EFFECT OF MID-CYCLE $PGF_2\alpha$ AND GnRH AT AI ON CONCEPTION RATE, PLASMA PROGESTERONE AND BIOCHEMICAL PROFILE IN 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 progesterone, protein and cholesterol profile at different time intervals post-treatment/AI. Nine out of 10 cows responded within 82.67±4.22 hrs to 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. The plasma progesterone levels in mid-cycle $PGF_2\alpha$ protocol on day of treatment, AI and day 21 post-AI were 6.25±1.41, 0.60±0.10 and 9.53±1.66 ng/ml ($P<0.01$) in conceived and 1.93±0.11, 0.67±0.27 and 0.88±0.13 ng/ml in non-conceived animals. The P_4 levels in AI + GnRH protocol on day 0 (AI) and 21 post-AI were 0.47±0.23, and 9.75±2.63 ng/ml ($P<0.01$) in conceived and 0.43±0.08 and 0.69±0.11 ng/ml in non-conceived cows. The variation in P_4 between conceived and non-conceived animals was also significant ($P<0.01$) on the day of treatment in mid cycle PG group. The P_4 levels in normal cyclic control cows on day 0 and 21 post-AI were 0.24±0.04 and 10.48±1.34 ng/ml ($P<0.01$) in conceived cows and 0.41±0.15 and 0.63±0.11 ng/ml in non-conceived cows, respectively. In mid-cycle $PGF_2\alpha$ protocol, the plasma total cholesterol concentrations in conceived and non-conceived animals were 155.20±14.40 vs 230.25±7.74 mg/dl ($P<0.05$) and and total protein 12.39±0.25 vs 12.16±0.28 g/dl, with significant variation between sampling days in cholesterol only. In AI + GnRH protocol and in normal cyclic control groups, no significant variation was found in total cholesterol and total protein levels between sampling days and between pregnancy status. In general, both the treatment protocols improved conception rates and plasma P_4 profile in repeat breeding cows, though there was no significant influence on plasma protein and cholesterol profile.

KEYWORDS: Crossbred cows, repeat breeding, controlled breeding techniques, fertility, plasma progesterone, biochemical profile.

INTRODUCTION

Dairy farming is one of the most important agricultural activities in India. High reproductive performance is an essential requirement to ensure maximum livestock production and satisfactory economic return (Baruselli *et al.,* 2012). Cattle productivity depends largely on reproductive efficiency and is often measured by offspring per breeding animal per unit of time. Repeat breeding syndrome leads to loss of time and economy of dairy farms (Modi *et al*., 2011). 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 oestrus detection errors, endocrine dysfunctions, ovulatory defects, uterine infection, gamete quality etc, and thereby

poor fertilization rates and/or early embryonic deaths. The overall prevalence of repeat breeding was recorded as 28.31 per cent by Bhat *et al*. (2012) in Kashmir valley. Goley and Kadu (1995) reported that prostaglandin increased the conception rate in repeat breeders. GnRH, hCG and/ or progesterone analogues have been successful to sustain early pregnancy and improve conception rate in repeat breeding bovines (Sreenan and Diskin, 1983; Kim *et al*., 2007). 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 progesterone, protein and cholesterol in repeat breeding crossbred cows.

MATERIALS 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 during the months of November 2012 to March 2013. Problem breeders were confirmed by rectal palpation twice 10 days apart and were subjected to mid-cycle $PGF_2\alpha$ or AI + GnRH treatment protocol (10 each). They were regularly followed for a period of at least 3 months post-treatment. All infertile animals identified were dewormed using Ivermectin 70 mg s/c and by providing medicated concentrate mixture of Amul (fenbendazole 3 g/kg feed). Owners of the ear-marked animals were also supplied with 1 kg mineral mixture (Amul brand) for supplementing to their animals @ 25-30 g/day. 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.

The repeat breeding cows of average BCS (2.75 to 3.50) selected without visible and palpable genital abnormalities were treated initially once with i/m injection of 3.0 g enrofloxacin (Inj. Conflox, 15 ml, Concept Pharmaceuticals) to check invisible genital infection, if any. Ten repeat breeding cows with mature palpable mid-cycle CL on either of the ovaries were treated with i/m injection of $PGF_2\alpha$ 25 mg (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 i/m injection of GnRH 20 μg (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.

Blood samples were collected from jugular vein in heparinised vacutainers thrice or twice depending upon treatment protocol, i.e., 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 progesterone concentrations were estimated by employing standard RIA technique of Kubasic *et al*. (1984). Labelled antigen (I^{125}), antibody coated tubes and standards were procured from Immunotech, France. The sensitivity of assay was 0.1 ng/ml. The coefficients of variation for intra-and inter-assay were 5.4 and 9.1 per cent, respectively. Plasma total cholesterol was estimated by CHOD/PAP method and total protein by Biuret method using standard procedures and assay kits procured from Crest Bio-systems, Goa, with the help of Chemistry Analyzer (Mindray, BS 120).

Animals conceived at induced/first estrus were taken as pregnant/conceived and the rests as non-pregnant/non-conceived group. The observations/data on oestrus behaviour/response, conception rates and blood profiles of plasma P_4, cholesterol and protein were analyzed statistically using standard procedures (CRD, t-test) within and between groups for the effect of period and pregnancy status.

RESULTS AND DISCUSSION

Oestrus response and Conception rate in repeat breeding cows

Out of 10 repeat breeding crossbred cows treated with mid-cycle $PGF_2\alpha$ injection, 9 (90%)

responded with behavioural oestrus within 82.67±4.22 hrs. Of these 6 cows exhibited prominent oestrus and 3 showed moderate oestrus signs. These findings are in close agreement with Kharche and Srivastava (2002), Patel *et al*. (2010) and Rajesh Kumar *et al.* (2011).

Table 1: Effect of Mid-cycle PGF_2a injection and GnRH injection at the time of AI on fertile oestrus interval (days) and conception rates to first three cycles and overall in repeat breeding crossbred cows

Group	No. of Cows	Per cent Oestrus Response	PG Injection to Oestrus Interval (hrs)	Conception Rate (%)			
				Induced/ First Oestrus	Second Cycle	Third Cycle	Overall of 3 Cycles
Mid-cycle PGF_2a	10	90.00 (9)	82.67±4.22 (n=9)	40.00 (4/10)	50.00 (3/6)	33.33 (1/3)	80.00 (8/10)
AI + GnRH	10	100.00 (10)	--	30.00 (3/10)	42.86 (3/7)	25.00 (1/4)	70.00 (7/10)
Normal Cyclic	10	100.00 (10)	--	50.00 (5/10)	60.00 (3/5)	0.00 (0/2)	80.00 (8/10)

Figures in parentheses indicate number of cows.

The conception rates in mid-cycle $PGF_2\alpha$ protocol at induced/first, second, third cycle and overall of 3 cycles were 40.00, 50.00, 33.33 and 80.00 (8/10) per cent, respectively. The corresponding figures with AI + GnRH protocol were 30.00, 42.86, 25.00 and 70.00 (7/10) per cent, respectively. In normal cyclic control group, the corresponding conception rates were 50.00, 60.00, 0.00 and 80.00 (8/10) per cent (Table 1). These findings closely resembled with the reports of Rayos *et al*. (1995), Kumar *et al*. (2000), Ranganekar *et al*. (2002) and Rajesh Kumar *et al*. (2009). 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 and those of many of the above researchers clearly support 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 and these results from mid-cycle $PGF_2\alpha$ injection were somewhat similar to the results in normal cyclic control group. 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 Progesterone Profile:

The mean plasma progesterone levels on day of PG injection, day of induced oestrus/AI and on day 21 post-AI in cows subjected to mid-cycle $PGF_2\alpha$ treatment were 3.66±0.87, 0.64±0.16 and 4.34±1.53 ng/ml, respectively. The values were significantly ($P<0.05$) higher on day 0 and day 21 post-AI as compared to day of induced oestrus/AI (Table 2). Significantly ($P<0.01$) higher mean plasma P_4 level on day 21 post-AI in conceived than non-conceived cows (9.53±1.66 vs. 0.88±0.13 ng/ml) is conceivable due to establishment of pregnancy and maintenance of CL in conceived

Table 2: Plasma progesterone concentration (ng/ml) 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	6.25*±1.41	0.60±0.10	9.53**±1.66	5.46**±1.29
	Non-conc	6	1.93±0.11	0.67±0.27	0.88±0.13	1.16±0.17
	Overall	10	3.66^{b}±0.87	0.64^{a}±0.16	4.34^{b}±1.53	2.88±0.65
AI+ GnRH	Conceived	3	--	0.47±0.23	9.75**±2.63	5.11±2.38
	Non-conc	7	--	0.43±0.08	0.69±0.11	0.56±0.07
	Overall	10	--	0.44±0.08	3.40±1.54	1.92±0.83
Normal Cyclic Control	Conceived	5	--	0.24±0.04	10.48**±1.34	5.36*±1.82
	Non-conc	5	--	0.41±0.15	0.63±0.11	0.52±0.10
	Overall	10	--	0.32^{a}±0.08	5.55^{b}±1.76	2.93±1.05

*P<0.05, **P<0.01 between conceived and non-conceived status within the group.
Means bearing uncommon superscripts within the row differ significantly (P < 0.05).
Day-0 = Day of treatment, D-AI = Day of AI, D-21 = Day 21 post-AI.

animals. These observations are in close agreement with the reports of Chander *et al.* (2002), Kavani *et al.* (2007) and Rajesh Kumar *et al.* (2009). Significantly higher plasma P_4 noted at the initiation of PG treatment proved that all the cows selected were in mid-luteal phase and hence responded to PG injection with luteolysis and expression of oestrus within 3-4 days (Table 1, 2).

In cows subjected to AI + GnRH treatment, the mean plasma progesterone levels on day of AI and day 21 post-AI were 0.44±0.08 and 3.40±1.54 ng/ml, respectively. In normal cyclic group, the corresponding P_4 concentrations were 0.32±0.08 and 5.55±1.76 ng/ml, respectively (Table 2). The mean plasma P_4 levels on day 21 post-AI were found to be significantly (P<0.01) higher in conceived than non-conceived cows in both PG treated (9.75±2.63 vs. 0.69±0.11 ng/ml) and normal cyclic group (10.48±1.34 vs. 0.63±0.11 ng/ml), because of maintenance of CL and pregnancy among conceived cows and luteolysis with return to oestrus in non-conceived cows. The values on the day of AI were however almost same in all the groups. These findings corroborated with the reports of Muhammad *et al.* (2000), Willard *et al.* (2003) and Patel *et al*. (2005, 2006).

Plasma Total Cholesterol Profile:

The mean plasma total cholesterol concentrations on day of PG injection, day of induced oestrus/ AI and on day 21 post-AI in mid-cycle PG protocol did not differ significantly. However, the levels were consistently and significantly (P<0.01) higher in non-conceived than the conceived cows at all the periods including the overall pooled mean (230.25±7.74 vs. 155.20±14.40 mg/dl; Table 3). Similarly, the mean cholesterol concentrations on day of AI and day 21 post-AI in AI + GnRH treatment protocol and in normal cyclic control group were also more or less same. However, the overall mean value of cholesterol in GnRH treated conceived cows was non-significantly higher than in non-conceived cows (205.24±11.53 vs.191.91±15.61 mg/dl) and the same was the trend in normal cyclic group (219.47±22.15 vs. 192.80±16.79 mg/dl). Further, the levels of plasma total

cholesterol were almost same in normal cyclic control and repeat breeding (GnRH treated) groups. The levels neither differed significant between conceived and non-conceived groups nor between sampling days. These findings are in accordance with the reports of Srivastava and Sahni (2000), Ahmad *et al*. (2004) and Rajesh Kumar *et al.* (2009).

Table 3: Plasma total cholesterol 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	160.71±24.70	148.91±30.82	155.98±26.41	155.20±14.40
	Non-conc	6	226.38*±15.53	237.00*±14.00	227.37*±12.64	230.25**±7.74
	Overall	10	200.11±16.63	201.77±19.97	198.81±16.80	200.23±9.96
AI + GnRH	Conceived	3	--	201.55±15.57	208.93±20.21	205.24±11.53
	Non-conc	7	--	191.91±22.84	191.90±22.84	191.91±15.61
	Overall	10	--	194.80±16.37	197.01±16.66	195.91±11.37
Normal Cyclic Control	Conceived	5	--	218.48±36.08	220.41±30.09	219.47±22.15
	Non-conc	5	--	200.08±25.17	185.51±24.68	192.80±16.79
	Overall	10	--	209.28±20.96	202.96±19.25	206.12±13.87

*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 Total Protein:

The mean plasma total protein levels on day of PG injection, day of induced oestrus/AI and on day 21 post-AI in mid-cycle PG protocol and even in treated conceived and non-conceived cows were practically similar (12.15±1.50 to 12.65±0.29 g/dl). The mean total protein values on day of AI and day 21 post-AI in repeat breeding cows of AI + GnRH group were 11.82±0.27 and 12.03±0.36 g/dl, respectively, and the values for conceived and non-conceived animals were 12.29±0.51 and 11.76±0.23 g/dl, respectively. The corresponding values for normal cyclic group were 12.33±0.20 and 12.77±0.33 g/dl, and 12.35±0.36 and 12.76±0.15, respectively. Moreover, the plasma protein levels were not different between normal cyclic and repeat breeding cows. The values neither varied significant between days nor between conceived and non-conceived animals. These findings resembled with the reports of Srivastava and Sahni (2000), Patel *et al.* (2005, 2006), Dhami *et al.* (2007) and Rajesh Kumar *et al.* (2009). The present mean values of plasma protein in repeat breeding and normal cyclic animals were however relatively higher than those reported by some of the above researchers.

It is thus, inferred that both the treatment protocols improved conception rates and plasma P_4 profile in repeat breeding cows, though the plasma protein and cholesterol profile was not influenced significantly by these therapies.

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