BLOOD PLASMA BIOCHEMICAL CHANGES AND CONCEPTION RATE IN ANOESTRUS BUFFALOES TREATED WITH VARIOUS HORMONE PROTOCOLS

ABSTRACT

To study certain blood biochemical parameters (total cholesterol, total protein, calcium and inorganic phosphorus) at various periods of treatment.Total 40 anestrous buffalo heifers with history of anoestrus were selected from the semi-arid area of Banaskanth and randomly divided into four equal groups. (1)Group-I:Heatsynch protocol (n=10), (2)Group-II:Ovsynch protocol(n=10), (3)Group-III: Doublesynch protocol (n=10) and (4)Group-IV: Control(n=10) followed by fixed time artificial insemination in treated buffalo heifers and insemination at spontaneous estrus in buffalo heifers of group-IV.The overall conception rate following 3 consecution services was maximum in Group II (50%) followed by Group III (40%), Group I (30%) and Group IV (30%). The blood plasma was collected at the time of initiation of treatment (P1), at the time of inj. $PGF_{2\alpha}$ (P2), at the time of 1st FTAI (P3) and day 21st post insemination (P4) for the estimation of biochemical parameters.The blood biochemical profiles include total protein (range: 4.54± 0.19 to 6.11± 0.52 g/dl) which in all group did not show any significant difference during the different period but significant ($P<0.05$) difference between the groups was noticed. Similarly calcium and phosphorus found ranging between 5.73± 0.20 to 9.53± 0.95 mg/dl and 5.46± 0.22 to 6.95± 0.30 mg/dl, respectively in all four groups. The cholesterol estimated values (range: 34.12 ± 1.75 to 41.97± 2.43 mg/dl) were found not-significantly varying between the groups as well as among the different periods.Heatsynch, Ovsynch and Doublesynch protocol can be successfully used for the induction of estrus in true anoestrus buffalo heifers with Better conception rate would be achieved following Ovsynch protocol in buffalo heifers.

1. INTRODUCTION

The buffalo farmers usually face challenges in detecting estrus as buffaloes have a tendency to show silent estrus and anestrous. Prolonged postpartum acyclicity and anestrous are also responsible for huge economic losses to buffalo breeders. The incidence of anestrous is higher (56.0%) in buffalo heifers than in cow heifers (36.0%) (Ullah *et al.*, 2006).Anestrous is one of the most commonly occurring reproductive problems in cattle and buffalo of India. 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. In heifers, it poses a herd problem possibly due to low plane of nutrition, stress of seasonal transition or extremes of climatic conditions. Seren *et al.* (1993) and Ohashi (1994) reported that widespread use of AI in buffalo is still limited due to relatively low expression of estrus behavior in buffaloes. This consideration indicates need for estrus synchronization using fixed time insemination for implementation of breeding

programs in buffaloes (Ali and Fahmy, 2007).To improve reproductive efficiency, several protocols of estrus induction and ovulation synchronization have been developed. These procedures are based on manipulating the Corpus luteum, either to induce premature luteolysis using prostaglandins or to prolong the luteal phase using progestogens. The keys for successful estrus synchronization are closely synchronized rapid decline in circulating progestin concentrations and synchronous growth and ovulation of a viable follicle. Estrus synchronization has many advantages and is becoming mandatory in modern animal husbandry practices in indigenous cattle and buffaloes, which are known for anoestrus and silent estrus.

Biochemical constituents of blood have great diagnostic value in evaluating the physiological status as well as to improve post-partum fertility in female. Lack of protein or insufficient intake of protein was considered to be a cause of failure or delay in estrus cycle (Roberts, 1971). Cholesterol is synthesized from acetate and is an essential precursor for steroid hormone synthesis. Mineral like calcium and phosphorus have influence on certain enzyme system and affects reproductive efficiency (Dhoble and Gupta, 1986), which might be reflected in lower blood level of them. Lack of calcium and phosphorus upsets the proper functioning of genital organs (Acharya, 1960). Looking to the above fact the comparative study of various synchronization protocols and their influence on plasma biochemicals in anestrous buffalo heifers was decided.

2. MATERIAL AND METHODS

2.1 Locationand Climatic Of the Experimental Area

The study was carried out at Banaskantha district.The district is situated in the north western part of the Gujarat state and lies between north latitudes 23°33' & 24°25'and east longitude 71°07' & 73°02'. The district has semi-arid climate. The recorded maximum and minimum temperature was 32.1 ºC and 15.5 ºC. Whereas, Relative humidity were 70.5 and 46.15 per cent, respectively during the period of study.

2.2 EXPERIMENTAL PROTOCOL

2.2.1 Selection of the buffalo heifer

A total 40 Anestrous buffalo heiferswith the history of anestrous were selected from villages of Banaskantha milk-shed areas of Gujarat. The buffalo heifers were screened gynaeco-clinically for their reproductive status. Detailed history and rectal palpation findings were recorded. The anestrous buffalo heifers having small, smooth, inactive ovaries with normal genitalia and no palpable CL on either ovary, relaxed uterine horn was selected for study. The reproduction status of their buffalo heifers were re-examined after 10 days for conformation the condition. All these selected buffalo heifers were dewormed.

2.2.2 Grouping of buffalo heifers.

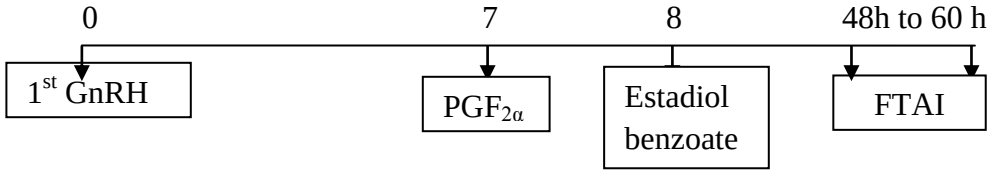
All the 40 buffalo heifers were randomly divided in four groups as follow.

2.2.2.1 Group-I (HeatSynch Protocol, n=10)

The Selected buffalo heifers of this group were administered with Intramascular Inj. of 10 µg of GnRH analogue i.e. Buserelin Acetate 10 µg (Receptal , @ 2.5 ml, Intervet India Pvt Ltd, New delhi) on day 0, followed by Inj. of 500 µg $PGF_2\alpha$ analogue i.e. Cloprostenol sodium (Estrumate, @ 2 ml, Intervet India Pvt Ltd) and estradiol benzoate 1 mg I/M (Sigma, USA) on

days 7 and 8, respectively, and were fix time artificial inseminated(FTAI) twice at 48 and 60 hrs post-Estradiol injection.

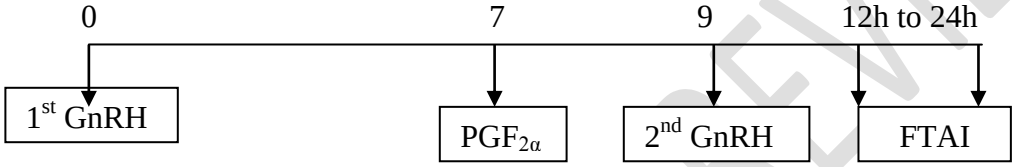
Heatsynch Protocol with Time AI



2.2.2.2 Group-II (Ovsynch Protocol, n=10):

The selected buffalo heifers of this group were administered with Intramascular Inj. of 10 µg of Buserelin acetate on day 0, Inj. of 500 µg Cloprostenol sodium, on day 7 and second Inj. of 10 µg of Buserelin acetate on day 9 followed by fix time artificial insemination twice at 12 and 24 hrs later.

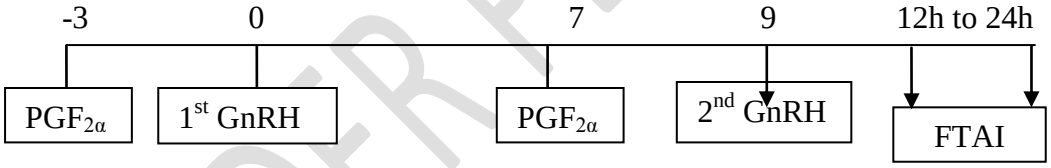
Ovsynch Protocol with Time AI



2.2.2.3 Group-III (Double Synch Protocol, n=10):

The buffalo heifers of this group were administered with Inj. of 500 µg Cloprostenol sodium on -3 day (3 days before the first injection of GnRH), I/M followed Inj. of 10 µgBuserelin acetate on day 0, Inj. of 500 µg Cloprostenol sodium on 7th day and second Inj. of 10 µg Buserelin acetateon 9th day, the buffalo heifers were fix time artificial inseminate twice at 12 and 24 hrs later following the second injection of buserelin acetate.

Doublesynch Protocol with Time AI



2.2.2.4 Group-IV (Anoestrus control, n=10):

The selected buffalo heifers of this group were injected with normal saline 5 ml, i.m on days 0, 7 and 9 and were observed for onset of natural estrus and artificial insemination was performed on detection of estrus. The group served as untreated control.

2.3 INDUCTION OF ESTRUS AND ESTRUS INTENSITY

The buffalo heifers were closely observed for the exhibition of estrus symptoms at the time of fix time insemination. The intensity of induce estrus was recorded based on the expressions of estrus symptoms. Estrus symptoms of group IV were recorded at the spontaneous estrus. The estrus intensity of selected buffalo heifers were monitored on the basis of their behavior changes i.e. bellowing, excitement, off feed, estrus mucus discharge as well as edema, erythema and wetness of vulva.

2.4 CONCEPTION RATE / FERTILITY RESPONSE

The buffalo heifers which did not show signs of estrus following the treatment were examined per rectally for pregnancy diagnosis on 60th day post insemination to confirm the pregnancy. The buffalo heifers which did not conceive at the fixed time insemination were re-inseminated up to two subsequent estrus cycles. The conception rate up to 3rd insemination was recorded.

2.5 BLOOD PLASMA COLLECTION

2.5.1 Method of Blood plasma collection

The 5 ml blood samples from buffalo heifers under treatment were collected in heparinized vials through jugular vein puncture. The samples were centrifuged at 3000 rpm for 15 min and plasma was separated out and was stored deep freeze at −20°C until analyzed.

2.5.2 Schedule of Blood Plasma Collection

Blood plasma collection was as under;

P1 = before initiation of treatment

P2 = at the time of injection of $PGF_{2\alpha}$

P3= on the day of 1st timed artificial insemination

P4 = on the 21st day post insemination.

The blood samples from control group were collected on 0, 7, 1st (at spontaneous estrus) insemination and 21st day post insemination.

2.6 BLOOD PLASMA BIOCHEMICALS

Total cholesterol, total protein, calcium and phosphorus were quantified in blood plasma by using diagnostic kits (Coral Clinical System (India) Pvt. Ltd., Goa) and analyzed by using Bio-chemistry Analyzer RX-50V (Micro Lab, India).

2.7 STATISTICAL ANALYSIS OF DATA

The data collected were suitably tabulated and analyzed following standard statistical method shown by Steel and Torrie (1981). While the test of significance between and within the treatment groups and periods for biochemical profile were made by factorial completely randomized block design and Duncan's new multiple range test. The Duncan's new multiple range test was performed at 5 per cent level of significance.

3 RESULTS AND DISCUSSION

3.1 Intensity and Induction of Estrus

Intensity of estrus was recorded on fix time insemination in different groups of treatment. Different estrus symptoms were bellowing, excitement, off-feed, estrus mucus discharge as well as edema, erythema and wetness of vulva. The finding (bellowing, excitement, mucus discharge and swollen) of present study are similar of Mohan *et al.* (2009) in buffaloes following Heatsynch protocol.The recorded estrus intensity at the time of artificial Insemination in Group-I was 90 per cent (9/10), Group-II express 80per cent (8/10), In Group-III estrus induction was 80per cent (8/10), Whereas in group-IV (control anestrous buffalo heifer) it was50 per cent (5/10).Neglia *et al.* (2003) and Atanasov *et al.* (2011) observed mucus discharge in buffaloes at the time of AI following Ovsynch protocol. Similar Malik *et al.* (2011) and Yotov *et al.* (2012) also observed 40 and 66.7 per cent anestrous buffaloes showing estrus mucus discharge following Ovsynch protocol. Karen and Darwish, (2010) observed

estrus mucus discharge in cyclic heifers and non-cyclic cows following Ovsynch protocol. Hoque *et al.* (2014) observed diverse intensity of estrus signs water buffaloes with different protocols like Ovsynch, modified Ovsynch and Double-ovsynch.

When the records of all 40 buffalo heifers were pooled it was observed that 75 per cent (30/40 buffalo heifer) heifers showed induction of estrus. In Heatsynch protocol total 90 per cent (9/10) buffalo heifer showed estrus symptoms at the time of insemination. Similar result was recorded by Buhecha *et al.* (2016) in anestrues buffaloes. However, 100 per cent estrus was recorded following heatsynch protocol in buffalo by Ali *et al.* (2012) and Mohan *et al.* (2014). In Ovsynch protocol total 80 per cent (8/10) buffalo heifers showed estrus induction at the time of insemination. Similar result was recorded by Jabeen *et al.* (2012), Thorat *et al.* (2012), Savalia *et al.* (2014) and Buhecha *et al.* (2016) in anoestrus buffaloes. In Doublesynch protocol total 80 per cent (8/10) buffalo heifer showed estrus symptoms at the time of insemination. Similar results were recorded by Ozturk *et al.* (2010) in dairy cow. However, 100 per cent estrus was recorded following doublesynch protocol in buffalo by Mirmahmoudi and Prakash, (2012). In control group total 50 per cent (5/10) buffalo heifer showed estrus symptoms at different time interval within 90 days. Whereas, Lower estrus response was observed by Naikoo *et al.* (2010), Parmar *et al.* (2012), Nakrani *et al.* (2014) and Buhecha *et al.* (2016) in anestrous buffaloes. In present study maximum estrus induction was obtained in Heatsynch group followed by Ovsynch and Doublesynch group.

3.2 Conception rate

The buffalo heifers of treatment group were inseminated at fix time and the control group was inseminated at spontaneous estrus by semen of buffalo bull. The buffalo heifers which failed to conceive at 1st insemination were re-insemination for two subsequent estrus. The recorded overall conception rate of group-I, II, III and IV was 30.00, 50.00, 40.00 and 30.00 per cent, respectively. The 1st insemination conception rate of group I, II, III&IV was 10.00, 20.00, 20.00 and 10.00 per cent, respectively. Whereas the similar fingers for 2nd insemination conception rate was 22.22, 25.00, 12.50 and 22.22 per cent, respectively. Following the third insemination recorded conception rate was 0, 16.66, 14.28 and 0 per cent, respectively in group I, II, III and IV.

In present study 30 per cent conception rate was achieved by using Heatsynch protocol in anestrous buffalo heifers. Similar conception rate was obtained by Mohan *et al.* (2009) using Heatsynch protocol during summer and winter season in anestrous buffaloes. Almost similar conception rate was obtained by Buhecha *et al.* (2016) in buffaloes using Heatsynch protocol. However, higher (80%) conception rate was reported by Cevik *et al.* (2010) in cows. Conception rate was 50 per cent following Ovsynch protocol in anestrous buffalo heifers. Similar results were obtained by Berber *et al.* (2002), Naikoo *et al.* (2010) in buffalo. Derar *et al.* (2012) obtained 62.5 per cent conception rate in buffalo heifers which was higher than the reported in present study. Ramakrishnan *et al.* (2012) obtained 66.66 per cent conception in post-partum anestrous Gir cow.In Doublesynch protocol obtained conception rate was 40 per

cent in anestrous buffalo heifers of present study. Higher conception rate was obtained by Mirmahmoudi and Prakash, (2012) in anestrous buffaloes. Ozturk *et al.* (2010) reported 72 per cent conception rate in anestrous dairy cow following Doublesynch protocol. In control group overall conception rate was 30 per cent in anestrous buffalo heifers. Contrary to the present study higher conception rate (Kumar *et al.*, 2008; Nakrani *et al.*, 2014; Buhecha *et al.*, 2016) in anestrous buffaloes and Lower conception rate (10.00%) in buffaloes (Yotov *et al.*, 2012) was observed.

3.3 BIOCHEMICAL PROFILE

3.3.1 Total Protein (g/dl)

The concentrations of total protein found at various periods in four different groups are presented in table.1 and figure 1.

Table 1: Total protein concentrations (g/dl) in blood plasma at various periods in groups of anoestrus buffalo heifers (Mean ± SEm).

Period / Groups	Total protein (g/dl)			
	P1	P2	P3	P4
Group-I	4.630 ± 0.15^{d}	4.901 ± 0.27^{d}	4.549 ± 0.19^{d}	5.077 ± 0.22^{c}
Group-II	5.243 ± 0.21^{b}	5.489 ± 0.26^{b}	5.123 ± 0.13^{c}	5.594 ± 0.18^{b}
Group-III	4.849 ± 0.25^{cd}	4.884 ± 0.22^{cd}	5.719 ± 0.38^{b}	4.706 ± 0.31^{d}
Group-IV	5.902 ± 0.20^{a}	6.073 ± 0.24^{a}	6.112 ± 0.52^{a}	5.990 ± 0.49^{a}

Note: - Means bearing different superscripts differ significantly ($P<0.05$) within Columns.

In the present study the mean plasma total protein level on different period in buffalo heifers under Heatsynch, Ovsynch and Doublesynch revealed that the profile did not vary significantly but significant different was observed between groups. It shows close agreement with Savalia *et al.* (2014), Nakrani *et al.* (2014), Buhecha *et al.* (2016) in anestrous buffaloes. Contrary to the present finding Kumari *et al.* (2015) reported significant difference between before and after Ovsynch protocol in responded Murrah buffaloes, however this difference has not been observed in non-responded groups. Higher total protein level was recorded in Anestrous buffaloes (Ali *et al.*, 2012 and Nakrani *et al.*, 2014) and anestrus cow (Dhami *et al.*, 2015) was in agreement with the present study.

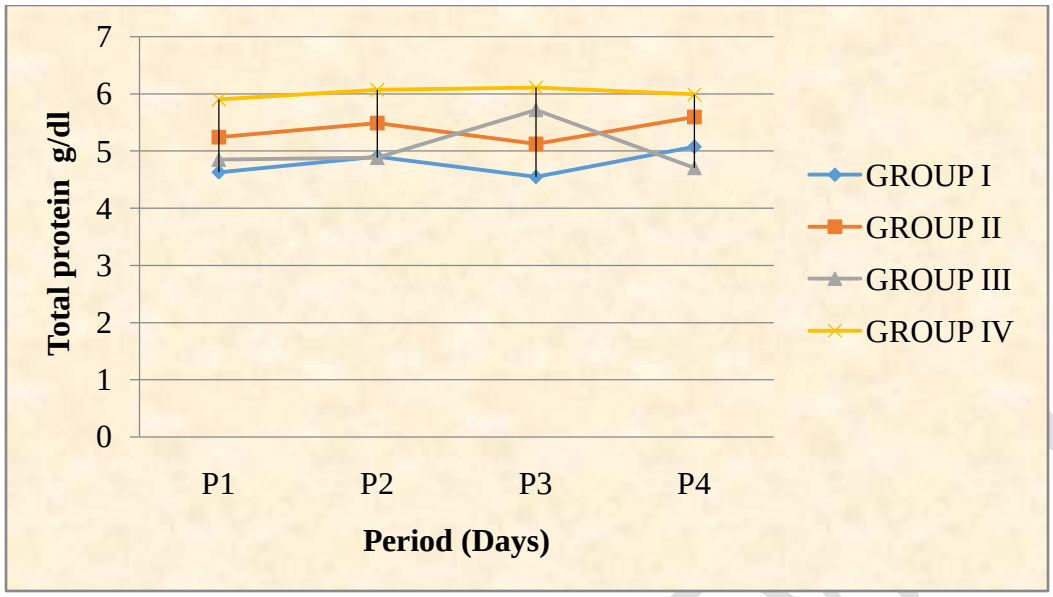


Fig.1: Total protein levels (g/dl) at various periods in different treatment groups.

3.3.2 Total Cholesterol (mg/dl)

The concentrations of total cholesterol was found at various periods in four different groups are presented in table.2and figure 2.

Table 2:TotalCholesterol concentration (mg/dl) in blood plasma at various periods in different groups of anoestrus buffalo heifers (Mean + SEm).

Period / Group	Total cholesterol (mg/dl)			
	P1	P2	P3	P4
Group-I	37.75±1.46	36.96±1.62	35.20±1.02	39.61±1.54
Group-II	36.07±2.64	35.89±1.29	35.17±2.21	41.97±2.43
Group-III	37.26±2.26	39.77±3.80	39.81±4.34	34.12±1.75
Group-IV	38.98±0.81	40.30±1.66	41.56±3.36	37.88±0.93

In the present study mean level of plasma total cholesterol level recorded in buffalo heifers under treatment of Heatsynch, Ovsynch and Doublesynch protocol did not showed significant different in profile between period of blood collection. Similar result was reported by Parmar *et al.* (2012), Savalia *et al.* (2014) and Buhecha *et al.* (2016) in anoestrus buffaloes. Kumari *et al.* (2015) reported the total cholesterol level increase in responded buffaloes after treatment of Ovsynch protocol.

Total cholesterol concentration remained fluctuating 41.97 ± 2.43 mg/dl to 34.12 ± 1.75 mg/dl in all four groups of buffalo heifers during different period. Similar total cholesterol level was recorded by Ghuman *et al.* (2011). Higher total cholesterol concentration was recorded by Parmar *et al.* (2012), Savalia *et al.* (2014) and Buhecha *et al.* (2016) in anestrous buffalo.

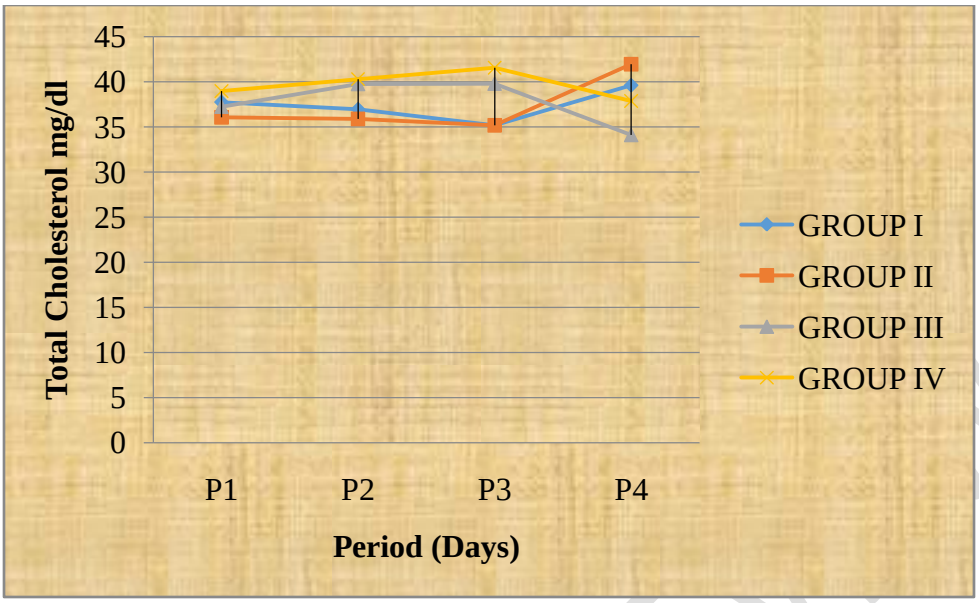


Fig. 2: Total cholesterol levels (mg/dl) at various periods in different treatment groups

3.3.3 Calcium (mg/dl)

The concentrations of calcium found at various periods in four different groups are presented in table.3 and figure 3.

Table 3:Calcium concentrations (mg/dl) in blood plasma at various periods in different groups of buffalo heifers (Mean± SEm).

Period / Group	Calcium (mg/dl)			
	P1	P2	P3	P4
Group-I	5.97±0.27^{c}	5.73±0.20^{c}	5.95±0.22^{d}	6.02±0.22^{c}
Group-II	7.77±0.84^{a}	7.16±0.74^{ab}	7.31±0.89^{c}	7.94±0.84^{b}
Group-III	8.33±0.79^{a}	7.85±0.66^{a}	9.53±0.95^{a}	8.80±0.83^{a}
Group-IV	6.81±0.16^{bc}	7.01±0.26^{ab}	8.24±1.01^{b}	8.48±0.65^{ab}

Note: - Means bearing deferent superscripts differ significantly (P<0.05) within column.

The plasma calcium level obtained in anestrous buffalo heifer during Heatsynch, Ovsynch and doublesynch protocols did not differed significant within group. Similar results were obtained by Buhecha *et al.* (2016) in anestrous buffaloes following PRID, Ovsynch and Heatsynch protocol in anestrous buffaloes. Ali *et al.* (2013) reported significant difference in calcium concentration on day 0 and 10th day in anestrous buffaloes following Ovsynch, Heatsynch and CIDR.

The concentration of plasma calcium in anestrous buffalo heifers during study was obtained in rang of 5.73±0.20 to 9.53±0.95 mg/dl in all groups. Similar results were obtained by (Jayachandran *et al.*, 2013 and Kumar *et al.*,

2016) in anestrous buffaloes. Kumari *et al.* (2015) reported higher calcium concentration during Ovsynch protocol in anestrous buffaloes. Higher calcium concentration was recorded by Paul *et al.*, (2000) in anestrous Nili Ravi buffaloes. Ahmed *et al.* (2010) reported 9.07±0.10 mg/dl in delayed buffalo heifers.

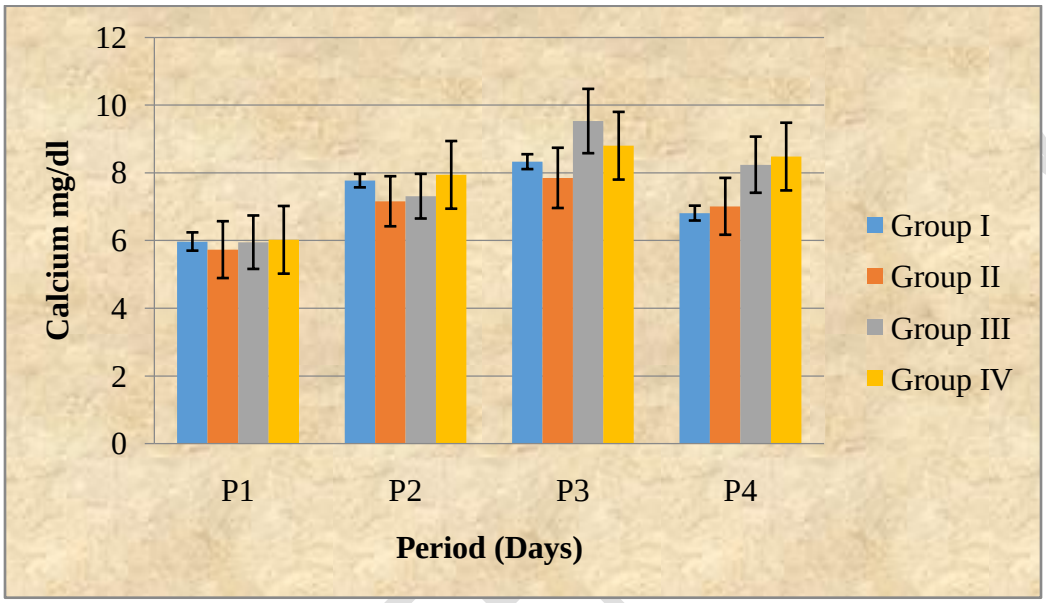


Fig.3: Calcium levels (mg/dl) at various periods in different treatment groups

3.3.4 Inorganic Phosphorus (mg/dl)

The concentrations of phosphorus found at various periods in four different groups are presented in table 4 and figure 4.

Table 4: Inorganic phosphorus concentration in blood plasma at various periods in different groups of buffalo heifers (Mean ± SEm).

Period Group	Phosphorus (mg/dl)			
	P1	P2	P3	P4
Group-I	5.54±0.27 ^{c}	5.88±0.30^{bc}	5.29±0.28^{c}	5.19±0.24^{c}
Group-II	5.76±0.28^{bc}	5.81±0.35^{c}	5.66±0.33^{bc}	6.11±0.44^{ab}
Group-III	6.95±0.30^{a}	6.81±0.41^{a}	6.54±0.24^{a}	6.31±0.45^{a}
Group-IV	5.91±0.26^{bc}	6.01±0.39^{bc}	5.91±0.35^{b}	5.46±0.22^{ab}

Note: - Means bearing different superscripts differ significantly (P<0.05) within column.

The plasma inorganic phosphorus level was obtained in anestrous buffalo heifers during Heatsynch, Ovsynch and doublesynch protocols did not revealed significant difference within groups. Similar result obtained by Buhecha *et al.* (2016) in anestrous buffaloes following PRID, Ovsynch and Heatsynch protocol

in anestrous buffaloes.Ali *et al.* (2012) reported significant difference in inorganic phosphorus concentration on day 0 and 10th day in anestrous buffaloes following ovsynch, heatsynch and CIDR. Parmar *et al.* (2012) reported the inorganic phosphorus level significantly higher at induce estrus than pre-treatment in post-partum anoestrus buffaloes. Kumar *et al.* (2015) reported not-significant different in inorganic phosphorus level in Murrah buffaloes before and after ovsynch protocol.

The concentration of plasma inorganic phosphorus in anoestrus buffalo heifers during study was obtained in rang (5.73±0.20 to 9.53±0.95 mg/dl) in all groups. Similar result obtained by (Ahlawat *et al.* 2010; Butani *et al.*, 2011 and Kumar *et al.*, 2016) in anestrous buffaloes. Higher phosphorus concentration was recorded by Paul *et al.* (2000) in anestrous Nili Ravi buffaloes. Ahmed *et al.* (2010) reported 9.07±0.10 mg/dl in delayed buffalo heifers. Also lower concentration of inorganic phosphorus reported in acyclic Murrah buffaloes (Kumar *et al.*, 2010), anestrous buffaloes (Chaurasia *et al.*, 2010; Jayachandran *et al.*, 2013).

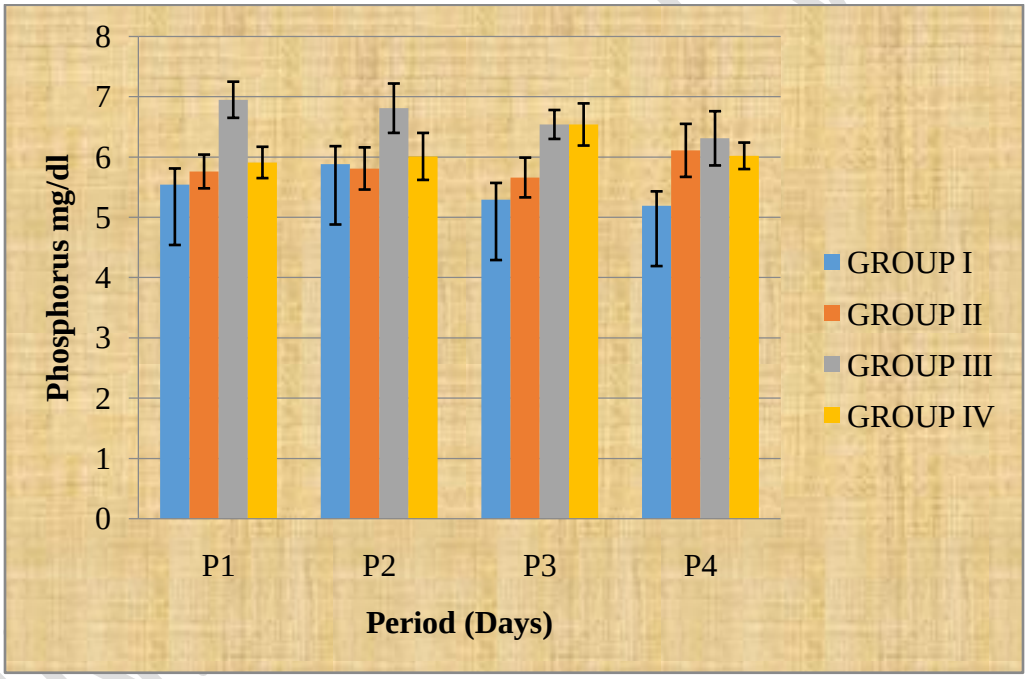


Fig. 4: Phosphorus levels (mg/dl) at various periods in different treatment groups

SUMMARY AND CONCLUSIONS

The study included the response of different hormonal protocols like Heatsynch (Group I), Ovsynch (Group II) and Doublesynch (Group-III) for the induction of estrus, intensity of estrus, conception rate, plasma biochemical (total protein, total cholesterol, calcium and phosphorus). The recorded estrus induction was 90, 80 and 80 per cent in Group I, II and III, respectively. The buffalo heifers were inseminated at fixed time with good quality of buffalo bull

semen and the overall conception rate following 3 consecution services was maximum in Group II (50%) followed by Group III (40%) and I (30%).The total protein was found significantly ($P<0.05$) different between the groups. The cholesterol estimated values were found not-significantly varying between the groups as well as among the different periods. The levels of calcium in all the four groups were estimated and statistical analysis revealed the significant ($P<0.05$) difference in calcium concentration among the groups. The estimation value of Inorganic phosphorus was differed significantly ($P<0.05$) among the groups but not within the periods. Hence, the present study concluded that the Heatsynch, Ovsynch and Doublesynch protocol can be successfully used for the induction of estrus in true anoestrus buffalo heifers. Comparatively better conception rate could be achieved following Ovsynch protocol followed by Doublesynch and Heatsynch protocol in buffalo heifers. The alteration of blood biochemical like total protein, total cholesterol, calcium and phosphorus did not show any specific effect due to any of the synchronization protocol.

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