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Efficacy of Estrus Synchronization Protocols during Summer and Winter Seasons together with Biochemical and Minerals Profile in Anoestrus Crossbred Cows

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Abstract

The estrus induction and fertility response along with biochemical and minerals profile of >90 days postpartum anoestrus crossbred cows following use of different estrus synchronization protocols was investigated during summer and winter seasons. The true anoestrus cows selected (n=43 in summer, n=40 in winter) were randomly treated with Ovsynch, Heatsynch and PRID protocols keeping 10 animals as control in both the seasons. During summer season, the estrus induction response with Ovsynch, Heatsynch and PRID protocol was 80, 90 and 80 %; while in winter season, the response was 100% with all three protocols. In summer season, the conception rates with Ovsynch, Heatsynch and PRID protocols at induced estrus were 30, 20 and 20 %and those of 3 cycles 60, 40 and 50 %, respectively, while in winter season, the conception rates at induced estrus were 45.45, 27.27 and 36.36 % and overall of 3 cycles 72.72, 45.45 and 63.64%, respectively. The overall mean plasma concentrations of total cholesterol (mg/dl) in anoestrus cows under Ovsynch, Heatsynch and PRID protocols were 171.57±4.75, 190.97±4.90 and 184.93±3.10 during winter and 163.73±4.96, 162.87±11.24 and 177.63±7.98 during summer, respectively. The corresponding values for plasma total protein (g/dl) during winter season were 7.77±0.11, 7.90±0.12and7.51±0.14, and in summer season 7.90±0.14, 8.02±0.67 and 8.32±0.10, respectively. The cholesterol levels were insignificantly lower and protein levels were significantly higher in summer than winter season, particularly in non-conceived cows. Among the minerals profiles, i.e. calcium, phosphorus and magnesium, only the calcium levels were significantly higher during winter and in conceived cows as compared to counter parts. In conclusion, the results showed that estrus synchronization with fixed time insemination protocols can augment fertility in anoestrus crossbred cows under favorable as well as heat stress condition, and the response is better with Ovsynch and PRID protocols without influencing much the blood metabolic profile.

Key words: Crossbred cows, Anoestrus, Season, Synchronization protocols, Biochemical profile, Conception rate.

Introduction

Acyclicity leading to low reproductive efficiency is one of the most important problems in dairy animals. Heat stress is a major contributing factor in the low fertility of dairy cows inseminated in the late summer months. Lower conception rates are consistently observed in summer months compared to winter months (De Rensis *et al.,* 2002; Alnimer *et al.,* 2002 and 2009). Heat stress decreases intensity and duration of estrus expression and increases incidences of anoestrus and silent ovulation via direct actions on the reproductive tract as well as by indirect effects mediated by alterations in energy balance and metabolism. Biochemical profile plays a key role in diagnosis of an array of productive and reproductive disorders in different livestock species (Niazi *et al.,* 2003). The minerals also play an intermediate role in the action of hormones and enzymes at the cellular level. The absence of an efficient and accurate method of estrus detection is a major factor limiting the reproductive performance of lactating dairy cattle. Synchronization of estrus in cattle can facilitate the use of artificial insemination by reducing the time needed for detection of estrus compared to cattle entering estrus spontaneously. Since the use of shade, fans or evaporative cooling reduces, but does not eliminate the fertility problems associated with heat stress; additional reproductive strategies are needed to counteract the adverse effects of heat stress. So, the present study was conducted with the aim to evaluate and compare the response of various estrus synchronization protocols together with biochemical and minerals profile during summer and winter seasons in anoestrus crossbred cows under field conditions.

Materials and Methods

This investigation was conducted during March to June (summer) and November to February (winter) of 2015-16 in the villages of milk shed areas of Panchamrut Dairy, Godhra and Amul Dairy, Anand, Gujarat using three estrus synchronization protocols, viz., (i) Ovsynch (ii) Heatsynch, and (iii) Triu-B/PRIDon 43 anoestrus crossbred cows during winter (n=11 in each protocol) and 40 anoestrus cows during summer seasons (n=10 each), keeping 10 cows as untreated anoestrus controls in both the seasons. The anoestrus animals were identified by palpating smooth, small and inactive ovaries on two occasions 10 days apart. All the infertile animals identified were dewormed using Inj. Ivectin, 10 ml, s/c (100 mg Ivermectin, IIL), and were treated once initially with Inj. Alphos-40, 10 ml, i/m (Sodium Acid Phosphate, 400 mg/ml, Pfizer) and Inj. Vetacept, 10 ml, i/m (Vit. AD_3E, Concept Pharma), and Minotas bolus (Intas Pharma) @ 1 bolus per day, for 7 days. Fix timed AI (FTAI) was performed as per the protocol and cows detected in estrus subsequent to FTAI were re-inseminated up to 3 cycles.In non-return cases the pregnancy was confirmed per rectum 60 days of last AI.

Blood samples were collected from jugular vein thrice in heparinized vaccutainers for treatment cycle, i.e. on day 0 - just before treatment, on day 9/10 - induced estrus/FTAI and on day 21 post-AI. The samples were centrifuged at 3000 rpm for 15 minute and plasma separated out was stored deep frozen at -20°C with a drop of merthiolate (0.1%) until analyzed. The concentrations of plasma total protein (Biuret method), total cholesterol (CHOD/PAP method),calcium (Arsenazol-III method), inorganic phosphorus (Modified Goummori's method) and plasma magnesium (Calmagite method) were estimated using standard procedures and assay kits procured from Crest Bio-systems, Goa, with the help of Chemistry Analyzer (Mindray, BS 120). The data on plasma profile were analyzed statistically by using ANOVA on SPSS software version 20.00, while estrus response and conception rates were compared by chi-square test.

Results and Discussion

Estrus induction and Conception rates

During winter season, all the anoestrus cows (100.00 %) under each of the 3 protocols exhibited induced estrus within 48-72 hrs from the time of $PGF_2\alpha$ injection, while in summer the estrus induction responses with Ovsynch, Heatsynch and Triu-B were 80.00, 90.00 and 80.00 %,respectively. During winter and summer seasons, the conception rates obtained at induced/first estrus with Ovsynch protocol were 45.55 and 30.00 %, and overall of 3 cycles 72.72 and 60.00 %, respectively. The corresponding values with Heatsynch protocol were 27.27 and 20.00 % at induced estrus, and

45.45 and 40.00 %overall, and for Triu-B, the CRs were 36.36 and 20.00% at induced estrusand overall of 3 cycles as 63.64 and 50.00 %, respectively. Apparently the results were higher during winter season as compared to summer with all 3 protocols, but did not differ statistically. Similar seasonal influence has been reported earlier in cattle by Alnimer *et al.* (2002) and De Rensis *et al.* (2002) and in buffaloes with significant seasonal difference by Mungad *et al.* (2016). In untreated anoestrus controls, out of 10 cows in each season, only 2 cows (20 % each) expressed spontaneous estrus and conceived either at first or second cycle, giving overall conception rate of only 20 % in winter and 10 % in summer season, respectively. The overall 2-3 times higher conception rates obtained using synchronization protocols in anoestrus crossbred cows during both the seasons, in comparison to hardly 20.00 % in control group, are suggestive of beneficial effect of using hormones in achieving higher pregnancy rates in infertile cattle.

The 100% estrus induction response obtained during winter season under all three protocols is in close agreement with Buhecha *et al.* (2015), Dhami *et al.* (2015) and Patel *et al.* (2013) in crossbred cows, and Bhoraniya *et al.* (2012[a]) in Kankrej cows. The present conception rates obtained at induced estrus and overall of 3 cycles in winter season with Ovsynch protocol compared well with the results of Buhecha *et al.* (2015), Dhami *et al.*(2015), Abubaker *et al.* (2013) and Ahmadi and Ghaiseri (2007), while the results during summer season coincided well with Alnimer *et al.*(2002, 2009). However, much lower pregnancy rates of 20.83 to 37.50 % have been reported by others during summer (Ahmadi and Ghaiseri, 2007; Ahuja *et al.,* 2005; De Rensis *et al.,* 2002).

The present conception rate of 27.27 % at induced estrus with Heatsynch protocol in winter season is in line with Kasimanickam *et al.* (2005), but comparatively higher than 15.51 to 18.00 % reported by Bhoraniya *et al.* (2012[a]) and Leyva and Mellado (2009). The results (36.36%) obtained using Triu-B protocol also concurred well with Buhecha *et al.* (2015) in anoestrus crossbred cows using same protocol, and also to the reports with CIDR by others (Abubaker *et al.,* 2013; Narenji Sani *et al.* (2011). However, the overall conception rate of 63.64 % obtained with Triu-B is higher than 43.80 to 53.85 % reported by Buhecha *et al.* (2015) in crossbreds and Bhoraniya *et al.* (2012[a]) in zebu cattle.

Plasma Biochemical profile

The plasma total cholesterol levels did not show any significant variation between days in any of the protocols during summer or winter season, and the values were higher during winter than summer season (Table 1). Alameen and Abdelatif (2012) also reported higher value of total cholesterol in winter than summer (157.67±39.77 vs 137.92±30.08 mg/dl) in non-pregnant crossbred cows. Bhoraniya *et al.* (2012[b]) and Patel *et al.* (2013) found non-significant variations in the cholesterol levels between day 0, 7, 9 (AI) and on day 20 post-AI in Ovsynch and CIDR treated anoestrus Kankrej and HF crossbred cows, respectively. The conceived animals had non-significantly higher plasma total cholesterol than non-conceived ones under all three protocols during winter season, but during summer season this trend was inversed in Heatsynch and Triu-B protocols. Buhecha *et al.* (2015) reported non-significantly higher value of plasma total cholesterol in conceived than non-conceived cows. The probable reason behind the lower levels of circulatory plasma cholesterol concentrations recorded on day 21 post-AI in conceived cows is utilization of cholesterol in progesterone synthesis as cholesterol is the immediate precursor of steroid hormones and a continuous synthesis of progesterone is needed in maintaining pregnancy in animals, thereby utilization of greater amount of plasma cholesterol. Exposure of dairy cows to hot environment produces reduction in the rates of metabolism and feed intake, which is the probable reason behind lower value of plasma cholesterol level during summer and because of that secretion of sex steroids under heat stress decreases as a result of decreased steriodogenic activity of ovary and the animals undergo a state of anoestrus or silent estrus (Abeni *et al.,* 2007).

The plasma total protein values were apparently higher during summer as compared to the winter season on various days, but differed significantly ($p<0.05$) particularly with Triu-B protocol (Table

Table 1: Plasma total cholesterol concentrations (mg/dl) in anoestrus conceived and non-conceived crossbred cows during winter and summer seasons on different days of various estrus synchronization treatments/AI (Mean± SE)

Sea-son	Estrus induction protocol	Status	No.	Days from treatment/AI			Overall
				D-0	D-AI	D-21 post-AI	
Winter	Ovsynch	Conceived	5	164.54±13.29	150.82±12.57	172.59±15.36	162.65±7.75
		Non-conc	6	178.44±5.43	178.88±13.11	179.73±9.94	179.01±5.43
		Overall	**11**	**172.12±6.70**	**166.12±9.75**	**176.48±8.43**	**171.57±4.75^{p}**
	Heatsynch	Conceived	3	177.66±7.65	180.36±5.68	170.86±4.97	176.29±5.31
		Non-conc	8	196.61±9.96	199.45±9.51	202.37±13.00	196.48±6.12
		Overall	11	**191.44±7.80**	**187.69±7.06**	**193.78±10.87**	***190.97±4.90^{q}**
	Triu-B	Conceived	4	180.52±13.02	164.83±8.53	185.69±7.76	177.01±5.89^{a}
		Non-conc	7	193.30±3.38	183.95±8.72	191.11±3.08	189.45±3.24^{b}
		Overall	**11**	**188.65±5.16**	**177.00±6.74**	**189.14±3.30**	**184.93±3.10^{q}**
Summer	Ovsynch	Conceived	3	160.67±7.75	180.67±17.17	157.33±18.11	166.22±8.38
		Non-conc	7	166.14±12.26	162.14±11.78	159.71±9.92	162.67±6.22
		Overall	**10**	**164.50±8.65**	**167.70±9.61**	**159.00±8.13**	**163.73±4.96**
	Heatsynch	Conceived	2	165.50±6.50	187.50±24.50	184.00±19.56	179.00±7.95
		Non-conc	8	155.87±24.72	158.25±25.90	162.37±24.70	158.84±13.86
		Overall	**10**	**157.80±19.56**	**164.10±21.11**	**166.70±19.71**	***162.87±11.24**
	Triu-B	Conceived	2	171.50±10.50	180.50±6.50	193.00±33.08	181.67±5.57
		Non-conc	8	178.87±15.29	171.50±18.21	179.50±19.95	176.62±9.92
		Overall	**10**	**177.40±12.21**	**173.30±14.45**	**182.20±15.90**	**177.63±7.98**

Means bearing uncommon superscripts within column (p, q) and subgroup (a, b) for a season differ significantly (p<0.05) and those with asterisk (*) within column differ between season (p<0.05); D-0 = Day of starting the treatment.

2). Das *et al.* (2014) recorded significantly higher value of total plasma protein in summer season as compared to winter season (10.24±0.35 vs. 9.25±0.26 g/dl) in lactating Kankrej cows. The conceived cows under Triu-B group had significantly (p<0.05) higher plasma protein in comparison to non-conceived ones in both the seasons. However, during winter season, the levels of plasma total protein between the conceived and non-conceived cows were statistically the same in Heatsynch and Ovsynch protocols, and in summer these were significantly higher in non-conceived than conceived cows. Dhoble *et al.* (2004) and Patel *et al.* (2013) also documented higher values of plasma total protein on the day of estrus in conceived as compared to non-conceived cows. The values of plasma total protein found at day '0' of treatment (anoestrus condition) is in the line with the observations reported by Buhecha *et al.* (2015) and Mahour *et al.* (2011), whereas higher values were reported by Dhami *et al.* (2015) and apparently lower values by Bhoraniya *et al.* (2012[b]) and Dhami *et al.* (2007) in anoestrus cows during winter season. Heat stressed cows have increased plasma urea nitrogen levels compared with the thermoneutral controls. This higher level of protein in circulation added with increased ammonia has detrimental effect on early embryo and its implantation. This higher circulating plasma protein may be because of feeding practice and it could be one of the reasons in early embryonic death and subsequent loss of pregnancies. Feeding high protein diets has also been shown to increase vaginal and uterine concentrations of ammonia and binding of LH to ovarian receptor sites has been shown to be inhibited by increased blood urea (Alnimer *et al.,* 2002).

Table 2: Plasma total protein concentrations (g/dl) in anoestrus conceived and non-conceived crossbred cows during winter and summer seasons on different days of various estrus synchronization treatments/AI (Mean± SE)

Season	Estrus induction protocol	Status	No.	Days from treatment/AI			Overall
				D-0	D-AI	D-21 post-AI	
Winter	Ovsynch	Conceived	5	8.12±0.50	7..60±0.12	7.99±0.50	7.90±0.22
		Non-conc	6	7.77±0.20	7.58±0.15	8.23±0.27	7.86±0.13
		Overall	**11**	**7.64±0.22**	**7.74±0.12**	**7.93±0.20**	**7.77±0.11**
	Heatsynch	Conceived	3	7.81±0.43	7.48±0.15	7.93±0.36	7.93±0.20
		Non-conc	8	7.92±0.31	7.74±0.21	8.05±0.29	7.90±0.15
		Overall	11	**7.97±0.25**	**7.70±0.16**	**8.04±0.24**	**7.90±0.12**
	Triu-B	Conceived	4	8.25±0.59	8.15±0.34	7.96±0.45	8.12±0.25[a]
		Non-conc	7	6.69±0.26	7.34±0.16	7.48±0.21	7.17±0.14[b]
		Overall	**11**	***7.26±0.36**	***7.63±0.15**	***7.65±0.16**	***7.51±0.14**
Summer	Ovsynch	Conceived	3	7.30±0.48	7.31±0.47	7.68±0.52	7.43±0.25[a]
		Non-conc	7	8.13±0.37	8.08±0.26	8.21±0.22	8.11±0.24[b]
		Overall	**10**	**7.88±0.31**	**7.85±0.24**	**7.99±0.21**	**7.90±0.14**
	Heatsynch	Conceived	2	6.99±0.67	7.37±0.99	7.43±0.81	7.27±0.37[a]
		Non-conc	8	8.20±0.33	8.10±0.37	8.31±0.20	8.21±0.17[b]
		Overall	**10**	**7.97±0.32**	**7.96±0.34**	**8.14±0.23**	**8.02±0.67**
	Triu-B	Conceived	2	8.80±0.64	8.92±0.37	8.79±0.49	8.84±0.22[a]
		Non-conc	8	8.18±0.16	8.16±0.21	8.25±0.19	8.20±0.10[b]
		Overall	**10**	***8.30±0.18**	***8.31±0.20**	***8.36±0.18**	***8.32±0.10**

Means bearing uncommon superscripts within column subgroups (a, b) for a season and astrike (*) within column differ significantly (P<0.05) between season; D-0 = Day of starting the treatment

Plasma Minerals Profile

The concentrations of plasma calcium, phosphorus and magnesium in anoestrus cows under Ovsynch, Heatsynch and Triu-B protocol during winter as well as summer seasons did not reveal any significant variation between days/periods, i.e. anoestrus, induced estrus and day 21 post-AI (Table 3). This was in accordance with the reports of Buhecha *et al.* (2015) and Patel *et al.* (2013) using same protocols. During both summer and winter seasons, the overall mean concentration of calcium on day 21 post-AI was found to be higher in conceived than non-conceived cows in all three protocols, but differed significantly only in Ovsynch protocol (Table 4). As compared to the present findings, Buhecha *et al.* (2015) documented relatively higher values of plasma calcium in anoestrus crossbred cows during winter season, while Alameen and Abdelatif (2012) found lower level of calcium during both summer and winter (7.92±2.11 and 7.85±2.10 mg/dl) in non-pregnant crossbred dairy cows.

In winter season, the mean plasma inorganic phosphorus levels obtained in anoestrus cows were in close collaboration with Muneer *et al.* (2013), while lower values were documented by Buhecha *et al.* (2015). During summer season, the values of plasma inorganic phosphorus obtained were higher than those reported by Alameen and Abdelatif (2012). In contrast to this, Das *et al.* (2014) documented significantly higher level of phosphorus in summer than winter season (5.29±0.15 vs 3.74±0.11 mg/dl).The levels of plasma magnesium found in anoestrus crossbred cows were in close collaboration with Dutta *et al.* (2001) and Ceylan *et al.* (2008). However, much lower level of 1.88±0.37 mg/dl was reported by Alameen and Abdelatif (2012). In brief, hormone therapy of estrus synchronization did not influence the plasma mineral profile.

Table 3: Plasma minerals profile (mg/dl) in anoestrus crossbred cows during winter and summer seasons on different days of various estrus synchronization treatments/AI (Mean± SE)

Plasma Trait	Season	Protocol	Days from treatment/AI			Overall
			D-0	D-AI	D-21 post-AI	
Calcium	Winter (n=11)	Ovsynch	8.66±0.25	8.51±0.37	8.69±0.23	8.62±0.16
		Heatsynch	9.03±0.22	8.90±0.23	8.75±0.30	*8.91±0.14
		Triu-B	8.78±0.32	9.09±0.46	9.17±0.27	*9.02±0.20
	Summer (n=10)	Ovsynch	8.73±0.30	8.97±0.37	9.05±0.34	8.92±0.19[q]
		Heatsynch	8.15±0.92	8.41±0.80	7.99±0.28	*8.18± 0.16[p]
		Triu-B	7.99±0.14	8.20±0.12	8.17±0.19	*8.12±0.08[p]
Phosphorus	Winter	Ovsynch	6.18±0.11	6.14±0.13	6.27±0.13	6.19±0.07
		Heatsynch	6.09±0.12	6.15±0.14	6.36±0.15	6.20±0.08
		Triu-B	6.15±0.19	6.21±0.15	5.75±0.15	6.04±0.10
	Summer	Ovsynch	6.61±0.30	6.60±0.27	6.55±0.27	6.59±0.15
		Heatsynch	6.76±0.13	6.62±0.16	6.97±0.31	6.78±0.12
		Triu-B	6.80±0.21	6.79±0.25	6.88±0.23	6.82±0.13
Magnesium	Winter	Ovsynch	2.40±0.12	2.33±0.09	2.39±0.08	2.37±0.06
		Heatsynch	2.47±0.06	2.29±0.06	2.41±0.12	2.39±0.06
		Triu-B	2.29±0.09	2.34±0.11	2.33±0.10	2.32±0.05
	Summer	Ovsynch	2.19±0.13	2.12±0.14	2.07±0.13	2.12±0.07[p]
		Heatsynch	2.15±0.82	2.22±0.12	2.20±0.00	2.19±0.06[pq]
		Triu-B	2.37±0.10	2.17±0.15	2.56±0.23	2.36±0.10[q]

Means bearing uncommon superscripts within column (a, b) for a season and astrike (*) between season differ significantly (P<0.05); D-0 = Day of starting the treatment.

Table 4: Mean (±SE) plasma minerals concentrations (mg/dl) during winter and summer seasons in conceived and non-conceived anoestrus cows under different synchronization protocols

Season	Estrus induction protocol	Pregnancy Status	No	Calcium	Phosphorus	Magnesium
Winter	Ovsynch	Conceived	5	8.80±0.22[a]	6.19±0.09	2.47±0.16[a]
		Non-conceived	6	8.54±0.21[b]	6.20±0.12	2.40±0.09[b]
		Overall	**11**	**8.62±0.16**	**6.19±0.07**	**2.37±0.06**
	Heatsynch	Conceived	3	8.84±0.18	6.24±0.15	2.49±0.10
		Non-conceived	8	8.96±0.17	6.19±0.10	2.36±0.05
		Overall	**11**	***8.91±0.14**	**6.20±0.08**	**2.39±0.06**
	Triu-B	Conceived	4	9.14±0.26	6.08±0.17	2.26±0.86
		Non-conceived	7	8.95±0.28	6.01±0.12	2.35±0.70
		Overall	**11**	***9.02±0.20**	**6.04±0.10**	**2.32±0.05**
Summer	Ovsynch	Conceived	3	9.61±0.37[a]	6.93±0.20	2.36±0.12[a]
		Non-conceived	7	8.62±0.19[b]	6.44±0.20	2.02±0.87[b]
		Overall	**10**	**8.92±0.19[q]**	**6.59±0.15**	**2.12±0.07[p]**
	Heatsynch	Conceived	2	8.60±0.35	6.61±0.13	2.39±0.92
		Non-conceived	8	8.08±0.17	6.82±0.15	2.14±0.64
		Overall	**10**	***8.18± 0.16[p]**	**6.78±0.12**	**2.19±0.06[pq]**
	Triu-B	Conceived	2	8.08±0.16	7.38±0.31[a]	2.56±0.09
		Non-conceived	8	8.13±0.10	6.68±0.13[b]	2.33±0.12
		Overall	**10**	***8.12±0.08[p]**	**6.82±0.13**	**2.36±0.10[q]**

Means bearing uncommon superscripts within column (p, q) and subgroup (a, b) differ significantly (p<0.05) within season and those with astrike (*) differ between seasons.

Conclusion

The use of estrus synchronization protocols, viz., Ovsynch, Heatsynch and PRID during winter season and only Ovsynch and/or PRID during summer season gave acceptable conception rates without significantly altering the biochemical and minerals profile. Higher plasma protein and lower cholesterol during summer was associated with poor estrus synchronization and conception results. Hence all three protocols can be used to solve the problem of winter anoestrus and only PRID during summer anestrus in crossbred cows under field condition.

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