INFLUENCE OF MID-CYCLE PG THERAPY ON FERTILITY AND PLASMA PROFILE OF PROGESTERONE AND BIOCHEMICAL CONSTITUENTS IN REPEAT BREEDING COWS AND BUFFALOES

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ABSTRACT

A study was conducted under field conditions in Amul and Panchamrut milk-shed areas of Gujarat on 30 postpartum cows and buffaloes each of average body condition score without any visible or palpable genital abnormalities. These comprised of 20 repeat breeding (10 PG treated and 10 untreated control) and 10 normal cyclic control animals in each class. The aim was to evaluate and compare the fertility response as well as plasma progesterone and biochemical profile of repeat breeders to i/m injection of $PGF_2\alpha$ 25 mg at mid-cycle with fix timed AI (FTAI) twice 72 and 96 hrs later (Gr-I), with those of untreated repeat breeder (Gr-II) and normal cyclic (Gr-III) controls. The oestrus induction response obtained with mid-cycle PG treatment in both cows and buffaloes was 100 % within mean intervals of 53.79±2.18 and 61.16±5.21 h, respectively, from the day of PG injection. The conception rates at induced/first oestrus and overall of 3 cycles in cows were 50 and 70%, and in buffaloes 60 and 70.00 %, respectively. The corresponding first service and overall conception rates in untreated repeat breeder cows were 20 and 30 % and buffaloes 20 and 40 %, respectively, and in normal cyclic control cows 50 and 80% and in buffaloes 40 and 70%. The repeat breeding cows and buffaloes had significantly ($P<0.01$) higher plasma progesterone (4.73±0.37 and 4.43±0.43 ng/ml) at the time of $PGF_2\alpha$ injection suggesting that they all had mid-cycle functional CL on the ovary. This PG injection caused rapid luteolysis and drastic ($P<0.01$) reduction in plasma progesterone concentrations within next 72 hrs when FTAI was done. The levels further rose significantly by day 21 post-AI due to establishment of pregnancy in 50-60 per cent of treated animals. The levels of plasma protein, cholesterol, calcium and phosphorus were not influenced by treatment or reproductive status of animals, except that among cows the minerals were significantly higher in normal cyclic than repeat breeders. Thus, the results with mid-cycle PG protocol in repeat breeding cows and buffaloes were better or at par with normal cyclic controls (50-80%), as against only 20-40% in untreated repeat breeding control groups. Hence, the mid-cycle $PGF_2\alpha$ treatment protocol can be used in repeat breeding cows and buffaloes under field conditions, provided animals have a palpable CL at the time of PG treatment and are not bred during previous cycle.

KEY WORDS : Repeat breeding, Cow, Buffalo, $PGF_2\alpha$ treatment, Conception rate, Progesterone profile.

INTRODUCTION

High reproductive performance in dairy animals is an essential requirement to ensure maximum livestock production and satisfactory economic return (Baruselli *et al.,* 2012). However, repeat breeding syndrome in dairy animals causes heavy economic loss to the dairy farmers (Modi *et al*., 2011). There are apparently several reasons for the repeat breeder syndrome, such as oestrus detection errors, endocrine dysfunctions, ovulatory defects, uterine infection, poor gamete quality

etc, and thereby poor fertilization rates and/or early embryonic deaths. No single treatment is likely to alleviate the condition in every herd or animal, but the hormonal therapies give good results in classical repeat breeders in absence of other causes of conception failure. Therefore, many field veterinarians are now adopting several innovative management technologies in routine farm practices to optimize and maintain a high degree of breeding efficiency in dairy herds. $PGF_2\alpha$ and its analogues have a luteolytic effect between day 5 and 17 (luteal phase) of bovine estrous cycle (Rowson *et al*., 1972), and by employing these agents subsequent endocrine events closely resembles those of the normal cycle. Prostaglandins were also reported to increase the conception rate in repeat breeder bovines (Goley and Kadu, 1995; Savalia *et al.,* 2013; Patel *et al.,* 2014; Dhami *et al*., 2015). The present study was therefore planned to evaluate how the mid-cycle $PGF_2\alpha$ treatment influences the conception rate and plasma profile of progesterone and biochemical constituents in repeat breeding cows and buffaloes under field conditions.

MATERIAL AND METHODS

The study was carried out in villages of Amul and Panchamrut milk-shed areas of Gujarat during November 2013 to February 2014. The animals owned by farmers were selected through organizing sexual health control camps. The animals having apparently healthy mucus discharge with regular cyclicity and bred 3 or more times previously at regular intervals with good quality frozen-thawed semen, yet failed to conceive were screened gynaeco-clinically for their reproductive status and genital health. In all 30 pluriparous postpartum cows and buffaloes each of average body condition score; 20 repeat breeders and 10 normal cyclic control cows and buffaloes each were included in the study. These were regularly followed for a period of at least 3 cycles, if not settled. The animals in spontaneous or induced oestrus were inseminated using good quality frozen-thawed semen by the trained inseminators of the concerned Milk Unions together with i/m injection of 3.0 g enrofloxacin (Inj. Flobac 30 ml, Intas Pharma) to check invisible genital infection, if any. All the selected animals were also dewormed by administering ivermectin 100 mg s/c (Inj. Ivectin 10 ml, IIL). The owners of the ear-marked animals were supplied with multi-minerals bolus (Minotas, Intas Pharma) for supplementing to their animals for 7 days. Problem breeders were confirmed by rectal palpation twice 10 days apart, and were subjected to the following therapeutic regime.

Ten repeat breeding crossbred cows and buffaloes each (Gr.-I) 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, Pfizer Animal Health) and fix timed AI (FTAI) was done twice at 72 and 96 hours later. The other 10 repeat breeding cows and buffaloes (Gr.-II) with clear standing oestrus were inseminated and followed for next three cycles without $PGF_2\alpha$ therapy as repeat breeder controls. Moreover, 10 cows and buffaloes (Gr.-III) detected in oestrus first time spontaneously within 90 days postpartum and inseminated using good quality frozen–thawed semen without $PGF_2\alpha$ treatment, served as normal cyclic/fertile controls. Blood samples were collected from all these animals on the day of PG injection, on the day of induced/spontaneous oestrus and on day 21-post-AI for estimation of plasma progesterone by RIA techniques (Kubasic *et al.,* 1984) and biochemical constituents like total protein, total cholesterol, calcium and phosphorus using standard procedures and assay kits on an autoanalyzer.

Animals in all the groups once inseminated were followed for recurrence of oestrus and in non-return cases pregnancy was confirmed per rectum 60 days post-AI. Oestrus response and conception rate as well as interval from PG treatment to induced oestrus were calculated and compared between groups. The plasma profiles of progesterone and various biochemical constituents studied were analyzed statistically using ANOVA and critical difference test.

RESULTS AND DISCUSSION

Effect of Mid-Cycle $PGF_2\alpha$ Treatment:

Out of 10 repeat breeding crossbred cows and buffaloes each treated with mid-cycle $PGF_2\alpha$ injection, 100 per cent animals responded with behavioural oestrus within mean intervals of 53.79±2.18 and 61.16±5.21 h, respectively, due to rapid luteolysis, with more than 50 % expressing prominent signs. These findings were also authenticated and correlated with the plasma progesterone profile evaluated by RIA in the present study (Table 2) and from earlier studies (Savalia *et al.,* 2013; Patel *et al.,* 2014). The nature of oestrus induced with mid-cycle PG injection was almost similar to that in normal cyclic cows and buffaloes.

A comparable oestrus response of 85 to 95 % has been documented following mid cycle PG injection in cows and buffaloes by Totewad *et al.* (2007), Savalia *et al.* (2013) and Patel *et al.* (2014), while lower oestrus response of around 65 % (Sathiamoorthy *et al.,* 2007; Patel *et al.,* 2009) was found by others. Prostaglandin $F_2\alpha$ (25 to 30 mg) and its analogues (500 µg) induce luteolysis and were found to induce ovulatory oestrus and improve reproductive efficiency in subfertile cows and buffaloes (Rao and Rao, 1979; Khasatiya *et al.*, 2008; Patel *et al.*, 2014). The luteolytic action is most potent between day 5 and 17 of the bovine cycle and most of the animal show ovulatory oestrus within 3-4 days of treatment (Rowson *et al.,* 1972). The oestrus induction intervals recorded following mid-cycle $PGF_2\alpha$ injection in present study were comparable to those reported earlier ranging from 54.4±7.6 to 71.33±6.38 h in suboestrus and/or repeat breeding bovines by Sathiamoorthy *et al.* (2007), Savalia *et al.* (2013) and Patel *et al.* (2014), while delayed oestrus response between 3 and 4 days has been noted by others (Totewad *et al.,* 2007).

The conception rates in cows treated with mid-cycle $PGF_2\alpha$ injection were 50, 20, 25 and 70 per cent at induced/first oestrus, second, third cycle and overall of 3 cycles, respectively. The corresponding figures in buffaloes were 60, 25, 00 and 70 per cent. These results were closely comparable to the conception rates obtained in normal cyclic controls in the respective species (Table 1), and were achieved within mean intervals of 18 to 22 days from PG injection among total conceived animals. This 50-60 % first service conception rate found following PG induced oestrus in the present study corroborated well with earlier reports of Rao and Rao (1979), Sathiamoorthy *et al.* (2007) and Dhami *et al.* (2015). Similarly, 70 % overall conception rates obtained in repeat breeding cows and buffaloes with PG treatment compared favourably with reports of Patel *et al.* (2014) and Patel *et al.* (2009). In contrast to these, Khasatiya *et al.* (2008) observed 100 per cent overall conception rate among PG treated suboestrus Surti buffaloes. The beneficial effect of mid-cycle PG injection could be due to better synchrony of endocrine events leading to timely ovulation and strengthening of luteal function in repeat breeding crossbred cows and buffaloes.

The variations observed in oestrus induction response and fertility with PG injection in different studies could be due to differences in the stage of oestrus/estrous cycle at the time of treatment, product potency, oestrus detection efficiency, nutritional status, general and genital health, breeding time, quality of semen used, season/climate, and luteal insufficiency leading to embryonic mortality etc. 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 infection free repeat breeding conditions in cows and buffaloes, since these drugs induce ovulatory oestrus following luteolysis.

Effect PG Treatment on Plasma progesterone Profile

Significantly ($P<0.01$) higher mean plasma progesterone (4.73±0.37 and 4.43±0.43 ng/ml) concentrations observed among repeat breeding cows and buffaloes on the day of mid-cycle $PGF_2\alpha$ injection suggested that they all had functional CL on the ovary at that time. This PG injection caused rapid luteolysis and drastic ($P<0.01$) reduction in plasma progesterone concentrations within next

Table 1: Effects of mid-cycle PG treatment on estrus induction and conception rate in repeat breeding cows and buffaloes

Animal Species	Reprod-uctive Status	Treatment Protocols	No.	PG Inj. to Oestrus Interval (hrs)	PG Inj. to Fertile Oestrus Interval (days)	Conception Rate (%)			
						Induced/ First Oestrus	Second Cycle	Third Cycle	Overall of 3 Cycles
Cross-bred Cows	Repeat Breeder	Mid-cycle PG Inj.	10	53.79±2.2 (n=10)	132.91 ±4.4*	50.00 (5/10)	20.00 (1/5)	25.00 (1/4)	70.00 (7/10)
		Untreated Control	10	--	187.57 ±12.8*	20.00 (2/10)	13.75 (1/8)	00.00 (0/7)	30.00 (3/10)
	Normal Cyclic	Cyclic Control	10	--	98.77 ±6.84*	50.00 (5/10)	40.00 (2/5)	33.33 (1/3)	80.00 (8/10)
Buffaloes	Repeat Breeder	Mid-cycle PG Inj.	10	61.16±5.2 (n=10)	127.26 ±5.4*	60.00 (6/10)	25.00 (1/4)	00.00 (0/3)	70.00 (7/10)
		Untreated Control	10	--	168.53 ±11.6*	20.00 (2/10)	13.75 (1/8)	14.28 (1/7)	40.00 (4/10)
	Normal Cyclic	Cyclic Control	10	--	105.67 ±7.44*	40.00 (4/10)	33.33 (2/6)	25.00 (1/4)	70.00 (7/10)

*Service period for overall conceived animals.

Table 2. Plasma progesterone concentrations (ng/ml) in normal cyclic and in repeat breeding cows and buffaloes on different days of PG treatment/AI

Animal species	Reprod-uctive Status	Treatment protocol	Status	No.	Days from treatment/AI			
					Day of PG treatment	Day of AI	Day 21 Post-AI	Overall
Cross-bred cows	Repeat Breeder	Mid-cycle PG Injection	Conceived	5	4.66±0.50	0.71±0.43	6.09±0.61	3.82±0.67
			NC	5	4.79±0.60	0.55±0.14	4.27±0.57	3.20±0.57
			Overall	**10**	**4.73±0.37^{b}**	**0.63±0.22^{a}**	**5.18±0.50^{b}**	**3.51±0.43^{y}**
	Normal Cyclic	Control	Conceived	4	--	0.57±0.34	5.39±0.67	2.68±0.83
			NC	6	--	0.35±0.23	2.73±0.37	1.57±0.61
			Overall	**10**	--	**0.43±0.17^{a}**	**4.68±0.45^{b}**	**2.56±0.36^{xy}**
Buffaloes	Repeat Breeder	Mid-cycle PG Injection	Conceived	6	4.40±0.65	0.51±0.10	5.26±0.56	3.39±0.57
			NC	4	4.48±0.55	0.62±0.09	3.03±1.20	2.71±0.62
			Overall	**10**	**4.43±0.43^{b}**	**0.55±0.07^{a}**	**4.37±0.66^{b}**	**3.12±0.42^{x}**
	Normal Cyclic	Control	Conceived	4	--	0.16±0.03	3.86±0.47^{x}	2.01±0.73
			NC	6	--	0.61±0.18	1.18±0.52^{y}	0.90±0.28
			Overall	**10**	--	**0.43±0.13^{a}**	**2.26±0.56^{b}**	**1.34±0.35^{y}**

NC=non-conceived; Means bearing uncommon superscripts within the row (a,b,c) and column (x, y) differ significantly (P<0.05).

72 hrs when FTAI was done. The levels further rose significantly by day 21 post-AI due to establishment of pregnancy in 50-60 per cent of treated animals (Table 1, 2). The levels of plasma P_4 thus on the day of beginning of treatment protocol helped delineate the reproductive and endocrine status of the animals and thereby predicting the possible response to the therapy. One or two non-conceived repeat breeding cows and buffaloes had plasma P_4 levels between 4 and 7 ng/ml on day 21 post-AI suggesting long cycle and/or EED later on, and thus making the difference in P_4 between conceived and non-conceived groups insignificant (Table 1). Gustafsson *et al.* (1986) opined that hormonal asynchrony starts before or early in oestrus presumably leading to improper hormonal sequential changes responsible for the elevated embryonic loss during the first 3 weeks after AI in repeat breeding animals. The present findings corroborated well with those of Patel *et al.* (2014) in repeat breeding cows using similar treatment protocols. Kumar *et al.* (2009), Butani *et al.* (2011) and Savalia *et al.* (2013) reported comparable serum progesterone values at the time of oestrus in repeat breeding and normal cyclic cows and buffaloes.

Effect PG Treatment on Plasma Biochemical Constituents

The levels of plasma total protein, total cholesterol, calcium and inorganic phosphorus were neither influenced by treatment nor by the reproductive status of animals, except that among cows the minerals were significantly higher in normal cyclic than repeat breeders. Moreover, the cholesterol values in all groups of cows were almost double than those in buffaloes suggestive of species variation (Table 3). Patel *et al.* (2005) reported non-significantly lower values of serum cholesterol

Table 3. Mean values of plasma protein, cholesterol, calcium and phosphorus concentrations in repeat breeders (PG treated) and normal cyclic cows and buffaloes

Animal species	Reproductive Status	Treatment protocol	Status	No.	Plasma profile			
					Total Cholesterol (mg/dl)	Total Protein (g/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)
Cross-bred cows	Repeat Breeder	Mid-cycle PG Injection	Concd	5	130.96±5.55	10.83±0.16	9.08±0.14	3.63±0.07
			NC	5	129.73±2.63	10.99±0.14	9.14±0.15	3.82±0.06
			Overall	**10**	**130.34±3.01**	**10.91±0.10**	**9.11±0.10**	**3.72±0.05**
	Normal Cyclic	Control	Concd	4	134.14±8.47	10.78±0.18	9.28±0.26	4.75±0.31
			NC	6	139.26±4.54	10.97±0.21	9.62±0.30	4.23±0.29
			Overall	**10**	**137.78±4.36**	**10.61±0.14**	**9.42±0.24**	**4.43±0.21**
Buffaloes	Repeat Breeder	Mid-cycle PG Injection	Concd	6	58.34±2.19	9.03±0.18	8.30±0.17	4.02±0.15
			NC	4	63.42±1.79	8.76±0.20	8.41±0.15	3.62±0.11
			Overall	**10**	**60.37±1.55**	**8.92±0.14**	**8.34±0.12[a]**	**3.86±0.11[a]**
	Normal Cyclic	Control	Concd	4	62.93±3.90	8.44±0.35	9.37±0.33	5.14±0.21
			NC	6	66.26±4.10	8.96±0.16	9.13±0.22	5.11±0.13
			Overall	**10**	**64.93±2.87**	**8.75±0.18**	**9.22±0.18[b]**	**5.12±0.11[b]**

NC=non-conceived;

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

in conceived than non-conceived cows. Savalia *et al.* (2013) recorded significantly (P<0.05) lower plasma total cholesterol concentrations in conceived than non-conceived buffaloes consistently over the periods studied indirectly reflected its role in steroidegenesis, particularly progesterone synthesis in conceiving buffaloes. Kapadiya and Siddiquee (2013) documented significantly higher plasma cholesterol and protein concentration on day of oestrus as compared to day 12 of estrous cycle in repeat breeding Mehsana buffaloes. Further, the insignificant variations noted in plasma protein and cholesterol of repeat breeding and normal cyclic cows and buffaloes under study however contradicted the previous significant differences recorded by Akhtar *et al.* (2010), Butani *et al.* (2011) and Patel *et al.* (2014). Lodhi *et al.* (1998) opined that the Murrah buffaloes having high level of total protein had good reproductive performance. However, Gentile *et al.* (1978) opined that serum protein level was not related with fertility in dairy cows. Kumar *et al.* (2009) and Butani *et al.* (2011) found significantly higher serum total protein concentrations in cycling as compared to repeat breeding cows and buffaloes. Patel *et al.* (2005) reported the mean plasma calcium level to be significantly lower in $PGF_2\alpha$ treated repeat breeder HF cows as compared to untreated control group. Patel *et al.* (2009) reported the mean values of plasma calcium for postpartum buffaloes under $PGF_2\alpha$ treatment and control group to be 9.98 ± 0.04 and 9.17 ± 0.04 mg/dl (P<0.05), that is somewhat similar to the trend in present findings. Kumar *et al.* (2009) and Butani *et al.* (2011) reported serum calcium and phophorus levels to be non-significantly lower in repat breeding than normal cycling cows and buffaloes, which is in contrast to the present findings.

CONCLUSION

The conception rates obtained in the present study in repeat breeding cows and buffaloes using $PGF_2\alpha$ injection were better or at par with normal cyclic control group, due to ovulatory oestrus induced by PG therapy in a pre-determined time when fixed time insemination was performed, though it had no influence on plasma biochemical and mineral profile. 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 cows and buffaloes under field conditions.

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