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Research Article

BIOCHEMICAL PROFILE IN RELATION TO THE EFFECT Of PGF2α, MINERAL MIXTURE AND PROGESTERONE ON RESUMPTION OF CYCLICITY ON POST-PARTUM ANESTRUS BUFFALOES LOCATED IN AND AROUND DANAPUR (BIHAR)

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Abstract- Total of 24 buffaloes were selected randomly and divided into four groups (six animals in each group) to observe the effect of Duraprogen at different dosages on resumption of cyclicity. Animals of group C (Control) were treated with normal saline (2 ml) on day 39, 43, 47 and 51 while those under Group T1, T2 and T3 were treated with Duraprogen (Progesterone, 250 mg) intramuscular on day 39. The animals of group T1 were further treated with Duraprogen (250 mg) on day 41, 43, 45, 47 and 49 (*i.e.*, on alternate day). The animal of group T2 were further treated with Duraprogen (250 mg) on day 43, 47 and 51 (*i.e.*, three day interval) while the animals of group T3 were further treated on day 46 (*i.e.,* six day interval). Blood samples were collected at day 0, day 8 (after PGF2α treatment), day 39 (post mineral mixture treatment), on day 43, 47 and 51 or on the day of estrus. Lutalyse and mineral mixture treated buffaloes. The mean value of serum calcium, SGOT and SGPT on day 51/estrus did not differ significantly with anestrus (*i.e.*, day 0), post Lutalyse and mineral mixture treated buffaloes. The resumption of cyclicity was highly significant in group T1 in which repeated dosages of Duraprogen (250 mg) *i.e.*, on alternate days were administered. Duraprogen had significant effect in resuming estrus cyclicity in post-partum anestrus buffaloes, repeated dosages of Duraprogen *i.e.*, 250 mg (1 ml) on alternate day had better effect than administered at three day interval or at six day interval.

Keywords- Postpartum anestrus, Mineral mixture, PGF2α, Cyclicity, Buffalo, Progesterone

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Introduction

Dairying provides millions of small marginal farmers and landless labours means for their subsistence. India has the largest holding of bovine population, about one third of the world's population and about half of the Asian population. India is now the largest milk producer in the world producing about 84.6 million tons of milk per annum (2001-02) [1]. Buffalo alone accounts for 53% of total milk production in India. So, its importance cannot be ignored in dairy industry. Full genetic potential in terms of milk production from buffalo can be explored only when the reproduction is normal. In India delayed puberty, acyclicity after attaining puberty, and post-partum anestrus which lead to prolong inter calving period are major causes of poor reproductive efficiency in cattle and buffaloes. Real anestrus with inactive, smooth, small and round and flat ovarian condition is a major limiting factor in greater utilization of artificial insemination for rapid improvement of livestock productivity. This also results in loss of production and increased cost of maintenance. The problem of anestrus has been recognized as having moderate to high incidence affecting the fertility of the animal vis-à-vis economy of the farmer [2,3]. Anestrus is one of the most commonly occurring reproductive problems in cattle and buffalo in India, affecting livestock productivity and enterprise to a great extent. Buffalo has higher incidences of functional anestrus than cattle as the post-partum estrus interval is longer and especially during summer. The problem is more severe in sub urban and rural areas of the country.

It is a functional disorder of the reproductive cycle which is characterized by absence of overt signs of estrus manifested either due to lack of expression of estrus or failure of its detection. Anestrus is observed in post pubertal heifers, during pregnancy, lactation and in early postpartum period in adult animals. Reproductive failures such as anestrum, repeat breeding and pathological condition of the genital tract suggest the nutritional deficiencies, hormonal imbalance and deranged enzymatic activity affect the normal reproductive behavior of the animal, causing serious morphological and physiological alterations [4]. Nutritional deficiencies and excesses may cause infertility. They may act via the hypothalamus and anterior pituitary thus influencing the production of gonadotropins or directly on the ovaries, thus influencing oogenesis and endocrine function. Buffalo has higher incidences of functional anestrus than cattle as the post-partum estrus interval is longer. Besides breed and climate, management and nutrition also play vital role in determining the reproductive disorder in cattle and buffalo. Reproductive failures such as anestrum, repeat breeding and pathological condition of the genital tract suggest the nutritional deficiencies, hormonal imbalance and deranged enzymatic activity affect the normal reproductive behavior of the animal, causing serious morphological and physiological alterations [4]. Nutritional deficiencies and excesses may cause infertility. They may act via the hypothalamus and anterior pituitary thus influencing the production of gonadotropins or directly on the ovaries.

Thus, influencing oogenesis and endocrine function. Deficiencies of certain blood constituents *i.e.,* macro and micro nutrients, may cause reproductive disorder [5]. Changes in the hormonal and biochemical milieu are also responsible for the anestrus condition. Protein deficiency is usually accompanied by phosphorus deficiency which may be in the feed and fodders and also in the soil [4, 6]. Biochemical profile can indicate the nutritional status of the animal and thus will further help in the diagnosis and management of anestrus. Hormonal treatments have been tested for their ability to induce puberty and post-partum estrus. These treatments usually consist of progestogen and/ or prostaglandin. Progestogen treatment alone does not always induce ovulation although progesterone releasing intravaginal device (PRID) has been shown to induce cyclicity in dairy cow during early post-partum period [7]. Considering above mentioned point in view, it is proposed to investigate the effect of nutritional supplement (Mineral mixture- at the dose rate of 30 gms per animal per day up to one month) and hormonal preparations (repeated dosages of Duraprogen *i.e.,* 250 mg (1 ml) on alternate day) and their effect on blood biochemical changes in the treatment of anestrus and as a managemental aid for better reproductive efficiency in rural buffaloes

Materials and Methods

Area of investigation

The present study was done in and around the rural area of Danapur situated on western proximity of Patna. The experiment was based upon small and unorganized dairy units popularly known as 'Khatals'.

Geogrpaphy and climatological description of the location

Danapur is located 25º36' North (latitude) and 85º06' East (longitude) at an altitude of about 60 meters from mean sea level. The total annual rainfall ranges from 100-120 cm and the maximum temperature goes above 38º C during May-June. The rainy season is hot-humid and winters are cold.

Primary survey

At first, the private dairy units distributed in the study area consisting of non-descript buffaloes were enumerated through a "door to door" survey method. Only those buffaloes, which did not show estrus upto one year after parturition were selected for this investigation. The selected buffaloes were examined per-rectally for their reproductive status. The animal showing infectious conditions like pyometra, metritis etc. were not selected. The faecal test of each selected animals was done for three consecutive days and deworming was done for infested animals accordingly. Selected animals were free from ectoparasites. Their rectal temperature, respiration rate, pulse rate and ruminal motality rate were also recorded and those within the normal range were selected. All the animals included in this experiment were apparently healthy, with no apparent sign of health problem.

Hormones and chemicals

Fenbendazole in the trade name of Panacur was purchased from local market for deworming of experimental animals. Prostaglandin (PGF2α) manufactured by Pharmacia N.V./S.A. Puurs-Belgium under trade name Lutalyse containing 5 mg per ml. DinoprostTromethamin, marketed by Novartis India Ltd., Animal Health Sector, 14 J Tata Road, Mumbai – 400020 was purchased and used in the experiment. The Progesterone in the trade name of Duraprogen having 17-α-Hydroxyprogesterone Caproate, 250 mg. per ml., manufactured by AgvetUnichem a division of Unichem Laboratories Limited Unichem Bhawan Prabhat Estate S.V. Road, Jogeshwari (West), Mumbai - 400102 was received as gift from the Unichem Laboratories Limited for the purpose. Mineral mixture in the trade name of Agrimin having Copper 312 mg., cobalt 45 mg., Magnesium 2.114 gm., Iron 979 mg., Zinc 2.130 mg., Iodine 156 mg., DL-Methionine 1.920 gm., L-lysine monohydrochloride 4.4gm., Calcium 30 % and Phosphorous 8.25 % per kg. marketed by Agrivet Farm Care Division, Glaxosmithkline Pharmaceutical Ltd., Dr. Annie Besant Road, Mumbai, 400025 was received as gift for the experiment. Among blood biochemical profile serum total cholesterol, serum calcium, serum inorganic phosphorous, Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) were analysed by standard methods from standard manufacturers and purchased from local market.

Experimental design

Preparation of the animal

Selected buffaloes having normal physique and reproductive status were subjected to deworming prior to this study with broad spectrum anthelmintic Panacur (Fenbendazole) at dose rate of 1.5 gm once. The faecal examination of all the buffaloes were done on three consecutive days *i.e.,* 13 to15 days after Panacur administration and found negative for parasitic infestation were taken for the experiment. The selected buffaloes were treated with LutalyseTM (PGF2α analogue) at the dose rate of 25 mg, intramuscular per animal. The day of administration of the drug was taken as day 0. The animals showing signs of estrus after the treatment were discarded from present investigation and allowed for insemination. The LutalyseTM treated anestrus buffaloes were subjected to mineral mixture treatment from day 8 to day 38. Mineral mixture (AgriminTM) were added at the dose rate of 30 gm once daily in their ration. Buffaloes showing signs of estrus during mineral mixture treatment (*i.e.,* up to day 38) were also discarded from further investigation and were allowed for A.I. On day 39, all the mineral mixture treated animals which did not show signs of estrus were subjected to further investigation.

Grouping of the animal

Out of 38 animals 2 buffaloes who had shown signs of estrus have been dropped and they were discarded from further investigation. Thus, out of 36 animals, only 24 left for investigation were selected randomly and divided into four groups having six animals in each group [Table-1]. The animals of Control group were treated with 2 ml normal saline (N.S.) on day 39, 43, 47 and 51 while the animals of Group T1, T2 and T3 were treated with 1 ml DuraprogenTM (250 mg) intramuscularly on day 39. The animals of Group T1 were further treated with 1 ml DuraprogenTM on day 41, 43, 45, 47 and 49 (*i.e.,* on alternate day). While those under Group T2 were further treated with 1 ml DuraprogenTM on day 43, 47 and 51 (*i.e.,* three day interval) and those under Group T3 were further treated on day 46 (*i.e.,* six days interval). Blood samples from all treated animals were collected on day 39, 43, 47 and 51 and/or the day when animal showed sign of estrus.

Table-1 Grouping of the animals for treatment

Groups	No. of animals	Treatment
Group Control	6	Injection of normal saline 2 ml I/M on every 4th day (i. e. 39th, 43th, 47th and 51th)
Group T1	6	Injection of Duraprogen*250 mg (1ml) I/M on alternate day (*i.e.,* day 39th, 41st, 43rd, 45th, 47th and 49th)
Group T2	6	Injection of Duraprogen*250 mg (1ml) I/M on every 4th day (*i.e.,* day 39th,43rd, 47th and 51st)
Group T3	6	Injection of Duraprogen*250 mg (1ml) I/M on every 7th day after administration of 1st injection (*i.e.,* day 39th and 46th)

*DuraprogenTMhaving 17-α-Hydroxyprogesterone Caproate

Statistical analysis

Data were analysed for averages and standard error as described by [8]. Analysis of variance for comparison of various traits between groups was done. The model [8] was used as follows :

$$Yij = \mu + £i + eij$$

Yij is the measurement of jth individual of the ith group.
μ is the overall population mean.
£i is the effect of ith group.
eij is the random error assumed to be normally and independently distributed with mean "0" and variance σ^2e *i.e.,* NID (0, σ^2e).

Results and Discussion

Blood biochemical profile

The blood biochemical parameters of 38 selected buffaloes prior to treatment (*i.e.,* on day 0) are presented in the [Table-2]. The mean value of total serum cholesterol, serum calcium, serum inorganic phosphorous, SGPT and SGOT were found to be 96.78±12.56 mg%, 9.76±0.78 mg%, 4.10±0.44 mg%, 40.55±2.89 mlu/ml and 27.98±4.46 mlu/ml respectively. The mean value of serum total cholesterol recorded were lower than that of the findings of Amanullah, *et al*., (1997) [9]; Latif, *et al*., (1993) [10]; Patel, *et al*., (1994) [11]; but higher than the findings recorded by Umesh, *et al*., (1995) [12] all in anestrus buffaloes. The probable cause of difference in the mean value of cholesterol may be due to different agro-climatic factors and difference in vegetation in different area of investigation. The mean value of calcium and phosphorous recorded in our experiment was 9.76±0.78 and 4.10±0.44 respectively and it was more or less same as recorded [9,11]. The mean value of SGOT recorded was 27.98±4.46 higher than the findings of Latif, *et al*., (1993) [10].

Effect of Lutalyse

All 38 anestrus buffaloes selected for this study and subjected to PGF2α (LutalyseTM) treatment as per schedule, on observation for seven continuous days showed no sign of estrus [Table-6].

Effect of Lutalyse on cyclicity

Experimental buffaloes subjected to Lutalyse treatment showed no signs of estrus [Table-6] confirming absence of any palpable corpus luteum

Table-2 *Mean values of serum biochemical constituents in anestrus buffaloes on 0-day.*

Serum constituents	Mean ± S. E. -38
Serum Total Cholesterol (mg %)	96.78±12.56
Serum Calcium (mg%)	9.76±0.78
Serum Inorganic Phosphorous (mg%)	4.10±0.44
SGPT (mlu/ml)	40.55±2.89
SGOT (mlu/ml)	27.98±4.46

Figure in parenthesis are number of observations

The findings revealed that administration of prostaglandin did not leave any influence on the reproductive status of the buffaloes. Prostaglandin is well known to have a potent luteolytic effect and intramuscular administration of 25 mg prostaglandin in the animals with palpable corpus luteum cause the degeneration of the CL within 24 to 48 hrs. and return of estrus within 3 to 5 days post treatment [13]. Present investigation findings were not in accordance with the findings of Dhoble and Gupta, (1987) [14]. The variation in findings may be attributed to difference in dose rate and difference in frequency of administration. The other possible reason is that all the buffaloes under investigation were in true anestrus as they were not having luteal tissue of the ovary.

Effect of Lutalyse on the blood biochemical profile

Blood from all the buffaloes treated with Lutalyse were collected on day '8' of the treatment. The mean value of different blood biochemical constituents were analysed and compared with their pre 'Lutalyse' treated blood biochemical profile. The results are presented in [Table-3]. Result revealed that there was no significant difference in the level of serum total cholesterol, serum calcium, serum inorganic phosphorous, SGPT and SGOT in pre and post Lutalyse treated animals. [15] reported higher level of inorganic phosphorous in control and PGF2α treated buffaloes which were contrary to the findings of this study.

Effect of Mineral Mixture

Since no animal responded to PGF2α treatment hence all 38 LutalyseTM treated animals were subjected to mineral mixture treatment from day '8' to day '38'. The animals were supplied with mineral mixture (AgriminTM) as per program continuously for 30 days. The animals were kept under close observation for estrus detection. Routine visit and guidance to the owners for proper detection of estrus was adopted. The effect of mineral mixture was noted and analysed for further investigation.

Table-3 *Mean values of serum biochemical constituent of anestrus buffaloes pre and post Lutalyse treatment.*

Serum constituents	Period	
	0 – day	8 – day
	-38	-38
	Mean ± S.E.	Mean ± S.E.
1. Serum Total Cholesterol (mg %)	96.78±12.56	101.24±9.78
2. Serum Calcium (mg%)	9.76±0.78	9.97±0.94
3. Serum Inorganic Phosphorous (mg%)	4.10±0.44	3.90±0.49
4. SGPT (mlu/ml)	40.55±2.89	38.43±4.72
5. SGOT (mlu/ml)	27.64±4.46	29.08±3.91

Effect of Mineral Mixture on cyclicity

After supplementation of mineral mixture, out of 38 animals under treatment only 2 animals exhibited signs of estrus. . Both the animals showed the sign of estrus on day 37 of treatment. The signs of estrus were moderate. Both the animals were put to natural service. Amongst the two, only one buffalo conceived [Table-6].

Effect of Mineral Mixture on blood biochemical profile

Blood from all the buffaloes treated with mineral mixture were collected on day '39'. The mean value of different blood biochemical constituents wereanalysed and compared with the pre 'mineral mixture' treated blood biochemical profile. The results were presented in [Table-4]. Result revealed that there was no significant difference in the level of serum total cholesterol (104.08±13.94 mg%), serum calcium (10.28±1.32 mg%), serum inorganic phosphorous (4.23±0.95 mg%), SGPT (39.97±2.23 mlu/ml) and SGOT (30.30±4.32 mlu/ml) after the mineral mixture treatment.

The result revealed that [Table-4] 2 animals in estrus showed significant increase in mean value of serum total cholesterol (148.56±11.22 mg%) and serum inorganic phosphorous (5.33±0.32 mg%) while, serum calcium (10.68±1.22 mg%), SGPT (40.24±1.59 mlu/ml) and SGOT (31.14±2.72 mlu/ml) did not differ significantly from the animals in anestrus status similar significant increase in serum total cholesterol level in estrus animal were also reported by [9],[11].[12] but the findings of Latif, *et al*., (1993) [10] was different. The significant increase in serum inorganic phosphorous level in estrus animal were also found by Bhatia, *et al*., (1985) [16] and Umesh, *et al*., (1995) [12] but differed from the findings of Agrawal, *et al*., (1985) [17] and Amanullah, *et al*., (1997) [9]. The findings of serum calcium level is in agreement with the findings of Samad, *et al*., (1980) [18] and Umesh, *et al*., (1995) [12] but differed to the findings of Bhatia, *et al*., (1985) [16] who had reported lower level of Calcium in anestrus animals.

Table-4 *Mean ± S.E. of serum biochemical profile of buffaloes after continuous feeding of mineral mixture.*

SN	Serum constituents	8th day	39th day	
		n = 38	Anestrus	Day of estrus
			n = 36	n = 2
1	Serum Total Cholesterol (mg %)	101.24±9.78	104.08±13.94	148.56**± 11.22
2	Serum Calcium (mg%)	9.97±0.94	10.28±1.32	10.68±1.22
3	Serum Inorganic Phosphorous (mg%)	3.90±0.49	4.23±0.95	5.33**±0.32
4	SGPT (mlu/ml)	38.43±4.72	39.97±2.23	40.24±1.59
5	SGOT (mlu/ml)	29.08±3.91	30.30±4.32	31.14±2.72

** *(P<0.01)*

The mean values of blood biochemical profile observed on day '39' of all the four groups 2 animals were separately recorded. The group wise mean value of different blood constituents is given in [Table-5]. All the groups differed non-significantly with respect to their mean value of blood constituent.

Effect of Duraprogen on cyclicity

The effect of Duraprogen on cyclicity was summarized in [Table-5] and [Table-6]. In control, no animal showed sign of estrus. In group T1, animals treated with Duraprogen*250 mg I/M on alternate day (*i.e.,* day 39, 41, 43, 45, 47 and 49), five out of 6 animals showed signs of estrus.

Table-6 *Response of anaestrus buffaloes to PGF2α and Progesterone administration on resumption of estrus cyclicity*

Treatment	No. of Animal	No. of Animal showing sign of estrus	Intensity of estrus	Insemination	Remark
PGF2α (LutalyseTM) treatment @ 25 mg (5ml) I/M per animal	38	0	-		
Mineral Mixture* @ 30 gm per animal for one month	38	2	Moderate	Natural	Pregnant (1)
Group C (Control) Injection of Normal saline I/M	6	0	-		
Group T1 -Injection of Duraprogen*250 mg (1ml) I/M on alternate day (*i.e.,* day 39th, 41st, 43rd, 45th, 47th and 49th)	6	5	Moderate to strong	Natural	Pregnant (3)
Group T2 -Injection of Duraprogen*250 mg (1ml) I/M on every 4th day (*i.e.,* day 39th,43rd, 47th and 51st)	6	3	Moderate to strong	Natural	Pregnant (3)
Group T3 - Injection of Duraprogen*250 mg (1ml) I/M on every 7th day after administration of 1st injection (*i.e.,* day 39th & 46th)	6	1	Moderate	Natural	Pregnant (1)

Table-8 *Mean ± SE of Serum Total Cholesterol Level (mg/dl) in Buffaloes*

Animal gr. (No. of Animals)	Day '39' (anestrus)	Day '43'/ estrus	Day '47'/ estrus	Day '51'/ estrus
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Control Gr. (6)	105.37 ± 6.84 a	101.25 ± 8.43 a	106.43 ± 6.38 a	107.39 ± 8.95 a
Group T1 (6)	107.48± 10.32 a	139.46± 13.43 b	156.73± 8.96 c	157.45± 9.42 c
Group T2 (6)	109.37 ± 7.88 a	132.43± 7.76 b	148.46± 7.70 c	151.44± 10.33 c
Group T3 (6)	104.44 ± 9.43 a	131.03 ± 11.23 b	144.97± 8.11 c	143.35± 6.76 c

Two animals showed signs of estrus on day 44 while other three showed the estrus sign on day 45, 46 and 49. The intensity of estrus were moderate in 2 animals and strong in 3 animals. In group T2, where animals were treated with Duraprogen*250 mg I/M on every 4th day (*i.e.,* day 39, 43, 47, 51), three out of 6 animals showed signs of estrus. Two animals showed sign of estrus on day 46 and one on day 50. The intensity of estrus was moderate in 2 animals and strong in 1 animals. In group T3, where animals were treated with Duraprogen*250 mg I/M on every 7th day after administration of 1st injection (*i.e.,* day 39 & 46), one out of 6 animals showed sign of estrus on day 43 and the intensity was moderate.

Table-5 *Serum biochemical profile of different groups buffaloes on day '39' i.e., prior to Duraprogen treatment.*

Group	Serum constituents				
	Serum Total Cholesterol	Serum Calcium	Serum Inorganic Phosphorous	SGPT	SGOT
Control group(6)	105.37± 6.84	10.23±0.98	4.13± 0.65	37.83± 2.25	29.89± 4.86
Group T1 (6)	107.48±10.32	10.02±1.21	4.46± 0.76	38.40± 2.50	30.46± 3.97
Group T2 (6)	109.37± 7.88	10.13±1.07	4.25± 0.48	38.66± 2.91	31.11± 2.87
Group T3 (6)	104.44±9.43	9.47±1.40	4.32± 0.81	37.97± 1.96	30.39± 5.01

Effect of Duraprogen on cyclicity

The effect of Duraprogen on cyclicity was summarized in [Table-5] and [Table-6]. In control, no animal showed sign of estrus. In group T1, animals treated with Duraprogen*250 mg I/M on alternate day (*i.e.,* day 39, 41, 43, 45, 47 and 49), five out of 6 animals showed signs of estrus. Two animals showed signs of estrus on day 44 while other three showed the estrus sign on day 45, 46 and 49. The intensity of estrus were moderate in 2 animals and strong in 3 animals. In group T2, where animals were treated with Duraprogen*250 mg I/M on every 4th day (*i.e.,* day 39, 43, 47, 51), three out of 6 animals showed signs of estrus. Two animals showed sign of estrus on day 46 and one on day 50.

The intensity of estrus was moderate in 2 animals and strong in 1 animals. In group T3, where animals were treated with Duraprogen*250 mg I/M on every 7th day after administration of 1st injection (*i.e.,* day 39 & 46), one out of 6 animals showed sign of estrus on day 43 and the intensity was moderate. Detection of estrus in 1 out of 6 buffaloes in Group T3 indicates that the injection of 250 mg progesterone might not have been sufficient to modulate the hypothalamo hypophyseal-gonadal axis. Thakur *et al*., 1989 and Kumar *et al*., 2000 reported successful induction of estrus in anestrous buffaloes with administration of 500 mg of Progesterone and Estradiol combination, while [19] induced estrus in anestrous buffaloes with only Progesterone; supports our observation of induction of estrus in 1 out of 6 buffaloes under group T3 treated with high dose of Progestrone. However, attempt to induce estrus in buffalo could not achieve height because the experiment was conducted in the months of April to June, the period which is well known to keep buffalo away from breeding. Animals under Group T2 though received four injections each of 250 mg progesterone at 3rd day interval only three animals were detected in estrus. This might be due to continuous administration of higher doses of progesterone. Detection of estrus in 5 (83.33%) out of 6 buffaloes of Group T1 receiving 250 mg of progesterone on alternate day, suggests sensitization of hypothalamo-hypophyseal-gonadal axis to release its respective hormones ultimately to trigger the mechanism of folliculogenesis and subsequent fertile estrus.

The positive response of consistent administration of even lower dose of progesterone through preantral route release of progesterone through intra-vaginal implant and ear implant, might expert depressing effect on hypothalamo-hypophyseal-gonadal axis; and their withdrawal released the very axis from the negative effect and thereby set to function for release the tropic hormones indirectly or directly responsible for folliculogenesis expression of estrus symptom and ovulation [13].

Table-7 *Effect of Duraprogen on anestrus buffaloes*

Group	No. of animals	No. of animals in estrus	No. of animals in anestrus	Day on which sign of estrus observed
Group Control	6	0	6	-
Group T1	6	5	1	Day 44, 44, 45, 46, 49
Group T2	6	3	3	Day 46, 46, 50
Group T3	6	1	5	Day 43

Effect of Duraprogen on Blood Biochemical Profile

On day 39th, all the mineral mixture treated animals which did not showed signs of estrus were subjected to further investigation. Out of 38 animals 2 buffaloes showed sign of estrus during mineral mixture treatment were discarded from further investigation. Out of 36 animals, 24 animals were selected randomly and divided into four groups having six animals in each group. The animals of Control group were treated with normal saline on day 39,43,47 and 51 while the animals of Group T1, T2 and T3 were treated with DuraprogenTM at the dose rate of 250 mg per ml. intramuscularly on day 39. The animals of Group T1 were further given Duraprogen on day 41, 43, 45, 47 and 49 (*i.e.,* on alternate day). The animals of Group T2 were further treated with that on day 43, 47 and 51(*i.e.,* three day interval) while the animals of Group T3 were further treated on day 46 (*i.e.,* six day interval). Blood samples were collected on day 43, 47, and 51 and/or the day when animal showed sign of estrus. The findings of blood biochemical profile were recorded and analysed.

Total Serum Cholesterol

Blood from all the buffaloes treated with mineral mixture were collected on day 8, 39, 43, 47 and 51 or on the day of estrus. The results are presented in [Table-8]. Result revealed that there is significant difference (P<0.05) in the level of serum total cholesterol in all the treatment groups with respect to control group. The comparison of mean value of total serum cholesterol is shown in [Table-8]. Result revealed that on day 43, 47 and 51/day of estrus, there is significant increase in mean level of serum total cholesterol. On day 43, Group T1 has the highest mean serum cholesterol (139.46±13.43 mg%) followed by Group T2 (132.43±7.76 mg%) and Group T3 (131.03±11.23 mg%). All the treatment groups differ significantly with control group. However, they did not vary within themselves. On day 48, the trend of increase in mean cholesterol level was almost same as on day 43 but Group T1 (156.73±8.96 mg%), Group T2 (148.46±7.70 mg%) and Group T3 (144.97±8.11 mg%) had significantly higher level of serum total cholesterol than day 43. This height was non significantly different from that of day 43. There was no significant increase in mean serum cholesterol level on day 51 with respect to day 47. The trend of mean value total serum cholesterol was same as on day 43 and 47. Group T1 (157.45±9.42 mg%) had the highest mean serum cholesterol followed by Group T2 (151.44±10.33 mg%) and Group T3 (143.35±6.76 mg%).

A similar finding was obtained by Amanullah, *et al*., (1997) [9]; Latif, *et al*., (1993) [10]; Patel, *et al*., (1994) [11]; Umesh, *et al*., (1995) [12]. By over viewing of data of serum total cholesterol concentration of different group of animals just before the administration of prostaglandin in experimental and control group animals (day 0) revealed that the values of serum total cholesterol in all groups fell in the range from 84.35 mg% to 112.29 mg%. The pre-treatment values of serum total cholesterol of the animals may be taken as the normal basal serum total cholesterol concentration of anestrus buffaloes maintained under village management system. Some investigators had reported a little lower serum total cholesterol concentration in dry anestrus [12, 20] and anestrus repeat breeding buffaloes [21], while some other investigators had reported a little higher mean values of serum total cholesterol during anestrus period [12], than that of the pre-treatment value recorded during the present experimentation. Moreover, the pre-treatment values of the serum total cholesterol estimated in buffaloes during present experiment was similar to the values of serum total cholesterol reported during anestrus period in buffaloes by several investigators [11]. The serum total cholesterol concentration detected in anestrus buffaloes on day 0 just before PGF2α/normal saline injections in different experimental and control groups of present experiment fell within the reference basal range of serum cholesterol concentration in bovines. In different groups even the pre-treatment serum total cholesterol concentration estimated in buffaloes was similar to the serum total cholesterol concentration reported during anestrus period in crossbred cows [22], in Jersey cow [23], in Deoni cows [24]. However, a higher circulating concentration of serum total cholesterol during anestrus period in lactating crossbred Jersey cow [25] and Friesean and crossbred dairy cows [26] have also been reported.

The buffaloes of Group T1, Group T2 and Group T3 that were having higher progesterone concentration on day '40' were detected in estrus between 4 and 9 days in Group T1 and between 6 and 8 days in Group T2 of last Duraprogen injection. The buffaloes of Group T1 receiving 1 ml (250 mg) Duraprogen on alternate days although were having similar higher serum total cholesterol concentration exhibited estrus between day 4 of 2ndDuraprogen injection and similarly the buffaloes of Group T2 having higher serum cholesterol on day Duraprogen injection also exhibited the estrus between 6 and 8 days after the two doses of progesterone treatment. The reproductive behaviour of these two groups revealed clearly that these buffaloes were although having developing follicle on the ovary might be secreting elevated level of estradiol but could not exhibit the overt sign of estrus and it took 6-8 days after first Duraprogen injection for exhibition of estrus. It might be due to suppression of the mechanism responsible for the final development of graffian follicles, secretion of higher concentration of estradiol by the developed follicle and changes in female genitalia and behaviour of the buffaloes due to the decrease in estradiol-progesterone ratio in the circulation and imbalance in the co-ordination in the functioning of hypothalamo–hypophyseal–gonadal system required for bringing the animal in estrus [27]. The observation suggest that in both the groups the mechanism responsible for steroid metabolism and its disintegration might have taken 5 to 9 days to reduce the concentration of progesterone in circulation increasing the estradiol–progesterone ratio to bring the animal to estrus. The dose related response of progesterone administration has also been observed during present experimentation in terms of synchronization of estrus by progesterone treatment in anestrus buffaloes. It has been observed through present experimentation, that the administration of 250 mg progesterone to buffaloes as a single injection did not have any influence on estrus cyclicity. It may be presumed that administration of single injection of 250 mg progesterone might not be sufficient to sensitize the hypothalamo–hypophyseal–gonadal system to establish co-ordination in the organs to integrate the functional activity therein. As minimum threshold level of any hormone is required to activate its target organ [27]. The injection of 500 mg progesterone twice have influenced the system similar to the single dose responsible to bring the animals to estrus cyclicity even then the excess higher dose of progesterone does not have positive response to bring the animal to estrus. The observation of present experimentation suggest that either 250 mg progesterone followed with 500 mg of progesterone or 500 mg progesterone either as single or double injection in anestrus buffaloes are sufficient to set the possible mechanism to bring the animal to estrus. However, before drawing any conclusion on the efficacy of the synchronization of estrus in buffaloes by using prostaglandin and progesterone combination various trial of progesterone at different doses and frequency on large number of animals are required. The abruptly higher concentration of serum cholesterol detected in cycling animals than in the noncycling ones have suggested that intrinsic mechanism like implication of hypophyseal and gonadal hormones in conjunction with thyroxin and corticoids [28], contributed for elevation of cholesterol for synthesis of steroid hormones during the phase of ovarian activity. The higher concentration of serum total cholesterol estimated on the day of estrus in buffaloes of present experiment is similar to the observation already reported on concentration of serum total cholesterol during anestrus and estrus phase of cattle [29, 24] and buffaloes [9,12].

Total Serum Calcium and Total Inorganic Phosphorous

Total serum Ca and P estimated in the blood of all the buffaloes grouped and classified as per schedule (on day 39, 43, 47, 51 or on day of estrus) are presented in the [Table-9] and [Table-10] respectively. On day 43, 47 and 51, the group T2 appeared to have higher blood serum Ca levels in comparison to that of other groups (T1and T3) but statistical analysis revealed no significant difference (P<0.05) between them at all day wise and group wise. The comparison of mean value of Serum inorganic phosphorous of all groups are presented in [Table-10]. Result revealed that there is significant difference in the level of Serum inorganic phosphorous in all the treated groups with respect to control group on day 47 and 51. Result revealed that the mean value of Serum inorganic phosphorous on day 47 and 51/day of estrus, showed significant increase in mean Serum inorganic phosphorous level. The mean value on day 43 did not differ significantly with control group. On day 43, Group T1 (4.89±0.84 mg%) had the highest mean Serum inorganic phosphorous followed by Group T3 (4.81±0.99 mg%) and Group T2 (4.76±0.33 mg%). All the treatment groups differed significantly with control group.On day 47, there is significant increase in mean Serum inorganic phosphorous level from the day 43. Group T1 (5.99±0.58 mg%) has the highest mean Serum inorganic phosphorous which did not differ significantly with the mean value of Group T2 (5.52±0.41 mg%) but Group T3 (5.15±0.28 mg%) had significantly higher level of Serum inorganic phosphorous than day 43.There was no significant increase (P<0.05) in mean serum inorganic phosphorous level on day 51 with respect to day 47. The trend of mean value serum inorganic phosphorous was same as on day 47. Group T1 (6.03±0.69 mg%) had the highest mean serum inorganic phosphorous followed by Group T2 (5.73±0.61 mg%)and Group T3 (5.25±0.75 mg%).The non-significant difference (P<0.05) in the mean value of total serum calcium was in accordance with the findings of Amanullah, *et al*., (1997) [9]; Patel, *et al*., (1994) [11]; Umesh, *et al*., (1995) [12] but differed with the findings of Bhatia, *et al*., (1985) [16].

The significant difference in the mean value of total inorganic phosphorous was in accordance with the findings of Bhatia, *et al*., (1985) [16] and Umesh, *et al*., (1995) [12] but differed with the findings of Umesh, *et al*., (1995) [12] and Amanullah, *et al*., (1997) [9]. Eltohamy, *et al*., (1989) [30] observed that the low phosphorous level may be accountable for infertility. The level of circulating serum calcium and inorganic phosphorous largely depended upon age and breed of animal, calcium and phosphorous content of the ration, temperature and season of the year, internal endocrine factors and health status of the animals. The report available on the serum calcium and inorganic phosphorous at different ages and stages of reproduction are variable.

Table-9 *Mean ± SE of Serum Calcium Level (mg/dl) in Buffaloes*

Animal gr. (No. of Animals)	Day '39' (anestrus)	Day '43'/ estrus	Day '47'/ estrus	Day '51'/ estrus
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Control Gr. (6)	10.23 ± 0.98 a	10.15 ± 1.02 a	10.37 ± 0.97 a	10.44 ± 1.20 a
Group T1 (6)	10.02 ± 1.21 a	10.25 ± 0.89 a	9.69 ± 1.20 a	10.19 ± 1.13 a
Group T2 (6)	10.13 ± 1.07 a	10.40 ± 1.33 a	10.20 ± 1.01 a	10.26 ± 1.41 a
Group T3 (6)	9.47 ± 1.40 a	9.68 ± 1.26 a	9.96 ± 1.08 a	9.54 ± 0.96 a

Mean values with similar superscript did not differ significantly (P<0.05).

Table-10 *Mean ± SE of Serum Inorganic Phosphorus Level (mg/dl) in Buffaloes*

Animal gr. (No. of Animals)	Day '39' (anestrus)	Day '43'/ estrus	Day '47'/ estrus	Day '51'/ estrus
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Control Gr. (6)	4.13 ± 0.65 a	4.10 ± 0.53 a	4.05 ± 0.47 a	4.15 ± 0.44 a
Group T1 (6)	4.46 ± 0.76 a	4.89 ± 0.84ab	5.99 ± 0.58 b	6.03 ± 0.69 b
Group T2 (6)	4.25 ± 0.48 a	4.76 ± 0.33ab	5.52 ± 0.41 b	5.73 ± 0.61 b
Group T3 (6)	4.32 ± 0.81 a	4.81 ± 0.99ab	5.15 ± 0.28 b	5.25 ± 0.75 b

Mean values with similar superscript did not differ significantly (P<0.05).

The similar pre-treatment (day 0) values of serum calcium and phosphorous concentration observed in all the groups of buffaloes suggest that the feeding and managemental (health, status and internal endocrine) factors were nearly identical in all the animals. Although the animals selected for present investigation were maintained under village management system by the different farmers within the radius of about 10 km of Danapur, it reflects that the animal management practices of that locality are ideal and feeding system are proper. The administration of prostaglandin and progesterone did not have any effect on serum calcium and phosphorous concentration during the experimental period, revealed that prostaglandin and progesterone administration does not have any influence directly or indirectly a mechanism responsible for the maintenance of calcium and phosphorous in circulation. By over viewing the serum calcium concentration of individual animals, it revealed that the serum calcium concentration on the day of estrus were also similar to the serum calcium concentration recorded on day 0, day 8, day 39, day 43, day 47 and day 51/estrus. This was the reason that the mean values of serum calcium on the day of estrus was similar to the mean value of serum calcium recorded on day 0, day 8, day 39, day 43, day 47 and day 51/estrus but serum inorganic phosphorous concentration of individual animals, revealed that the serum inorganic phosphorous concentration on the day of estrus were significantly higher to the serum inorganic phosphorous concentration recorded on day 0, day 8, day 39, day 43, day 47 and day 51. There is no report of the effect of prostaglandin and progesterone administration on serum calcium and phosphorous concentration available to relate the values of serum calcium and phosphorous recorded during post treatment period of day 0, day 8, day 39, day 43, day 47, and day 51/estrus of present experiment. However, some investigators have reported higher calcium and phosphorous at estrus than anestrus period in cows [16, 31] and buffaloes, similarly few reports are also available which suggest higher calcium and phosphorous in cycling ones than in noncycling [24, 31] and buffaloes [32].Cyclic periods without alteration in serum calcium concentration in cattle stand reported [24,33]. However, the maintenance of similar concentration of serum calcium and phosphorous in circulation during pre and post treatment anestrus period and at estrus of present investigation agrees with the non elevation of serum calcium and phosphorous concentration already reported in buffaloes [9, 11,21]. The values of serum calcium and phosphorous estimated in buffaloes of present experiment is nearly similar to the values of serum calcium and phosphorous concentration reported by the other investigators in cattle [24] and buffaloes [11,22,34].

Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT)

Since more than six decades attempts have been made to introduce the values of enzymes in domestic animals with view to establish normal activity of different enzymes during different status of health and production from birth to entire reproductive life. Estimation of serum concentration of different enzyme activities in normal intermediary metabolism have become increasingly useful to relate the levels of the enzymatic activity with the necrosis, degeneration or regeneration of tissue cells supposed to be rich source of enzyme. During the course of formation of these enzymes and during its activity in the tissue cells some fractions of enzymes spill over and/or escape from the cells into the blood stream. The quantity of the enzymes activity in the serum has been considered to be directly proportional to its rate of formation and the activity in the synthesizing cells. Thus, the activities of enzyme in the serum determine the physiological and pathological status of the cells in which enzymes are synthesized and remain active.

The estimation report on SGOT, SGPT in blood from all the buffaloes treated with mineral mixture on day 43, 47 and 51 or on the day of estrus have been presented in [Table-11]. The comparison of mean value of SGPT is shown in the [Table-11]. There is no significant difference in mean value of SGPT day '39' (39.97±2.23 mlu/ml.) with the mean value of SGPT recorded post Duraprogen treatment *i.e.,* on day 43, 47 and 51/day of estrus. On day 43, Group T2 (38.90±2.81 mlu/ml.) had higher mean SGPT but did not differ significantly with Group T3 (38.40±2.33 mlu/ml.) and Group T1 (37.96±1.81 mlu/ml.). Similarly, on day 47, Group T2 (39.02±2.12 mlu/ml.) had higher mean level followed by Group T1 (38.47±2.70 mlu/ml.) and Group T3 (37.98±1.99 mlu/ml.). On day 51, Group T2 (38.88±2.21 mlu/ml.) had higher mean level followed by Group T1 (38.25±2.29 mlu/ml.) and Group T2 (38.23±1.85 mg%). The mean value of SGPT did not differ significantly throughout the course of investigation *i.e.,* from day 0 to day 52.

Table-11 *Mean ± SE of SGPT Level (mlu/ml) in Buffaloes*

Animal gr. (No. of Animals)	Day '39' (anestrus)	Day '43'/ estrus	Day '47'/ estrus	Day '51'/ estrus
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Control Gr. (6)	37.83 ± 2.25 a	37.45 ± 1.89 a	37.86 ± 2.11 a	38.32 ± 2.04 a
Group T1 (6)	38.40 ± 2.50 a	37.96 ± 1.81 a	38.47 ± 2.70 a	38.25 ± 2.29 a
Group T2 (6)	38.66 ± 2.91 a	38.90 ± 2.81 a	39.02 ± 2.12 a	38.88 ± 2.21 a
Group T3 (6)	37.97 ± 1.96 a	38.40 ± 2.33 a	37.98 ± 1.99 a	38.23 ± 1.85 a

Mean values with similar superscript did not differ significantly (P<0.05).

Table-12 *Mean ± SE of SGOT Level (mlu/ml) in Buffaloes*

Animal gr. (No. of Animals)	Day '39' (anestrus)	Day '43'/ estrus	Day '47'/ estrus	Day '51'/ estrus
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Control Gr. (6)	29.89 ± 4.86 a	30.15 ± 1.63 a	30.20 ± 1.81 a	30.42 ± 1.85[a]
Group T1 (6)	30.46 ± 3.97 a	30.31 ± 2.68 a	29.35 ± 2.25 a	29.75 ± 2.32 a
Group T2 (6)	31.11 ± 2.87 a	30.25 ± 2.56 a	30.66 ± 2.65 a	31.47 ± 2.61 a
Group T3 (6)	30.39 ± 5.01 a	30.15 ± 3.25 a	29.86 ± 1.95 a	30.56 ± 2.20 a

Mean values with similar superscript did not differ significantly (P<0.05).

The comparison of mean value of SGOT has been presented in [Table-12]. There is no significant difference in mean value of SGOT at day '39' (30.30±4.32 mlu/ml.) with the mean value of SGOT recorded post Duraprogen treatment *i.e.,* on day 43, 47 and 51/day of estrus. On day 43, Group T1 (30.31±2.68 mlu/ml.) had non-significantly higher mean SGOT level followed by Group T2 (30.25±2.56 mlu/ml.) and Group T3 (30.15±3.25 mlu/ml.). On day 47, Group T2 (30.66±2.65 mlu/ml.) had higher mean level followed by Group T3 (29.86±1.95 mlu/ml.) and Group T1 (29.35±2.25 mlu/ml.).

On day 51, Group T2 (31.47±2.61 mlu/ml.) had higher mean level followed by Group T3 (30.56±2.20 mlu/ml.) and Group T1 (29.75±2.32 mlu/ml.). The mean value of SGOT did not differ significantly throughout the course of investigation *i.e.,* from day 0 to day 51. Among the enzymes the transaminases are the primary enzymes which are normally concerned with the transfer of α-amino group of either aspartic acid or alanine of α-ketoglutaric acid resulting in formation of oxaloacetic acid and pyruvic acid respectively. It has been observed that SGOT activity is significantly elevated after induced hepatic necrosis by administering carbon tetra chloride in horse, cow, pig and dog but marked elivation in SGPT was confirmed in dogs only [35]. Animals of the present experiment were apparently healthy though under rural management system and thus the SGOT and SGPT activity detected on day 0 could be taken as the normal basic values for the buffaloes. Maintenance of similar SGOT and SGPT activities in the experimental buffaloes from day 0 suggested to the end of the experiment that the physical and physiological status of buffaloes of these four groups were similar and these factors were unaffected by administration of Progesterone.

Conclusion

On critical analysis it may be concluded that mineral mixture feeding at the dose rate of 30 gms per animal per day up to one month had non-significant effect on maintaining reproductive rhythm during summer season. Repeated dosages of progesterone (Duraprogen 250 mg, 1 ml) on alternate day had better effect than administered at three-day interval or at six-day interval. There is significant increase of serum cholesterol and serum inorganic phosphorous level in the animal showing sign of estrus. There is no significant difference in the level of serum calcium, SGOT and SGPT in post-partum anestrus as well as buffaloes showing sign of estrus.

Application of research: It has a potential for newer diagnostic and therapeutic applications in the future.

Research Category: Bovine, Reproductive disorders, Hormonal therapy
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