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Response on cyclicity evidenced by serum progesterone profile in post partum crossbred cows treated with GNRH PGF2A

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Abstract
The experiment was conducted on 24 post partum crossbred cows at private dairy farm and divided into three treatment (TG) and control (C) group (6 each) were monitored for serum progesterone on 14th,24th,27th,38th, 41st and 42nd of post partum following GnRH and $PGf_2\alpha$ and in combination treatment TG-1 (GnRH - $PGf_2\alpha$), TG-2 (GnRH –N.S), TG-3 (N.S - $PGf_2\alpha$) and control (N.S – N.S) on day 14th and 24th with fixed timed artificial insemination by injecting $PGf_2\alpha$ on day 38th in all the groups(except control) and reveals that mean serum concentration progestrone in TG-I,TG-II,TG-III and control1.74 ± 0.11, 1.72 ± 0.10, 1.70±0.09, 2.74±0.04 ng/ml. 4 in TG-I, 2 in TG-II,3 out of 6 treated cows in TG-III showed <1ng/ml progesterone concentration on day 41st and 42nd indicating that these animals exhibited oestrus and cyclity during the period of Fixed timed AI. Whereas none of the animals in control group showed <1ng/ml progesterone concentration on day 41st and 42nd indicating that these animals did not exhibit oestrus and cyclity during the period of Fixed timed AI. The data of mean serum progesterone concentration were also compared between and within treatment and control groups using Analysis of variance. There was a significant difference in serum progesterone concentration within and between the groups ($P \leq 0.01$).

Keywords: artificial insemination, crossbred cows, cyclicity, dairy farm, oestrus

Introduction
Endocrine mechanisms are the most important aspects of postpartum reproductive performance. Progesterone in cyclic animals acts as a regulator of diestrus period, because as soon as the corpus luteum fails to secrete progesterone, development of follicles begins leading to pro-estrus phase. Moreover, cows loosing body condition and undergoing negative energy balance in early postpartum period usually from prolonged anestrus or subfertility condition leading to extended calving interval [6]. Progesterone is a steroid hormone, the first biologically active compound in the steroid biosynthesis pathway. Its concentration in an animal's body reflects the stage of the reproductive cycle, pregnancy and ovarian disorders. The mean level of serum progesterone was found to be ranging from 0.23 to 5.92ng/ml in normal fertile cow [5]. Radioimmunoassay (RIA) was developed to measure progesterone and monitors the ovarian activity of dairy animals. Progesterone monitoring is an accurate method for assessing typical and atypical ovarian function in postpartum cows. In buffaloes, progesterone profiles have been studied in postpartum periods [3, 9] concluded that induction of oestrous in postpartum anoestrus in buffaloes with Crester ear implant+ RnRH+ PGF2α hormonal therapy and exhibition of first postpartum oestrus and observed first post partum oestrus (days) in 42.38± 11.09 and their conception rate (%) 83.33.

Materials and Methods
The experiment were conducted on 24 postpartum cross bred cows at Organized Private Dairy Farm located at Jabalpur of 3-5 parity having normal parturition and divided into three treatment (TG) and control (C) group (6 each) were monitored for serum progestrone on 14th, 24th, 27th, 38th, 41st and 42nd of post partum following GnRH and $PGf_2\alpha$ treatment TG-1 (GnRH - $PGf_2\alpha$), TG-2 (GnRH –N.S), TG-3 (N.S - $PGf_2\alpha$) and control (N.S – N.S) on day 14th 250µg I/m (Receptal® containing 0.0042mg/ml of Buserelin acetate) and 24th 500µg I/m (Juramate® containing 250mcg/ml of cloprostenol sodium) with fixed timed artificial insemination by injecting $PGf_2\alpha$ on day 38th 500µg I/m in all the groups (except control).

The animals of both the treatment and control groups were followed and compared for their cyclicity and serum profile of progesterone on 14, 24, 27, 38, 41 and 42 days of post partum and fixed timed artificial insemination done on 41 and 42 days of post partum. The serum samples were stored at -20ºC till assay. The serum progesterone assays were determined by using the quantitative determination of progesterone concentration serum was made by ELISA method using kits supplied by united Biotech. Inc. Mountain View, USA. The progesterone EIA is based on the principle of competitive solid phase enzyme immunoassay. The sample competes with enzyme- labeled progesterone for a fixed and limited number of antibody sites on the microtiter wells. The data were analyzed statistically using one way ANOVA and Duncan's multiple range test by [7].

Results and Discussion

The mean serum progesterone concentration in TG–I, TG-II,TG-III and control on day-14th, 24th, 27th, 38th, 41st and 42nd of postpartum in each six crossbred cows 1.6±0.22, 1.6±0.25, 1.8±0.11, 1.7±0.28, 1.9±0.30 2.0±0.22 (ng/ml) respectively with the overall mean concentration value 1.74 ± 0.11 ng/ml. 1.4±0.19, 1.9±0.18, 1.6±0.25, 1.9±0.09, 1.6±0.11, 2.0±0.31 respectively with the overall mean concentration value of 1.72 ± 0.10 ng/ml. 1.66±0.10, 1.5±0.33, 1.5±0.42, 1.8±0.20, 1.7±0.13, 2.2±0.20 respectively with the overall mean concentration value1.70±0.09 ng/ml. 2.7±0.13, 3.0±0.04, 2.8±0.19, 2.7±0.03, 2.6±0.08, 2.6±0.62 respectively with the overall mean concentration value 2.74±0.04 ng/ml (Table 1.4).The data of mean serum progesterone concentration were also compared between and within treatment and control groups using Analysis of variance (Table 1.5). There was a significant difference in serum progesterone concentration within and between the groups ($P \leq 0.01$).

A perusal of Table 1. reveals that progesterone concentration in animals of TG-I showed a declining trend from day 38 onwards in animal numbers 411,284,453 and 699 showing the effect of $PGF_2\alpha$ administration on day38postpartum.However no decline in progesterone concentration in animal numbers 799 and 20 was evident on administration of $PGF_2\alpha$ on day 38. This implies that the corpora lutea of responding animals were vulnerable to $PGF_2\alpha$ treatment. The two non-responding animals although having a corpus luteum as evidenced by high progesterone (1.98 and 1.89 ng/ml) on day 38 were not at responsive stage to $PGF_2\alpha$ treatment. In a study by [2]. Luteolytic properties of cloprostenol was demonstrated by a significantly greater number of cloprostenol treated cows experiencing a progesterone drop from >1ng to < 1ng between day 24 to day28 postpartum than cows not treated with cloprostenol [8]. Determine the efficacy of GnRH and $PGF_2\alpha$ for inducing early post partum partum estrul activity, hastening uterine involution and enhancing ovulation in lactating dairy cows. Reproductive function was monitored through day 58 PP blood samples obtained 3 times week for serum progesterone analysis. On day 58 PP, an ovulation was initiated as follows: GnRH given on day 0, $PGF_2\alpha$ administered on day 7, GnRH given again on day 9 and artificial insemination conducted 16h after the 2nd GnRH injection. InbothT-2 andT-3 serums progesterone falls sharply after the $PGF_2\alpha$ injection at 25 days PP [1]. Determine whether a single injection of a GnRH analogue would ovulate the first postpartum dominant follicle. Blood samples were collected daily for progesterone measurement to confirm ovulation shows low progesterone concentration to determine the duration of the first oestrous cycle [4]. investigate the impacts of GnRH treatment on post-partum productive and reproductive performance of buffaloes. Blood samples were collected twice weekly from each buffalo cow for determination of progesterone (P4). The group-I of buffaloes achieved the least (P<0.01) calving interval (CI) and days open (DO) as compared with buffalo groups with control. However, GnRH treatment had significant on Postpartum concentrations of P4 were significantly (P<0.05) greater in animals in group –I than that in buffaloes with control throughout the experimental months. GnRH treatment increased significantly (P<0.05) postpartum. Similar trend was also observed with present study.

Out of the four responded animals, three animals (No.411,284,699) were declared to be pregnant on day 45 post insemination giving an overall conception rate of 75%.Since fixed time A.I. was done and estrus observation was not done, the animals not conceiving might be asynchronous with the insemination period.

A perusal of Table 1.1 reveals that progesterone concentration in animals of TG-II showed a declining trend from day 38 onwards in animal numbers 369 and 15 showing the effect of $PGF_2\alpha$ administration on day 38 postpartum. However no decline in progesterone concentration in animal numbers 343,353,360 and 225 was evident on administration of $PGF_2\alpha$ on day 38. This implies that the corpora lutea of responding animals were vulnerable to $PGF_2\alpha$ treatment. The four non-responding animals although having a corpus luteum as evidenced by high progesterone (1.12, 1.63, 1.25and 1.56 ng/ml) on day 38 was not at responsive stage to $PGF_2\alpha$ treatment.

Out Of the two responded animals, one animal (No.15) was pregnant on day 45 post insemination giving an overall conception rate of 50%. Since fixed time A.I. was done and estrus observation was not done, the animal not conceiving might be asynchronous with the insemination period.

A perusal of Table 1.2 reveals that progesterone concentration in animals of TG-III showed a declining trend from day 38 onwards in animals' numbers 425, 94 and 336 showing the effect of $PGF_2\alpha$ administration on day 38 postpartum. However no decline in progesterone concentration in animal numbers 41, 35 and 61 was evident on administration of $PGF_2\alpha$ on day 38. This implies that the corpora lutea of responding animals were vulnerable to $PGF_2\alpha$ treatment. The three non-responding animals although having a corpus luteum as evidence of high progesterone (1.39, 1.68 and 2.75 ng/ml) on day 38 was not at responsive stage to $PGF_2\alpha$ treatment.

Out of the three responded animals, two animals (No.425 and 336) were declared to be pregnant on day 45 post insemination giving an overall conception rate of 75%.Since fixed time A.I. was done and estrus observation was not done, the animal not conceiving might be asynchronus with the insemination period.

In control group all the animals were not subjected to $PGF_2\alpha$ treatment on day 38. A perusal of the progesterone profile on day 38 onwards up to day 42 (Table 1.3) also shows no definite trend in decline of progesterone from day 38 onwards. This shows that none of animals of the control group were heading towards spontaneous estrus. This is also evident by the fact that none of these animals conceived to fixed time A.I. on day 41 and 42.

Comparing the overall trend in progesterone profile between the treatment groups as compared to control group it can be observed that on days of sampling from day 14 till day 42 the control group animals had high progesterone level on all days

of sampling as compared to all the samples from treatment group. This shows that the treatment effects were evident in all the animals of treatment group as compared to those in the control group.

The data of mean serum progesterone concentration were also compared between and within treatment and control groups by using Analysis of variance in Table No.1.5.However there was a significant difference were recorded in serum progesterone concentration within and between the groups ($P < 0.01$).

Therefore low progesterone in treatment cows as compared to the control animals collaborates with the trend observe in the above study.

Legends

Table 1: Progesterone (ng/ml) concentration in Group-I at different time interval.

	Days					
Animal No.	**14**	**24**	**27**	**38**	**41**	**42**
411*	1.32	1.41	2.49	2.63	0.71	0.85
284*	1.89	1.91	1.93	2.91	0.077	0.61
453*	1.85	1.95	2.34	2.91	0.76	0.91
699*	1.84	1.93	1.93	2.45	0.96	0.87
799**	1.31	1.91	1.93	1.98	1.89	2.12
20**	1.97	1.31	1.68	1.89	2.32	2.88
*Responded (n=4)\Mean±SE	1.72±0.28	1.8±0.11	2.17±0.22	2.72±0.03	0.62±0.14	0.81±0.18
**Not responded (n=2) Mean±SE	1.64±0.22	1.61±0.25	1.80±0.20	1.93±0.09	2.10±0.31	2.5±0.20

Table 1.1: Progesterone (ng/ml) concentration in Group-II at different time interval.

	Days					
Animal No.	**14**	**24**	**27**	**38**	**41**	**42**
343**	1.25	1.55	1.59	1.12	1.14	1.52
369*	2.45	2.26	2.04	2.57	1.29	0.65
15*	2.14	1.68	1.61	2.45	0.77	0.67
353**	2.35	2.45	2.58	1.63	1.34	1.21
360**	2.34	2.15	1.05	1.25	1.54	1.34
225**	2.78	2.98	1.65	1.56	1.15	1.98
*Responded (n=2)Mean±SE	2.29±0.13	1.97±0.18	1.82±0.21	2.51±0.13	1.03±0.19	0.66±0.20
**Notresponded (n=4)Mean±SE	2.18±0.20	2.28±0.19	1.71±0.28	1.39±0.42	1.29±0.25	1.51±0.41

Table 1.2: Progesterone (ng/ml) concentration in Group-III at different time interval.

	Days					
Animal No.	**14**	**24**	**27**	**38**	**41**	**42**
425*	1.97	1.98	1.58	2.70	0.67	0.59
94*	1.60	1.69	1.72	1.96	1.26	0.76
336*	1.72	1.82	1.63	1.70	0.97	0.93
41**	2.15	2.02	2.28	1.39	1.76	1.45
35**	2.76	1.87	1.12	1.68	1.37	1.14
61**	1.71	2.46	2.56	2.75	1.87	1.56
*Responded (n=3)Mean±SE	1.76±0.12	1.83±0.13	1.64±0.28	2.12±0.22	0.96±0.13	0.76±0.10
**Notresponded (n=3)Mean±SE	2.20±0.21	2.11±0.20	1.98±0.09	1.94±0.30	1.66±0.10	1.38±0.17

Table 1.3: Progesterone (ng/ml) concentration in Group-IV (control) at different time interval.

	Days					
Animal No.	**14**	**24**	**27**	**38**	**41**	**42**
297	2.25	2.54	2.86	2.72	2.87	2.92
67	2.89	2.91	2.96	3.10	3.26	3.12
07	1.96	2.60	2.92	2.98	3.12	3.20
13	2.18	2.67	2.68	2.76	2.84	2.98
21	2.32	2.41	2.65	2.68	2.71	2.81
201	2.38	2.41	2.65	2.72	2.78	2.84
*NotResponded (n=6)Mean±SE	2.33±0.23	2.59±0.12	2.78±0.13	2.82±0.20	2.93±0.21	2.97±0.04

Table 1.4: Serum progesterone concentration (ng/ml) (Mean ± SE) at different time interval in treated and control postpartum crossbred animals.

Groups	**Days**						**overall mean**
	14	**24**	**27**	**38**	**41**	**42**	
IV (control)	2.7±0.13	3.0±0.04	2.8±0.19	2.7±0.03	2.6±0.08	2.6±0.62	2.74±0.04[a]
I	1.6±0.22	1.6±0.25	1.8±0.11	1.7±0.28	1.9±0.30	2.0±0.22	1.74±0.11[b]
II	1.4±0.19	1.9±0.18	1.6±0.25	1.9±0.09	1.6±0.11	2.0±0.31	1.72±0.10[b]
III	1.66±0.10	1.5±0.33	1.5±0.42	1.8±0.20	1.7±0.13	2.2±0.20	1.70±0.09[b]

TG-I (GnRH - $PGf_2\alpha$), TGII (GnRH –N.S), TG-III (N.S-$PGf_2\alpha$) control-IV (N.S – N.S)

Table 1.5: Analysis of variance for serum Progesterone in different treated and control groups in post partum crossbred cows.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-value
Between group within interval	3	28	9.35	32.54**
Between interval within group	20	6	0.29	0.90
Within interval	120	38	0.32	

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