Scholars Journal of Agriculture and Veterinary Sciences **e-ISSN 2348–1854**
Sch J Agric Vet Sci 2014; 1(4B):299-304 **p-ISSN 2348–8883**

An International Publisher for Academic and Scientific Resources) DOI: 10.36347/sjavs.2014.v01i04.028

Effect of Controlled Breeding Techniques on Fertility and Plasma Profile of Biochemical and Mineral Constituents in Anoestrus Buffaloes

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Abstract: This study was planned to evaluate the fertility response and plasma profile of biochemical and mineral constituents in 55 postpartum (>90 days) anoestrus rural buffaloes treated with three standard hormonal protocols (CIDR, Ovsynch and Crestar, n=15 each), keeping a group of untreated anoestrus control (n=10) and a group of normal cyclic control (n=10). All the 15 (100 %) buffaloes in each group under CIDR, Ovsynch and Crestar protocols exhibited induced oestrus within mean intervals of 65.00±1.55, 69.46±1.04 and 46.00±1.37 hrs, respectively, from $PGF_2\alpha$ injection. The conception rates obtained at induced oestrus with CIDR, Ovsynch and Crestar protocols were 46.67, 53.33 and 33.33 per cent, respectively. The corresponding overall conception rates of three cycles were observed to be 66.67, 73.33 and 60.00 per cent. In untreated anoestrus control (n=10), only 2 buffaloes exhibited spontaneous oestrus within 90 days of follow up and one conceived. The pooled conception rates of all three treatment groups at induced oestrus and overall of 3 cycles were 44.44 and 66.67 per cent, respectively. These were nearer to those in normal cyclic control group (40.00 and 70.00 %). Further, in the normal cyclic control group, the overall mean plasma total cholesterol, total protein, calcium and inorganic phosphorus concentrations were 64.93±2.87 mg/dl, 8.75±0.18 g/dl, 9.22±0.18 mg/dl, and 5.12±0.11 mg/dl, respectively. The influence of treatment days (0, 7, 9 (AI) or day 21 post-AI) was not significant for any of the traits in any of the groups. The levels of all these traits, except cholesterol, were significantly higher in normal Cyclic Control group than the values found in all three treatment groups of anoestrus animals, which were at par. The conceived buffaloes had insignificantly lower values of total cholesterol and significantly ($p<0.05$) higher protein as compared to non-conceived buffaloes in all the three protocols and even in cyclic control group, but no such trend was noted for either plasma calcium or phosphorus profile. It was concluded that Ovsynch and/or CIDR protocols could be a better option to improve fertility in anoestrus buffaloes without altering blood biochemical and mineral status under field conditions.

Keywords: Buffalo, Anoestrus, Treatment protocols, Fertility rate, Biochemical & Mineral profile

INTRODUCTION

Various hormonal preparations and protocols are being practised by the field veterinarians to treat postpartum anoestrus in dairy animals, but the results are inconsistent. The variable results obtained following hormonal treatments by different workers may be largely due to varying nutritional and ovarian status at the start of treatment, endocrine events, faulty management and uterine infection apart from product potency, closeness to its deposition in vascular structures, and the quality of breeding services and its follow up provided in treated animals [1-3]. Use of hormonal protocols like Ovsynch, CIDR and Crestar induces and synchronizes the oestrus/ovulation and thus improves the conception rates and establishes cyclicity in acyclic buffaloes, thereby achieving ideal calving interval. The plasma protein, cholesterol and mineral profile denote nutritional status of animals and are related with their fertility. The cholesterol being precursor of steroid hormones play an important role in steroideogenesis; while calcium tones up the genitalia, and protein and inorganic phosphorus are involved at cellular level in metabolic processes. Hence, this study was planned to evaluate the comparative efficacy of CIDR, Ovsynch and Crestar protocols for fertility enhancement and to see their influence on plasma biochemical and mineral profile in anoestrus rural buffaloes.

MATERIALS AND METHODS

This study was carried out during breeding season on 55 postpartum (>90 days) anoestrus buffaloes and 10 normal cyclic buffaloes of average BCS selected from tribal villages of Mahisagar district of Gujarat. The buffaloes were initially screened gynaeco-clinically for their reproductive status as cyclic, anoestrus or

repeat breeders by organizing special sexual health camps. Anoestrus buffaloes were re-confirmed by rectal palpation of small smooth inactive ovaries 10 days later. All the selected buffaloes were initially dewormed using Ivermectin, 100 mg s/c. Owners of the ear-marked animals were supplied with multi-mineral boluses (Bolus-Minotas, Intas Pharma) for oral supplemention for 7 days. The anoestrus buffaloes were then randomly subjected to different standard estrus induction/ synchronization protocols (viz., CIDR, Ovsynch and Crestar, n=15 each) with fix timed AI [3, 4]. 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 oestrus were inseminated using good quality frozen-thawed semen, and in nor-return cases pregnancy was confirmed per rectum 60 days of last AI.

All the hormonally treated control buffaloes were studied for their reproductive status and plasma protein, cholesterol, calcium and inorganic phosphorus profile on day 0 - just before treatment, on day 7 - at the time of $PGF_2\alpha$ administration, on day 9 - induced oestrus/ FTAI and on day 21 post-AI by sampling the blood in heparinized vials. The blood samples were centrifuged at 3000 rpm for 15 min. and plasma separated out was stored deep frozen at -20°C with a drop of merthiolate (0.1%) until analyzed. Plasma profiles of total protein, cholesterol, calcium and inorganic phosphorus were estimated by using standard procedures and kits procured from Analytical Technologies Pvt. Limited, Baroda, on chemistry analyzer.

The data on conception rate (by Chi square test) and plasma profile of biochemical and mineral constituents (ANOVA) were analyzed statistically [5] using online SAS software.

RESULTS AND DISCUSSION

Fertility Response to Synchronization Protocols

Hundred per cent buffaloes in each group under CIDR, Ovsynch and Crestar protocols exhibited induced oestrus with prominent, moderate or weak oestrus signs within mean intervals of 65.00±1.55, 69.46±1.04 and 46.00±1.37 hrs, respectively, from the time of $PGF_2\alpha$ injection. The conception rates obtained at induced oestrus in buffaloes under CIDR, Ovsynch and Crestar protocols were 46.67, 53.33 and 33.33 per cent, respectively. The corresponding overall conception rates of three cycles were 66.67, 73.33 and 60.00 per cent, respectively. All the treated anoestrus buffaloes became regular cycling and exhibited normal estrus within 18-21 days post-treatment if not settled at induced estrus.

In untreated Anoestrus Control group (n=10), only 2 buffaloes exhibited spontaneous oestrus within 90 days of follow up and one conceived at first AI (CR, 50.00 %) at 157 days postpartum. The pooled conception rates of all three treatment groups (n=45) at induced oestrus and overall of 3 cycles were 44.44 and 66.67 per cent, respectively. In normal Cyclic Control group (n=10), the corresponding conception rates were 40.00 and 70.00 per cent, respectively. These results indicated the positive contributory role of handling the problem of acyclicity in buffaloes with estrus synchronization protocols like CIDR, Ovsynch and Crestar for their induction of oestrus/ovulation and making them pregnant to the levels nearly at par with normal cyclic control buffaloes without waiting for months together to see them cycling. These findings are in accordance with or even better than those documented in several earlier studies [1, 3, 4, 6-11].

Plasma total Protein and Total Cholesterol

The mean levels of plasma total cholesterol and total protein recorded on day 0, 7, 9 (AI) of treatment and on day 21 post-AI in buffaloes under CIDR, Ovsynch and Crestar protocols, and on day of AI and day 21 post-AI in normal cyclic buffaloes are presented in Tables 1 and 2. Among the 10 untreated anoestrus control buffaloes only 2 buffaloes exhibited estrus after a long time and one of them conceived, and rest all remained acyclic till 90 days of follow up, hence their data are not tabulated.

The results in Table 1 did not reveal significant variations in plasma total cholesterol profile between days/periods of the treatment in any of the groups. But the pooled values irrespective of periods were apparently higher in non-conceived than conceived buffaloes of all 4 groups. Similar results of cholesterol were observed in anoestrus buffaloes by Savalia *et al.* [3] with values of 92.27±6.04 and 79.96±2.17 mg/dl in non-conceived and conceived anoestrus buffaloes, respectively, treated with CIDR protocol, but in Ovsynch group no such difference was found. However, other researchers [2, 12] reported that the conceiving buffaloes had significantly higher levels of plasma cholesterol as compared to non-conceiving ones.

The non-significantly ($p>0.05$) higher plasma total cholesterol concentrations observed in non-conceived than conceived buffaloes consistently over the periods studied directly reflected its role in steroidegenesis, particularly in progesterone synthesis in conceiving buffaloes, thereby reducing the circulatory cholesterol levels in them. Earlier the higher mean plasma total cholesterol levels at induced oestrus and 22nd day post-AI than that of pre-treatment level in GnRH treated anoestrus buffaloes have been documented [13], and the high level of cholesterol increased the oestrogen synthesis resulting in manifestation of heat [14]. In the present study plasma total cholesterol profile in anoestrus and cyclic buffaloes was more or less similar. The higher levels of cholesterol (mg/dl) in cyclic as compared to acyclic buffaloes are however reported by previous workers [15-18]. Present findings with respect to trend of

cholesterol profile over the induced cycle corroborated with those of Khasatiya *et al*. [25] in Surti buffaloes, who did not find significant difference in the levels of plasma total cholesterol between fertile and infertile cycles in buffaloes.

The mean plasma protein profile also did not vary significantly between sampling days (0, 7, 9 (AI) and day 21 post-AI) in any of the protocols/groups studied. However, the concentrations were significantly ($p<0.05$) higher in conceived than non-conceived buffaloes of all the treatment protocols, while in normal cyclic control group the trend was reverse with the mean values of 8.44±0.35 g/dl in conceived and 8.96±0.16 g/dl in non-conceived buffaloes ($p<0.05$), with the overall pooled mean value of 8.75±0.18 mg/dl, which was significantly higher as compared to values obtained in all three treatment groups (Table 2). More or less comparable findings were reported by Savalia *et al*. [3] in buffaloes and Patel *et al.* [19] in crossbred cows with CIDR and Ovsynch protocol and in normal cyclic control groups also. Parmar [2], however, observed inverse trend with comparable values of plasma protein in conceived and non-conceived buffaloes under both the protocols. In one earlier study the conceived buffaloes had higher plasma total protein than non-conceived ones on the day of oestrus/AI [12].

In the present study significantly ($p<0.05$) higher mean levels of plasma total proteins were observed in normal cyclic buffaloes as compared to anoestrus ones. These observations are similar to the findings reported by many of the earlier workers in buffaloes [15-18, 20]. Gentile *et al.* [21] opined that serum protein level was not related with fertility in dairy cows. However, as has been noted in the present study Lodhi *et al*. [20] also opined that the Murrah buffaloes having high level of total protein had good reproductive performance.

Table1: Plasma total cholesterol concentrations (mg/dl) in anoestrus and cyclic buffaloes on different days of treatment/AI under various oestrus induction protocols

Group	Status	No.	Days of Treatment/AI				
			D-0	D-7	D-9 (AI)	D-21 post-AI	Overall
CIDR	Conceived	7	67.84±8.81	72.12±7.88	67.46±7.14	65.89±6.60	68.33±3.63
	Non-conceived	8	77.54±3.42	71.94±3.44	70.24±3.24	80.44±3.77	75.04±1.81
	Overall	15	73.01±4.51	72.02±3.95	68.94±3.62	73.65±4.03	71.92±1.98
Ovsynch	Conceived	8	63.64±5.26	65.33±6.72	64.44±4.81	63.77±4.69	64.29±2.58
	Non-conceived	7	65.52±5.00	66.52±4.91	68.02±6.80	69.68±6.16	67.43±2.74
	Overall	15	64.52±3.53	65.88±4.11	66.11±3.96	66.52±3.75	65.76±1.87
Crestar	Conceived	5	61.46±1.95	65.06±2.64	66.62±3.57	66.00±3.06	64.79±1.39
	Non-conceived	10	64.48±5.96	67.32±6.12	67.54±5.02	67.97±4.43	66.83±2.62
	Overall	15	63.47±3.97	66.57±4.10	67.23±3.47	67.31±3.06	66.15±1.80
Normal Cyclic Control	Conceived	4	--	--	63.85±4.83	62.02±6.87	62.93±3.90
	Non-conceived	6	--	--	67.54±6.03	64.98±6.09	66.26±4.10
	Overall		--	--	66.06±3.95	63.80±4.35	64.93±2.87

D-0 = Day of starting the treatment, D-7 = Administration of PG, D-9 = Fixed time AI, D-21 = Day 21 post-AI. The variations between periods and between pregnancy statuses were not significant.

Table 2: Plasma total protein concentrations (g/dl) in anoestrus and cyclic buffaloes on different days of treatment/AI under various oestrus induction protocols

Group	Status	No.	Days of Treatment/AI				
			D-0	D-7	D-9 (AI)	D-21 (post-AI)	Overall
CIDR	Conceived	7	8.52±0.29	8.52±0.19	8.49±0.22	8.56±0.28	8.52±0.12 ^{a}
	Non-C	8	8.02±0.22	7.84±0.26	7.88±0.23	7.86±0.24	7.90±0.11 ^{b}
	Overall	15	8.25±0.19	8.15±0.18	8.17±0.17	8.19±0.20	8.19±0.09 ^{x}
Ovsynch	Conceived	8	8.27±0.21	8.17±0.28	8.28±0.27	8.13±0.28	8.21±0.12 ^{a}
	Non-C	7	8.21±0.27	8.09±0.27	8.04±0.29	8.02±0.28	8.09±0.13 ^{b}
	Overall	15	8.24±0.16	8.13±0.19	8.17±0.20	8.08±0.19	8.15±0.09 ^{x}
Crestar	Conceived	5	8.44±0.43	8.54±0.40	8.51±0.39	8.51±0.42	8.50±0.19 ^{a}
	Non-C	10	7.93±0.15	7.93±0.16	7.91±0.17	7.91±0.16	7.92±0.08 ^{b}
	Overall	15	8.10±0.18	8.14±0.18	8.11±0.18	8.11±0.19	8.12±0.09 ^{x}
Normal Cyclic Control	Conceived	4	--	--	8.51±0.42	8.36±0.63	8.44±0.35 ^{b}
	Non-C	6	--	--	8.82±0.24	9.11±0.21	8.96±0.16 ^{a}
	Overall		--	--	8.70±0.22	8.81±0.29	8.75±0.18 ^{y}

D-0 = Day of starting the treatment, D-7 = Administration of PG, D-9 = Fixed time AI, D-21 = Day 21 post-AI. Non-C = Non-conceived; Means bearing uncommon superscripts within the column for a group differ significantly.

Plasma Calcium and Phosphorus

The data present in Tables 3 and 4 did not reveal significant variations in plasma calcium and inorganic phosphorus concentrations of buffaloes between days/periods of the treatments (0, 7, 9 (AI) and day 21 post-AI) with CIDR, Ovsynch and Crestar protocols, and even in normal cyclic control group. Similarly the variations in plasma calcium and inorganic phosphorus levels between conceived and non-conceived groups were not significant, though the values were little higher in conceived buffaloes in most groups. Further, the overall mean values of plasma calcium and inorganic phosphorus obtained in anoestrus buffaloes under CIDR, Ovsynch and Crestar protocols were statistically similar and were significantly ($p<0.05$) lower than that in normal cyclic buffaloes (9.22±0.18 mg/dl calcium and 5.12±0.11 mg/dl phosphorus). Very similar observations have been recently made by researchers using CIDR and Ovsynch protocols in anoestrus cows and buffaloes, including normal cyclic control groups [2, 3, 19, 22]. Similarly, Parmar *et al.* [13] observed non-significantly higher mean plasma calcium concentration at induced oestrus as compared to values at 22nd day post-AI and pre-treatment for GnRH treated anoestrus buffaloes. Savalia *et al.* [20] also obtained higher mean calcium levels in conceived as compared to non-conceived buffaloes under CIDR, Ovsynch and even normal cyclic control groups, which are to some extent comparable with the present findings.

Table 3: Plasma calcium concentrations (mg/dl) in anoestrus and cyclic buffaloes on different days of treatment/AI under various oestrus induction protocols

Group	Status	No.	Days of Treatment/AI				
			D-0	D-7	D-9 (AI)	D-21 post-AI	Overall
CIDR	Conceived	7	8.39±0.20	8.63±0.31	8.77±0.28	8.60±0.29	8.60±0.13
	Non-C	8	8.39±0.17	8.14±0.18	8.34±0.28	8.84±0.34	8.43±0.13
	Overall	15	8.39±0.12	8.37±0.18	8.54±0.20	8.73±0.22	8.51±0.09 [a]
Ovsynch	Conceived	8	8.78±0.15	9.04±0.17	8.68±0.16	8.74±0.20	8.81±0.08
	Non-C	7	8.30±0.60	8.39±0.44	8.80±0.52	8.81±0.49	8.57±0.25
	Overall	15	8.56±0.29	8.74±0.23	8.74±0.25	8.77±0.24	8.70±0.12 [a]
Crestar	Conceived	5	8.60±0.44	8.40±0.26	8.15±0.22	8.28±0.25	8.35±0.14
	Non-C	10	9.17±0.32	8.81±0.41	8.80±0.27	8.86±0.23	8.91±0.15
	Overall	15	8.98±0.26	8.67±0.28	8.58±0.21	8.67±0.18	8.72±0.11 [a]
Normal Cyclic Control	Conceived	4	--	--	9.24±0.45	9.50±0.54	9.37±0.33
	Non-C	6	--	--	9.10±0.27	9.16±0.38	9.13±0.22
	Overall		--	--	9.15±0.23	9.29±0.30	9.22±0.18 [b]

D-0 = Day of starting the treatment, D-7 = Administration of PG, D-9 = Fixed time AI, D-21 = Day 21 post-AI. Non-C = Non-conceived; Means bearing uncommon superscripts within the column differ significantly ($p< 0.05$).

Table 4: Plasma inorganic phosphorus concentrations (mg/dl) in anoestrus and cyclic buffaloes on different days of treatment/AI under various oestrus induction protocols

Group	Status	No.	Days of Treatment/AI				
			D-0	D-7	D-9 (AI)	D-21 post-AI	Overall
CIDR	Conceived	7	4.16±0.15	4.16±0.24	4.01±0.20	4.02±0.16	4.09±0.09
	Non-C	8	3.90±0.19	4.12±0.28	4.04±0.14	4.07±0.20	4.03±0.10
	Overall	15	4.02±0.13	4.13±0.18	4.03±0.11	4.04±0.13	4.06±0.07 [a]
Ovsynch	Conceived	8	4.04±0.33	4.15±0.24	3.92±0.35	4.32±0.40	4.11±0.16
	Non-C	7	3.83±0.19	3.70±0.30	3.77±0.18	3.83±0.13	3.78±0.10
	Overall	15	3.94±0.19	3.94±0.19	3.85±0.20	4.09±0.23	3.96±0.10 [a]
Crestar	Conceived	5	4.35±0.41	3.90±0.40	3.66±0.24	3.81±0.29	3.93±0.17
	Non-C	10	3.99±0.20	3.80±0.24	3.76±0.22	3.96±0.17	3.88±0.10
	Overall	15	4.12±0.19	3.83±0.20	3.73±0.16	3.91±0.14	3.89±0.09 [a]
Normal Cyclic Control	Conceived	4	--	--	4.88±0.31	5.41±0.23	5.14±0.21
	Non-C	6	--	--	5.08±0.10	5.14±0.25	5.11±0.13
	Overall		--	--	5.00±0.13	5.25±0.17	5.12±0.11 [b]

D-0 = Day of starting the treatment, D-7 = Administration of PG, D-9 = Fixed time AI, D-21 = Day 21 post-AI. Non-C = Non-conceived; Means bearing uncommon superscripts within the column differ significantly ($p< 0.05$).

The marginal deficiency of phosphorus is opined to be enough to cause disturbances in pituitary-ovarian axis, without manifesting specific systemic deficiency symptoms [23]. Parmar *et al.* [13] and Savalia *et al.* [3] 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 22 post-AI in anoestrus or suboestrus buffaloes. The present insignificant differences observed in plasma inorganic phosphorus profile between different phases of the cycle and even conceived and non-conceived groups corroborated with the earlier reports in non-suckled dairy cows [24], and in Nili-Ravi and Surti buffaloes [26, 25] . Moreover, the significantly higher mean plasma phosphorus levels observed in the present study in normal cyclic control buffaloes than the anoestrus ones have also been documented in different breeds of buffaloes by earlier workers [3, 19, 20, 24] from different parts of the country.

CONCLUSION

It can be inferred that the hormonal protocols used, particularly Ovsynch & CIDR protocol, improved conception rates in anoestrus buffaloes under field condition, but did not influence the plasma profile of biochemical and mineral constituents significantly.

ACKNOWLEDGEMENT

We gratefully acknowledge the authorities of AAU, Anand and Panchamrut Dairy, Godhra for providing facilities and permission granted to take up this work under milk shed area of Panchamrut Dairy, and also the inseminators of the concerned villages under study for technical support in the follow up work.

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