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EFFECT OF DIFFERENT HORMONAL PROTOCOLS ON FERTILITY, PLASMA PROGESTERONE AND BIOCHEMICAL PROFILE IN CONCEIVING AND NON-CONCEIVING INFERTILE BUFFALOES IN TRIBAL AREA OF DAHOD DISTRICT IN GUJARAT

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Abstract: The experiment was carried out in milk shed area of Panchamrut dairy on 22 postpartum buffaloes, 15 repeat breeding and 7 normal cyclic under field condition with to evaluate their response to Mid-cycle $PGF_2\alpha$ and AI + GnRH by monitoring estrus induction rate, conception rate and Plasma progesterone profile following time interval post-treatment/AI. Cent percent buffaloes responded within 78.25 ± 2.40 hrs to Mid-cycle $PGF_2\alpha$. The first service and overall conception rate of 3 cycles were 37.50 and 62.50 % in Mid-cycle $PGF_2\alpha$; 28.57 and 57.14 % in AI + GnRH; and 42.85 and 71.42 % in normal cyclic control group. The variation in progesterone levels between conceived and non-conceived buffaloes was also significant ($P < 0.01$) on 21 Post-AI in Mid-cycle $PGF_2\alpha$. There were no significant differences in plasma total cholesterol profile between days/periods of the treatment with Mid-cycle $PGF_2\alpha$ and AI + GnRH protocols or even in normal cyclic group or animals. The values were found to be higher in conceived than non-conceived buffaloes of Mid-cycle $PGF_2\alpha$ group of buffaloes (78.28 ± 16.08 vs. 59.20 ± 4.52 mg/dl), while higher in non-conceived as compared to conceived buffaloes of AI + GnRH treated buffaloes (59.43 ± 5.00 vs. 55.13 ± 0.64 mg/dl) on day '21' post-AI whereas in control animals no statistically significant differences were found between conceived and non-conceived group (58.42 ± 8.28 vs. 59.78 ± 8.02 mg/dl) on day '21' post-AI. The total protein concentrations in conceived and non-conceived buffaloes under Mid-cycle $PGF_2\alpha$ and AI + GnRH protocols were inverse and insignificant while in normal cyclic control buffalo's non-significant difference was found. In general, both the protocols improve conception rates and plasma progesterone level in repeat breeding buffaloes, though there was no significant influence on plasma protein and cholesterol profile.

Keywords: Mid-cycle $PGF_2\alpha$, AI + GnRH, Plasma progesterone, biochemical profile.

INTRODUCTION

The buffaloes are considered superior to crossbred cows with respect to milk production and feed conversion efficiency. Efficient dairying and breeding demand that an animal shall give

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birth to a healthy calf every year and be in milk for at least 300 days. Efforts should therefore be made to enhance fertility in dairy animals by narrowing their dry period to the barest minimum of 60 to 90 days. Thus, fertility of milch animals appears to play a major role in dairy farm economics. The productivity of buffaloes, however, remains low largely due to poor management of health, nutrition and breeding (Rane *et al*., 2003).

One of the most important and commonly encountered sub-fertile conditions in buffalo which plays a vital role in dairy farm economics is repeat breeding. The condition may occur due to defects in gametes, failure of gametic encounters, endocrine dysfunction, nutritional defects etc., which ultimately leads to either fertilization failure or early embryonic death. To overcome this inconvenient phenomenon in dairy farms GnRH and prostaglandin F2α (PGF2α) have been used in different combinations to improve the reproductive efficiency in lactating dairy cows and buffaloes (Hegazy, 2001). GnRH administered at the time of insemination and after the insemination may bring the pre-ovulatory LH peak back again and synchronize ovulation with insemination.

MATERIAL AND METHODS

The study was undertaken on 15 repeat breeding buffaloes and 7 normal cyclic buffaloes kept as control in different villages of Dahod district. Repeat breeding buffaloes without any visible or palpable genital abnormalities were initially treated with 20% Enrofloxacin (Inj. Conflox @ 15 ml, Concept Pharmaceuticals Ltd.) to check invisible infection, if any. They were divided into two subgroups at random and treated as under.

Mid-cycle $PGF_2\alpha$ group: This protocol consists of only one injection of $PGF_2\alpha$: Pragma, 2.0 ml, (Intas Pharmaceuticals Ltd., Ahmedabad). Blood samples were collected from the buffaloes for estimation of plasma progesterone levels on day 0, day 3 i.e. the day of AI and finally on day 21 post-insemination.

AI + GnRH group: This protocol consist of only one injection GnRH. Blood samples were collected from the buffaloes for estimation of plasma progesterone levels on day 0, along with AI and finally on day 21 post-insemination.

Normal Cyclic Control group: Normal cyclic animals were inseminated on the day of detection of oestrus.

Blood samples were collected from jugular vein in heparinized vaccutainers twice or thrice depending upon treatment protocols from the buffaloes for estimation of plasma progesterone and biochemical profile on day 0, on day 3 (i.e. the day of AI) and finally on day 21 post-insemination. While from normal cyclic animals blood samples were collected on the day of

estrus and 21 days post-AI. Plasma progesterone concentration was estimated by employing standard RIA technique of Kubasic *et al.* (1984). Labelled antigen of assay was 0.1 ng/ml. plasma total cholesterol was estimated by CHOD/PAP method and total protein by Biuret method using standard procedure and assay kits procured from Crest Bio-system, Goa, with the help of chemistry Analyzer (Mindray, BS 120).

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

RESULTS AND DISCUSSION

Table 1.1 Effect of Mid-Cycle $PGF_2\alpha$ and GnRH injection at the time of AI on estrus induction and conception rate in repeat breeding Buffaloes.

Treatment Group	No. of Buff.	Per Cent estrus Response	Intensity of Estrus Signs		
			Prominent	Moderate	Weak
Mid-Cycle $PGF_2\alpha$	8	100.00 % (8/8)	50.00 % (4)	37.50 % (3)	12.5 % (1)
AI + GnRH	7	100.00 % (7/7)	42.86 % (3)	42.86 % (3)	14.28 % (1)
Normal Cyclic Control	7	100.00 % (7/7)	57.14 % (4)	42.86 % (3)	00.00 % (0)

Figures in parentheses indicate number of animals.

Table 1.2 Effect of Mid-cycle $PGF_2\alpha$ injection and GnRH injection at the time of AI on estrus induction response, PG injection to induced estrus and fertile estrus intervals and conception rates to AIs at first/treatment cycle and overall of three cycles in repeat breeding buffaloes.

Group	No. of Buff.	Per cent Estrus Response	PG Injection to Estrus Interval (hrs)	PG Injection to Fertile Estrus Interval (days)	Conception Rate (%)			
					Induced / First Cycle	Second Cycle	Third Cycle	Overall of 3 Cycles
Mid-cycle $PGF_2\alpha$	8	100.00% (8/8)	78.25±2.40 (n=8)	15.00 ± 8.01 (n=5)	37.50% (3/8)	20.00% (1/5)	25.00 % (1/4)	62.50% (5/8)
AI+GnRH	7	100.00% (7/7)	--	--	28.57% (2/7)	40.00% (2/5)	00.0% (0/3)	57.14% (4/7)
Normal Cyclic Control	7	100.00% (7/7)	--	94.00 ± 5.35* (n=5)	42.85% (3/7)	25.00% (1/4)	33.33 % (1/3)	71.42% (5/7)

Figures in parentheses indicate number of cows, *Service period

Out of 8 repeat breeding buffaloes treated with Mid-cycle $PGF_2\alpha$, 8(100%) responded with behavioural estrus within 78.25 ±2.4 hrs. Of these 4 buffaloes show prominent estrus and 3 show moderate estrus signs. Butani *et al.* (2009) and Singh *et al.* (1996) found 100.00 per cent estrus response following $PGF_2\alpha$ treatment in subestrus buffaloes and cows respectively. This is similar to the present findings (100.00 %). Comparably slightly lower estrus response of 83.33 per cent has been reported following mid-cycle $PGF_2\alpha$ injection by Dhami *et al.* (2006) and Khasatiya *et al*. (2008). While lower estrus response of 62.00 per cent reported by Sathiamoorty *et al*. (2008).

In the $PGF_2\alpha$ treated (mid-cycle) group 37.50 per cent conception rate found following PG induced estrus was lower than the findings of Gupta *et al.* (2008) in buffaloes (53.00%). Similarly the overall conception rate of 62.5 % observed in this group compared favourably with 64.3 % reported by Gupta *et al.* (2008). Others however reported lower conception rate of 40.00 and 45.10 per cent in subestrus buffaloes in similar trials Butani *et al.* (2009) and Sathiamoorty *et al*. (2008). Dhami *et al.* (2006), Khasatiya *et al*. (2008) observed 100.00 per cent conception rates and Singh *et al.* (1996) observe 83.33 per cent overall conception rates in postpartum, subestrus buffaloes and cows which is contrary to the present findings. Further, 28.57 per cent first service conception rate obtained with GnRH treatment at the time of AI in repeat breeder buffaloes was lower than the earlier reports of around 55.00 and 52.63 percent in river buffaloes by Batavani and Eliasi (2004) and Rajesh Kumar *et al*. (2011). The present study 57.14 per cent conception rate obtained under GnRH treated animals are at par with Butani *et al.* (2009) in repeat breeder buffaloes.

Based on the results found in the present study, it could be concluded that the application of mid-cycle $PGF_2\alpha$ injection can be used as a good tool for induction of estrus as well as improvement of conception rate in repeat breeding buffaloes.

Plasma progesterone concentrations:

Table 1.3 Plasma progesterone concentrations (ng/ml) in repeat breeding buffaloes on different days of treatment/AI under various estrus induction protocols.

Treatment protocol	Status	No.	Days from treatment/AI			Overall
			D-0(T)	D-AI	D-21 post-AI	
Mid cycle PG	Conceived	3	3.63 ± 1.56	0.11 ± 0.00	3.50^{**} ± 0.56	2.41 ± 0.75
	Non-conc.	4	2.68 ± 0.93	0.11 ± 0.00	0.54 ± 0.24	1.11 ± 0.45
	Overall	7	3.09^{b} ±	0.11^{a} ±0.00	1.81^{ab} ±0.65	1.67 ± 0.42

			0.80			
AI + GnRH	Conceived	2	--	0.25 ±0.03	3.00 ± 0.60	1.63 ± 0.83
	Non-conc.	5	--	0.65 ± 0.29	1.11 ± 0.51	0.88 ±0.30
	Overall	7	--	0.54 ± 0.21	1.65 ± 0.51	1.09 ±0.31
Normal Cyclic Control	Conceived	3	--	0.11 ± 0.00	3.92^{**} ±0.66	2.01 ± 0.90
	Non-conc.	4	--	0.33 ± 0.09	0.57 ± 0.26	0.45 ± 0.13
	Overall	7	--	0.23^{a}± 0.07	2.00^{b} ± 0.73	1.12 ± 0.43

**P<0.01significantly differ 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.

The higher mean plasma progesterone concentration of 3.09 ± 0.80 ng/ml recorded on day 0, i.e day of initiation of PG treatment, suggested that the repeat breeding buffaloes selected were in true luteal phase or mid-cycle when $PGF_2\alpha$ was injected. The plasma P_4levels decreased drastically and significantly within 72 hrs of PG injection, i.e on day of induced estrus/AI, with behavioural estrus in cent per cent of buffaloes proving successful luteolysis with a single dose of 25 mg $PGF_2\alpha$. The mean plasma P_4 levels (ng/ml) on day 21 post-AI was found to be significantly (P<0.01) higher in conceived than non-conceived buffaloes (3.50 ± 0.56 vs. 0.54 ± 0.24 ng/ml). This indicated better ovulatory estrus and maintenance of luteal function due to establishment of pregnancy in former group and return to next estrus with luteolysis of CL in most buffaloes by day 21 post-estrus/AI in later group. However, no significant differences were noted on initiation of treatment and at induced estrus between conceived and non-conceived buffaloes.

The levels were significantly (P<0.05) higher on day 21 post-AI as compared to day of estrus/AI in all the groups. The results of plasma progesterone in AI + GnRH group suggested that the animals when inseminated and simultaneously treated with GnRH were in true estrus with basal level of plasma progesterone (0.54 ± 0.21 ng/ml). Further relatively higher plasma P_4 noted on day 21 post-AI in non-conceived group (1.65 ± 0.51 ng/ml) indicated that these animals returned to next estrus at varying and delayed interval probably due to prolonged luteal activity, long cycle or early embryonic mortality beyond day 17. However, it was not studied with extended period of P_4 assay or other alternative methods like ultrasonography.

In normal cyclic control group, the mean progesterone concentration on day of AI and day 21 post-AI were 0.11 ± 0.00 and 3.92 ± 0.66 ng/ml for conceived (n=3) animals and 0.33 ± 0.09

and 0.57 ± 0.26 ng/ml for non-conceived (n=4) buffaloes, with overall means of 0.23 ± 0.07 and 2.00 ± 0.73 ng/ml ($P<0.05$). The P_4 values were identical on day of estrus /AI in conceived and non-conceived buffaloes (0.11 ± 0.00 vs. 0.33 ± 0.09) but on day 21 post-AI, it was significantly ($P<0.05$) higher for conceived than non-conceived buffaloes.

The present findings agreed well with the findings of Kavani *et al.* (2007) who reported weekly significantly higher P_4 levels ($P<0.01$) in fertile (n=10) than the infertile (n=12) cycles on day 21 (3.70 ± 0.28 vs. 0.36 ± 0.08 ng/ml), but not at oestrus (0.32 ± 0.07 vs. 0.24 ± 0.05 ng/ml). Bugalia and Sharma (1990) also observed basal plasma progesterone concentration at the time of insemination in repeat breeding cows (0.35 ± 0.5 ng/ml), as has been noted in the present study on repeat breeding buffaloes under $PGF_2\alpha$ and AI + GnRH protocols. Butani *et al.* (2011) reported serum progesterone values at the time of estrus as 0.45 ± 0.05 and 0.36 ± 0.08 ng/ml, respectively, in repeat breeding and normal cyclic buffaloes, which is in line with present findings in repeat breeding and normal cyclic control group.

Plasma Total Cholesterol: The review of data presented in Table 1.4 reveal that there were no significant differences in the plasma total cholesterol profiles between days/periods of the treatment with $PGF_2\alpha$ and AI + GnRH protocols or even in normal cyclic group, but the values were non-significantly higher in conceived than non-conceived buffaloes of $PGF_2\alpha$ (81.04 ± 6.17 vs. 62.70 ± 2.66 mg/dl) group. While in AI + GnRH and control group no significant differences were observed. Similarly, the overall pooled mean value of total cholesterol obtained in repeat breeder animals under $PGF_2\alpha$ treated group was higher (70.56 ± 3.59 mg/dl) than the pooled value recorded in AI + GnRH (57.60 ± 2.76 mg/dl) and normal cyclic buffaloes (60.61 ± 3.28 mg/dl).

Shrivastava and Kharche (1986) found serum cholesterol levels of 59.00 and 61.53 mg per cent in normal cycling and repeat breeder Murrah buffaloes. These results were in close agreement with the present study. In normal cyclic buffaloes (60.61 ± 3.28 mg/dl) and repeat breeding buffaloes treated with $PGF_2\alpha$ the levels were 70.56 ± 3.59 mg/dl, and (AI + GnRH) 57.60 ± 2.76 mg/dl. In contrary to present findings, Panchal (1995) reported overall higher mean plasma total cholesterol levels in repeat breeding buffaloes.

Sharma *et al.,* (2004) reported that in repeat breeding buffaloes the mean plasma cholesterol concentration was found to be higher on day of estrus in the control as well as progesterone supplemented groups. The concentration was found to be significantly lower on day 16 and 20 as compared to day-0. Singh *et al.,* (2004) reported significantly lower serum cholesterol

values in repeat breeder (196.64 ± 6.10 mg/dl) as compared to normal cyclic buffaloes (237.08 ± 4.99 mg/dl) which were higher than the present findings in repeat breeder (70.56 ± 3.59 and 57.60 ± 2.76 mg/dl) and normal cyclic (60.61 ± 3.28 mg/dl) buffaloes.

Table 1.4 Plasma total cholesterol concentrations (mg/dl) in repeat breeding buffaloes on different days of treatment/AI under various estrus induction protocols.

Treatment protocol	Status	No.	Days from treatment/AI			Overall
			D-0(T)	D-AI	D-21 post-AI	
Mid cycle PG	Conceived	3	82.16±8.96	82.70±10.60	78.28±16.08	81.04 ±6.17
	Non-conc	4	64.07 ±5.46	64.84 ±4.61	59.20 ± 4.52	62.70 ±2.66
	Overall	7	71.82 ± 5.77	72.50 ± 5.93	67.37 ± 7.60	70.56 ±3.59
AI + GnRH	Conceived	2	--	47.50 ± 3.68	55.13 ± 0.64	51.32 ±2.68
	Non-conc	5	--	60.81 ± 5.66	59.43 ± 5.00	60.12 ±3.50
	Overall	7	--	57.01 ± 4.68	58.20 ± 3.32	57.60 ±2.76
Normal Cyclic Control	Conceived	3	--	62.13 ± 6.38	58.42 ± 8.28	60.28 ±4.75
	Non-conc	4	--	61.95 ± 6.43	59.78 ± 8.02	60.86 ±4.80
	Overall	7	--	62.02 ± 4.2	59.2 ± 5.32	60.61 ±3.28

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

Plasma Total Protein: The review of data presented in Table 1.5 show that the total protein concentration was higher in non-conceived than conceived buffaloes in animals under $PGF_2\alpha$ (11.30 ± 0.30 vs. 11.18 ± 0.18 g/dl) and AI + GnRH (10.20 ± 0.37 vs. 9.84 ± 0.35 g/dl) protocol, while in normal cyclic control, the trend was reverse (10.52 ± 0.18 vs. 10.13 ± 0.16 g/dl). The overall mean value for normal cyclic buffaloes was 10.30 ± 0.13 g/dl, which was nearly similar to the value obtained in AI + GnRH protocol. However, no significant differences were observed in plasma total proteins profile between days/periods of the treatment or between conceived and non-conceived groups. The plasma protein profile in general was higher in repeat breeders under mid-cyclic PG protocol as compared to AI + GnRH treated even normal cyclic control group.

Gandotra *et al.,* (1993) reported that the values of total serum protein (g/100ml) in repeat breeder buffaloes (10.7 ± 0.55) did not vary significantly from that of normal buffaloes (10.8 ± 0.77), which is compatible with the present findings in AI + GnRH and normal cyclic control group (10.09 ± 0.28 vs. 10.30 ± 0.13 g/dl), but in $PGF_2\alpha$ protocol this values were

higher as compared to normal cyclic control (11.25 ± 0.18 vs. 10.30 ± 0.13g/dl). The present findings were however, in contrary to findings of Singh *et al.* (2004) and Butani *et al.* (2011). Kapadiya and Siddiquee (2013) recorded slightly higher plasma total protein value on day of estrus (6.70 ± 0.11 g/dl) as compared to day 12 (6.37 ± 0.32 g/dl) and day 24 (6.32 ± 0.09 g/dl) of estrus cycle in repeat breeding Mehsana buffaloes.

Tables 1.5 Plasma total protein concentration (g/dl) in anestrus buffaloes on different days of treatment/AI under various estrus induction protocols.

Treatment protocol	Status	No.	Days from treatment/AI			Overall
			D-0(T)	D-AI	D-21 post-AI	
Mid cycle PG	Conceived	3	11.15 ± 0.44	11.35 ±0.36	11.04 ± 0.24	11.18 ±0.18
	Non-conc	4	11.17 ± 0.82	11.42 ± 0.36	11.33 ± 0.40	11.30 ±0.30
	Overall	7	11.16 ± 0.47	11.39 ± 0.24	11.20 ± 0.24	11.25 ±0.18
AI + GnRH	Conceived	2	--	9.46 ±0.38	10.22 ± 0.24	9.84 ±0.35
	Non-conc	5	--	10.30 ± 0.67	10.09 ± 0.39	10.20 ±0.37
	Overall	7	--	10.06 ± 0.49	10.12 ± 0.29	10.09 ±0.28
Normal Cyclic Control	Conceived	3	--	10.67 ± 0.26	10.38 ± 0.28	10.52 ±0.18
	Non-conc	4	--	10.25 ± 0.25	10.01 ± 0.21	10.13 ±0.16
	Overall	7	--	10.43 ± 0.19	10.17 ± 0.17	10.30 ±0.13

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

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