. (2022). Functions of manganese in reproduction. Animal Reproduction Science. 106924.

Sartori, R., Drum, J.N. and Prata, A.B. (2019). Fertility and Infertility in *Bosindicus*. In: Veterinary Reproduction and Obstetrics. [Noakes, D.E., Parkinson, T.J. and England G.C.W. (eds.)], Elsevier; pp: 500-509.

Wilde, D. (2006). Influence of macro and micro minerals in the peri-parturient period on fertility in dairy cattle. Animal Reproduction Science. 96(3-4): 240-249.

Wiltbank, M.C., Souza, A.H., Carvalho, P.D., Cunha, A.P., Giordano, J.O., Fricke, P.M., Baez, G.M. and Diskin, M.G. (2014). Physiological and practical effects of progesterone on reproduction in dairy cattle. Animal. 8(s1): 70-81.