RESEARCH ARTICLE

Plasma Trace Minerals Profile and Fertility without and with Estrus Synchronization Therapy at Day 90 Postpartum in Suckled Kankrej Cows

Mehrajuddin Naikoo[1]*, Arjun .J. Dhami[2], Ammu Ramakrishnan[3], Bharat S. Divekar[4]

Abstract

The study was aimed at assessing the plasma trace minerals profile at 10 days interval up to 90 days postpartum, followed by estrus induction and fertility response together with mineral profiling till day 40 post-FTAI in anestrus/subestrus suckled Kankrej cows (n=36) of an organized farm using five different treatment protocols and a normal cyclic control group (6 cows in each group). The mean plasma levels of zinc, iron, copper, cobalt, and manganese did not vary significantly until 90 days postpartum in most groups, except Ovsynch and Ovsynch + CIDR and Cosynch. The estrus induction response with Ovsynch, CIDR, Ovsynch + CIDR, Cosynch, and $PGF_2\alpha$ treatment protocols initiated at day 90-92 postpartum was 66.66, 83.33, 50.00, 66.66, and 66.66 %, and the conception rates at induced estrus in anestrus/subestrus cows were 16.66, 33.33, 16.66, 50.00 and 50.00 %, respectively. In the standard cyclic control group, the conception rate at the first cycle was 33.33 %. The above treatment protocols influenced none of the minerals studied. The values of trace minerals varied insignificantly for entire post-treatment periods even within and between conceived and non-conceived groups, with a few exceptions. There was no association between plasma trace minerals profile and estrus response or fertility after treatment. It was concluded that CIDR, Cosynch, and $PGF_2\alpha$ protocols were better than others for estrus induction and conception rate in anestrus/subestrus zebu cows. Although they did not influence the plasma trace minerals (Zn, Fe, Cu, Co, and Mn) profile, perhaps due to well-nourished animals on the organized farm, it helped to infer that the selected animals were maintained under the optimum nutritional regime.

Keywords: Estrus synchronization, Kankrej cows, Postpartum period, Subfertility, Trace minerals.

Ind J Vet Sci and Biotech (2021): 10.21887/ijvsbt.17.2.7

Introduction

The Kankrej, a dual-purpose zebu cattle, is known for its endurance but is a slow breeder with very late maturity, prolonged postpartum anestrus/subestrus, long calving interval, and strong mothering instinct is leading to suppression of postpartum ovarian activity. These peculiarities necessitate scientists to initiate appropriate steps to improve their reproductive efficiency from all these angles. Fixed time artificial insemination (FTAI) protocols such as Ovsynch, CIDR, Cosynch, and Doublesynch have been developed to decrease reliance on estrus detection in reproductive management programs and to improve herd fertility (Pursley *et al.,* 1995; Mohankrishana *et al.,* 2010; Bhoraniya *et al.,* 2012; Patel *et al.*, 2014; Buhecha *et al.,* 2015; Chaudhary *et al.,* 2018). Moreover, minerals play an intermediate role in hormones and enzymes at the cellular level in an integrated fashion. Trace elements affect various physiological activities as they function as cofactors, activators of enzymes, or stabilizers of secondary molecular structures. Trace minerals are absorbed only minimally across the rumen epithelium but are absorbed mainly through the small intestine (Wright *et al.,* 2008). Deficiency or excess of mineral elements like P, Cu, and Zn is associated with subnormal fertility and anestrus condition (Hidiroglou, 1979). The literature on postpartum endocrine and minerals profile and fertility without and with hormonal treatments

[1]Department of Veterinary Gynaecology and Obstetrics, Faculty of Veterinary Science and AH, SKUAST- Kashmir, Srinagar-190 006, Jammu and Kashmir, India.

[2]Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and AH, Anand Agricultural University, Anand - 388 001, Gujrat, India.

[3]Kerala Livestock Development Board, Manuthy, Trichur, Kerala, India.

[4]Directorate of Extension Education, Anand Agricultural University, Anand - 388 001, Gujrat, India.

Corresponding Author: Mehrajuddin Naikoo, Department of Veterinary Gynaecology and Obstetrics, Faculty of Veterinary Science and AH, SKUAST- Kashmir, Srinagar-190 006, Jammu and Kashmir, India, e-mail: drmehraj@gmail.com

How to cite this article: Naikoo, M., Dhami, A.J., Ramakrishnan, A., & Divekar, B.S. (2021). Plasma Trace Minerals Profile and Fertility without and with Estrus Synchronization Therapy at Day 90 Postpartum in Suckled Kankrej Cows. Ind J Vet Sci and Biotech, 17(2): 35-41.

Source of support: Nil

Conflict of interest: None.

Submitted: 23/01/2021 **Accepted:** 29/04/2021 **Published:** 25/06/2021

at 3 months postpartum is meagre in zebu cows (Bhoraniya *et al.,* 2012; Ammu *et al.,* 2013; Naikoo *et al.,* 2016). Hence, the present study was planned to evaluate the postpartum plasma trace minerals profile till day 90 and to see whether it can be influenced further by different estrus synchronization protocols in postpartum anestrus/subestrus suckled Kankrej cows concurrent to fertility improvement.

MATERIALS AND METHODS

Selection and Management of Animals

The study was approved by the Institutional Animal Ethics Committee of the University. A total of 36 healthy pluriparous parturients suckled Kankrej cows of the University Farm, Anand, Gujrat, India were selected for this study. They were monitored from the day of calving till 140 days postpartum. The animals were maintained identically under a loose housing system and received green fodder (20 kg/animal/day), hay (5-6 kg/animal/day), compounded concentrate mixture (40 % of milk weight) mixed with the mineral mixture (50 g/animal/day - Amul brand) and had free access to pure, wholesome drinking water. Reproductive/ovarian status of these cows was assessed by palpation per rectum of the genitalia on three occasions, each at 10 days intervals beginning at day 70 postpartum, which revealed most of them (n=30) to be in anestrus/ subestrus condition. Cows were inseminated only after 60 days of calving if found in estrus. Based on the reproductive status, the anestrus and subestrus cows were randomly distributed at day 90-92 postpartum into five treatment groups, each with 6 animals and one group (six animals) exhibiting normal estrus within 80 days postpartum served as normal cyclic control. The anestrus animals (Group-I to III) so selected were treated with controlled breeding techniques, *viz.,* Ovsynch, CIDR, and Ovsynch + CIDR protocols (Naikoo *et al.,* 2016), and subestrus estrus animals (Group- IV and V) were treated with Cosynch (Ammu *et al.,* 2012) and double PG injection 11 days apart protocols (Patel *et al.,* 2014).

All the cows were bred during spontaneous or induced estrus and were followed for pregnancy or estrus recurrence. The cows returned to estrus were re-inseminated, and pregnancy was confirmed per rectum 60 days after the last AI. Out of the total 36 animals under study, the cows conceived at first AI, irrespective of treatment groups, were classified as conceived or pregnant (n=12), and the remaining cows (n=24), which returned to the next estrus following AI, were taken as non-conceived cows.

Blood Sampling and Trace Minerals Estimation

Blood samples were collected in heparinized vacutainers through jugular vein puncture from all the animals on the day of calving and after that at 10 days intervals up to the initiation of treatment protocols around day 90-92 postpartum, or AI in case of cyclic control animals. The blood sampling was later rescheduled based on the treatment protocols or AI, *i.e.*, on day of first treatment (day 0), day of $PGF_2\alpha$ injection (day 7), day of induced/spontaneous estrus and AI (day 9/11), and then on day 10, 20, 30 and 40 post-AI. The samples were immediately centrifuged at 3000 rpm for 15 minutes, and the plasma samples were stored at -20°C with a drop of 0.01 % sodium merthiolate until analyzed.

The blood plasma samples (1 ml each) were wet digested with 5 ml volume of the di-acid mixture (Perchloric acid: Nitric acid, 1:4) on a hot plate according to the method of Krishna and Ranjhan (1980). The clear transparent residues were diluted in triple glass distilled water, and the final volume was made to 5 ml. These aliquots were then used to estimate trace elements, *viz.,* iron, zinc, copper, cobalt, and manganese on an Atomic Absorption Spectrophotometer (Model-3110, Perkin Elmer).

Statistical Analysis

The Chi-square test compared the estrus induction response and conception rates of different estrus synchronization protocols. The data on plasma trace minerals profile was analyzed using a completely randomized design, Duncan's new multiple range test, and 't' test through an online SAS system of statistical analysis.

RESULTS AND DISCUSSION

The results obtained on plasma trace minerals profile till 90 days postpartum and after that with different estrus synchronization protocols in anestrus/ subestrus suckled Kankrej cows are presented in Tables 1 and 2. The mineral profile of conceived and non-conceived animals from calving till day 140 postpartum is shown in Figure 1.

Plasma Trace Minerals Profile

The mean plasma zinc levels in Kankrej cows under Ovsynch, CIDR, Ovsynch+CIDR, Cosynch, and $PGF_2\alpha$ protocols and Control group fluctuated insignificantly between different intervals postpartum, except in Ovsynch and Cosynch protocols wherein the period effect was significant ($p<0.05$). The mean values of zinc also did not vary significantly between different groups at any of the intervals postpartum, and the values fluctuated unevenly within the normal physiological limits (Table 1). The present findings corroborated with Ammu *et al*. (2013) report on the same line in Gir cows. Akhtar *et al.* (2009) reported a non-significant ($p>0.05$) decrease in zinc concentration between 1st and 2nd week after parturition, whereas a significant increase ($p<0.05$) in zinc concentration took place during 3rd week after parturition. There was no clear trend in the plasma levels of zinc overall or in any of the estrus induction/ synchronization protocols studied before, during, and after treatment/AI, indicating that the hormone protocols used did not influence the plasma zinc levels in cows under study. Almost similar findings were reported by Ammu *et al.* (2013), who also noted that the zinc profile was neither influenced by any of the estrus synchronization

Table 1: Plasma zinc and iron levels (ppm) postpartum in different groups of Kankrej cows before and after various estrus synchronization protocols (Mean ± SE)

PPD	*Group–I (Ovsynch)*	*Group–II (CIDR)*	*Group-III (Ovs+CIDR)*	*Group-IV (Cosynch)*	*Group-V ($PGF_2\alpha$)*	*Group-VI (Control)*
Plasma zinc concentration						
0$	0.75 ± 0.09^{a}	0.74 ± 0.06	0.75 ± 0.08	0.84 ± 0.08^{a}	0.70 ± 0.09	0.76 ± 0.11
10	0.63 ± 0.07^{ab}	0.61 ± 0.06	0.72 ± 0.03	0.75 ± 0.05^{abc}	0.69 ± 0.07	0.79 ± 0.06
20	0.70 ± 0.09^{ab}	0.64 ± 0.07	0.67 ± 0.06	0.70 ± 0.04^{abc}	0.77 ± 0.06	0.71 ± 0.07
30	0.70 ± 0.06^{ab}	0.68 ± 0.07	0.63 ± 0.08	0.60 ± 0.02^{c}	0.66 ± 0.06	0.82 ± 0.06
40	0.67 ± 0.03^{ab}	0.72 ± 0.06	0.72 ± 0.05	0.75 ± 0.04^{abc}	0.80 ± 0.08	0.64 ± 0.06
50	0.62 ± 0.06^{ab}	0.70 ± 0.05	0.66 ± 0.08	0.77 ± 0.04^{abc}	0.66 ± 0.08	0.66 ± 0.03
60	0.61 ± 0.07^{ab}	0.73 ± 0.08	0.69 ± 0.03	0.64 ± 0.06^{bc}	0.72 ± 0.09	0.68 ± 0.06
70	0.47 ± 0.05^{b}	0.71 ± 0.06	0.72 ± 0.07	0.76 ± 0.04^{abc}	0.65 ± 0.11	0.71 ± 0.08
80	0.55 ± 0.09^{ab}	0.62 ± 0.05	0.71 ± 0.02	0.82 ± 0.05^{ab}	0.69 ± 0.09	0.67 ± 0.06
90	0.71 ± 0.05^{a}	0.76 ± 0.06	0.73 ± 0.02	0.72 ± 0.06^{abc}	0.72 ± 0.10	0.76 ± 0.70
D 0*	0.60 ± 0.11^{ab}	0.66 ± 0.03	0.69 ± 0.05	0.74 ± 0.07^{abc}	0.73 ± 0.07	0.68 ± 0.09
D 7/11**	0.58 ± 0.09^{ab}	0.69 ± 0.09	0.75 ± 0.05	0.72 ± 0.06^{abc}	0.75 ± 0.07	-
D of AI	0.68 ± 0.09^{ab}	0.68 ± 0.04	0.69 ± 0.04	0.81 ± 0.04^{ab}	0.57 ± 0.04	0.76 ± 0.05
D10 PAI	0.74 ± 0.06^{a}	0.76 ± 0.06	0.68 ± 0.07	0.72 ± 0.06^{abc}	0.64 ± 0.06	0.80 ± 0.05
D20 PAI	0.65 ± 0.05^{ab}	0.72 ± 0.04	0.73 ± 0.05	0.76 ± 0.04^{abc}	0.75 ± 0.06	0.76 ± 0.09
D30 PAI	0.68 ± 0.05^{ab}	0.65 ± 0.06	0.65 ± 0.07	0.72 ± 0.07^{abc}	0.63 ± 0.07	0.80 ± 0.07
D40 PAI	0.68 ± 0.03^{ab}	0.79 ± 0.04	0.65 ± 0.03	0.74 ± 0.07^{abc}	0.76 ± 0.06	0.83 ± 0.05
Plasma iron concentration						
0$	3.56 ± 0.36	3.47 ± 0.25^{ab}	3.34 ± 0.24	3.25 ± 0.20	3.10 ± 0.10^{abc}	3.96 ± 0.44
10	3.47 ± 0.23	3.23 ± 0.18^{abc}	3.11 ± 0.20	3.15 ± 0.28	3.18 ± 0.25^{abc}	3.52 ± 0.32
20	3.35 ± 0.41	3.55 ± 0.17^{ab}	3.23 ± 0.22	3.42 ± 0.33	2.87 ± 0.22^{bc}	3.05 ± 0.25
30	3.72 ± 0.28	2.89 ± 0.11^{bc}	2.87 ± 0.16	3.40 ± 0.27	3.09 ± 0.24^{abc}	3.32 ± 0.25
40	3.82 ± 0.40	2.52 ± 0.10^{c}	2.94 ± 0.11	3.40 ± 0.32	3.40 ± 0.20^{abc}	3.15 ± 0.18
50	3.28 ± 0.39	3.02 ± 0.23^{bc}	3.04 ± 0.17	3.47 ± 0.28	2.94 ± 0.13^{bc}	3.13 ± 0.47
60	3.68 ± 0.19	2.97 ± 0.19^{bc}	3.06 ± 0.14	3.32 ± 0.28	3.02 ± 0.17^{abc}	3.42 ± 0.19
70	3.58 ± 0.47	3.17 ± 0.31^{abc}	3.32 ± 0.16	2.89 ± 0.06	3.08 ± 0.26^{abc}	3.53 ± 0.21
80	3.77 ± 0.43	3.47 ± 0.14^{ab}	2.97 ± 0.11	3.31 ± 0.41	3.58 ± 0.12^{a}	3.13 ± 0.20
90	3.79 ± 0.25	3.61 ± 0.25^{ab}	3.06 ± 0.13	3.37 ± 0.13	2.82 ± 0.97^{bc}	3.32 ± 0.33
D 0*	3.42 ± 0.31	3.33 ± 0.28^{ab}	3.17 ± 0.18	3.14 ± 0.17	2.91 ± 0.08^{bc}	3.18 ± 0.14
D 7/11**	3.49 ± 0.41	3.24 ± 0.32^{abc}	3.42 ± 0.15	3.18 ± 0.22	3.02 ± 0.13^{abc}	-
D of AI	3.31 ± 0.16	3.47 ± 0.25^{ab}	3.18 ± 0.28	3.24 ± 0.22	2.78 ± 0.25^{bc}	3.32 ± 0.09
D10 PAI	3.57 ± 0.35	3.32 ± 0.32^{ab}	3.41 ± 0.26	3.17 ± 0.14	3.19 ± 0.23^{abc}	3.34 ± 0.33
D20 PAI	3.26 ± 0.31	3.92 ± 0.29^{a}	3.42 ± 0.23	3.42 ± 0.32	2.75 ± 0.14^{c}	3.41 ± 0.29
D30 PAI	3.27 ± 0.25	3.36 ± 0.26^{ab}	3.25 ± 0.20	3.17 ± 0.10	3.19 ± 0.20^{abc}	3.31 ± 0.25
D40 PAI	3.90 ± 0.29	3.52 ± 0.27^{ab}	3.17 ± 0.14	3.31 ± 0.22	3.41 ± 0.23^{ab}	3.27 ± 0.26

Gr-I,II,III = Anestrus cows, Gr-IV,V = Subestrus, and Gr-VI = Normal cyclic control.
PPD= postpartum days, 0$ = day of calving, * day of first treatment, ** day of PG injection. Synchronization treatment was initiated on day 90-95 postpartum. PAI= Post-AI.
Means bearing uncommon superscripts within the column differ significantly ($p < 0.05$).

protocols (Ovsynch, Cosynch, CIDR) used nor between periods pre and post-treatment/AI, except at estrus.

The mean plasma iron concentrations fluctuated significantly between periods only in CIDR and $PGF_2\alpha$ groups. Like zinc, the iron profile was neither influenced by any of the estrus synchronization protocols used nor between periods pre-and post-treatment/AI, except at day 40 postpartum, where the mean iron level was significantly lower in CIDR group than in the Control and other groups (Table 1). Similar findings were reported by Ammu *et al.* (2013) in Gir cows. In

Table 2: Plasma copper and manganese levels (ppm) postpartum in different groups of Kankrej cows before and after various estrus synchronization protocols (Mean ± SE)

PPD	*Group–I (Ovsynch)*	*Group–II (CIDR)*	*Group–III (Ovs+CIDR)*	*Group-IV (Cosynch)*	*Group-V ($PGF_2α$)*	*Group-VI (Control)*
Plasma copper concentration						
0$	0.78 ± 0.09^{ab}	0.73 ± 0.09	0.82 ± 0.04^{ab}	0.80 ± 0.07	0.73 ± 0.06	0.81 ± 0.07
10	0.78 ± 0.08^{ab}	0.76 ± 0.03	0.82 ± 0.05^{ab}	0.75 ± 0.05	0.55 ± 0.06	0.78 ± 0.07
20	0.69 ± 0.05^{abc}	0.77 ± 0.09	0.68 ± 0.05^{abcd}	0.73 ± 0.04	0.63 ± 0.08	0.82 ± 0.07
30	0.70 ± 0.05^{abc}	0.80 ± 0.06	0.75 ± 0.05^{abcd}	0.62 ± 0.04	0.63 ± 0.05	0.73 ± 0.08
40	0.65 ± 0.08^{abc}	0.77 ± 0.05	0.85 ± 0.03^{a}	0.71 ± 0.06	0.69 ± 0.05	0.72 ± 0.09
50	0.83 ± 0.05^{a}	0.68 ± 0.09	0.68 ± 0.07^{abcd}	0.65 ± 0.04	0.66 ± 0.07	0.77 ± 0.08
60	0.64 ± 0.05^{abc}	0.79 ± 0.07	0.68 ± 0.03^{abcd}	0.74 ± 0.03	0.67 ± 0.07	0.73 ± 0.07
70	0.60 ± 0.05^{bc}	0.83 ± 0.06	0.67 ± 0.05^{abcd}	0.77 ± 0.06	0.63 ± 0.04	0.67 ± 0.04
80	0.70 ± 0.06^{abc}	0.75 ± 0.07	0.65 ± 0.05^{bcd}	0.74 ± 0.06	0.69 ± 0.04	0.64 ± 0.06
90	0.83 ± 0.03^{a}	0.79 ± 0.06	0.73 ± 0.04^{abcd}	0.79 ± 0.06	0.67 ± 0.04	0.63 ± 0.08
D 0*	0.56 ± 0.08^{c}	0.72 ± 0.09	0.73 ± 0.06^{abcd}	0.72 ± 0.05	0.62 ± 0.05	0.67 ± 0.03
D 7/11**	0.60 ± 0.04^{bc}	0.77 ± 0.10	0.78 ± 0.07^{abc}	0.67 ± 0.04	0.65 ± 0.03	-
D of AI	0.62 ± 0.05^{bc}	0.72 ± 0.10	0.62 ± 0.06^{bcd}	0.69 ± 0.05	0.72 ± 0.04	0.78 ± 0.04
D10 PAI	0.63 ± 0.03^{bc}	0.78 ± 0.10	0.77 ± 0.04^{abcd}	0.65 ± 0.05	0.67 ± 0.05	0.70 ± 0.05
D20 PAI	0.62 ± 0.03^{bc}	0.84 ± 0.10	0.62 ± 0.09^{cd}	0.68 ± 0.04	0.81 ± 0.07	0.65 **± 0.07**
D30 PAI	0.67 ± 0.04^{abc}	0.80 ± 0.08	0.63 ± 0.08^{bcd}	0.63 ± 0.07	0.72 ± 0.08	0.68 ± 0.05
D40 PAI	0.66 ± 0.06^{abc}	0.80 ± 0.08	0.58 ± 0.06^{d}	0.71 ± 0.05	0.72 ± 0.06	0.75 ± 0.03
Plasma manganese concentration						
0$	0.22 ± 0.04^{bcd}	0.28 ± 0.04	0.23 ± 0.04^{bc}	0.29 ± 0.04	0.25 ± 0.24	0.23 ± 0.03
10	0.25 ± 0.05^{abcd}	0.22 ± 0.04	0.37 ± 0.03^{a}	0.30 ± 0.06	0.27 ± 0.04	0.31 ± 0.03
20	0.25 ± 0.07^{abcd}	0.22 ± 0.02	0.25 ± 0.01^{abc}	0.25 ± 0.05	0.25 ± 0.04	0.25 ± 0.02
30	0.31 ± 0.04^{abc}	0.23 ± 0.04	0.17 ± 0.01^{c}	0.21 ± 0.05	0.24 ± 0.02	0.30 ± 0.04
40	0.36 ± 0.03^{a}	0.27 ± 0.04	0.27 ± 0.05^{abc}	0.27 ± 0.05	0.27 ± 0.03	0.27 ± 0.05
50	0.35 ± 0.05^{ab}	0.20 ± 0.04	0.20 ± 0.03^{bc}	0.26 ± 0.02	0.24 ± 0.05	0.23 ± 0.02
60	0.30 ± 0.02^{abc}	0.22 ± 0.33	0.32 ± 0.02^{ab}	0.28 ± 0.03	0.24 ± 0.04	0.21 ± 0.01
70	0.29 ± 0.05^{abcd}	0.24 ± 0.03	0.20 ± 0.03^{bc}	0.26 ± 0.04	0.20 ± 0.02	0.27 ± 0.03
80	0.21 ± 0.02^{cd}	0.29 ± 0.05	0.20 ± 0.02^{bc}	0.17 ± 0.03	0.27 ± 0.04	0.26 ± 0.04
90	0.31 ± 0.01^{abc}	0.26 ± 0.04	0.27 ± 0.02^{abc}	0.22 ± 0.05	0.20 ± 0.03	0.29 ± 0.04
D 0*	0.16 ± 0.03^{d}	0.23 ± 0.03	0.22 ± 0.04^{bc}	0.25 ± 0.04	0.21 ± 0.03	0.22 ± 0.03
D 7/11**	0.24 ± 0.04^{abcd}	0.27 ± 0.05	0.26 ± 0.06^{abc}	0.25 ± 0.04	0.27 ± 0.04	-
D of AI	0.22 ± 0.03^{bcd}	0.27 ± 0.03	0.22 ± 0.02^{bc}	0.25 ± 0.04	0.19 ± 0.04	0.29 ± 0.03
D10 PAI	0.21 ± 0.04^{cd}	0.26 ± 0.03	0.25 ± 0.02^{abc}	0.27 ± 0.02	0.22 ± 0.03	0.29 ± 0.03
D20 PAI	0.23 ± 0.04^{abcd}	0.28 ± 0.03	0.26 ± 0.04^{abc}	0.26 ± 0.04	0.17 ± 0.03	0.27 ± 0.04
D30 PAI	0.31 ± 0.05^{abc}	0.27 ± 0.04	0.25 ± 0.04^{abc}	0.25 ± 0.05	0.23 ± 0.02	0.31 ± 0.03
D40 PAI	0.27 ± 0.02^{abcd}	0.22 ± 0.04	0.32 ± 0.04^{ab}	0.27 ± 0.02	0.28 ± 0.03	0.23 ± 0.02

Gr-I,II,III = Anestrus cows, Gr-IV,V = Subestrus, and Gr-VI = Normal cyclic control.
PPD= postpartum days, 0$ = day of calving, * day of first treatment, ** day of PG injection. Synchronization treatment was initiated on day 90-95 postpartum. PAI= Post-AI.
Means bearing uncommon superscripts within the column differ significantly ($p < 0.05$).

contrast to the present findings, Akhtar *et al.* (2009) found a significant difference in the relationship between iron level and fertility in cows. Patel *et al.* (2010) also found significantly higher iron values in normal fertile than repeat breeding Mehsana buffaloes at 0, 10, and 20 days post-estrus.

The plasma copper profile was also neither influenced by any estrus induction and synchronization protocols used nor between periods pre-and post-treatment/AI, except in Ovsynch and Ovsynch + CIDR groups (Table 2). Similar findings were reported by Ammu *et al.* (2013) in Gir cows.

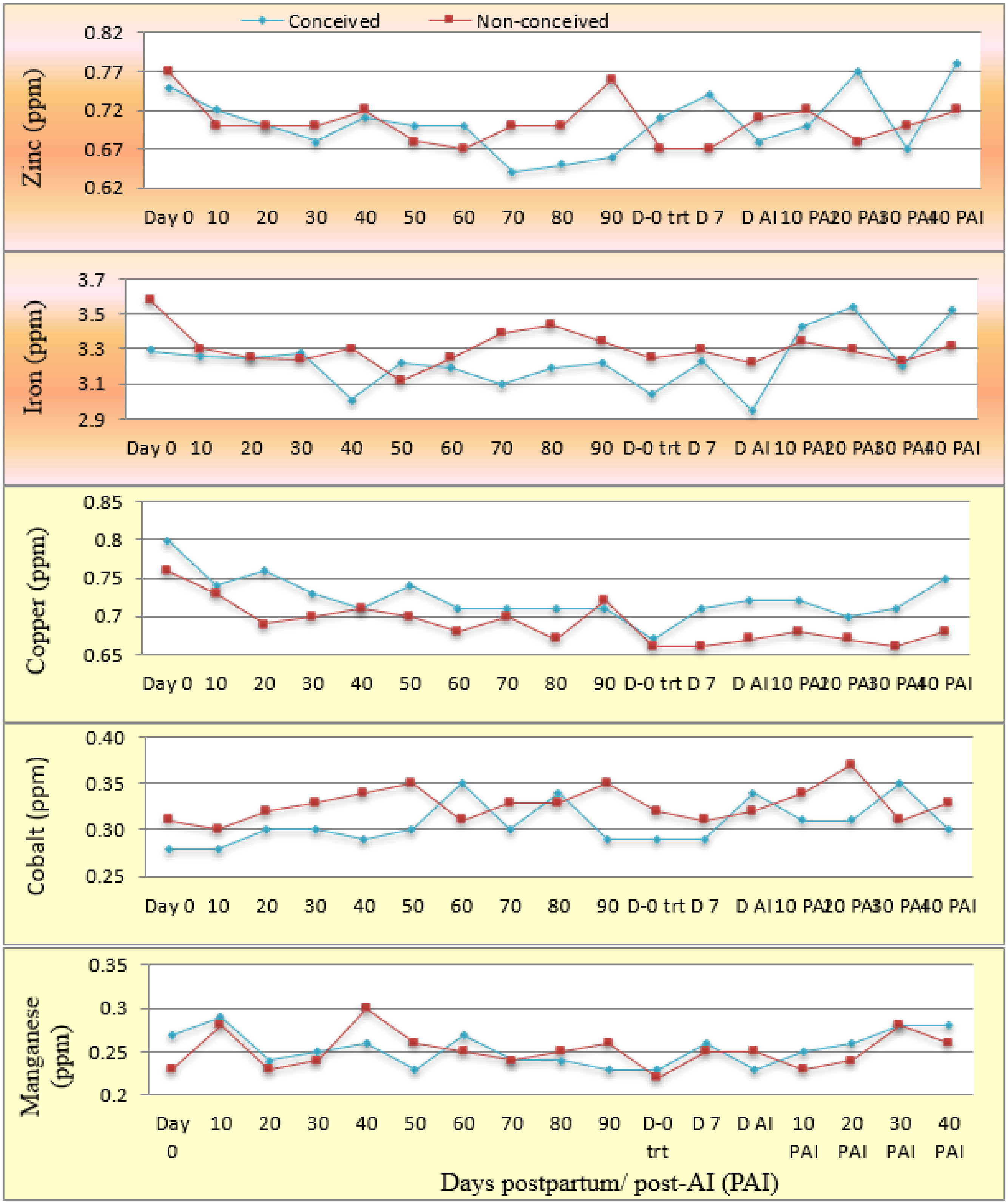

Fig. 1: Plasma minerals profile at 10 days interval postpartum (0-90 days), at treatment (day 90, 106) and post-AI (day 0-40) in conceived and non-conceived Kankrej cows

Das *et al.* (2002) reported significantly lower levels of copper in repeat breeder cows than normal cyclic cows. Akhtar *et al.* (2009) found significantly lower ($p<0.05$) copper at parturition, which increased significantly after that ($p<0.05$) following three weeks postpartum. Patel *et al.* (2006) and Butani *et al.* (2009) also noted a similar non-significant influence of GnRH and $PGF_2\alpha$ treatment on plasma trace minerals profile in both anestrus and subestrus cows and buffaloes when used at day 42-49 postpartum or at later stages.

The mean plasma cobalt concentrations fluctuated non-significantly between different intervals postpartum in the narrow range of 0.30±0.05 to 0.39±0.02 ppm among the six groups. The values were almost constant from calving till 90 days and even 140 days postpartum. Moreover, the values did not differ significantly between treatment and control groups at any of the intervals postpartum. Patel *et al.* (2006) and Butani *et al.* (2009) reported a similar non-significant influence of GnRH and $PGF_2\alpha$ treatment on plasma trace minerals profile when used for estrus induction in cows and buffaloes at day 42-49 postpartum or at later stages.

The mean concentrations of manganese did not vary significantly between different intervals postpartum in most groups, except Ovsynch and Ovsynch + CIDR groups. Moreover, there was no significant difference between groups at any of the intervals postpartum, including the post-treatment period, except on the day of treatment, where the values in Ovsynch group were significantly lower than other groups (Table 2). Patel *et al.* (2006) and Butani *et al.* (2009) reported a similar non-significant influence of GnRH & $PGF_2\alpha$ treatment on plasma trace minerals profile in postpartum anestrus and subestrus cows and buffaloes. Similarly, Ammu *et al.* (2013) found non-significant fluctuations in the mean plasma manganese concentrations, including control, CIDR, Ovsynch, and Cosynch groups, between different intervals postpartum and even up to 40 days post-AI.

Response to Estrus Synchronization Protocols

The ovulatory estrus was induced in 66.66, 83.33, 50.00, 66.66 and 66.66 % of cows under Ovsynch, CIDR, Ovsynch + CIDR, Cosynch, and $PGF_2\alpha$ protocols, respectively, as confirmed by the presence of CL on the ovary 12 days later. Comparable or higher ovulatory response has been reported with one or more of these protocols by many earlier researchers (Mohankrishna *et al.,* 2010; Khade *et al.,* 2011; Ammu *et al.,* 2012; Bhoraniya *et al.,* 2012; Hadiya *et al*., 2015; Chaudhary *et al*., 2018) in cyclic and/or acyclic zebu cattle.

The conception rates obtained during the induced estrus under Ovsynch, CIDR, Ovsynch + CIDR, Cosynch, and $PGF_2\alpha$ protocols and in control group were 16.66, 33.33, 16.66, 50.00, 50.00, and 33.33 %, respectively. In similar experiments with Ovsynch, Mohankrishna *et al.* (2010) recorded conception rates of 30 and 20 % in Sahiwal cows and heifers, respectively. On the contrary, higher conception rates of 50 % each in Gir and Kankrej cows with Ovsynch were recorded by Khade *et al.* (2011), Ammu *et al.* (2012), and Bhoraniya *et al.* (2012), and with CIDR protocol (50.0 to 66.6%) by Khade *et al.* (2011), Bhoraniya *et al.* (2012) and Hadiya *et al.* (2015). The conception rate at the induced estrus in Ovsynch plus CIDR protocol was at par with Ovsynch alone and was lower than in other groups and even the control group. There was no beneficial effect of combining Ovsynch with CIDR, and in fact, it reduced the efficacy of CIDR (Naikoo *et al.,* 2016). However, higher conception rates with this combination were reported earlier by Khade *et al.* (2011) in anestrus Gir heifers. The present findings suggest that the CIDR insert alone over Ovsynch or its combination in anestrus and subestrus suckler Kankrej cows improved synchrony of estrus occurrence and considerably enhanced conception rate.

Trace Minerals Profile in Conceived and Non-conceived Groups

The mean plasma levels of all trace minerals fluctuated non-significantly between different intervals postpartum in both conceived and non-conceived groups, except that iron, which varied significantly ($p<0.05$) between intervals in the conceived group. There was no significant difference between the conceived and non-conceived groups at any intervals postpartum in any of these traits (Fig.1). This could be because the mineral supplementation and regular supply of green legumes was a standard feature on the farm under study, which might have created plasma levels slightly higher than the critical limits required for normal reproduction and fertility in dairy animals. The present observations supported the previous findings on a similar line in Gir cows by Ammu *et al.* (2013). They reported that the overall mean plasma zinc and iron levels varied insignificantly from 0.78 ± 0.06 to 0.98 ± 0.06 and 2.86 ± 0.19 to 3.35 ± 0.20 ppm between 10 days interval for the first 90 days postpartum in Gir cows, and similar were the trends in conceived and non-conceived cows of control, CIDR, Ovsynch and Cosynch groups post-AI. Das *et al.* (2002) also reported non-significant variation in manganese levels between repeat breeder and normally cycling cows. However, Patel *et al.* (2010) found significantly higher levels of blood plasma zinc, iron, copper, and manganese, but not the cobalt, in normal fertile buffaloes compared to repeat breeding ones at day 0, $10^{th,}$ and 20^{th} post-estrus. Butani *et al.* (2009) and Patel *et al.* (2014), and Savalia *et al.* (2020) did not find significant variations in trace minerals profile between conceived and non-conceived animals following the use of similar treatment protocols. In Jafarabadi buffaloes of an organized farm supplemented with bypass fat and minerals also revealed similar non-significant variation in trace minerals profile of postpartum period and in conceived-nonconceived groups, except zinc and copper, which were higher in the conceived group (Vala *et al.,* 2019).

Conclusions

Under the optimal nutritional management of cows on the farm, the plasma levels of trace minerals (Zn, Fe, Cu, Co, Mn) did not reveal any association with the reproductive status, and there was no consistent trend for these elements between different groups/periods studied. However, the profile helped to conclude that the selected animals were maintained under an optimum nutritional regime. Moreover, none of the five estrus synchronization protocols used influenced the plasma trace minerals profile. Good estrus induction/synchronization response and improvement in conception rates were noted using different Ovsynch, CIDR, Cosynch, and $PGF_2\alpha$ in postpartum anestrus/ subestrus Kankrej cows. However, the insignificant variations noted in trace minerals profile during postpartum/post-treatment periods in both cyclic and acyclic/ subestrus cows in the study suggest that the postpartum fertility is regulated not only by nutritional and mineral status, but by a complex of many more factors, including climate, suckling etc. which need to be explored. Therefore, further studies with frequent blood sampling of lactating suckled and weaned cows are warranted on the increased number of cows to elucidate suckling stimulus to be the significant factor of subestrus and anestrus in zebu cows.

Acknowledgements

We thank the Principal and Dean of College of Veterinary Science and Animal Husbandry, AAU, Anand, Gujrat, India and the AAU authorities for the facilities provided for this work.

References

Akhtar, M.S., Farooq, A.A., & Mushtaq, M. (2009). Serum trace mineral variation during pre and post partum period in Nili-Ravi buffaloes. *Journal of Animal and Plant Science, 19*(4), 182-184.

Ammu Ramakrishnan; Dhami, A.J., Naikoo, M., Parmar, B.C., & Divekar, B.S. (2012). Estrus induction and fertility response in postpartum anestrus Gir cows. *Indian Journal of Animal Reproduction, 33*(1), 37-42.

Ammu Ramakrishnan; Dhami, A.J., Patel, S.B., Patel, K.P., & Pande, A.M. (2013). Postpartum plasma profile of macro-micro minerals in Gir cows conceiving and non-conceiving following estrus synchronization treatments. *Gujarat Agricultural Universities Research Journal, 38*(1), 49-56.

Bhoraniya, H.L., Dhami, A.J., Naikoo, M., Parmar, B.C., & Sarvaiya, N.P. (2012). Effect of estrus synchronization protocols on plasma progesterone profile and fertility in postpartum anoestrus Kankrej cows. *Tropical Animal Health and Production, 44*(6), 1191-1197.

Buhecha, K.V., Dhami, A.J., Hadiaya, K.K., Parmar, C.P., Parmar, S.C., & Patel, J.A. (2015). Influence of Triu-B, Ovsynch and Heatsynch protocols on estrus induction, conception and biochemical and mineral profile in anoestrus crossbred cows. *Indian Journal of Veterinary Sciences and Biotechnology, 11*(2), 65-71.

Butani, M.G., Dhami, A.J., Ramani, V.P., Savalia, F.P., & Patel, M.D. (2009). Influence of hormonal and non-hormonal therapies on fertility and serum mineral profile of conceiving and non-conceiving anoestrus buffaloes. *Indian Journal of Field Veterinarians, 5*(2), 59-67.

Chaudhary, N.J., Patel, D.M., Dhami, A.J., Vala, K.B., Hadiya, K.K., & Patel, J.A. (2018). Effect of doublesynch and estradoublesynch protocols on estrus induction, conception rate, plasma progesterone, protein and cholesterol profile in anestrus Gir heifers. *Veterinary World, 11*(4), 542-548.

Das, P., Biswas, S., Ghosh, T.K.. & Haldar, S. (2002). Micronutrient status of dairy cattle in new alluvial zone of West Bengal. *Indian Journal of Animal Sciences, 72*(2), 171-173.

Hadiya, K.K., Dhami, A.J., Nakrani, B.B., & Lunagariya, P.M. (2015). Estrus induction, follicular dynamics and fertility response to mid-cycle PGF2α, CIDR and Ovsynch protocols in subfertile Gir and crossbred cows. *Indian Journal of Animal Reproduction, 36*(1), 23-27.

Hidiroglou, M. (1979). Trace element deficiency and fertility in ruminants. A review. *Journal of Dairy Science, 62,* 1195-1206.

Khade, N.B., Patel, D.M., Naikoo, M., Dhami, A.J., Sarvaiya, N.P., & Gohel, M.M. (2011). Estrus induction in pubertal anestrous Gir heifers using different hormone protocols. *Indian Journal of Field Veterinarians, 7*(1), 4-8.

Krishna, O.P., & Ranjhan, S.K. (1980). *Laboratory Manual for Nutrition Research*. Vikash publishing house private limited, New Delhi, India. pp 83-84.

Mohankrishna, Mishra, U.K., Mishra, O.P., Khan, J.R., & Prakash, B.S. (2010). Efficacy of Ovsynch protocol with fixed time insemination in anestrous Sahiwal cows and heifers. *Indian Veterinary Journal, 87,* 297-298.

Naikoo, M., Dhami, A.J., & Ammu Ramakrishnan (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

Patel, K.R., Dhami, A.J., Savalia, K.K., Hadiya, K.K., & Pande, A.M. (2014). Influence of mid-cycle PG treatment and GnRH at AI on plasma minerals profile in conceiving and non-conceiving repeat breeding crossbred cows. *Indian Journal of Veterinary Sciences and Biotechnology, 10*(2), 86-92.

Patel, P.A., Siddiquee, G.M., Nakhashi, H.C. & Suthar, B.N. (2010). Blood plasma trace elements in repeat breeding Mehsana buffaloes. *Indian Journal of Animal Reproduction*, *31*(1), 7-9.

Patel, P.M., Dhami, A.J., Savaliya, F.P. and Ramani, V.P. (2006). Postpartum plasma profile of certain trace elements in Holstein Friesian cows with and without hormone therapy under tropical climate. *Indian Journal of Animal Reproduction*, *27*(2), 19-24.

Pursley, J.R., Mee, M.O., & Wiltbank, M.C. (1995). Synchronization of ovulation in dairy cows using $PGF_2\alpha$ and GnRH. *Theriogenology, 44*(7), 915-923.

Savalia, K.K., Dhami, A.J., Hadiya, K.K. and Ramani, V.P. (2020). Effect of controlled breeding techniques on plasma trace minerals profile in conceiving and not-conceiving anoestrus and repeat breeding buffaloes. *Gujarat Agricultural Universities Research Journal, 45*(1), 22-34.

Vala, K.B., Dhami, A.J., Kavani, F.S., Ramani, V.P. and Parmar, S.C. (2019). Influence of bypass fat and mineral supplementation during transitional period on plasma trace minerals profile and postpartum fertility in Jafarabadi buffaloes. *Indian Journal of Veterinary Sciences and Biotechnology, 15*(1), 45-49

Wright, C.L., Spears, J.W., & Webb, Jr.K.E. (2008). Uptake of zinc from zinc sulfate and zinc proteinate by ovine ruminal and omasal epithelia. *Journal of Animal Sciences, 86,* 1357-1363.