MONITORING POSTPARTUM PLASMA MINERALS PROFILE (Ca, P and Mg) AND FERTILITY WITHOUT AND WITH ESTRUS SYNCHRONIZATION THERAPIES AT DAY 90 IN SUCKLED ANESTROUS/SUBESTROUS KANKREJ COWS

Mehrajuddin Naikoo, A.J. Dhami, Ammu Ramakrishnan, B.C. Parmar and B.S. Divekar

Department of Animal Reproduction Gynaecology and Obstetrics,

College of Veterinary Science and Animal Husbandry, AAU, Anand - 388 001, India

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Corresponding Author : drmehraj@gmail.com

ABSTRACT

The present study was aimed at assessing the postpartum ovarian activity through clinical monitoring and plasma macro-minerals profile at 10 days intervals from calving till 140 days postpartum and fertility following various estrus synchronization therapies viz. Ovsynch, CIDR, Ovsynch + CIDR, Cosynch and $PGF_2\alpha$ around day 90-95 postpartum in 30 suckled anestrous/ subestrous (6/treatment) Kankrej cows of an organized farm. Six normal cyclic cows were kept as control. The variations between 10 days interval were significant in some of the groups for mean values of plasma calcium (CIDR & Control), phosphorus (Ovsynch, Ovsynch + CIDR & Control) and magnesium ($PGF_2\alpha$ & Control) studied from calving till 90 days postpartum. Estimations of plasma minerals were also done on day 0, 7, 9/10 (AI) and then at 10 days interval up to day 40 post-AI following use of above estrus synchronization treatments. None of the protocols used, viz, Ovsynch, CIDR, Ovsynch + CIDR, Cosynch and $PGF_2\alpha$ significantly influenced the plasma calcium, phosphorus and/or magnesium concentrations, though the estrus response (66.66, 83.33, 50.00, 66.66 and 66.66 %) and conception rate at induced estrus (16.66, 33.33, 16.66, 50.00 and 50.00 %, respectively) and overall of 3 cycles (33.33, 50.00, 33.33, 50.00 and 50.00 %) varied between them. The first service and overall 3 cycles conception rates in normal cyclic control group were 33.33 and 50.00 %, respectively. Among the conceived and non-conceived groups of Kankrej cows, the values of calcium and phosphorus varied insignificantly between intervals from day of calving to day 140 postpartum (10-40 post-AI), except for calcium in non-conceived group, and magnesium varied significantly between days in both conceived and non-conceived groups. The levels however did not vary between conceived and non-conceived groups at any of the intervals postpartum/post-AI. It was concluded that neither the levels of plasma calcium, inorganic phosphorus and magnesium were good indicators of normal or abnormal reproductive status, nor they were influenced by different estrus induction protocols used in Kankrej cows, and that CIDR, Cosynch and $PGF_2\alpha$ protocols were better in terms of estrus induction response and conception rate in anestrous/ subestrous zebu cows.

KEYWORDS: Postpartum, Kankrej cows, Macro-minerals, Anestrous, Subestrous, Estrus Synchronization protocols.

INTRODUCTION

The Kankrej is a dual purpose - powerful draft and milch - breed of zebu cattle. The animals are slow breeder with very late maturity, prolonged postpartum anestrous/subestrous and thereby calving interval, and strong mothering instinct 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 and Cosynch have been developed to decrease reliance on estrus detection in reproductive management programmes and to improve herd fertility (Mohan Krishana *et al*., 2010; Keskin *et al.*, 2011; Bhoraniya *et al.,* 2012[b]). Further, minerals play an intermediate role in the action of hormones and enzymes at cellular level in an integrated fashion. Besides working as a cofactor or activator of enzyme systems the elements like calcium have been found to sensitize for the action of hormones. Deficiency or excess of mineral elements are associated with subnormal fertility and anestrous condition (Moddie, 1965). The present study was thus intended to evaluate the postpartum plasma macro-minerals profile till day 90, and to see whether it varies in the postpartum anestrous/subestrous suckled Kankrej cows concurrent to fertility improvement following use of different estrus synchronization protocols.

MATERIALS AND METHODS

A total of 36 postpartum suckled Kankrej cows of the University Farm, Anand were selected for this study. Blood sampling of these cows was done at every 10 days interval from calving till 140 days postpartum. The reproductive/ovarian status of these cows was assessed by palpation per rectum of the genitalia on three occasions, each at 10 days interval beginning at day 70 postpartum, which revealed most of them to be in anestrous/subestrous condition. These cows were randomly distributed at day 90-95 postpartum into five groups, each with 6 animals and one group (six animals) served as normal cyclic control.

Among the animals so selected, six anestrous cows each were treated with three standard estrus induction/synchronization protocols, viz., Ovsynch (Gr-I), CIDR (Gr-II) and Ovsynch plus CIDR protocol (Gr-III) as per Khade *et al.* (2011) and Bhoraniya *et al.* (2012[b]) with fixed time AI 22 hrs after last GnRH or PG injection. Similarly, six subestrous cows each (having silent estrus/ovulation till 90 days postpartum) were treated with either Cosynch (Gr-IV) (Ammu *et al*., 2012) or double PG injections 11 days apart protocol (Gr-V) with fixed time AI, and six normal healthy cows exhibiting first pronounced estrus and having palpable follicle/CL on the ovaries within 3 months postpartum were included as normal cyclic control group (Gr-VI).

All these cows were inseminated during spontaneous or induced estrus and were followed for pregnancy or repeat estrus/AI for two more cycles. In non-return cases pregnancy was confirmed per rectum 60 days post-AI. Out of 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 next estrus following AI, were taken as non-conceived cows.

Blood samples were collected in heparinized vacutainers through jugular vein from all these animals on the day of calving and thereafter at 10 days intervals up to the initiation of treatment protocol in anestrous/subestrous animals around day 90 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/ natural estrus (day 9/11, AI), 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 by adding a drop of 0.01 % sodium merthiolate. Plasma calcium, inorganic phosphorus and magnesium concentrations were estimated by employing standard procedures and assay kits procured from Crest Biosystems, Goa, with the help of Chemistry Analyzer BS 120 (Mindray).

The data generated on plasma mineral profile were analyzed statistically using CRD and Duncan's NMRT through an online package of SAS system of statistical analysis to test variation in the mean values of particular trait within the group between periods and between groups within the period (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The reproductive tempo and fertility improvement through various therapeutic means has been the prime goal of any economically viable dairy entrepreneur. The results of postpartum plasma macro-

Table 1. Postpartum plasma calcium and phosphorus levels (mg/dl) at 10 day interval in different groups of Kankrej cows before and after various estrus synchronization protocols

Postpartum Days	Group–I (Ovsynch)	Group–II (CIDR)	Group-III (Ovs+CIDR)	Group-IV (Cosynch)	Group-V (PGF_2a)	Group-VI (Cyclic control)
Plasma calcium (mg/dl)						
0$	9.34±0.59	9.66±0.50^{ab}	10.44±0.52	10.27±0.61	8.85±0.87	9.01±0.66^{abc}
10	8.87±0.46	8.39±0.30^{cd}	8.95±0.20	9.38±0.60	8.56±0.80	8.98±0.29^{abc}
20	8.84±0.38	9.01±0.31^{bcd}	8.67±0.33	9.41±0.45	8.19±0.36	9.41±0.66^{abc}
30	8.63±0.29	8.24±0.39^{d}	8.82±0.45	9.10±0.23	8.25±0.69	9.14±0.45^{abc}
40	9.56±0.46	8.20±0.28^{d}	9.21±0.24	8.70±0.28	8.58±0.83	8.52±0.41^{bc}
50	9.02±0.54	9.51±0.42^{abc}	8.77±0.28	8.81±0.23	8.78±0.84	8.26±0.27^{c}
60	8.77±0.14	8.77±0.27^{bcd}	8.80±0.35	9.24±0.21	8.98±0.65	9.22±0.31^{abc}
70	8.18±0.16	9.14±0.25^{abcd}	9.51±0.42	9.04±0.32	9.06±0.62	9.20±0.30^{abc}
80	9.09±0.45	9.01±0.26^{bcd}	9.20±0.20	9.41±0.71	8.85±0.63	10.08±0.41^{a}
90	9.36±0.36	9.16±0.25^{abcd}	9.80±0.71	9.00±0.45	8.34±0.60	9.70±0.36^{ab}
D 0*	8.90±0.51	9.38±0.33^{abcd}	9.84±0.29	9.79±0.37	8.77±0.48	9.18±0.29^{abc}
D 7/11**	9.19±0.71	8.88±0.50^{bcd}	9.49±0.55	9.20±0.26	8.65±0.49	-
D 9/10 (AI)	9.46±0.27	9.62±0.48^{abc}	9.51±0.52	9.61±0.19	8.63±0.55	9.07±0.44^{abc}
10 PAI	9.19±0.34	9.17±0.44^{abcd}	9.69±0.45	9.49±0.41	8.31±0.42	9.54±0.27^{abc}
20 PAI	9.24±0.20	9.61±0.41^{abc}	8.91±0.28	9.28±0.31	8.97±0.34	9.57±0.35^{abc}
30 PAI	9.06±0.42	10.29±0.31^{a}	9.77±0.38	9.22±0.40	9.00±0.49	9.51±0.41^{abc}
40 PAI	9.19±0.24	9.74±0.32^{ab}	9.65±0.24	9.46±0.32	9.88±0.73	10.09±0.40^{a}
Plasma inorganic phosphorus (mg/dl)						
0$	4.88±0.25^{bc}	4.81±0.15	4.90±0.15^{ab}	5.38±0.29	4.83±0.29	4.74±0.22^{b}
10	5.31±0.11^{abc}	5.19±0.18	4.93±0.19^{ab}	5.85±0.60	5.13±0.19	5.93±0.38^{ab}
20	5.15±0.20^{abc}	5.33±0.14	4.49±0.49^{b}	5.24±0.19	5.13±0.26	6.14±0.48^{a}
30	5.47±0.15^{ab}	5.06±0.20	5.59±0.33^{a}	5.93±0.14	5.06±0.33	5.84±0.50^{ab}
40	5.16±0.20^{abc}	5.43±0.24	5.57±0.16^{a}	5.44±0.30	5.59±0.44	5.38±0.22^{ab}
50	5.59±0.20^{ab}	4.79±0.21	5.61±0.29^{a}	5.48±0.10	4.66±0.38	5.63±0.45^{ab}
60	5.06±0.24^{abc}	5.07±0.20	5.69±0.27^{a}	5.47±0.15	4.78±0.28	5.56±0.24^{ab}
70	5.02±0.16^{abc}	4.96±0.20	5.27±0.22^{ab}	5.76±0.27	5.10±0.22	5.50±0.32^{ab}
80	4.89±0.17^{bc}	5.02±0.15	5.42±0.18^{ab}	5.22±0.23	5.47±0.19	5.31±0.26^{ab}
90	4.74±0.16^{c}	5.15±0.11	5.46±0.47^{a}	4.92±0.24	5.39±0.20	5.38±0.14^{ab}
D 0*	4.97±0.12^{abc}	5.23±0.09	5.36±0.19^{ab}	5.42±0.50	5.40±0.45	5.74±0.46^{ab}
D 7/11**	5.00±0.15^{abc}	5.07±0.24	5.37±0.27^{ab}	4.98±0.30	5.40±0.37	-
D 9/10 (AI)	5.35±0.18^{abc}	4.90±0.21	5.37±0.24^{ab}	5.47±0.29	5.11±0.70	5.99±0.31^{a}
10 PAI	5.61±0.42^{ab}	5.01±0.22	5.20±0.10^{ab}	5.63±0.29	4.73±0.24	5.88±0.43^{ab}
20 PAI	5.66±0.35^{a}	5.37±0.26	5.12±0.45^{ab}	5.63±0.10	5.01±0.16	5.43±0.28^{ab}
30 PAI	5.33±0.19^{abc}	5.15±0.26	5.71±0.20^{a}	5.29±0.11	5.06±0.18	5.30±0.17^{ab}
40 PAI	4.70±0.19^{c}	4.74±0.15	5.60±0.10^{a}	5.43±0.20	5.05±0.39	5.82±0.45^{ab}

0$ = day of calving, * day of first treatment, ** day of PG injection.

Synchronization treatment was initiated on day 90-95 postpartum.

Means bearing uncommon superscripts within the column differ significantly ($P<0.05$).

minerals profile monitored at 10 days interval till day 90, around use of different hormonal protocols to induce/synchronize estrus and then till 40 days post-AI in anestrous/ subestrous suckled Kankrej cows are presented in Tables 1 and 2, and those of conceived and non-conceived groups are illustrated in Figure 1.

Plasma calcium profile

The mean plasma calcium levels fluctuated non-significantly between 10 days intervals postpartum in most groups for first 90 days, except in CIDR treated and control group, wherein the values varied significantly between periods ($P<0.01$). The plasma calcium levels were apparently high at calving, which dropped steeply within 10-20 days postpartum in all the groups (Table 1). These findings corroborated with the earlier observations of Ammu *et al.* (2013) on similar lines in postpartum Gir cows. Further, Kumar *et al.* (2009) reported significantly higher ($P<0.05$) serum calcium in normal cyclic than repeat breeding crossbred cows (9.66 ± 0.27 vs 8.76 ± 0.25 mg/dl), while Singh *et al.* (2007) reported significantly lower concentration of serum calcium in cyclic Hariana cows as compared to anestrous cows. Patel and Dhami (2005) reported that the calcium levels neither differed significantly between different days nor between fertile-infertile cycles at any of the intervals post-breeding in postpartum HF cows.

Further, none of the oestrus induction/ synchronization protocols, viz, Ovsynch, CIDR, Ovsynch + CIDR, Cosynch and $PGF_2\alpha$ used in the present study influenced the plasma calcium concentration, and the values in all the treated and control groups were almost same before, during and after AI at spontaneous or induced estrus (Table 1). These observations coincided well with those of Bhoraniya *et al.* (2012[a]), Ammu *et al.* (2013) and Savalia *et al.* (2013), who also could not see any variation in serum calcium levels before, during and after use of CIDR, Ovsynch and Cosynch protocols and control group in Kankrej and Gir cows and Surti buffaloes. Further, the calcium profile fluctuated significantly ($P<0.05$) between intervals postpartum/post-AI only in non-conceived group of cows (Fig. 1), the level was the highest on the day of calving and the lowest on day 30 postpartum. These observations coincided with the reports of Patel and Dhami (2005) and Ammu *et al.* (2013) in HF and Gir cows with use of some of the similar hormonal protocols.

Plasma inorganic phosphorus profile

The mean plasma inorganic phosphorus levels in Ovsynch, CIDR, Ovsynch + CIDR, Cosynch, $PGF_2\alpha$ and Control groups fluctuated between different intervals postpartum. However, only the values of Ovsynch, Ovsynch + CIDR and Control groups differed significantly between intervals ($P<0.05$). The mean values of phosphorus were lowest on the day of calving, rose steeply by day 10 postpartum and then remained almost constant over 90 days postpartum and even thereafter during various treatment and post-AI periods till days 140 postpartum in most groups (Table 1). Bhoraniya *et al.* (2012[a]), Ammu *et al.* (2013) and Savalia *et al.* (2013) also could not see any variation in serum inorganic phosphorus levels before, during and after use of CIDR and Ovsynch protocol in Kankrej cows and buffaloes. Raj *et al.* (2006) found identical phosphorus levels between cyclic and anestrous Sahiwal heifers. In the present study, plasma inorganic phosphorus profile remained unaltered between periods postpartum/ post-AI in both conceived and non-conceived cows (Fig. 1) as documented by above workers.

Plasma magnesium profile

The mean magnesium levels varied non-significantly between 10 days intervals in Ovsynch, CIDR, Ovsynch + CIDR and Control groups till day 90 postpartum. Neither the periods nor the treatment influenced the plasma magnesium levels in these groups. However, significant differences in the values of magnesium were observed between periods in Cosynch and PGF_2á groups up to day 90 postpartum and thereafter up to day 40 post-AI. The values were low on the day of calving and

Table 2. Postpartum plasma magnesium levels (mg/dl) at 10 day interval in different groups of Kankrej cows before and after various estrus synchronization protocols (Mean ± SE)

Postpartum Days	Group–I (Ovsynch)	Group–II (CIDR)	Group-III (Ovs+CIDR)	Group-IV (Cosynch)	Group-V (PGF_2a)	Group-VI (Cyclic control)
0$	2.93±0.14	2.99±0.18	2.81±0.22	2.75±0.13[c]	2.74±0.10[cd]	2.92±0.34
10	3.14±0.16	2.78±0.23	2.93±0.17	2.83±0.13[bc]	3.19±0.19[abcd]	2.79±0.19
20	3.02±0.11	2.81±0.16	2.71±0.14	2.96±0.16[abc]	3.16±0.14[abcd]	3.19±0.15
30	3.20±0.23	2.57±0.18	2.92±0.13	2.97±0.19[abc]	3.38±0.13[ab]	2.97±0.16
40	3.04±0.16	2.80±0.18	2.92±0.13	3.31±0.10[ab]	3.17±0.20[abcd]	3.21±0.17
50	3.01±0.19	3.15±0.20	2.53±0.19	2.94±0.14[abc]	3.28±0.15[ab]	2.92±0.13
60	3.10±0.09	3.03±0.22	3.04±0.14	3.10±0.13[abc]	3.54±0.12[a]	3.00±0.22
70	2.97±0.15	3.01±0.13	2.88±0.08	3.09±0.18[abc]	3.33±0.13[ab]	3.27±0.14
80	3.24±0.23	3.15±0.14	2.95±0.10	3.29±0.15[ab]	3.19±0.10[abcd]	3.29±0.14
90	3.01±0.05	3.22±0.14	3.07±0.11	3.26±0.09[abc]	3.33±0.14[ab]	3.25±0.15
D 0*	3.04±0.20	3.11±0.16	2.90±0.16	3.06±0.10[abc]	3.09±0.21[abcd]	2.92±0.21
D 7/11**	2.98±0.21	3.06±0.15	2.85±0.21	3.44±0.10[a]	2.97±0.18[bcd]	-
D 9/10 (AI)	3.23±0.20	3.15±0.11	3.23±0.24	3.33±0.20[ab]	2.68±0.18[d]	3.07±0.13
10 PAI	3.12±0.19	3.13±0.17	3.10±0.19	3.15±0.18[abc]	2.88±0.18[bcd]	3.08±0.07
20 PAI	3.27±0.08	3.07±0.15	3.22±0.12	3.38±0.10[a]	3.28±0.16[ab]	3.07±0.19
30 PAI	3.17±0.18	3.19±0.15	3.01±0.10	3.19±0.22[abc]	3.21±0.11[abc]	3.11±0.18
40 PAI	3.01±0.25	3.32±0.10	2.83±0.16	3.07±0.18[abc]	3.38±0.16[ab]	3.19±0.19

0$ = day of calving, * day of first treatment, ** day of PG injection.

Synchronization treatment was initiated on day 90-95 postpartum.

Means bearing uncommon superscripts within the column differ significantly ($P<0.05$).

increased around day 10-20 postpartum and fluctuated unevenly thereafter (Table 2). Ammu *et al*. (2013) reported that the mean magnesium levels varied non-significantly from 2.87 ± 0.07 to 3.05 ± 0.08 mEq/L at different intervals till day 90 postpartum in Gir cows and that the levels were not influenced by any of the estrus induction protocols subsequently used as has been noted by Savalia *et al.* (2013) in buffaloes also. The present findings support their observations. Moreover, the influence of intervals was significant ($P<0.05$) on plasma magnesium profile in both conceived and non-conceived groups of cows (Fig. 1), both at postpartum and post-AI periods.

The Kankrej cows under study did not show significant variation in macro-minerals profile over postpartum/post-treatment periods, possibly due to optimal nutritional and managerial conditions provided to all of them on the farm, thus preventing mineral deficiency or malnutrition upon onset of lactation or even therapy, and in fact lactation/suckling is perhaps the major inhibitory factor in onset of postpartum ovarian activity in zebu cattle.

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 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 (Ravikumar *et al*., 2007; Mohan Krishna *et al.,* 2010; Keskin *et al.,* 2011; Khade *et al.,* 2011; Ammu *et al.,* 2012 and Bhoraniya *et al.,* 2012[a,b]) in cyclic and/or acyclic cows.

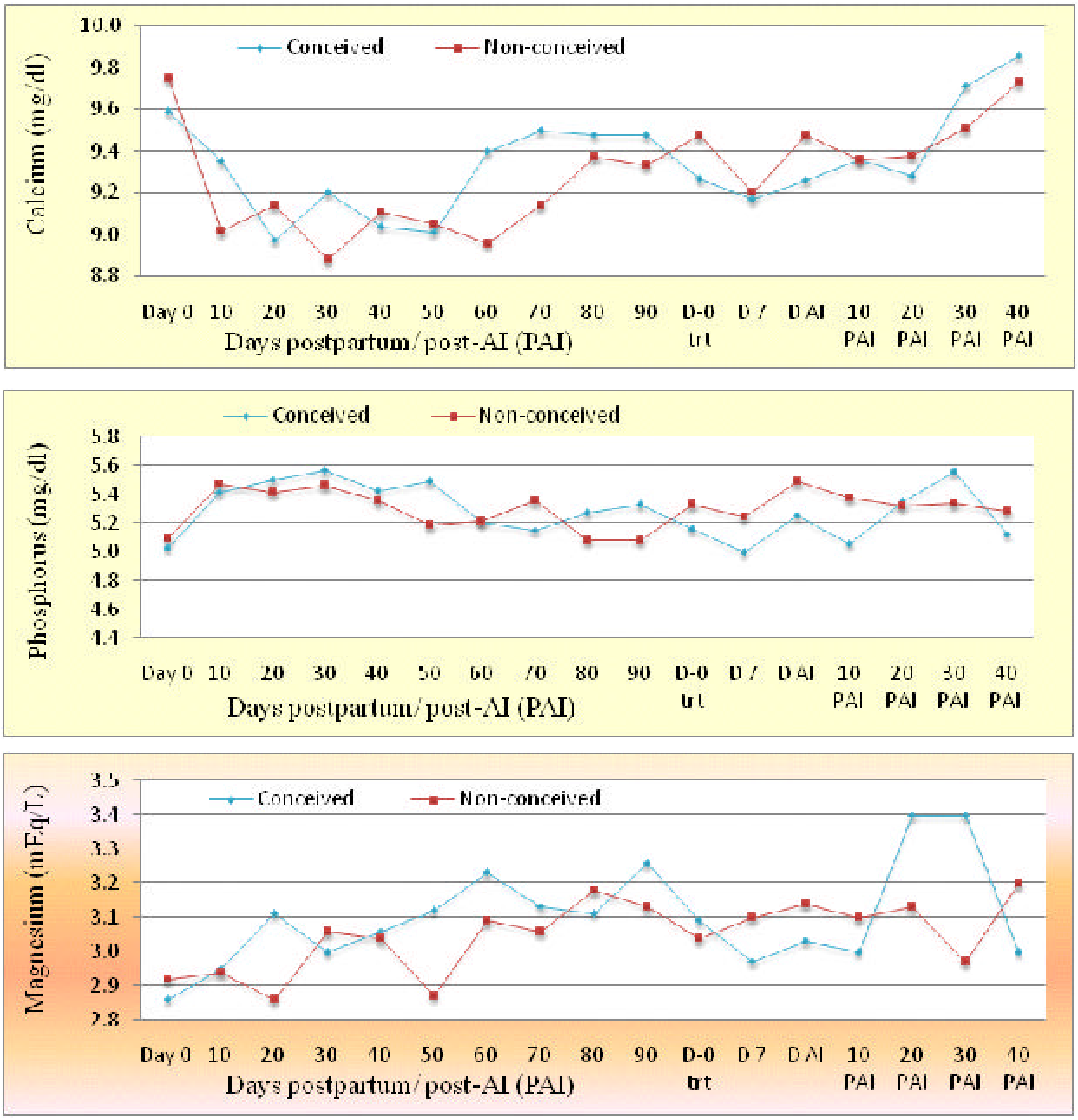

Fig. 1. Plasma minerals profile postpartum irrespective of groups (days 0-90), at synchronization treatment (day 90, 106) and at post-AI (day 100-140 postpartum) in conceived and non-conceived Kankrej cows.

The conception rates obtained during the induced estrus and overall of 3 cycles following Ovsynch protocol were 16.66 and 33.33 (2/6) %, respectively. The corresponding conception rates for CIDR protocol were 33.33 and 50.00 (3/6) %, Ovsynch + CIDR 16.66 and 33.33 (2/6) %; Cosynch protocol 50.00 and 50.00 (3/6) %, and double PG protocol 50.00 and 50.00 (3/6) %, respectively. The values for normal cyclic control group were 33.33 and 50.00 (3/6) %. The conception rates of CIDR were the same as in the normal cyclic control group. In similar experiments with Ovsynch, Ghallab *et al.* (2009) reported conception rate of 40 % in HF cows, while Mohan Krishna *et al.* (2010) recorded it as 30 and 20 % in Sahiwal cows and heifers, respectively. On the contrary, higher conception rates of 50 % each in Gir heifers and Kankrej cows with Ovsynch were recorded by Khade *et al.* (2011) and Bhoraniya *et al.* (2012[b]), respectively. The conception results of the present study with CIDR agreed with the findings of Naikoo *et al.* (2010). On the contrary, Khade *et al.* (2011) and Bhoraniya *et al.* (2012[b]) reported higher conception rates in Gir and Kankrej animals. Relatively lower conception rates of 26.00 to 42.74 % in cows were reported by Sathiamoorthy and Kathirchelvan (2010). The prolonged exogenous progesterone priming from CIDR device causes negative feedback effect on hypothalamo-hypophyseal-gonadal axis and increases receptors for gonadotropins on the ovaries followed by rebound on its sudden withdrawal causing stimulated FSH secretion, folliculogenesis and ovulation.

The conception rates at the induced estrus and overall of three cycles (16.66 & 33.33 %) for Ovsynch + CIDR combined protocol were at par with Ovsynch alone, and lower than in all other protocols including control group. Combining Ovsynch with CIDR in fact reduced the efficacy of CIDR. Although higher conception rates (55.71 and 66.66 %) with this combination were reported earlier by Fallah Rad and Ajam (2008) and Khade *et al.* (2011) in anestrous HF cows and anestrous Gir heifers, respectively.

The conception rate obtained with Cosynch protocol coincided with the results of Larson *et al.* (2006) in suckled beef cows (44 %). However, Ammu *et al.* (2012) found much higher conception rates of 72 % in suckled acyclic Gir cows, while lower conception rate of 28 % was noted by Ahuja *et al.* (2005) in anestrous lactating crossbred cows. Further, the estrus response and CRs obtained with double PG protocol in subestrous cows were in accordance with those of Gacche *et al.* (2002) and Mohanty *et al.* (2003). The possible reason for variation in results of different studies could be the reproductive status or stage of estrous cycle at the beginning of the protocol, apart from variations in nutrition, management, lactation, suckling stimulus, season, drug source, age, breed, and species etc.

CONCLUSIONS

The results of treatment approaches showed that for maintaining a high degree of breeding efficiency among lactating suckled zebu cattle herds of the tropical regions like India, advanced reproductive management strategies like estrus induction/ synchronization need to be adopted as CIDR, Cosynch and PG improved synchrony of estrus occurrence and considerably enhanced conception rate over Ovsynch or its combination in anestrous/subestrous suckler Kankrej cows. The levels of plasma calcium, inorganic phosphorus and magnesium were not good indicators of reproductive status, as there was no consistent trend for these elements between different groups/ periods studied.

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