INFLUENCE OF MID-CYCLE PG TREATMENT AND GNRH AT AI ON PLASMA MINERALS PROFILE IN CONCEIVING AND NON-CONCEIVING REPEAT BREEDING CROSSBRED COWS

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ABSTRACT

This study was carried out in AMUL milk shed area on 30 crossbred cows, comprising 20 repeat breeding and 10 normal cyclic ones to evaluate their clinical response to mid-cycle $PGF_2\alpha$ and AI + GnRH protocol (10 RB cows each) and to monitor plasma macro-micro minerals profile on day 0,7, 9 (AI) of treatment and on day 21 post-AI. Nine out of 10 cows exhibited estrus within 82.67±4.22 hrs of mid-cycle $PGF_2\alpha$ (25 mg, i/m) treatment. The first service and overall conception rates of 3 cycles were 40 and 80 % in mid-cycle $PGF_2\alpha$ group; 30 and 70 % in AI + GnRH (20 µg, i/m) group; and 50 and 80 % in normal cyclic control group. In normal cyclic control group, the overall mean values of plasma calcium, phosphorus and magnesium were 9.98 ± 0.04, 5.97 ± 0.10 and 3.14 ± 0.03 mg/dl, respectively. Almost same profile was observed for repeat breeders. The mean values of plasma zinc, iron, copper, cobalt and manganese concentration were 1.04 ± 0.03, 16.26 ± 0.31, 1.23 ± 0.03, 0.63 ± 0.03 µg/ml, and 0.19 ± 0.01 µg/ml, respectively. There is no significant difference observed in micro minerals profile among the animals under three groups, except zinc and copper, which were significantly ($P<0.05$) lower in repeat breeding than normal cyclic control cows, and none of the macro-micro minerals was influenced by days or pregnancy status in any of the groups. It was inferred that hormonal therapies particularly mid-cycle PG significantly improved conception rates in repeat breeding cows, but had no influence on the plasma minerals profile remarkably.

KEY WORDS: Crossbred cows, Repeat breeding, Mid-cycle PG, AI + GnRH, Fertility, Plasma minerals profile.

INTRODUCTION

High reproductive performance is an essential requirement for an efficient dairy farming with satisfactory economic return. Cattle and buffalo productivity depends largely on their reproductive efficiency which is often measured by inter-calving intervals. Repeat breeding syndrome leads to loss of time and economy of dairy farms. There are apparently several reasons for the repeat breeder syndrome and no single treatment is likely to alleviate the condition in every herd or animal. Repeat breeding in dairy cattle is associated with either failure of fertilization or early embryonic mortality. The uses of mid-cycle $PGF_2\alpha$ (Goley and Kadu, 1995; Patel *et al.,* 2014), GnRH/hCG at AI and progesterone analogues post-AI have been successful to improve conception rate and sustain early pregnancy in repeat breeding bovines (Sreenan and Diskin, 1983; Kim *et al*., 2007; Patel *et al.,* 2014). However, the literature on the influence of such hormonal therapies on plasma minerals profile is meager (Savalia *et al.,* 2013). Hence, the present study was planned to evaluate whether mid-cycle $PGF_2\alpha$ and AI + GnRH treatment protocols influence the conception rate and plasma profile of macro-micro minerals in repeat breeding crossbred cows.

MATERIAL AND METHODS

The study was undertaken on 20 classical repeat breeding and 10 normal cyclic/fertile (control) crossbred cows in villages of AMUL milk shed areas around Anand. The repeat breeders were confirmed by rectal palpation twice 10 days apart. All the animals selected were initially dewormed using Ivermectin 70 mg s/c, fenbendazole 3 g orally, and were given injection enrofloxacin 3.0 g i/m (Inj. Conflox, 15 ml, Concept Pharma) to check invisible genital infection, if any, with mineral mixture supplementation @ 25-30 g/day for 15 days (Patel *et al.,* 2014). Ten repeat breeding cows with mature palpable mid-cycle CL on either of the ovaries were then injected with $PGF_2\alpha$ 25 mg i/m (Inj. Lutalyse, 5 ml) and FTAI was done twice at 72 and 96 hours later. Another 10 repeat breeding cows with clear standing oestrus and large ovaries were inseminated with simultaneous injection of GnRH 20 μg i/m (Inj. Receptal, 5 ml). Ten cows detected in oestrus spontaneously within 3 months postpartum with normal healthy genitalia were inseminated without any treatment, and served as normal cyclic/fertile control. Animals once inseminated were followed for recurrence of oestrus regularly for 3 cycles, and in non-return cases pregnancy was confirmed per rectum 60 days post-AI.

Blood samples were collected from jugular vein in heparinised vacutainers on day of $PGF_2\alpha$ injection, at induced/ spontaneous oestrus (AI) and on day 21 post-AI. The samples were centrifuged at 3000 rpm for 15 minutes, and the plasma separated out was stored deep frozen at -20°C with a drop of merthiolate (0.1%) until analyzed. Plasma calcium, inorganic phosphorus and magnesium concentrations were estimated by employing standard procedures and assay kits procured from Crest Bio-systems, Goa with the help of Chemistry Analyzer (Mindray, BS 120). The wet digested plasma samples (nitric acid:perchloric acid, 4:1) were used to determine micro-minerals, viz, zinc, iron, copper, cobalt and manganese on Atomic Absorption Spectrophotometer (Oser, 1979). Animals conceived at induced/first estrus were taken as pregnant/conceived and the rests as non-pregnant/ non-conceived group. The observations/data on conception rates and blood profiles of plasma minerals within and between groups were analyzed using standard statistical procedures (CRD, t-test).

RESULTS AND DISCUSSION

Oestrus Response and Conception Rate in Repeat Breeding Cows

In the present study, 90 % of repeat breeding crossbred cows treated with mid-cycle $PGF_2\alpha$ injection, responded with behavioural oestrus within 82.67±4.22 hrs. This was in close agreement with the reports of Kharche and Srivastava (2002) and Kumar *et al.* (2011). The conception rates in mid-cycle $PGF_2\alpha$ protocol at induced/first, second and overall of 3 cycles were 40.00, 50.00 and 80.00 %, respectively. The corresponding figures with AI + GnRH protocol were 30.00, 42.86 and 70.00 %. In normal cyclic control group, the corresponding conception rates were 50.00, 60.00 and 80.00 %, respectively. The details have been discussed earlier (Patel *et al.*, 2014). These findings closely corroborated with the reports of Ranganekar *et al*. (2002) and Kumar *et al*. (2009, 2011). The beneficial effect of mid-cycle PG injection and GnRH at AI could be due to better synchrony of endocrine events leading to timely ovulation and strengthening of luteal function in repeat breeding crossbred cows. The present findings clearly support the views of earlier research that $PGF_2\alpha$ analogues have definite standing in successful management of suboestrus and repeat breeding conditions in cows, since these drugs induce mostly ovulatory oestrus following luteolysis. The results obtained using $PGF_2\alpha$ injection were better than the results from GnRH injection at the time of AI. Thus, the application of mid-cycle $PGF_2\alpha$ injection can be used as a good tool for induction of fertile oestrus as well as enhancement of conception rate in repeat breeding crossbred cows at par with untreated normal cyclic cows.

Plasma Minerals Profile of Repeat Breeding Crossbred Cows

The plasma profiles of macro-micro minerals obtained in repeat breeding cows under Mid-cycle PG and AI + GnRH treatments as well as in normal cyclic control group on different days overall and in conceived and non-conceived cows are presented in Tables 1-3.

Table 1: Plasma calcium concentration (mg/dl) in repeat breeding crossbred cows on different days of treatment/AI under various treatment protocols

Treatment protocol	Status	No.	Days from treatment/AI			Overall
			D-0 (T)	D-AI	D-21post-AI	
Mid cycle PG	Conceived	4	9.18±0.37	9.80±0.17	9.76±0.25	9.58±0.17
	Non-conc	6	9.86±0.29	9.96±0.14	9.90±0.19	9.90±0.12
	Overall	10	**9.59±0.24**	**9.90±0.11**	**9.84±0.14**	**9.78±0.10**
AI + GnRH	Conceived	3	--	10.05±0.21	9.80±0.06	9.93±0.11
	Non-conc	7	--	10.23±0.21	9.78±0.09	10.00±0.13
	Overall	10	**--**	**10.18^{b}±0.16**	**9.79^{a}±0.07**	**9.98±0.10**
Normal Cyclic Control	Conceived	5	--	10.04±0.11	9.98±0.06	10.01±0.06
	Non-conc	5	--	9.89±0.08	10.03±0.09	9.96±0.06
	Overall	10	**--**	**9.97±0.05**	**10.00±0.05**	**9.98±0.04**

*P<0.05, **P<0.01 between conceived and non-conceived status within the group.

Day-0 = Day of treatment, D-AI = Day of AI, D-21 = Day 21 post-AI.

Table 2: Plasma phosphorus concentration (mg/dl) in repeat breeding crossbred cows on different days of treatment/AI under various treatment protocols

Treatment protocol	Status	No.	Days from treatment/AI			Overall
			D-0 (T)	D-AI	D-21post-AI	
Mid cycle PG	Conceived	4	6.01±0.09	6.00±0.22	5.95±0.23	5.99±0.10
	Non-conc	6	6.17±0.37	6.10±0.40	6.17±0.17	6.15±0.18
	Overall	10	**6.11±0.22**	**6.06±0.24**	**6.08±0.14**	**6.08±0.11**
AI + GnRH	Conceived	3	--	5.30±0.24	5.70 ±0.35	5.50±0.21
	Non-conc	7	--	5.49±0.28	5.49±0.37	5.49±0.22
	Overall	10	**--**	**5.43±0.20**	**5.56±0.28**	**5.49±0.16**
Normal Cyclic Control	Conceived	5	--	5.91±0.15	5.82±0.18	5.87±0.11
	Non-conc	5	--	6.20±0.27	5.93±0.24	6.07±0.18
	Overall	10	**--**	**6.06±0.15**	**5.87±0.14**	**5.97±0.10**

*P<0.05, **P<0.01 between conceived and non-conceived status within the group.

Day-0 = Day of treatment, D-AI = Day of AI, D-21 = Day 21 post-AI.

Plasma Macro-minerals Profile

The mean plasma calcium levels (mg/dl) in repeat breeding cows on the day of PG injection, day of induced oestrus/AI and on day 21 post-AI did not vary significantly and so also between conceived and non-conceived animals, and the overall mean was 9.78 ± 0.10 mg/dl. These findings are in contrast with those of Jain and Pandita (1995), who reported higher mean plasma calcium levels in normal cyclic and $PGF_2\alpha$ treated cows at oestrus than before oestrus /treatment. Patel and Dhami (2005) recorded higher overall mean value of calcium for conceived than non-conceived cows. Dutta *et al.* (2001) observed that the serum calcium level was significantly higher (P<0.01) in normal cyclic (10.73 ± 0.08 mg %) than repeat breeding cows of Assam (9.95 ± 0.18 mg %). The mean plasma calcium concentrations in cows of AI + GnRH treatment group on day of AI and day 21 post-AI were 10.18 ± 0.16 and 9.79 ± 0.07 mg/dl (P<0.05), respectively, with a grand mean of 9.98 ± 0.10 mg/dl. Almost similar were the values in normal cyclic control group. The values however did not vary significantly between conceived and non-conceived cows in any of the groups (Table 1). These findings of calcium are in contrast with those of Kumar *et al.* (2009), who reported significantly (P<0.05) higher serum calcium in normal cyclic than repeat breeding cows (9.66 ± 0.27 vs. 8.90 ± 0.21 mg/dl). The present profile of plasma calcium in repeat breeding animals is higher as compared to that reported by Dutta *et al.* (2001), Kumar *et al.* (2009) and Patel *et al*. (2011), whereas Mishra *et al.* (2007) recorded relatively lower value.

The grand mean plasma inorganic phosphorus concentrations in repeat breeding cows covered under mid-cycle $PGF_2\alpha$ and AI + GnRH groups and in normal cyclic controls were 6.08 ± 0.11, 5.49 ± 0.16 and 5.97 ± 0.10 mg/dl (P<0.05), respectively. The plasma inorganic phosphorus values neither varied significantly between sampling days nor between conceived and non-conceived groups, in any of the groups (Table 2). These findings on the effect of PGF2 alpha are similar to those of Jain and Pandita (1995). Dutta *et al.* (2001) and Cetin *et al.* (2002) observed significantly higher (P<0.01) serum inorganic phosphorus in fertile/normal cyclic than in repeat breeding cows. However, the values reported by Cetin *et al.* (2002) and Mahour *et al.* (2011) were relatively lower, whereas Mishra *et al.* (2007) and Kumar *et al.* (2009) reported higher values as compared to the present observations.

The grand mean magnesium values of repeat breeding cows under mid-cycle $PGF_2\alpha$ and AI + GnRH protocol and in normal cyclic control group were 3.20 ± 0.03, 3.12 ± 0.03 and 3.14 ± 0.03 mg/dl, respectively. The values did not vary significantly between conceived and non-conceived animals and between various sampling days, except in control group where the levels in non-conceived animals were consistently higher with significant difference in the pooled values (Table 3). Dutta *et al.* (2001) observed significantly higher (P<0.01) serum magnesium level in normal cyclic than in repeat breeding cows, while Kumar *et al.* (2009) reported inversed trend between normal cyclic and repeat breeding crossbred cows. Further Kumar *et al.* (2009) and Parmar *et al.* (2013) observed that the magnesium concentrations were significantly lower for GnRH treated cows than those in untreated control groups and the mean plasma levels between conceived and non-conceived group were also reported to be identical.

Plasma Micro-Minerals Profile

The grand mean levels of plasma zinc in repeat breeding cows under mid-cycle $PGF_2\alpha$ and AI + GnRH treatment, and in normal cyclic control group were 0.84 ± 0.06, 0.87 ± 0.05 and 1.04 ± 0.03 µg/ml (P<0.05), respectively. The values of plasma zinc and iron did not differ significantly between various days of sampling or between conceived and non-conceived animals in any of the treatment and control groups. However, zinc was significantly lower in repeat breeding than normal cyclic control. Iron was significantly (P<0.05) higher on day 21 post-AI as compared to day of AI in AI + GnRH group (16.36 ± 0.35 vs. 15.19 ± 0.40 µg/ml). Further, the plasma iron status was similar in repeat breeding and normal cyclic control groups (Table 3). The pooled mean values of

Table 3: Plasma magnesium (Mg/dl) and trace minerals (µg/dl) concentration in repeat breeding crossbred cows under different treatment in relation to normal cyclic controls

Treatment protocol	Status	No	Magnesium	Zinc	Iron	Copper	Cobalt	Manganese
Mid cycle PG	Conceived	4	3.22 ±0.05	0.86 ±0.11	15.88 ±0.27	1.15 ±0.10	0.65 ±0.02	0.21 ±0.02
	Non-conc	6	3.18 ±0.04	0.82 ±0.07	15.55 ±0.26	1.04 ±0.06	0.62 ±0.03	0.19 ±0.01
	Overall	10	3.20 ±0.03	0.84 ±0.06	15.68 ±0.19	1.09 ±0.05	0.63 ±0.02	0.20 ±0.01
AI + GnRH	Conceived	3	3.14 ±0.07	0.85 ±0.08	15.50 ±0.34	0.95 ±0.06	0.65 ±0.08	0.20 ±0.02
	Non-conc	7	3.11 ±0.04	0.87 ±0.05	15.89 ±0.40	1.04 ±0.08	0.65 ±0.04	0.20 ±0.03
	Overall	10	3.12 ±0.03	0.87 ±0.04	15.77 ±0.29	1.01 ±0.06	0.65 ±0.04	0.20 ±0.02
Normal Cyclic Control	Conceived	5	3.09 ±0.01	1.02 ±0.05	15.91 ±0.42	1.18 ±0.06	0.68 ±0.04	0.19 ±0.02
	Non-conc	5	3.19 ±0.06	1.05 ±0.05	16.62 ±0.45	1.28 ±0.03	0.58 ±0.04	0.20 ±0.01
	Overall	10	3.14 ±0.03	1.04 ±0.03	16.26 ±0.31	1.23 ±0.03	0.63 ±0.03	0.19 ±0.01

Day-0 = Day of treatment, D-AI = Day of AI, D-21 = Day 21 post-AI. The effect of pregnancy status (conceived vs. non-conceived) was not significant for any of the traits within the group/protocol. The period effect (Sampling days) was also insignificant, hence data are not shown in table.

plasma copper in repeat breeding cows under mid-cycle $PGF_2\alpha$ and AI + GnRH group and in normal cyclic control group were 1.09 ± 0.05, 1.01 ± 0.06 and 1.23 ± 0.03 µg/ml ($P<0.05$), respectively, and those of cobalt 0.63 ± 0.02, 0.65 ± 0.04 and 0.63 ± 0.03 µg/ml. The cobalt concentrations neither differed significantly between various days of sampling nor between conceived and non-conceived animals in any of the groups, and even between normal cyclic and repeat breeding treated groups (Table 3). The value of plasma copper was significantly ($P<0.05$) higher on day 21 post-AI in non-conceived cows as compared to conceived ones (1.28 ± 0.04 vs. 1.15 ± 0.04 µg/ml), and the levels in normal cyclic cows were significantly higher than in repeat breeding treated cows. The grand mean values of plasma manganese in cows under three groups were 0.20 ±0.01, 0.20 ± 0.02 and 0.19 ± 0.01 µg/ml, respectively, which also did not vary between conceived and non-conceived groups, between sampling days or between repeat breeding and normal cyclic cows.

The present findings are in close agreement with those of Manickam *et al.* (1977) in repeat breeding cows. Parmar *et al.* (1986) reported that there was no significant variation in the serum levels of any trace elements studied between periods either in normal or repeat breeding animals. Prasad *et al.* (1989) estimated average serum levels of trace elements during repeat breeding and reported similar results. The present values of micro-minerals were relatively lower than those reported by Parmar *et al.* (1986) and Kalita *et al.* (1999), and higher than those documented by Prasad *et al.* (1989), Singh and Pant (1998), Patel *et al*. (2007), Parmar *et al.* (2013) and Savalia *et al.* (2013) in normal cyclic and repeat breeding cows. Further, the present findings with respect to effect of PG or GnRH treatment coincided with the observations of Patel *et al*. (2007) in HF cows. Parmar *et al.* (2013) also recorded significantly ($P<0.05$) higher values of plasma zinc, iron and copper in

GnRH treated repeat breeder cows as compared to control group, and the values of zinc, iron, copper and manganese were significantly higher (P<0.01) in conceived than non-conceived group.

It is thus, inferred that both the treatment protocols, mid-cycle PG and AI + GnRH, improved conception rates in repeat breeding cows, though the plasma minerals profile was not influenced significantly by these therapies.

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