PLASMA CALCIUM, PHOSPHORUS AND MAGNESIUM PROFILE IN POSTPARTUM COWS FOLLOWING HORMONAL THERAPY*

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ABSTRACT

Recently calved HF cows (24) of University farm were monitored for weekly plasma profile of calcium, phosphorus and magnesium from the day of calving till 21st week postpartum following GnRH (Receptal) and PGF_2 alpha (Lutalyse) treatment at 7th week in anestrus and subestrus cows (6 each, keeping equal number as control), respectively. The plasma calcium levels in subestrus cows were significantly higher as compared to anestrus cows at 7th, 8th, 17th and 21st week postpartum, including overall mean (8.17±0.08 vs 7.72±0.08 mg/dl), while phosphorus levels were lower throughout the postpartum period, but varied significantly at calving and then at 1st-3rd and 13th week postpartum (overall mean 6.96±0.07 vs 7.12±0.06 mg/dl). The calcium and phosphorus levels were low at calving in all the groups, and increased linearly and significantly throughout the postpartum period in subestrus cows. Further, the cows of GnRH treatment group, in comparison to control, had significantly lower calcium levels at 1st, 5th, 8th and 19th weeks postpartum, but this trend was inversed between $PGF_{2\alpha}$ treatment and control groups. The mean plasma Ca:P ratio was higher throughout the postpartum period in subestrus cows as compared to anestrus cows, and it differed significantly at 13th, 17th-18th and 21st week postpartum. The magnesium levels of anestrus and subestrus cows (2.95±0.04 vs 3.14±0.01 mEq/L) did not differ significant at any of the intervals postpartum. Throughout the postpartum period magnesium levels were lower in GnRH treatment than the control group, but varied significantly only at 5th-10th and 13th-15th week postpartum, whereas it was higher in $PGF_{2\alpha}$ treatment than the control group, and differed significantly from calving to 4th week and again from 14th-20th week postpartum. The trend of plasma calcium, phosphorus levels reflected physiological/homeostatic mechanism of mineral metabolism and the hormone GnRH or $PGF_{2\alpha}$ treatment did not influence it.

Key words: GnRH, $PGF_{2\alpha}$, Calcium, Phosphorus, Magnesium, Postpartum cows.

INTRODUCTION

Nutritional inadequacy and negative energy balance in postpartum cows are the major causes for delayed resumption of ovarian activity, anestrus and subestrus in dairy animals (Butler *et al.*, 2000). Calcium plays an important role in utilization of cholesterol by mitochondria or by stimulating the conversion of pregnenoione to progesterone. GnRH stimulation of LH release from pituitary cells involves a Ca-dependent mechanism. Phosphorus is often associated with reproductive abnormalities in cattle through infertility. The role of calcium and phospholipid-dependent protein kinase and c-AMP-dependent protein kinase may be crucial in mediating hormone action. Magnesium is equally essential in all enzyme reactions catalyzed by ATP and in maintaining the physical integrity of RNA-DNA. No literature is available on monitoring the postpartum plasma profile of macro-minerals in exotic cattle born and reared under tropical climate. Hence, an attempt was made to monitor calcium, phosphorus and magnesium levels during early postpartum period with and without GnRH and $PGF_{2\alpha}$ treatment in HF cows under tropical farm management.

MATERIALS AND METHODS

This study was carried out over first 21 weeks postpartum on 24 healthy normally calved cows managed under routine feeding and housing protocol of HF Project, GAU, Anand. A group of 6 anestrus cows having small smooth inactive ovaries till day 48-49 postpartum were treated to induce ovarian activity with

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the single i/m injection of 0.02 mg (5 ml) Buserelin acetate (GnRH analogue, Receptal®, Intervet India Pvt. Ltd.) and 6 animals of similar nature were kept as control. Another group of 6 cyclic/subestrus animals (not exhibiting prominent estrus signs) was treated with single i/m injection of 25 mg (5 ml) Dinoprost tromethamin, THAM salt (Lutalyse®, Pharmacia & Upjohn), between day 48 and 55 postpartum upon confirming the presence of mature CL on either of the ovaries, and 6 animals of similar nature were kept as control.

The animals of both treatment and control groups were followed and compared for their clinical response, conception rate and weekly plasma profile of calcium, phosphorus and magnesium till 21st week postpartum. Cows exhibiting signs of estrus were bred only after 50 days of calving by AI using frozen-thawed semen and were palpated per rectum for pregnancy 45 days later. Blood samples were collected from all 24 cows at regular weekly interval from the day of calving till 21st week postpartum. The plasma samples were stored and frozen at -20°C until estimations. The plasma mineral profile was determined by using standard assay kits (crest Biosystems India Ltd., Goa) and an autoanalyzer. The data were analyzed statistically using Complete randomized design, Duncan's new multiple range test and Student's 't' test to know the weekly/ group variation, if any (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

The weekly mean plasma calcium, phosphorus, Ca:P ratio and magnesium levels of anestrus and subestrus cows (pooled over treatment & control groups) from the day of calving till 21st week postpartum are presented in Table.

The subestrus cows ($PGF_{2\alpha}$ group), compared to anestrus (GnRH group), had significantly ($P<0.01$) higher calcium level at 7th, 8th, 17th and 21st week postpartum, including the overall mean (8.17±0.08 vs 7.72±0.08 mg/dl). The calcium levels were lowest at calving in all the groups and then fluctuated non-significantly up to 21st week postpartum. GnRH treated cows had significantly lower calcium levels than those of control group at postpartum, including the overall mean (7.31±0.10 vs 8.11±0.11 mg/dl). In subestrus ($PGF_{2\alpha}$) group, the levels increased linearly after calving to reach significantly higher value of 8.69±0.38 mg/dl by 6th week postpartum and then fluctuated at the same level till 21st week postpartum (Table). These findings on calcium levels and its trend from calving till 21st week postpartum corroborated well with the reports of Belyea *et al.*, (1975) and Sato (1978). Deshpande *et al.*, (1998) reported significant increase in calcium level from day of calving to 7th, 14th and 21st day postpartum. Joe *et al.*, (1998) and Dutta *et al.*, (2001) recorded significantly higher values of calcium in cyclic cows as compared to anestrus cows. The role of calcium in sensitizing tubular genitalia for action of hormones is well established (Moddie and Robertson, 1962). The lower level of calcium at parturition and subsequently at estrus was thought to be due to high oestrogen levels, as oestrogen may change appetite in cows, hence diminished calcium intake and absorption (Sahukar *et al.*, 1984). The trend of gradual rise in plasma calcium level during postpartum period could be a homeostatic mechanism leading to elevated circulatory levels to meet the drain of calcium in milk during lactation (Sato, 1978).

The weekly mean plasma inorganic phosphorus levels in anestrus cows were higher throughout the postpartum period as compared to subestrus cows, but varied significantly at calving and then at 1st, 2nd, 3rd and 13th week postpartum and there was no significant difference between the overall pooled means of the two groups (6.96±0.07 vs 6.43±0.06 mg/dl. Further, the weekly mean phosphorus levels of all groups (treatment, control and pooled) fluctuated non-significantly between different intervals postpartum with relatively higher values in treated and control anestrus cows as compared to subestrus cows (Table). The mean inorganic phosphorus levels were lowest on the day of calving; increased a little for first two weeks and then fluctuated non-significantly between different weeks postpartum. These findings were in agreement with the report of Rowlands *et al.*, (1977). Deshpande *et al.*, (1998) noted marked decrease in serum inorganic phosphorus at calving followed by a little rise till 4th week. However, Jain and Pandita (1995) observed significantly lower phosphorus level ($P<0.05$) in control than $PGF_{2\alpha}$ treated crossbred cows. Hurley *et al.* (1980) suggested that the fertility of animals tended to be reduced if the inorganic phosphorus levels fall, while increased blood phosphorus level was related to the improvement of ovarian activity. Joe *et al.*, (1998) and Dutta *et al.*, (2001) observed significantly higher serum inorganic phosphorus in cyclic than anestrus cows.

The mean plasma Ca:P ratio in subestrus cows was higher throughout the postpartum period as compared to anestrus cows, but differed significantly at 13th, 17th, 18th and 21st week postpartum, including the overall pooled mean (1.29±0.01 vs 1.13±0.01).

Further, the weekly mean Ca:P ratio in GnRH/$PGF_{2\alpha}$ treatment, control and pooled groups varied insignificantly between weeks postpartum. The ratio was lower in cows of GnRH treatment than the control group throughout the postpartum period, but varied significantly only at 16th to 19th week postpartum; including the overall mean (1.04±0.02 vs 1.22±0.02). No such differences were found in $PGF_{2\alpha}$ treatment and control groups. In general, the ratio was found elevated at 7th - 8th week and again after 16th week postpartum compared to other intervals in all the groups (Table 2). Sahukar *et al.*, (1984) reported the ratio of Ca:P as 2:1, 1.5:1 and 2.5:1 at calving, estrus and at one month of pregnancy in cows. In the present study, the mean ratio observed in different groups of cows throughout the postpartum period was relatively low, particularly in anestrus group suggesting its adverse effect on reproductive efficiency of HF cows

There was no significant difference in the weekly mean magnesium levels of cows in anestrus and subestrus groups at any of the intervals postpartum, including the overall pooled values (2.95±0.04 vs 3.14±0.01 mEq/L). Moreover in all the groups, the weekly mean magnesium levels varied non-significantly from the day of calving till 21st week postpartum. The values were lower throughout the postpartum period in GnRH treatment group as compared to control, but varied significantly ($P<0.05$) only at 5th to 10th and 13th to 15th week postpartum, including the overall means (2.64±0.03 vs 3.26±0.05 mEq/L) and so also for $PGF_{2\alpha}$ treatment and its control groups, which differed significantly ($P<0.01$) from calving to 4th week and again from 14th to 20th week postpartum, including the overall mean (3.60±0.08 vs 2.66±0.04 mEq/L;). These findings are in agreement with the reports of Moddie and Robertson (1962), Sato (1978) and Deshpande *et al.*, (1998), where no significant change in magnesium level was recorded over the postpartum period in cows. However, Belyea *et al.*, (1975) recorded a gradual decrease in plasma magnesium levels for cows sampled at 4 days interval from parturition to 60 days postpartum. Kalita *et al.*, (1999) reported mean serum magnesium level to be significantly higher in normal cyclic cows as compared to repeat breeder and postpartum anestrus cows. It is postulated that all enzyme reactions, which are catalysed by ATP have an absolute requirement for magnesium. The magnesium and calcium has reciprocal relationship, but no endocrine gland has got specific primary regulatory effect over plasma magnesium level.

The present study concluded that the lowest levels of plasma calcium and phosphorus at calving followed by a gradual rise observed during the postpartum period could be a homeostatic mechanism to meet their drain in milk during lactation, and that a low plasma Ca:P ratio adversely affects the reproductive efficiency in cows. Hormone GnRH or $PGF_{2\alpha}$ therapy, however, did not influence the plasma profile of calcium, phosphorus or magnesium in exotic cows.

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Table : Weekly mean (± SE) plasma calcium and phosphorus profile (mg/dl) in postpartum anestrus and suboestrus cows (pooled over treated & control groups)

Postpartum period (in weeks	Ca : P ratio		Magnesium (mg/dl)	
	Anoestrus (GnRH T+C)	Suboestrus ($PGF_2\alpha$ T+C)	Anoestrus (GnRH T+C)	Suboestrus ($PGF_2\alpha$ T+C)
0	1.06±0.05	1.21 ± 0.09^{bcd}	3.03±0.18	2.95±0.20
1	1.13±0.06	1.13 ± 0.06^{d}	2.86±0.14	3.18±0.26
2	1.04±0.07	1.17 ± 0.06^{cd}	2.98±0.18	3.07±0.28
3	1.08±0.09	1.31 ± 0.07^{abcd}	3.03±0.18	3.26±0.27
4	1.05±0.09	1.24 ± 0.08^{abcd}	2.87±0.14	3.01±0.15
5	1.13±0.10	1.27 ± 0.07^{abcd}	2.86±0.16	3.31±0.21
6	1.15±0.13	1.31 ± 0.05^{abcd}	3.10±0.20	3.21±0.21
7 #	1.20±0.08	1.42 ± 0.08^{abc}	3.09±0.19	3.23±0.26
8	1.20±0.09	1.44 ± 0.08^{ab}	3.02±0.20	3.16±0.18
9	1.15±0.08	1.22 ± 0.06^{bcd}	2.96±0.15	3.15±0.21
10	1.30±0.08	1.24 ± 0.07^{abcd}	3.03±0.21	3.36±0.28
11	1.15±0.07	1.27 ± 0.05^{abcd}	2.79±0.19	3.00±0.20
12	1.22±0.07	1.27 ± 0.06^{abcd}	2.88±0.21	2.83±0.19
13	1.09±0.06 $$	1.35 ± 0.06^{abcd}	2.98±0.17	3.09±0.26
14	1.09±0.07	1.30 ± 0.09^{abcd}	3.00±0.17	3.08±0.22
15	1.07±0.06	1.25 ± 0.09^{abcd}	2.93±0.21	3.27±0.25

16	1.11±0.06	1.17±0.05^{cd}	2.92±0.14	3.05±0.19
17	1.08±0.08 $$	1.42±0.08^{abc}	2.96±0.15	3.19±0.32
18	1.17±0.09 $	1.46±0.08^{ab}	3.04±0.26	3.18±0.27
19	1.22±0.10	1.48±0.10^{a}	2.72±0.17	3.09±0.24
20	1.20±0.09	1.45±0.10^{ab}	2.87±0.18	3.32±0.30
21	1.10±0.07 $	1.38±0.07^{abcd}	3.02±0.13	3.15±0.35
Overall	**1.13±0.01 $$**	**1.29±0.01**	**2.95±0.04**	**3.14±0.05 $**
Treated	1.04±0.02	1.29±0.01	2.64±0.03	3.60±0.08
Control	1.22±0.02*	1.30±0.02	3.26±0.05*	2.66±0.04**

*0 = Day of calving; # Treatment day 49 PP; * P < 0.05, ** P < 0.01 between subgroups; $ P < 0.05, $$ P < 0.01 between anestrus & subestrus groups; T treated, C control.*
Means bearing superscript in common within a column do not differ significantly (P>0.05).