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Seasonal Influence on Efficacy of Ovsynch, Heatsynch and Triu-B Protocols in Terms of Estrus Response, Conception Rate and Biochemical Profile in Postpartum Anoestrus Buffaloes

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Abstract

This study was undertaken on 48 postpartum (>90 days) anoestrus buffaloes each in winter and summer seasons with the object to know the seasonal influence on efficacy of three estrus induction/ synchronization protocols towards fertility response and blood biochemical profiles. Twelve buffaloes each in treatment group I, II and III were subjected to Ovsynch, Heatsynch and Triu-B hormone protocols, respectively, while group IV served as untreated control. The estrus induction response varied from 75.00 to 83.33% during summer and 83.33 to 91.66% during winter season with these protocols. The conception rates obtained at induced estrus and overall of 3 cycles in anoestrus buffaloes were comparatively lower under all three protocols during summer than winter season (16.27 vs 41.67 & 25.00 vs 58.33% for Ovsynch; 16.27 vs 25.00 &25.00 vs 50.00% for Heatsynch, and 16.27 vs 41.67 & 33.33 vs 58.33%, respectively), and obviously higher than control group (overall CR 8.33 & 25.00%) in both the seasons. The overall mean plasma protein values were significantly higher ($p<0.01$) in winter season than summer season in all three groups, while inverse results were found with respect to seasons for total cholesterol under Ovsynch and Triu-B protocols. The effect of period and pregnancy status was non-significant within the protocol for both the traits in both the seasons, except the total protein under Ovsynch protocol in winter season was significantly higher in conceived than non-conceived group (7.96 ± 0.09 vs. 7.54 ± 0.11 g/dl; $p<0.05$). There was no influence of days or conceived and non-conceived status on the calcium, phosphorus and magnesium profile in any of the groups studied during both the seasons. However, significantly higher ($p<0.05$) overall mean plasma values for calcium and magnesium were observed in winter as compared to summer season in buffaloes of all three protocols. It was concluded that all three protocols can be used successfully during winter and Triu-B only during summer season to induce estrus and improve fertility in postpartum anoestrus buffaloes, and that the plasma metabolite profile was significantly altered by season, but not by the hormonal treatments or pregnancy status.

Key Words : Anoestrus Buffalo, Estrus Synchronization, Seasonal Influence, Conception Rate, Biochemical Profile.

Introduction

The blood biochemical studies in anoestrus buffaloes are very important to understand the

physiological and nutritional status of animals. Inadequate protein during pregnancy and lactation can impair synthesis of particular amino acids essential for gonadotrophins. Minerals are not only participating as cofactor, and activators of various enzymes in the carbohydrate and energy metabolism, but are also associated with normal reproductive behaviour in domestic animals. Minerals like calcium and phosphorus also influence the ability of animals to utilize other micro-minerals, and play an intermediate role in the action of hormones and enzymes at sub-cellular levels in an integrated fashion in the initiation of oestrus in animals. The calcium plays an important role in steroidogenesis especially progesterone production and secretion by granulose cell. Phosphorus is a universal constituent of living cells. Phosphorus deficiency induces irregular oestrus, anoestrus, decreases ovarian follicles and generally depresses fertility/conception rate. Total protein in blood serum helps in determining the reproductive status of animals. Cholesterol is the precursor of reproductive hormones and its concentration varies with different physiological status of animals. During pregnancy and at the start of lactation, changes in blood constituents occur (Hafez, 1993). Most of the macro and micro minerals are higher in the winter season both in the animal blood and forages compared to those during summer season indicating a direct plant-animal relationship (Chhabra *et al.,* 2015). Thus, the present investigation was carried out to evaluate the seasonal influence on success of three estrus synchronization protocols viz., Ovsynch, Heatsynch and Triu-B and their effect on plasma biochemical profile in anoestrus buffaloes.

Materials and Methods

The study was carried out under field conditions in Anand and Mahisagar districts of middle Gujarat. A total of 48 postpartum true anoestrus buffaloes, in each summer and winter season, that did not exhibit spontaneous estrus within 90 days postpartum constituted the experimental animals. All these animals 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_3E (Inj. Intavita @ 10 ml, Intas Pharma), and oral multi-minerals (Minotas, Intas Pharma) @ 1 bolus daily for 7 days. The anoestrus buffaloes were then randomly divided into 4 equal groups and were subjected to standard estrus induction/synchronization protocols with fixed time AI (TriU-B, Ovsynch and Heatsynch, n=12 each, Buhecha et *al*., 2016), keeping 4^{th} group as untreated control. Buffaloes in estrus (spontaneous or induced) were inseminated using good quality frozen-thawed semen and were followed for 2 more cycles, and in non-return cases pregnancy was confirmed per rectum 60 days of last AI.

During both the seasons, jugular blood samples were collected four times in heparinized vaccutainers from all buffaloes, i.e. on day 0 - just before treatment, on day 7 - at the time of $PGF_2\alpha$ administration, on day 9/10 - induced estrus/FTAI and on day 21 post-AI. Blood sampling for animals of control group was done on the day of spontaneous estrus if any, and on day 21 post-AI. The plasma separated out by centrifugation of blood samples 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, inorganic phosphorus and magnesium were estimated by using commercially available assay kits (Coral Clinical System, Goa) on biochemistry analyzer. The Chi-square test was used to compare estrus response and conception rates, while one way analysis of variance, DNMRT and 't' test were used for plasma profile of biochemical and mineral constituents (Snedecor and Cochran, 1994) using online SAS software version 20.00.

Results and Discussion

Estrus and Fertility Response

Out of 12 anoestrus buffaloes under Ovsynch, Heatsynch and Triu-B protocols during summer, 75, 83.33 and 83.33 per cent buffaloes exhibited induced estrus within 2-4 days of $PGF_2\alpha$ injection, which were comparable with Kajaysri et *al.* (2015) under Triu-B protocol but much higher than the findings of Azawi et *al.* (2012) as 35.00 per cent under Ovsynch protocol. Poor response during

summer season may be due to inherent susceptibility of buffaloes to environmental stress manifested mainly as poor expression of estrus signs. While in winter the corresponding oestrus induction response was 83.33, 91.66 and 83.33 per cent with prominent to mild estrus signs in harmony with the results of Buhecha et *al.* (2016[a]).

The conception rates obtained at induced and overall of 3 cycles in anoestrus buffaloes under Ovsynch protocol were 16.67 and 25.00 per cent during summer and 41.67 and 58.33 per cent during winter season, respectively. The corresponding values with Heatsynch protocol were 16.67 and 25.00 per cent in summer and 25.00, and 50.00 per cent in winter, while with Triu-B protocol the corresponding values were 16.67 and 33.33 per cent and 41.67 and 58.33 per cent, respectively. In contrast, the overall conception rates in untreated control animals were only 8.33 and 25.00 per cent during summer and winter season, respectively.

The conception rate obtained at induced oestrus during winter season under Ovsynch, Heatsynch and Triu-B protocols (41.67, 25.00 and 41.67 %) were quite higher than the results obtained by earlier researchers (Buhecha et *al.,* 2016[a]; Ali *et al*., 2012). Similarly, during summer relatively lower conception rate under Ovsynch (0.00%) and higher under CIDR (30.00%) have been documented by Jabeen (2013). Further, he obtained comparable overall conception rates to the present ones with Ovsynch (22.20%) and PRID/CIDR (40.00 %) protocols during low breeding season, but had relatively much better results with both the protocols (80.0 &81.8%) in peak breeding season. Warriach et *al.* (2008) found comparatively lower (36.30 vs. 30.40 %) overall conception rate under Ovsynch protocol with non-significant difference between two seasons. The overall higher conception rates obtained in present study with Heatsynch protocol during winter than summer (50.00 vs 25.00 %) was comparable with the report of Mohan *et al*. (2009) in winter. There was no published report under Heatsynch protocol involving both the seasons, hence findings of this study seems to be a base line reference data for future work.

Plasma Total Protein and Total Cholesterol

The mean plasma levels of total proteins and total cholesterol recorded on different days of treatment and on day 21 post-AI in buffaloes under three treatment protocols (Table 1 &2) revealed that the profile did not vary significantly between sampling days in any of the protocols/groups. Nakrani et *al.* (2014) and Buhecha et *al.* (2016[b]) also reported similar non-significant variations in the overall mean plasma protein and total cholesterol concentrations in buffaloes under Ovsynch, Heatsynch and CIDR and/or Crestar protocols.

The effect of pregnancy status was non-significant within the protocol for both the traits in both the seasons, except that the total protein under Ovsynch protocol in winter season was significantly higher in conceived than non-conceived group (7.96 ± 0.09 vs. 7.54 ± 0.11 g/dl; $p<0.05$). The results of the present study proved that the hormonal treatments used did not influence significantly the plasma total protein profile in dairy animals, and that higher plasma protein levels are indicative of better nutritional status, which favours the sound reproductive performance in animals.

Plasma protein and cholesterol levels did not vary between the protocols during summer season. Whereas the winter profile for total cholesterol (mg/dl) was significantly higher in Heatsynch protocol (104.54 ± 3.51; $P<0.05$) than Triu-B protocol (89.80 ± 2.16), and total protein value (g/dl) was significantly ($P<0.05$) higher in Triu-B protocol than the remaining two protocols. The overall mean plasma protein values were significantly higher ($P<0.01$) in winter than summer season in all three groups, while the values of cholesterol were significantly higher during summer than winter in Ovsynch and Triu-B protocols.

Plasma Minerals Profile

The results on levels of plasma calcium, phosphorus and magnesium concentrations obtained during summer and winter seasons on day 0 and 9/10 (AI) of treatment and on day 21 post-AI in buffaloes under Ovsynch, Heatsynch and TriU-B protocols are presented in Table 3. There was

Table 1: Plasma total protein concentrations (g/dl) in anoestrus buffaloes during summer and winter season overall and on day of initiation of estrus synchronization protocols, FTAI and day-21 post-AI

Season							
Summer	Ovsynch	Conceived	2	7.00±1.00	7.25±0.25	7.60±0.60	7.28±0.33
		Non-conc	10	7.18±0.25	7.05± 0.32	6.75±0.25	6.99±0.16
		Overall	12				
	Heatsynch	Conceived	2	7.00±1.00	7.25±0.25	7.00±0.10	7.37±0.23
		Non-conc	10	6.73±0.30	6.80±0.26	6.90±0.29	6.81±0.15
		Overall	12				
	Triu-B	Conceived	2	7.00±1.00	6.80±0.7	7.25±0.75	7.02±0.38
		Non-conc	10	7.01±0.06	6.93±0.16	7.01±0.12	6.98±0.07
		Overall	12				
Winter	Ovsynch	Conceived	5	8.04±0.19	7.99±0.12	7.85±0.19	7.96±0.09
		Non-conc	7	7.53±0.17	7.39±0.14	7.71±0.25	7.54±0.11
	Heatsynch	Conceived	3	7.87±0.16	7.67±0.26	7.54±0.26	7.69±0.13
		Non-conc	9	7.60±0.32	7.91±0.32	7.38±0.32	7.63±0.18
	Triu-B	Conceived	5	8.16±0.15	8.06±0.22	7.74±0.42	7.99±0.19
		Non-conc	7	7.90±0.23	8.28±0.19	8.62±0.16	8.26±0.13

*D-0 = Day of starting the treatment. Means bearing uncommon superscripts within column (x, y) and subgroup (p, q) differ significantly (P<0.05). *Significant (P<0.05) and **highly significant (P<0.01) between seasons within same protocol.*

no influence of days or conceived and non-conceived status on these minerals profile in any of the groups studied during both the seasons. However, significantly (p<0.05) lower overall mean calcium was obtained during winter in Ovsynch protocol compared to other two protocols. In contrast, Buhecha et *al.* (2016[b]) reported significantly higher (P<0.05) mean plasma calcium concentration in Ovsynch protocol (10.41 ± 0.07 mg/dl) as compared to the values of other treatment groups. Like ours, Butani et *al.* (2011), Parmar et *al.* (2012) and Savalia et *al.* (2014) also did not find variation in the mean plasma inorganic phosphorus levels on the day of GnRH and/or $PGF_2\alpha$ treatment, at induced estrus and on day 21 post-AI in anoestrus or suboestrus buffaloes. Kumari et *al.* (2015) found non-significant difference in the serum phosphorus values before and after treatment by Ovsynch protocol, which ranged between 6.6±0.35 and 9.6±1.03 mg/100 ml in responding and non-responding groups.

The values of plasma magnesium did not differ significantly between seasons, yet the trend clearly shows little higher values during winter than the summer season. Comparatively lower values than the present ones have been recently reported 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; Buhecha et *al.,* 2016[b]) from the same region of Gujarat state. Significantly higher (P<0.05) value of plasma magnesium on day 21 post-AI and overall were found in non-conceived than conceived buffaloes during summer season under Heatsynch protocol. Similar trend was

Table 2:Plasma total cholesterol concentrations (mg/dl) in anoestrus buffaloes during summer and winter season overall and on day of initiation of estrus synchronization protocols, FTAI and day-21 post-AI

Season	Estrus induction protocol	Status	No	Plasma protein on days of treatment/AI			Overall
				D-0	D-9/10 (FTAI)	D-21 post-AI	
Summer	Ovsynch	Conceived	2	113.70±17.00	117.30±4.00	111.50 ±13.00	114.17±8.35
		Non-conc	10	104.00 ±8.54	89.00±9.88	102.00±5.62	98.33 ±4.59
		Overall	**12**	**112.08±7.87**	**112.58±8.77**	**109.92 ±5.02**	***111.53±4.15**
	Heatsynch	Conceived	2	116.00±11.00	106.50±12.50	122.00±21.00	113.17±9.25
		Non-conc	10	98.00±6.49	93.20±6.36	100.60±7.19	97.27±3.81
		Overall	**12**	**102.67±6.39**	**96.25±6.43**	**105.83±7.63**	**101.58±3.83**
	Triu-B	Conceived	2	102.65±21.65	102.95±29.95	111.18±31.18	105.59±12.60
		Non-conc	10	101.50±6.57	97.80±5.88	105.80±7..61	101.70±3.80
		Overall	**12**	**101.69±6.04**	**98.66±6.12**	**106.70±7.38**	****102.35±3.71**
Winter	Ovsynch	Conceived	5	100.48±12.92	91.24±10.67	98.58 ±10.67	96.76±6.22
		Non-conc	7	102.72 ±11.28	100.50±11.47	97.14±9.51	100.12±5.93
		Overall	**12**	**101.79±8.12**	**96.64±7.81**	**97.74±6.79**	**98.72±4.27^{xy}**
	Heatsynch	Conceived	3	102.17±17.63	112.25±23.02	105.44±13.93	106.62±9.40
		Non-conc	9	96.46±6.69	104.73±5.52	110.35±6.27	103.85±3.61
		Overall	**12**	**97.89±6.25**	**106.61±6.46**	**109.13±5.54**	**104.54±3.51^{y}**
	Triu-B	Conceived	5	90.59±7.32	87.12±5.26	89.94±4.83	89.21±3.18
		Non-conc	7	87.59±4.90	86.65±4.97	96.39±5.53	90.21±2.98
		Overall	**12**	**88.84±4.00**	**86.85±3.47**	**93.70±3.77**	**89.80±2.16^{x}**

*D-0 = Day of starting the treatment. Means bearing uncommon superscripts within column (x, y) differ significantly (P < 0.05).*Significant (P<0.05) and **highly significant (P<0.01) between seasons within same protocol.*

reported by Savalia et *al.* (2013) in Ovsynch and CIDR group during winter season. The present non-significant differences observed in plasma magnesium profile between different phases of the cycle corroborated with the reports of Paul *et al.* (2000) and Butani et *al.* (2011). The imbalance of other minerals such as calcium, phosphorus and potassium may decrease magnesium absorption from the gut of ruminants.

Significantly higher (P<0.05) overall mean plasma values for calcium and magnesium were observed in winter as compared to summer season in buffaloes of all three treatment protocols. These higher plasma calcium levels in winter could be attributed to the significantly (p<0.01) higher levels of calcium found in winter fodder (0.77 ± 0.11 %) than summer fodder (0.46 ± 0.05 %; Chhabra *et al.,* 2015). Earlier, Behera *et al.* (2005) has also reported higher plasma calcium during winter season in sheep. Seasonal influence of estrus induction protocols like, Ovsynch, Heatsynch and Triu-B on biochemical and mineral profile could not be seen in the literature reviewed, hence provides a window for future research work.

Conclusions

Appreciably higher estrus response and conception rates were obtained in treated buffaloes as compared to controls during both summer and winter seasons, and also during winter than summer season for all three estrus synchronization protocols, suggesting their beneficial role in anoestrus buffaloes, particularly during winter or favourable breeding season. Significantly higher overall mean plasma values for calcium and magnesium were observed in winter as compared to summer season

Table 3: Plasma mineral concentrations (mg/dl) in anoestrus buffaloes during summer and winter season overall and on day of initiation of estrus synchronization protocols, FTAI and day-21 post-AI

Mine-rals							
Calcium	Ovsynch	Summer	12	7.86±0.17	7.98±0.24	8.06±0.21	7.96±0.12
		Winter	12	*8.46±0.15	8.49±0.21	8.46±0.17	**8.47±0.10^{x}
	Heatsynch	Summer	12	8.16±0.21	8.29±0.17	8.45±0.17	8.30±0.16
		Winter	12	*8.94±0.27	**8.99±0.15	8.93±0.18	**8.96±0.12^{y}
	Triu-B	Summer	12	8.07±0.18	8.16± 0.13	8.44±0.20	8.22±0.10
		Winter	12	**8.98±0.17	8.67±0.29	8.87±0.14	**8.84±0.12^{y}
Inorganic P	Ovsynch	Summer	12	5.89±0.16	5.92±0.13	5.87±0.16	5.89±0.08
		Winter	12	6.03±0.18	6.11±0.26	6.26±0.21	6.14±0.12
	Heatsynch	Summer	12	5.74±0.15	5.87±0.18	5.87± 0.18	5.83±0.10
		Winter	12	5.96±0.26	5.83±0.20	5.98±0.26	5.92±0.14
	Triu-B	Summer	12	5.79±0.21	5.84±0.24	5.99±0.21	5.87±0.12
		Winter	12	6.03±0.17	5.70±0.23	6.12±0.15	5.95±0.11
Magnesium	Ovsynch	Summer	12	1.76±0.18	1.68±0.13	1.78±0.17	1.74±0.09
		Winter	12	**2.48±0.18	**2.46±0.15	**2.75±0.18	**2.56±0.10
	Heatsynch	Summer	12	1.87±0.18	1.81±0.16	1.91±0.19	1.86±0.10
		Winter	12	**2.92±0.16	**2.75±0.13	**2.64±0.16	**2.77±0.09
	Triu-B	Summer	12	1.90±0.11	1.98±0.13	2.04±0.11	1.97±0.07
		Winter	12	**2.65±0.10	**2.65±0.10	**2.59±0.12	**2.67±0.06

*D-0 = Day of starting the treatment. Means bearing uncommon superscripts within column (x, y) differ significantly (P < 0.05) in same season. * Significant (P<0.05) and ** highly significant (P<0.01) between seasons within same protocol within column.*

in buffaloes of all three treatment protocols may be due to higher levels found in winter fodder than summer fodder maintaining the ratio of calcium and phosphorus required for optimum reproductive performance.

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