

## Research Article

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## Effect of Various Ovulation Synchronization Protocols on Estrus Response, Conception Rate and Blood Biochemical Profile in Anoestrus Buffaloes

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**Abstract** This study evaluated the fertility response and plasma profile of biochemical and mineral constituents in 46 postpartum anoestrus (>90 days) buffaloes treated under field conditions with TriU-B/PRID, Ovsynch and Heatsynch protocols (n=12 each), keeping untreated anoestrus control (n=10) and normal cyclic control (n=10) groups. The estrus induction response with 3 treatment protocols was 83.33, 83.33 and 91.66 % respectively within 2-3 days of PGF<sub>2</sub>α injection. The conception rates (CRs) obtained at induced estrus in buffaloes under TriU-B, Ovsynch and Heatsynch protocols were 25.00, 33.33 and 25.00 %, and those of overall three cycles' 50.00, 58.33 and 50.00 %, respectively, which were at par with normal cyclic control group (CR at first cycle 30.00 & overall of 3 cycles 50.00 %). Among untreated anoestrus control group, only two buffaloes (20%) exhibited estrus and conceived over 90 days follow up. The overall pooled mean plasma total cholesterol concentrations (mg/dl) in anoestrus buffaloes under TriU-B and in normal cyclic control group were the same (166.70±5.28 and 165.18±6.37) and significantly (P<0.01) higher than in Ovsynch (131.54±3.71) and lower than in Heatsynch (186.14±5.69) group. The value for total protein (g/dl) was significantly (P<0.05) lower in TriU-B (7.27±0.08) followed by other two groups (7.62±0.07 & 7.44±0.07) and highest in normal control group (7.98±0.10). The calcium was significantly higher (P<0.05) in Ovsynch (10.41±0.07 mg/dl) than other 3 groups (8.82±0.11 to 9.09±0.09 mg/dl), while inorganic phosphorus (3.99±0.10 to 4.29±0.13 mg/dl) did not vary between groups. Normal cyclic control buffaloes had significantly higher protein and lower calcium levels than in most treatment groups. The conceived buffaloes had apparently higher values of total cholesterol and protein as compared to non-conceived ones in all groups. In general, Ovsynch protocol was the best followed by TriU-B and Heatsynch for induction of estrus and improvement of fertility in anoestrus buffaloes.

**Keywords** *Anoestrus Buffalo; Estrus Synchronization Protocols; Blood Biochemical Profile; Fertility*

### 1. Introduction

In the recent years, considerable attention has been focused on reproductive endocrinology and blood biochemical and mineral profile as a means to identify specific reproductive and nutritional

problems and thereby to adopt appropriate therapeutic measures to augment fertility. Biochemical constituents of blood have great diagnostic value in evaluating the physiological status as well as in the clinical practice to improve postpartum fertility in female bovines. Use of hormonal protocols like TriU-B, Ovsynch and Heatsynch induces and synchronizes the estrus/ovulation and thus improves the conception rates and establishes cyclicity in acyclic buffaloes, thereby achieving ideal calving interval. Cholesterol is synthesized from acetate with a series of intermediate substances. It is an essential precursor for steroid hormone synthesis of testis, ovary and adrenal cortex. The thyroid hormones and estrogen influence cholesterol synthesis. Lack of protein or insufficient intake of protein was considered to be a cause of failure or delay in estrous cycle (Roberts, 1971). Protein deficiency is associated with retardation of the development of sex organs and may affect subsequently the reproductive performance (Herrick, 1977). Minerals like calcium, phosphorus and magnesium also influence the ability of animals to utilize other micro-minerals. The influence of these minerals on certain enzyme system may affect reproductive efficiency (Dhoble and Gupta, 1986), which might be reflected in lower blood level of them. Lack of minerals especially calcium and phosphorus upsets the proper functioning of the genital organs (Acharya, 1960). Hence, this study was planned to evaluate the comparative efficacy of TriU-B, Ovsynch and Heatsynch protocols for fertility enhancement and to see their influence on plasma biochemical and mineral profile in anoestrus rural buffaloes.

## 2. Materials and Methods

The present investigation was carried out under field conditions of Anand and Mahisagar districts of middle Gujarat and at Livestock Research Station, NAU, Navsari, South Gujarat. A total of 46 postpartum true anoestrus (>90 days) buffaloes and 10 normal cyclic control buffaloes that exhibited spontaneous estrus within 90 days postpartum constituted the experimental animals. All the infertile animals identified were dewormed using Inj. Ivermectin @ 100 mg s/c and were treated initially once with i/m injection of inorganic phosphorus (Inj. Alphos-40 @ 10 ml, Pfizer) and multivitamins AD<sub>3</sub>E (Inj. Vetacept @ 10 ml, Concept Pharma), and oral multi-minerals (Minotas, Intas Pharma) @ 1 bolus daily for 7 days. The anoestrus buffaloes were then randomly subjected to different standard estrus induction/synchronization protocols (TriU-B, Ovsynch and Heatsynch, n=12 each, Buhecha et al., 2016). Another 10 anoestrus animals were kept as anoestrus control and 10 normal cyclic buffaloes served as normal cyclic control group. Buffaloes in spontaneous or induced estrus were inseminated using good quality frozen-thawed semen. Buffaloes detected in estrus subsequent to FTAI were re-inseminated up to 2 cycles and in non-return cases pregnancy was confirmed per rectum 60 days of last AI.

Jugular blood samples were collected four times in heparinized vacutainers from true anoestrus animals, i.e. on day 0 - just before treatment, on day 7 - at the time of PGF2 $\alpha$  administration, on day 9/10 - induced estrus/FTAI and on day 21 post-AI. Blood sampling for two control groups of animals was done on the day of spontaneous estrus if any, and on day 21 post-AI. The plasma separated out by centrifugation of blood at 3000 rpm for 15 min was stored at -20°C with a drop of merthiolate (0.1%) until analyzed. Plasma total protein, cholesterol, calcium and inorganic phosphorus were estimated by using standard procedures and assay kits (Analytical Technologies Pvt. Limited, Baroda) on biochemistry analyzer. The Chi square test was used to compare conception rate while plasma profile of biochemical and mineral constituents was analyzed using one way analysis of variance and 't' test (Snedecor and Cochran, 1994) using online SAS software version 20.00.

### 3. Results and Discussion

#### 3.1. Estrus Induction and Fertility Response to Synchronization Protocols

In anoestrus buffaloes under TriU-B, Ovsynch and Heatsynch protocols, estrus induction response was 83.33, 83.33 and 91.66 % within mean intervals of  $69.30 \pm 0.80$ ,  $70.60 \pm 1.30$  and  $69.20 \pm 1.49$  h, from PG injection, with prominent to moderate estrus signs. The conception rates obtained at induced estrus in three protocols were 25.00, 33.33, and 25.00 %, and the overall conception rates of three cycles were 50.00, 58.33 and 50.00 %, respectively. Moreover, by 60 days of post-induction/AI in TriU-B, Ovsynch and Heatsynch protocols among treated non-conceived buffaloes 2 (16.66%), 3 (24.99%) and 4 (33.33%) buffaloes turned out to be anoestrus. In untreated anoestrus control group, out of 10 buffaloes only 2 buffaloes (20 %) expressed spontaneous estrus after 41 and 63 days of initiation of experiment and conceived giving overall conception rate of 20.00 %. In normal cyclic control group, the conception rate at first and overall of 3 cycles was 30.00 and 50.00 %, respectively.

The present findings of estrus synchronization rates with TriU-B (simulating CIDR) and Ovsynch were in accordance with the earlier results with CIDR and Ovsynch protocols by Campenile et al. (2005), Ali et al. (2012) and Kundalkar et al. (2014) in different breeds of buffaloes. TriU-B being newly launched product, no report on its use could be seen in the literature, except one of our own (Buhecha et al., 2016). Similarly, it was difficult to find report on use of Heatsynch protocol in buffaloes, except that of Mirmahmoudi et al. (2014), wherein the estrus induction response was relatively poor in both cyclic and acyclic buffaloes. The comparable conception rates at induced estrus and overall of three cycles in Ovsynch and CIDR protocols have been documented by Baruselli et al. (2003) as 52.50 and 28.20 %, Naikoo et al. (2010) as 50.00 and 50.00 % and Ali et al. (2012) as 60.00 and 33.33 %, respectively. However, relatively better results than the present ones were found earlier using similar protocols in buffaloes from Gujarat climate by Naikoo et al. (2010), Savalia et al. (2014) and Nakrani et al. (2014).

#### 3.2. Plasma Total Protein and Total Cholesterol

The mean levels of plasma total cholesterol recorded on day 0, 7, 9 (AI) of treatment and on day 21 post-AI in buffaloes under TriU-B, Ovsynch and Heatsynch protocols (Table 1) did not reveal significant differences in the profile between days/periods of the treatment in any of the groups, but it varied significantly between the groups, with inconsistent trend. Similar results were observed in anoestrus buffaloes under CIDR and Ovsynch protocols by Savalia et al. (2014). Parmar (2013) and Nakrani et al. (2014) reported that the influence of periods (day 0, 7, 9 of treatment and day 21 post-AI) and treatment groups (CIDR, Ovsynch and Crester protocols) in anoestrus buffaloes was not significant for the plasma total cholesterol profile. This may be due to difference in the nutritional status of animals and the assay kits and procedures followed. In the present study, at day 21 post-AI there were insignificantly low mean plasma total cholesterol levels in conceived as compared to non-conceived buffaloes. This trend was in line with the earlier observations of Savalia et al. (2014) and Nakrani et al. (2014), though their values were much lower than the present findings. Apparently or significantly lower plasma total cholesterol profile noted in anoestrus animals in some of the reports prove that the steroid hormone precursor cholesterol was not available in sufficient quantity to synthesize estrogen in the growing follicles triggering ovarian cyclicity and estrus in such animals.

**Table 1:** Plasma Total Cholesterol Concentrations (mg/dl) in Anoestrus Buffaloes on Different Days of Various Estrus Synchronization Treatments

Estrus Induction Protocol	Status	No.	Plasma Total Cholesterol Concentrations (mg/dl)				Overall
			D-0	D-7	D-9/10 (AI)	D-21 post-AI	
TriU-B	Conceived	3	149.65 ± 15.73	153.18 ± 10.61	150.11 ± 14.04	149.96 ± 19.64 <sup>p</sup>	150.73 ± 6.88 <sup>p</sup>
	Non-conc.	5	174.19 ± 12.02	176.61 ± 13.15	188.69 ± 13.28	191.23 ± 10.56 <sup>q</sup>	182.68 ± 5.82 <sup>q</sup>
	<b>Overall</b>	<b>8</b>	<b>161.92 ± 10.27</b>	<b>164.90 ± 8.99</b>	<b>169.40 ± 11.54</b>	<b>170.60 ± 12.94</b>	<b>166.70 ± 5.28<sup>y</sup></b>
Ovsynch	Conceived	4	143.32 ± 7.07	135.69 ± 10.50	123.83 ± 12.88	121.08 ± 10.24	130.98 ± 5.19
	Non-conc.	4	125.66 ± 8.18	143.18 ± 21.37	130.77 ± 2.70	128.79 ± 2.72	132.10 ± 5.46
	<b>Overall</b>	<b>8</b>	<b>134.49 ± 6.02</b>	<b>139.44 ± 11.11</b>	<b>127.30 ± 6.23</b>	<b>124.93 ± 5.12</b>	<b>131.54 ± 3.71<sup>x</sup></b>
Heatsynch	Conceived	3	185.18 ± 2.82	197.25 ± 4.24	196.67 ± 11.43	183.61 ± 3.56	190.68 ± 3.36
	Non-conc.	5	181.76 ± 20.49	167.85 ± 19.07	190.92 ± 20.64	193.16 ± 14.27	183.42 ± 8.92
	<b>Overall</b>	<b>8</b>	<b>183.04 ± 12.30</b>	<b>178.87 ± 12.68</b>	<b>193.08 ± 12.93</b>	<b>189.58 ± 8.78</b>	<b>186.14 ± 5.69<sup>z</sup></b>
Normal	Conceived	3	-	-	151.27 ± 6.81	149.51 ± 9.28	150.38 ± 5.16
Cyclic	Non-conc.	5	-	-	174.34 ± 9.33	173.77 ± 15.99	174.05 ± 8.72
Control	<b>Overall</b>	<b>8</b>	-	-	<b>165.69 ± 7.34</b>	<b>164.67 ± 10.97</b>	<b>165.18 ± 6.37<sup>y</sup></b>

Means bearing uncommon superscripts within column (x, y) and subgroup (p, q) differ significantly ( $P < 0.05$ ).

D-0 = Day of starting the treatment, D-7 = Administration of PG, D-9/10 = Fixed time AI, D-21 = Day 21 post-AI.

The mean plasma levels of total proteins recorded on different days of treatment and on day 21 post-AI in buffaloes under three treatment protocols (Table 2) revealed that the profile did not vary significantly between sampling days in any of the protocols/groups. Savalia et al. (2014) and Nakrani et al. (2014) also reported similar non-significant variations in the overall mean plasma protein concentrations in buffaloes under CIDR, Ovsynch and Crester protocols and between days of sampling. However, the overall mean concentrations were significantly ( $P < 0.05$ ) higher in conceived than non-conceived buffaloes in TriU-B and in normal cyclic control and insignificantly higher in Ovsynch and Heatsynch groups. Similar trend was also found by Savalia et al. (2014) and Nakrani et al. (2014). The results of the present study proved that the plasma total protein profile is not influenced significantly by the hormonal treatments used in dairy animals, and that higher plasma protein levels are indicative of better nutritional status/ nitrogen balance, which favours the sound reproductive performance in animals.

**Table 2:** Plasma Total Protein Concentrations (g/dl) in Anoestrus Buffaloes on Different Days of Various Estrus Synchronization Treatments

Estrus Induction Protocol	Status	No.	Plasma Total Protein Concentrations (g/dl)				Overall
			D-0	D-7	D-9/10 (AI)	D-21 post-AI	
TriU-B	Conceived	3	7.35 ± 0.14	7.25 ± 0.28	7.50 ± 0.32	7.66 ± 0.25	7.44 ± 0.12 <sup>q</sup>
	Non-conc.	5	7.17 ± 0.19	7.17 ± 0.24	7.01 ± 0.16	7.04 ± 0.10	7.10 ± 0.08 <sup>p</sup>
	<b>Overall</b>	<b>8</b>	<b>7.26 ± 0.11</b>	<b>7.21 ± 0.17</b>	<b>7.25 ± 0.19</b>	<b>7.35 ± 0.17</b>	<b>7.27 ± 0.08<sup>x</sup></b>
Ovsynch	Conceived	4	7.65 ± 0.18	7.51 ± 0.15	7.62 ± 0.22	7.66 ± 0.25	7.65 ± 0.09
	Non-conc.	4	7.67 ± 0.18	7.47 ± 0.27	7.85 ± 0.25	7.56 ± 0.25	7.61 ± 0.11
	<b>Overall</b>	<b>8</b>	<b>7.66 ± 0.12</b>	<b>7.49 ± 0.14</b>	<b>7.74 ± 0.16</b>	<b>7.61 ± 0.16</b>	<b>7.62 ± 0.07<sup>y</sup></b>
Heatsynch	Conceived	3	7.20 ± 0.24	7.13 ± 0.22	7.20 ± 0.22	7.65 ± 0.16	7.54 ± 0.11
	Non-conc.	5	7.64 ± 0.06	7.62 ± 0.26	7.50 ± 0.17	7.41 ± 0.17	7.45 ± 0.08
	<b>Overall</b>	<b>8</b>	<b>7.47 ± 0.12</b>	<b>7.44 ± 0.19</b>	<b>7.39 ± 0.13</b>	<b>7.50 ± 0.17</b>	<b>7.44 ± 0.07<sup>xy</sup></b>
Normal	Conceived	3	-	-	8.25 ± 0.11	8.39 ± 0.10	8.32 ± 0.07 <sup>q</sup>
Cyclic	Non-conc.	5	-	-	7.72 ± 0.19	7.83 ± 0.16	7.77 ± 0.12 <sup>p</sup>
Control	<b>Overall</b>	<b>8</b>	-	-	<b>7.92 ± 0.15</b>	<b>8.04 ± 0.15</b>	<b>7.98 ± 0.10<sup>z</sup></b>

Means bearing uncommon superscripts within column (x, y, z) and subgroup (p, q) differ significantly ( $P < 0.05$ ).

D-0 = Day of starting the treatment, D-7 = Administration of PG, D-9/10 = Fixed time AI, D-21 = Day 21 post-AI.

### 3.3. Plasma Calcium and Phosphorus

The results on levels of plasma calcium and phosphorus concentrations obtained on day 0, 7, 9/10 (AI) of treatment and on day 21 post-AI in buffaloes under TriU-B, Ovsynch and Heatsynch protocols, and on day of AI and day 21 post-AI in normal cyclic group are presented in Table 3.

**Table 3:** Plasma Calcium and Inorganic Phosphorus Concentrations (mg/dl) in Anoestrus Buffaloes on Different Days of Various Estrus Synchronization Treatments

Blood Parameter	Estrus Induction Protocol	No.	Plasma Calcium and Phosphorus Concentrations (mg/dl)				Overall
			D-0	D-7	D-9/10 (AI)	D-21 post-AI	
Plasma Calcium	TriU-B	8	9.12 ± 0.26	9.02 ± 0.30	9.04 ± 0.23	9.06 ± 0.27	9.06 ± 0.13 <sup>x</sup>
	Ovsynch	8	10.33 ± 0.08	10.32 ± 0.18	10.53 ± 0.11	10.47 ± 0.17	10.41 ± 0.07 <sup>y</sup>
	Heatsynch	8	9.06 ± 0.20	8.98 ± 0.24	9.07 ± 0.15	9.26 ± 0.15	9.09 ± 0.09 <sup>x</sup>
	Normal Cyclic	8	-	-	8.73 ± 0.15	8.92 ± 0.18	8.82 ± 0.11 <sup>x</sup>
Plasma Inorganic Phosphorus	TriU-B	8	3.81 ± 0.21	4.01 ± 0.25	4.20 ± 0.28	4.10 ± 0.13	4.03 ± 0.13
	Ovsynch	8	4.32 ± 0.31	4.25 ± 0.22	4.22 ± 0.27	4.37 ± 0.28	4.29 ± 0.13
	Heatsynch	8	3.98 ± 0.23	3.93 ± 0.23	3.85 ± 0.26	4.21 ± 0.10	3.99 ± 0.10
	Normal Cyclic	8	-	-	4.00 ± 0.17	4.21 ± 0.19	4.10 ± 0.12

Means bearing uncommon superscripts within column (x, y) for a trait differ significantly ( $P < 0.05$ ). D-0 = Day of starting the treatment, D-7 = Administration of PG, D-9/10 = Fixed time AI, D-21 = Day 21 post-AI.

These results did not reveal significant variations in plasma calcium concentrations of buffaloes between days/periods of any treatment and in normal cyclic control group. Similarly the variations in plasma calcium levels between conceived and non-conceived groups were not significant, though the levels were apparently higher in conceived than non-conceived animals. Further, the overall mean values of plasma calcium obtained in anoestrus buffaloes under TriU-B and Heatsynch protocols and in normal cyclic buffaloes were  $9.06 \pm 0.13$ ,  $9.09 \pm 0.09$  and  $8.82 \pm 0.11$  mg/dl, respectively, which were statistically similar. However, the mean plasma calcium concentration noted in Ovsynch protocol ( $10.41 \pm 0.07$  mg/dl) was significantly higher ( $P < 0.05$ ) as compared to the values of other treatment and control groups. Very similar observations have been recently documented by Savalia et al. (2013) and Nakrani et al. (2014) using CIDR in buffaloes, including normal cyclic control group. Further, Savalia et al. (2013) also obtained higher mean calcium levels in conceived as compared to non-conceived buffaloes under CIDR and Ovsynch protocols.

Further, the plasma concentrations of inorganic phosphorus in different groups closely followed the plasma calcium profile. The levels neither varied significantly between sampling days/periods in any of the treatment protocols and control group, nor between conceived and non-conceived buffaloes in any of the groups. The overall mean values of inorganic phosphorus obtained in anoestrus buffaloes under TriU-B, Ovsynch, Heatsynch and cyclic buffaloes were  $4.03 \pm 0.13$ ,  $4.29 \pm 0.13$ ,  $3.99 \pm 0.10$  and  $4.10 \pm 0.12$  mg/dl, respectively, which did not differ significantly among themselves. Very similar observations have been recently made by some of the workers using CIDR and Ovsynch protocols in anoestrus buffaloes, including normal cyclic control groups (Savalia et al., 2013; Nakrani et al., 2014) from the same institute. Bhaskaran and Abdulla Khan (1981) documented that the marginal deficiency of phosphorus is enough to cause disturbances in pituitary-ovarian axis, without manifesting specific systemic deficiency symptoms. Butani et al. (2011), Parmar et al. (2012) and Savalia et al. (2013) did not find appreciable variation in the mean plasma inorganic phosphorus levels on the day of GnRH and/or PG treatment, at induced oestrus and on day 21 post-AI in anoestrus or suboestrus buffaloes.

It was concluded that all the three protocols, viz, TriU-B, Ovsynch and Heatsynch used in true anoestrus buffaloes resulted into more than 83 % estrus induction with establishment of cyclicity and



good conception rates (50 to 58 %) without significantly altering the plasma biochemical and minerals profile suggestive of better scope for their use under field conditions.

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