Effect of Ovsynch and Mid-Cycle PGF$_2$$\alpha$ Treatment Protocols on Conception Rates and Plasma Biochemical and Minerals Profile in Repeat Breeding Cows and Buffaloes

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Publication Date: 10 March 2016

Article Link: [http://scientific.cloud-journals.com/index.php/IJAVST/article/view/Sci-421](http://scientific.cloud-journals.com/index.php/IJAVST/article/view/Sci-421)

Abstract

This study was conducted to evaluate the efficacy of Ovsynch and Mid-cycle PGF$_2$$\alpha$ protocols on conception rate and influence on plasma biochemical and mineral constituents in 20 each repeat breeding cows and buffaloes equally divided into Ovsynch and Mid-cycle PGF$_2$$\alpha$ protocols. Ten each healthy early postpartum cows and buffaloes served as normal cyclic controls. All the animals were inseminated at mid-estrus using good quality frozen-thawed semen and were kept under observation for next estrus or pregnancy. The first service conception rates at induced estrus obtained in repeat breeding cows and buffaloes were 50.00 and 40.00 % in Ovsynch protocol, and 40.00 and 50.00 % in mid-cycle PGF$_2$$\alpha$ treatment, respectively, and 40.00 % each in normal control group.

The overall mean values of total protein for conceived and non-conceived cows of Ovsynch protocol were 7.98±0.17 and 7.38±0.11 g/dl and for buffaloes 9.18±0.18 and 7.30±0.05 g/dl, respectively, being significantly higher in conceived ones in both the species. The mean cholesterol level in Ovsynch protocol was insignificantly higher in non-conceived than conceived cows (197.43±13.45 vs. 176.80±9.54 mg/dl). Contrary to this finding, in buffaloes it was higher in conceived than non-conceived group (149.43±9.02 vs. 135.65±9.81 mg/dl). No significant difference was observed in total protein and cholesterol profile between conceived and non-conceived groups of cows and buffaloes under Mid-cycle PG treatment. Also no significant differences were observed in values of calcium and inorganic phosphorus between days and between conceived and non-conceived groups of cows or buffaloes, apparently the values of both were higher in normal cyclic groups as compared to repeat breeders. The overall pooled values of calcium and phosphorus in cows under Ovsynch protocol were 9.17±0.12 and 4.44±0.10, and under Mid-cycle PGF$_2$$\alpha$ group 9.23±0.10 and 4.61±0.05 mg/dl, respectively, while in buffaloes the corresponding values under Ovsynch protocol were 9.88±0.10 and 4.94±0.05, and under Mid-cycle PGF$_2$$\alpha$ group 9.33±0.10 and 4.67±0.05 mg/dl, respectively. From the results, it can be inferred that hormonal therapies used significantly improved the pregnancy rates in repeat breeding cows and buffaloes under field conditions, without influencing the plasma biochemical and minerals profiles.

Keywords Repeat Breeding Bovines; Ovsynch; Mid-Cycle PGF$_2$$\alpha$ Treatment; Conception Rate; Biochemical & Mineral Profile
1. Introduction

Numerous studies have shown that repeat breeding is the second most prevalent reproduction disorder after anoestrus in dairy cows and buffaloes with the overall prevalence as high as 28.31 % (Bhat et al., 2012). Studies have shown that Ovsynch programme, with a strategic combination of GnRH (two injections) and PGF$_2$$\alpha$ (one injection), result in an acceptable pregnancy rate in cyclic cows (Pursley et al., 1995) and buffaloes (Baruselli et al., 1999). The recent reports demonstrated that the Ovsynch protocol resulted in increased conception rates by 21 per cent in repeat breeder dairy cows (Kasimanickam et al., 2005) and mid cycle PGF$_2$$\alpha$ treatment gave conception rate up to 70 per cent (Patel et al., 2014$^a$) without influencing the plasma biochemical and mineral profile. In view of the above fact, the present study was aimed to study the relative efficacies of two hormonal approaches in repeat breeding crossbred cows and buffaloes in terms of estrus response, conception rate and plasma biochemical and mineral profile.

2. Materials and Methods

2.1. Selection and Treatment of Animals

This study was conducted from November 2014 to April 2015 on repeat breeding dairy animals selected from the villages of Amul as well as Panchamrut milk shed areas of Gujarat and at Livestock Research Station, NAU, Navsari. Per rectum palpation of the reproductive tract was conducted in specific animals, which had been bred more than three times yet not conceived, to diagnose them as classical repeat breeders. Twenty each repeat breeding cows and buffaloes and 10 each normal cyclic early postpartum cows and buffaloes were selected for this study.

All animals identified were treated with s/c injection of ivermectin 100 mg (Inj. Neomec, 1 %, 10 ml, Intas Pharmaceuticals Pvt Ltd., Ahmedabad), i/m injections of inorganic phosphorus (Inj. Alphos-40, 10 ml, Zoetis), multivitamins AD3E (Inj. Intavita, 10 ml, Intas), and single shot enrofloxacin 3.0 g (Inj. Flobac SA, 10 %, 30 ml, Intas), 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 then randomly allotted to the following treatment protocols.

2.2. Ovsynch Protocol (Group-I)

Ten repeat breeding cows and buffaloes each with normal sized ovaries were put under Ovsynch protocol and were administered with i/m Inj. of Buserelin acetate 10 µg (Ovulanta, 2.5 ml, Vet Mankind) on day 0, Inj. PGF$_2$$\alpha$ 500 µg (Repregna, 2 ml, Vet Mankind) on day 7, and second i/m Inj. of Buserelin acetate was given on day 9, and fixed time AI (FTAI) was performed twice at 0 and 24 hrs later.

2.3. Mid-cycle PGF2$\alpha$ Treatment (Group-II)

Another ten repeat breeding cows and buffaloes each having mid-cycle palpable CL were put under Mid-cycle PGF$_2$$\alpha$ treatment and were given i/m Inj. of PGF$_2$$\alpha$ 500 µg (Repregna, 2 ml) and FTAIs were performed twice at 72 and 96 hrs later.

2.4. Normal Cyclic Control (Group-III)

Ten each normal cyclic cows and buffaloes exhibiting estrus within 90 days postpartum and bred without any treatment served as control.
2.5. Blood Sampling for Plasma Profile

Jugular blood samples were collected from 8 animals in heparinized vacutainers on day 0 (day of first GnRH inj.), day 7 (day of PGF₂α injection), day 9 (day of second GnRH inj./AI) of treatment and on day 21 post-Al in Ovsynch group, and on day of PGF₂α injection, day of induced estrus/AI and day 21 post-Al in Mid-cycle PGF₂α group, while in control group blood samples were obtained on day of spontaneous estrus/AI and 21 days post-Al. The blood samples were centrifuged and plasma stored at -20°C in deep freezer. The estimations of plasma protein, cholesterol, calcium and phosphorus were done using standard procedures and assay kits of Crest Biosystem, Goa on semi-autoanalyzer.

2.6. Statistical Analysis

The conception rates in different groups were compared by Chi-square test. The data on plasma biochemical and mineral profile were analyzed using one way analysis of variance and ‘t’ test to compare variation within and between group (Snedecor and Cochran, 1994).

3. Results and Discussion

3.1. Effect of Hormone Protocols on Fertility

All the repeat breeding cows and buffaloes (100 %) under both Ovsynch and mid-cycle PGF₂α treatment protocol exhibited induced estrus within 2 to 4 days from day of PGF₂α injection. These observations are in line with the response and intervals reported earlier by Sathiamoorthy et al. (2007), Khasatiya et al. (2008), Naikoo et al. (2010), Patel et al. (2014) and Nakrani et al. (2015) in cows and buffaloes.

The conception rates at induced (first) estrus following Ovsynch protocol in cows and buffaloes were 50.00 and 40.00 %, with some 20 to 33 % animals conceiving with 2nd and 3rd cycle post-treatment, thus giving the overall pregnancy rates of three cycles as 70.00 and 60.00 %, respectively. The conception rates in Mid-cycle PGF₂α treated repeat breeder cows and buffaloes were found to be 40.00 and 50.00 % at induced estrus, with some 16.66 to 25.00 % animals conceiving in 2nd and 3rd estrus, with the overall pregnancy rates of 60.00 and 70.00 %, respectively. In normal cyclic control cows and buffaloes also the conception rates at first AI were 40.00 and 40.00 % and those of overall of three cycles 70.00 and 60.00 %, respectively. The conception rates in treated cows and buffaloes improved significantly and were statistically at par with control groups. These findings of conception rates are in accordance with the earlier reports of 61 to 82 % by Tenhagen et al. (2004), Ali and Fahmy (2007), Biradar et al. (2014) and Nakrani et al. (2015). However, Paul and Prakash (2005), Karen and Darwish (2010) and Derar et al. (2012) recorded lower conception rates of 33.33, 18.00 and 22.71%, respectively, with Ovsynch protocol. The first service conception rates of 40-50% found for mid-cycle PGF₂α treated repeat breeders are little better than the reports of Sathiamoorthy et al. (2007) and Savaalia et al. (2014) in buffaloes and Patel et al. (2014) in crossbred cows. The present finding of 60.00 % overall pregnancy rate found in Mid-cycle PGF₂α treated repeat breeder cows is similar with the conception rate recorded by Patel et al. (2005). The pregnancy rate of the three services found as 70.00 % following PGF₂α induced estrus in buffaloes is however lower than the pregnancy rates reported by Dhami et al. (2009, 2014) and Khasatiya et al. (2008).

3.2. Plasma Protein and Cholesterol Profile

The mean total protein concentration was relatively lower in conceived than non-conceived cows under Ovsynch protocol (7.65±0.12 g/dl vs. 8.06±0.26 g/dl), while relatively higher level was found in buffaloes for the same protocol (9.41±0.05 g/dl vs. 9.27±0.10 g/dl). Also mean total protein concentrations of Mid-cycle PGF₂α inj. group in cows (7.39±0.12 g/dl) and buffaloes (7.32±0.07 g/dl)
were slightly higher in conceived than the non-conceived groups (7.28±0.11 and 7.19±0.07, respectively). Repeat breeder buffaloes treated with various hormonal therapies had relatively higher values of plasma total protein compared with normal cyclic buffaloes. No significant differences were observed in plasma total protein profiles between periods of the treatment. Higher plasma protein concentration was observed in conceived than non-conceived groups of both cows and buffaloes under Ovsynch protocol, but the difference was significant only in cows (Table 1).

**Table 1: Mean Plasma Total Protein Levels (G/DL) in Repeat Breeding Cows and Buffaloes under Ovsynch Protocol and Mid-Cycle PGF α Inj. on Different Days of Treatment/AI**

| Treatment Protocol | Status | No | Days from Treatment/AI | Overall |
|--------------------|--------|----|------------------------|---------|
|                    |        |    | D-0        | D-7        | D-Al  | D-21  |
| C                  | 5      | 7.93±0.39| 8.43±0.48 | 7.66±0.19 | 7.90±0.28 | 7.98±0.17 |
| NC                 | 3      | 7.41±0.13| 7.76±0.20 | 7.10±0.31 | 7.23±0.10 | 7.38±0.11 |
| Pooled             | 8      | 7.74±0.25| 8.18±0.32 | 7.45±0.18 | 7.65±0.21 | 7.75±0.13 |
| Mid Cycle          |        |    |            |            |       |       |
| PGF α inj.         | C      | 4  | 7.54±0.17 | 7.38±0.23 | 7.26±0.25 | 7.39±0.12 |
|                    | NC     | 4  | 7.26±0.27 | 7.40±0.17 | 7.19±0.12 | 7.28±0.11 |
|                    | Pooled | 8  | 7.40±0.16 | 7.39±0.13 | 7.22±0.13 | 7.34±0.08 |
| Normal Cyclic      |        |    |            |            |       |       |
| Control            | C      | 4  | 7.93±0.56 | 8.62±0.53 | 8.27±0.38 |
|                    | NC     | 4  | 7.74±0.15 | 7.69±0.35 | 7.71±0.18 |
|                    | Pooled | 8  | 8.53±0.27 | 8.15±0.34 | 7.99±0.21 |
| Ovsynch            |        |    |            |            |       |       |
| C                  | 4      | 8.83±0.52| 8.82±0.38 | 9.51±0.16 | 9.55±0.03 | 9.18±0.18 |
| NC                 | 4      | 7.26±0.11| 7.45±0.04 | 7.27±0.10 | 7.23±0.11 | 7.30±0.05 |
| Pooled             | 8      | 8.05±0.39| 8.13±0.31 | 8.39±0.43 | 8.39±0.44 | 8.24±0.19 |
| Mid Cycle          |        |    |            |            |       |       |
| PGF α inj.         | C      | 5  | 7.27±0.16 | 7.38±0.09 | 7.31±0.11 | 7.32±0.07 |
|                    | NC     | 3  | 7.36±0.17 | 7.12±0.11 | 7.11±0.08 | 7.19±0.07 |
|                    | Pooled | 8  | 7.30±0.11 | 7.28±0.08 | 7.23±0.08 | 7.27±0.05 |
| Normal Cyclic      |        |    |            |            |       |       |
| Control            | C      | 4  | 7.12±0.06 | 7.30±0.07 | 7.21±0.05 |
|                    | NC     | 4  | 7.06±0.07 | 7.16±0.05 | 7.11±0.04 |
|                    | Pooled | 8  | 7.09±0.04 | 7.23±0.05 | 7.16±0.04 |

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

Cetin et al. (2002) found relatively identical serum total protein levels at estrus in repeat breeder and fertile cows. Dhoble et al. (2004) found significantly higher levels of plasma total protein in pregnant (7.92 g/dl) than non-pregnant cows (7.71 g/dl), which is comparable with the present findings in cows and buffaloes in normal cyclic, Mid-cycle PGF α protocol, and Ovsynch protocol in buffaloes, while in cows under Ovsynch protocol, plasma total protein was lower in conceived than non-conceived animals. Parmar (2013) and Patel et al. (2014) reported much higher mean total protein concentration in non-conceived than conceived repeat breeder buffaloes (11.30±0.30 vs 11.18±0.40 g/dl) and cows (12.39±0.25 vs 12.16±0.28 g/dl) treated with Mid-Cycle PGF α inj. as compare to the present findings.

Like protein, there were no significant differences in the plasma total cholesterol profiles between days/periods of the treatment with Ovsynch protocol or Mid-cycle PGF α inj. group and also between conceived and non-conceived cows and buffaloes of any group, but the mean values at day 21 post-AI in non-conceived cows in Ovsynch protocol (197.43±13.45 mg/dl), Mid-cycle PGF α inj. group (217.58±12.05 mg/dl), and in normal cyclic group (211.47±5.85 mg/dl) were apparently higher as compared to those obtained in conceived groups (176.80±9.54 mg/dl, 200.89±10.51 mg/dl, and 191.96±8.62 mg/dl, respectively; Table 2).
Table 2: Mean Plasma Total Cholesterol Concentration (Mg/Dl) In Repeat Breeding Cows and Buffaloes under Ovsynch Protocol and Mid-Cycle PGF₂α Inj. on Different Days of Treatment/AI

| Treatment Protocol | Status | No. | Days from Treatment/AI | Overall |
|--------------------|--------|-----|------------------------|---------|
|                    |        |     | D-0 | D-7 | D-Al | D-21 |
| Cow Ovsynch        | C      | 5   | 163.29±22.52 | 179.23±26.46 | 185.50±18.49 | 179.29±10.66 | 176.80±9.54 |
|                    | NC     | 3   | 163.88±13.82 | 184.71±11.85 | 211.99±25.56 | 229.13±41.78 | 197.43±13.45 |
|                    | Pooled | 8   | 163.51±14.20 | 181.28±16.31 | 195.43±14.68 | 197.93±17.64 | 184.53±7.89  |
| Mid Cycle PGF₂α inj. | C   | 4   | 187.49±13.26 | --             | 207.26±9.97  | 209.72±17.12 | 200.89±10.51 |
|                    | NC     | 4   | 236.13±18.09 | --             | 200.30±17.86 | 216.33±27.12 | 217.58±12.05 |
|                    | Pooled | 8   | 211.81±13.87 | --             | 203.78±16.20 | 212.13±13.08 | 209.24±8.01  |
| Normal Cyclic Control | C    | 4   | --             | --             | 192.88±12.77 | 191.04±13.53 | 191.96±8.62  |
|                    | NC     | 4   | --             | --             | 217.99±7.19  | 204.95±8.92  | 211.47±5.85  |
|                    | Pooled | 8   | --             | --             | 205.44±8.28  | 197.99±7.95  | 201.71±5.63  |
| Buffalo Ovsynch    | C      | 4   | 149.98±16.00 | 161.65±19.72 | 142.32±18.84 | 143.76±23.43 | 149.43±9.02  |
|                    | NC     | 4   | 125.56±18.18 | 131.56±24.51 | 140.73±26.96 | 144.77±13.75 | 135.65±9.81  |
|                    | Pooled | 8   | 137.77±12.12 | 146.60±15.63 | 141.53±15.23 | 142.27±12.58 | 142.54±6.67  |
| Mid Cycle PGF₂α inj. | C   | 5   | 143.12±14.36 | --             | 148.69±9.41  | 129.54±1.98  | 140.45±5.75  |
|                    | NC     | 3   | 154.27±6.13  | --             | 143.79±8.62  | 148.72±4.36  | 148.93±6.33  |
|                    | Pooled | 8   | 147.30±9.04  | --             | 146.86±6.36  | 136.73±3.97  | 143.63±3.88  |
| Normal Cyclic Control | C    | 4   | --             | --             | 142.71±2.99  | 142.13±9.29  | 142.42±4.52  |
|                    | NC     | 4   | --             | --             | 146.77±3.77  | 150.19±4.95  | 148.48±2.95  |
|                    | Pooled | 8   | --             | --             | 144.74±2.35  | 146.16±5.11  | 145.45±2.72  |

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

Noble et al. (1977) and Chandrakar et al. (2003) observed low plasma total cholesterol level during fertile estrus compared to infertile estrus in buffaloes and cows, which corroborated with the present findings. The findings documented by Cetin et al. (2002) were also very much similar to the present observations. Chaudhari (2012) observed no significant difference between conceived and non-conceived crossbred cows under Ovsynch as well as Mid-cycle PGF₂α protocols, which corroborated well with the present findings. Similarly, no significant difference was observed in total cholesterol concentrations between conceived and non-conceived repeat breeding cows and buffaloes under mid-cycle PGF₂α protocol by Parmar (2013), Patel et al. (2014). The present findings thus support the observations of previous researchers.

3.3. Plasma Calcium and Phosphorus Profile

The plasma calcium and inorganic phosphorus levels presented in Table 3 and 4 did not reveal significant variations between days/periods of the treatment with Ovsynch and Mid-cycle PGF₂α protocol and in normal cyclic groups and even between conceived and non-conceived groups. There was no significant difference between treatment groups and control group and also no significant difference was observed between cows and buffaloes.

Table 3: Mean Plasma Calcium Levels (Mg/Dl) in Repeat Breeding Cows and Buffaloes under Ovsynch Protocol and Mid-Cycle PGF₂α Inj. on Different Days of Treatment/AI

| Treatment Protocol | Status | No. | Days from Treatment/AI | Overall |
|--------------------|--------|-----|------------------------|---------|
|                    |        |     | D-0 | D-7 | D-Al | D-21 |
| Cow Ovsynch        | C      | 5   | 8.58±0.53 | 9.41±0.20 | 9.33±0.24 | 9.29±0.33 | 9.15±0.18 |
|                    | NC     | 3   | 9.39±0.22 | 9.13±0.40 | 8.85±0.29 | 9.38±0.16 | 9.19±0.14 |
|                    | Pooled | 8   | 8.89±0.36 | 9.31±0.18 | 9.15±0.19 | 9.32±0.20 | 9.17±0.12 |
| Mid Cycle PGF₂α inj. | C   | 4   | 9.47±0.24 | --             | 9.42±0.23  | 9.55±0.10 | 9.48±0.11 |
|                    | NC     | 4   | 8.59±0.20 | --             | 9.25±0.25  | 9.10±0.25 | 8.98±0.15 |
|                    | Pooled | 8   | 9.03±0.22 | --             | 9.33±0.16  | 9.33±0.15 | 9.23±0.10 |
Ahlawat (2003) reported the mean calcium levels in conceiving and non-conceiving crossbred cows to be 9.09±0.25 and 8.59±0.38 mg/dl. These findings were similar to the present findings in both repeat breeding cows and buffaloes. The findings of Chandraker et al. (2003) in repeat breeder cows were also in close corroboration with the present findings. Parmar (2013) reported higher mean plasma calcium for postpartum buffaloes under PGF2α treatment than the control group (9.98±0.04 vs 9.17±0.04 mg/dl; P<0.05). Similar were the findings of Savalia et al. (2013) in repeat breeding buffaloes under Mid-cycle PGF2α treatment. Patel et al. (2014) reported mean plasma calcium levels of 9.58±0.17 and 9.90±0.12 mg/dl in conceived and non-conceived repeat breeder cows, which also closely corroborated with the present findings. Parmar (2013) reported the mean plasma calcium levels to be 8.66 ± 0.15 mg/dl and 8.57 ± 0.15mg/dl in non-conceived and conceived repeat breeding buffaloes under Mid-cycle PGF2α inj. group.

Fayez et al. (1992) reported that repeat breeder Egyptian buffaloes had significantly lower inorganic phosphorus concentration than normal animals. Parmar (2013) obtained much higher mean inorganic phosphorus concentration than normal animals.

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**Table 4: Mean Plasma Inorganic Phosphorus Levels (Mg/Dl) in Repeat Breeding Cows and Buffaloes under Ovsynch Protocol and Mid-Cycle PGF2α Inj. on Different Days of Treatment/AI**

| Treatment Protocol | Status | Days from Treatment/AI | Overall |
|--------------------|--------|------------------------|---------|
|                    |        | D-0       | D-7       | D-Al     | D-21     |
| Ovsynch             | C      | 4.40±0.35 | 4.61±0.38 | 4.20±0.30 | 4.65±0.13 | 4.47±0.15 |
|                     | NC     | 4.53±0.12 | 4.77±0.21 | 4.05±0.04 | 4.23±0.25 | 4.39±0.11 |
|                     | Pooled | 4.45±0.22 | 4.67±0.24 | 4.14±0.18 | 4.49±0.14 | 4.44±0.10 |
| Mid Cycle           | C      | 4.74±0.12 | 4.71±0.11 | 4.77±0.05 | 4.74±0.05 |         |
| PGF2α inj.          | NC     | 4.29±0.10 | 4.62±0.13 | 4.55±0.13 | 4.49±0.08 |         |
|                     | Pooled | 4.51±0.11 | 4.67±0.08 | 4.66±0.08 | 4.61±0.05 |         |
| Normal Cyclic       | C      | 4.76±0.26 | 4.90±0.15 | 5.06±0.10 | 5.04±0.05 | 4.94±0.08 |
| Control             | NC     | 4.94±0.11 | 5.03±0.12 | 4.82±0.20 | 4.97±0.09 | 4.94±0.06 |
|                     | Pooled | 4.85±0.13 | 4.96±0.09 | 4.94±0.11 | 5.01±0.05 | 4.94±0.05 |
| Ovsynch             | C      | 4.65±0.06 | 4.66±0.18 | 4.91±0.07 | 4.74±0.07 |         |
|                     | NC     | 4.51±0.00 | 4.57±0.10 | 4.61±0.05 | 4.56±0.03 |         |
|                     | Pooled | 4.60±0.05 | 4.63±0.11 | 4.79±0.07 | 4.67±0.05 |         |
| Mid Cycle           | C      | 4.53±0.23 | 4.76±0.13 | 4.64±0.13 |         |         |
| PGF2α inj.          | NC     | 4.94±0.05 | 4.85±0.07 | 4.90±0.04 |         |         |
|                     | Pooled | 4.73±0.13 | 4.80±0.07 | 4.77±0.07 |         |         |

C = conceived, NC = non-conceived; Day-0 = Day of treatment, D-Al = Day of AI, D-21 = Day 21 post-AI; Means bearing uncommon superscripts within the row (a,b) and column (p,q) (x,y) differ significantly (P<0.05).
phosphorus levels as 6.15±0.28 mg/dl and 7.25±0.50 mg/dl in non-conceived and conceived repeat breeding buffaloes under Mid-cycle PGF₂α injection group than the present findings. Patel et al. (2014) could not find significant variation in inorganic phosphorus concentration between various sampling days or between conceived and non-conceived groups of crossbred cows under Mid-cycle PGF₂α treatment, which is in line with the present findings.

From the results, it can be inferred that the application of both Ovsynch protocol and Mid-cycle PGF₂α injection are good tools for induction of timed estrus and ovulation and enhancement of pregnancy rate in repeat breeding cows and buffaloes without significantly influencing the plasma biochemical and mineral profile. However, looking to the results, cost and time involved, Mid-cycle PGF₂α treatment is much economic and cost effective as compared to Ovsynch protocol in handling problem breeders under field conditions.

**Acknowledgements**

We thank Dr. A.M. Thaker, Dean of the Veterinary Faculty, AAU, Anand for the funds and facilities provided for this work, and Dr. V.B. Kharadi, Research Scientist & Head of LRS, NAU, Navsari for kind cooperation and sparing few animals for this study.

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