

**EFFICACY OF AI +hCG AND POST-AI PROGESTERONE SUPPLEMENTATION FOR ENHANCING FERTILITY AND PLASMA PROGESTERONE AND BIOCHEMICAL PROFILE IN REPEAT BREEDING COWS AND BUFFALOES**

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**ABSTRACT**

This study was conducted to evaluate the efficacy of i/m Inj. of hCG1500 IU along with mid-estrus AI or Inj. hydroxy-progesterone caproate 500 mg on day 5<sup>th</sup> post-AI in 20 each repeat breeding cows and buffaloes in terms of conception rate and plasma progesterone and biochemical profile in comparison to normal cyclic controls. The conception rates in repeat breeding cows and buffaloes during progesterone supplemented cycle were 40 and 30 %, respectively, whereas 30 % cows and buffaloes each conceived under AI+hCG protocol and 40 % each in control groups. The overall 3 cycles' conception rates under AI+hCG groups were identical 60 % in both cows and buffaloes. The corresponding overall conception rates for cows and buffaloes under progesterone supplementation groups were 60 and 50 %, and in control groups 70 and 60 %, respectively. The mean plasma progesterone concentrations of cows and buffaloes on day of AI in all 3 groups were identical and basal. The values on day 21 post-AI in cows and buffaloes under AI+hCG protocol ( $4.23 \pm 1.87$  and  $2.89 \pm 1.18$  ng/ml), progesterone supplementation ( $2.62 \pm 1.03$  and  $2.50 \pm 0.77$  ng/ml) and normal cyclic ( $4.23 \pm 1.56$  and  $3.69 \pm 1.20$  ng/ml) group also did not vary significantly, but the conceived animals had significantly higher plasma progesterone than non-conceived animals in all groups. The plasma total protein, cholesterol, calcium and phosphorus profile neither varied significantly between days nor between conceived and non-conceived groups. In conclusion, both AI + hCG and post-AI progesterone injections, though did not influence the plasma progesterone or biochemical profile, effectively enhanced pregnancy rate in repeat breeding cows and buffaloes close to the normal cyclic control groups under field condition.

**KEYWORDS:** Crossbred cows, Buffaloes, Repeat breeding, hCG therapy, Progesterone supplementation, Conception rate, Plasma progesterone & biochemical profile

**INTRODUCTION**

Dairy farming is one of the most important agricultural activities in India. The profitable dairy farming is predominantly based on two major interrelated factors, viz., nutrition and reproduction. High reproductive performance is an essential requirement to ensure maximum livestock production and satisfactory economic return (Baruselliet *al.*, 2012). Despite technological advances in animal husbandry, numerous studies have shown that repeat breeding is still the most prevalent reproduction disorder in dairy cows and buffaloes. The overall prevalence of repeat breeding has been documented as high as 28.31 % by Bhat *et al.* (2012). This problem results in increased calving interval, decreased conception rates and decreased calf crop per animal. There are apparently several reasons for the repeat breeder syndrome and no single treatment is likely to alleviate the condition. However, GnRH/hCG and/or progesterone analogues have been successful to sustain early pregnancy and improve conception rates in repeat breeding bovines (Dhama *et al.*, 2015). Injections of GnRH/hCG at the time of AI (Dhama *et al.*, 2009, 2015) or depot preparation of progesterone (500 mg) on day 4-6 post-AI (Patel *et al.*, 2005) were found to improve fertility

significantly in apparently infection free repeat breeding cows and buffaloes. In view of the above fact, the present study was aimed to evaluate the relative efficacies of AI + hCG and post-AI progesterone supplementation in repeat breeding crossbred cows and buffaloes in terms of conception rate and plasmas progesterone and biochemical profile.

## MATERIALS AND METHODS

This study was conducted on 40 repeat breeding dairy animals (20 crossbred cows and 20 buffaloes) selected from the villages of Amul as well as Panchamrut milk shed areas of Gujarat from November 2014 to April 2015. Before the start of experiment, palpation of the reproductive tract per rectum was carried out and specific animals that had been bred three or more times and yet failed to conceive were diagnosed as repeat breeders.

All identified animals were treated once (on the day of diagnosis/first AI) with s/c injection of ivermectin 100 mg (Inj. Neomec, 1 %, 10 ml, Intas pharma) and i/m injection of single shot enrofloxacin 3.0 g (Inj. Flobac SA, 10 %, 30 ml, Intas pharma), inorganic phosphorus (Inj. Alphos-40, 10 ml, Zoetis, Mumbai) and multivitamins AD3E (Inj. Intavita, 10 ml, Intas Pharma), and were supplied with four multi-mineral boli (Garbhamin, Indian Immunologicals Ltd.) for PO use, one bolus on alternate day. The repeat breeder cows and buffaloes were randomly allotted to the following two treatment protocols.

**Group-I (AI+hCG):** Ten repeat breeding cows and buffaloes each in standing estrus were administered with i/m Inj. of hCG 1500 IU (Chorulon, 1500 IU, Intervet, MSD) simultaneous AI with good quality frozen thawed semen.

**Group-II (Post-AI Progesterone):** Ten repeat breeder cows and buffaloes each in standing estrus were inseminated with good quality semen and were administered with i/m inj. of hydroxyl progesterone 500 mg (Duraprogen 2.0 ml, Vet Care) on day 5 post-AI.

**Group-III (Normal control):** Ten cows and buffaloes detected in first estrous within three months postpartum and inseminated without hormone therapy served as control.

All the inseminated animals were followed for recurrence of estrus and rebreeding for next two cycles and pregnancy was confirmed per rectum in non-return cases 60 days post-AI. Jugular blood samples were collected twice, i.e. on day of spontaneous oestrus/AI and on day 21 post-AI, from 8 animals in each group for assay of plasma P<sub>4</sub> profile by RIA technique, and estimation of plasma protein, cholesterol, calcium and phosphorus using standard procedures and assay kits of Crest Biosystem, Goa on semi-autoanalyzer. The data were analyzed standard statistical procedure using Chi-square test, CRD and 't' test to compare variations within and between groups for each trait.

## RESULTS AND DISCUSSION

### Fertility Response to hCG/Progesterone Therapy

Identical conception rates of 30.00, 28.57, 20.00 and 60.00 % were obtained in both repeat breeding cows and buffaloes at AI+hCG treated estrus, post-treatment I and II estrus and overall of 3 cycles, respectively. The conception rates obtained during progesterone depot treatment cycle, post-treatment I and II cycle and overall of 3 cycles were 40.00, 16.66, 20.00 and 60.00 %, in cows and 30.00, 14.28, 16.66 and 50.00 % in buffaloes, respectively. In normal cyclic control group of cows, the conception rates at first, second, third estrus and overall of 3 cycles were 40.00, 33.33, 25.00 and 70.00 %, and in buffaloes 40.00, 16.66, 20.00 and 60.00 %, respectively. The improvement in fertility with two hormonal treatments was more or less statistically similar and to the extent of normal cyclic control groups in both cattle and buffaloes (Table 1).

The first service and overall conceptions obtained in crossbred cows under hCG treatment at AI corroborated with the reports of Ranganekar *et al.* (2002), Kumar *et al.* (2009) and Parmaret *al.* (2014) with the use of GnRH at AI. The present 50 % overall conception rate obtained in hCG treated

buffaloes closely concurred with the previous reports (Butani *et al.*, 2009; Savalia *et al.*, 2014; Dhama *et al.*, 2015) in repeat breeders. The present pregnancy rates with hCG in repeat breeder cows and buffaloes were however higher than 42 % obtained by others (Sianangama and Rajamahedran, 1992), but lower than 76 to 84 % reported by Ingawale *et al.* (2002) and Parmaret *et al.* (2014). The hCG has an established beneficial effect on fertilization, corpus luteum development, implantation of embryo and its survival.

**Table 1: Conception rates with different treatment protocols in repeat breeding cows and buffaloes and normal cyclic control groups**

Treatment Protocol	Species	No. of animals	Conception rates (%)			
			Treatment/ 1 <sup>st</sup> estrus	2 <sup>nd</sup> estrus	3 <sup>rd</sup> estrus	Overall
AI + hCG inj.	Cow	10	30.00	28.57	20.00	60.00
	Buffalo	10	30.00	28.57	20.00	60.00
Post-AI day-5 Inj. Progesterone	Cow	10	40.00	16.66	20.00	60.00
	Buffalo	10	30.00	14.28	16.66	50.00
Normal Cyclic Control	Cow	10	40.00	33.33	25.00	70.00
	Buffalo	10	40.00	16.66	20.00	60.00

The present 60.00 % overall conception rate obtained in repeat breeder cows under post-AI progesterone treatment was in agreement with the previous reports of 60 to 70 % by Awasthi *et al.* (2002) and Sharma *et al.* (2003). The present conception rate was however lower than 96.0 % documented by Umakanthan (1995). Our finding of 50 % overall conception rate in buffaloes with post-AI progesterone treatment corroborated well with observations of 60 and 50 % reported by Kumar *et al.* (2003) and Dhama *et al.* (2009), respectively, but was lower than 66.7 % obtained by Sharma *et al.* (2003).

The variations observed in fertility with both hCG and post-AI progesterone treatment in different studies are due to many factors such as stage of estrus/estrous cycle at the time of treatment, product potency, estrus detection efficiency, endocrine and nutritional status, general and genital health, breeding time and quality of semen used, season/climate, and luteal activity or sustainability leading to embryonic mortality post-breeding etc. The results obtained in the present study using both the approaches were at par with normal cyclic control groups. Thus, the application of hCG at AI or progesterone injection 5<sup>th</sup> day post-AI can be used as a good tool for enhancement of conception rate in repeat breeding cows and buffaloes under field conditions.

#### **Effect on Plasma Progesterone**

The plasma progesterone concentrations remained nearly same on the day of estrus/AI in both conceived and non conceived cows and buffaloes subjected to AI+hCG protocol, but differed significantly on day 21 post-AI. Similar was the trend and levels of plasma progesterone on day of estrus and on day 21 post-AI in conceived and non-conceived cows and buffaloes subjected to post-AI progesterone treatment. The mean plasma progesterone concentrations on day of estrus/AI and on day 21 post-AI in conceived cows of normal cyclic control group were 0.71±0.13 and 7.93±1.48 ng/ml, and in conceived buffaloes 0.49±0.08 and 6.68±0.90 ng/ml, respectively, but were identical at both the days in non-conceived animals of both the species. The overall mean concentrations of plasma progesterone were significantly (P<0.05) higher in conceived groups as compared to non-conceived cows and buffaloes in both treatment and control groups (Table 2).

**Table 2: Mean plasma progesterone levels (ng/ml) in repeat breeding bovines under AI+hCG and post-AI progesterone treated groups on day of treatment/AI and day 21 post-AI**

Species	Treatment Protocol	Status	No.	Days from treatment/AI		Overall
				D-AI	D-21	
Cow	AI + hCG inj.	Conceived	3	0.32±0.05	10.17 <sup>q</sup> ±2.09	5.24 <sup>q</sup> ±2.39
		Non-conc	5	0.78±0.09	0.66 <sup>p</sup> ±0.10	0.72 <sup>p</sup> ±0.07
		Overall	8	0.61 <sup>a</sup> ±0.10	4.23 <sup>b</sup> ±1.87	2.42 <sup>x</sup> ±1.02
	Post-AI D-5 Progesterone Inj	Conceived	3	0.65±0.33	6.00 <sup>q</sup> ±0.81	3.32 <sup>q</sup> ±1.26
		Non-conc	5	0.70±0.23	0.60 <sup>p</sup> ±0.15	0.65 <sup>p</sup> ±0.13
		Overall	8	0.68 <sup>a</sup> ±0.17	2.62 <sup>b</sup> ±1.03	1.65 <sup>x</sup> ±0.56
	Normal Cyclic Control	Conceived	4	0.71±0.13	7.93 <sup>q</sup> ±1.48	4.32 <sup>q</sup> ±1.53
		Non-conc	4	0.69±0.13	0.53 <sup>p</sup> ±0.13	0.61 <sup>p</sup> ±0.09
		Overall	8	0.70 <sup>a</sup> ±0.09	4.23 <sup>b</sup> ±1.56	2.47 <sup>x</sup> ±0.88
Buffalo	AI + hCG inj.	Conceived	3	0.51±0.25	6.87 <sup>q</sup> ±0.47	3.69 <sup>q</sup> ±1.44
		Non-conc	5	0.75±0.22	0.50 <sup>p</sup> ±0.05	0.62 <sup>p</sup> ±0.12
		Overall	8	0.66 <sup>a</sup> ±0.16	2.89 <sup>b</sup> ±1.18	1.77±0.64
	Post-AI D-5 Progesterone Inj.	Conceived	4	0.46±0.10	4.40 <sup>q</sup> ±0.57	2.43 <sup>q</sup> ±0.79
		Non-conc	4	0.47±0.21	0.60 <sup>p</sup> ±0.14	0.53 <sup>p</sup> ±0.12
		Overall	8	0.47 <sup>a</sup> ±0.11	2.50 <sup>b</sup> ±0.77	1.48±0.46
	Normal Cyclic Control	Conceived	4	0.49±0.08	6.68 <sup>q</sup> ±0.90	3.58 <sup>q</sup> ±1.24
		Non-conc	4	0.70±0.09	0.71 <sup>p</sup> ±0.01	0.70 <sup>p</sup> ±0.04
		Overall	8	0.59 <sup>a</sup> ±0.07	3.69 <sup>b</sup> ±1.20	2.14±0.70

Means bearing uncommon superscripts within the row (a, b) and column (p, q) (x, y) differ significantly ( $P < 0.05$ ). Day-0 = Day of treatment, D-AI = Day of AI, D-21 = Day 21 post-AI

Although Sianangama and Rajamahedran (1992) suggested that hCG induced CL secreted more progesterone than did spontaneously derived CL of similar nature and was associated with an increased frequency of large luteal cell, no such trend was noted in plasma progesterone in the present study. However, the higher conception rate (60.00 %) obtained in hCG treated animals may be due to this reason. The observations made in the present study are in contradiction to the report of Colazo *et al.* (2013) who obtained higher plasma progesterone concentration in post-AI progesterone supplemented than the non-supplemented cows. The values obtained in present study however corroborated with findings of Muhammad *et al.* (2000) and Colazo *et al.* (2013).

#### Effect on Plasma Total Protein and Cholesterol Profile

In both AI+hCG inj. and progesterone supplemented groups of repeat breeding cows and buffaloes, the overall mean total protein and cholesterol values neither varied significantly between days nor between the conceived and non-conceived groups, and cholesterol had inconsistent trend. In normal cyclic control groups, the overall mean total protein values were lower on day of AI than on day 21 post-AI in both cows (7.83±0.27 vs. 8.15±0.34 mg/dl) and buffaloes (7.09±0.04 vs. 7.23±0.05 mg/ml,  $P < 0.05$ ). The protein levels were higher on day 21 post-AI and also the overall in conceived than non-conceived cows and buffaloes both, but did not differ significantly. Similarly, the overall mean total cholesterol values in cows and buffaloes did not reveal clear trend, but was significantly higher in normal cyclic than repeat breeders (Table 3).

Cetin *et al.* (2002) found relatively identical and comparable serum total protein levels at estrus in repeat breeder and fertile cows. Dhoble *et al.* (2004) also found significantly higher levels of plasma total protein in pregnant (7.92 g/dl) than non-pregnant cows (7.71 g/dl). Parmaret *et al.* (2014)

found non-significant but relatively higher mean plasma total protein concentration in hCG treated ( $9.95\pm 0.69$  and  $10.12\pm 0.50$  g/dl) as well as 5<sup>th</sup> day post-AI progesterone supplemented ( $9.42\pm 0.21$  and  $9.35\pm 0.30$  g/dl) repeat breeder Gir cows on day of AI and on day 21 post-AI.

**Table 3: Overall mean plasma total protein, cholesterol, calcium and phosphorus concentrations in repeat breeding bovines under AI+hCG and post-AI progesterone treated groups**

Species	Treatment Protocol	Status	No.	Plasma protein (g/dl)	Plasma cholesterol (mg/dl)	Plasma calcium (mg/dl)	Plasma phosphorus (mg/dl)
Cow	AI + hCG inj.	Conceived	3	$7.68\pm 0.14$	$223.73\pm 2.74$	$9.70\pm 0.16$	$4.85\pm 0.08$
		Non-conc	5	$7.52\pm 0.09$	$232.89\pm 7.37$	$9.55\pm 0.14$	$4.77\pm 0.07$
		Overall	8	$7.58^{yz}\pm 0.08$	$229.45^y\pm 4.76$	$9.60^{xy}\pm 0.11$	$4.80^{xy}\pm 0.05$
	Post-AI D5 Progesterone Inj.	Conceived	3	$7.46^q\pm 0.28$	$181.82\pm 7.74$	$9.49\pm 0.06$	$4.74\pm 0.03$
		Non-conc	5	$6.73^p\pm 0.22$	$210.15\pm 11.57$	$9.35\pm 0.13$	$4.67\pm 0.07$
		Overall	8	$7.00^x\pm 0.19$	$199.53^x\pm 8.38$	$9.40^x\pm 0.86$	$4.70^x\pm 0.04$
	Normal Cyclic Control	Conceived	4	$8.27\pm 0.38$	$191.96\pm 8.62$	$9.65\pm 0.15$	$4.80\pm 0.09$
		Non-conc	4	$7.71\pm 0.18$	$211.47\pm 5.85$	$9.78\pm 0.96$	$4.96\pm 0.03$
		Overall	8	$7.99^z\pm 0.21$	$201.71^x\pm 5.63$	$9.71^y\pm 0.09$	$4.88^y\pm 0.05$
Buffalo	AI + hCG inj.	Conceived	3	$7.66\pm 0.06$	$129.42\pm 1.028$	$9.69\pm 0.15$	$4.85\pm 0.07$
		Non-conc	5	$7.47\pm 0.07$	$126.36\pm 7.43$	$9.34\pm 0.04$	$4.67\pm 0.03$
		Overall	8	$7.54^y\pm 0.06$	$127.51^x\pm 5.84$	$9.47\pm 0.07$	$4.74\pm 0.04$
	Post-AI D5. Progesterone Inj	Conceived	4	$7.79\pm 0.34$	$123.81\pm 4.54$	$9.34\pm 0.18$	$4.67\pm 0.09$
		Non-conc	4	$7.23\pm 0.30$	$128.14\pm 3.40$	$9.50\pm 0.11$	$4.75\pm 0.05$
		Overall	8	$7.51^y\pm 0.23$	$125.97^x\pm 2.80$	$9.42\pm 0.10$	$4.71\pm 0.05$
	Normal Cyclic Control	Conceived	4	$7.21\pm 0.05$	$142.42\pm 4.52$	$9.07\pm 0.15$	$4.64\pm 0.13$
		Non-conc	4	$7.11\pm 0.04$	$148.48\pm 2.95$	$9.68\pm 0.09$	$4.90\pm 0.04$
		Overall	8	$7.16^x\pm 0.04$	$145.45^y\pm 2.72$	$9.37\pm 0.12$	$4.77\pm 0.07$

Means bearing uncommon superscripts within the column for cow or buffalo differ significantly ( $P < 0.05$ ).

### Effect on Plasma Macro-Minerals (Ca, P) Profile

In repeat breeding cows and buffaloes under AI+hCG inj. group, the overall mean plasma calcium (9.60 mg/dl) and inorganic phosphorus (4.80 mg/dl) values were identical on day of AI and on day 21 post-AI (Table 3). The conceived animals had overall higher mean values of calcium and inorganic phosphorus than non-conceived ones. Similar was the trend in repeat breeding cows and buffaloes under progesterone inj. group, and in normal cyclic controls also, except that the levels of both calcium and phosphorus were little higher in conceived cows than non-conceived cows, while in buffaloes this trend was inversed. The plasma profile of none of these traits was influenced significantly by day of sampling or pregnancy status, but was higher in normal cyclic than repeat breeding cows.

Sharma and Dhama (2008) and Patel *et al.* (2014b) reported comparable mean plasma calcium levels in conceived and non-conceived repeat breeder buffaloes and cows. However, Parmar *et al.* (2014) documented higher mean values of plasma calcium and inorganic phosphorus on day of AI than day 21 post-AI in hCG treated and post-AI progesterone supplemented repeat breeding Gir cows and their values were comparatively much higher than the present findings in both the species.

From the results, it can be inferred that hormonal therapies used significantly improved pregnancy

rates in repeat breeding cows and buffaloes under field conditions, without influencing the plasma progesterone and metabolic or mineral profiles.

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