Influence of Controlled Breeding Techniques on Estrus Induction Response, Conception Rate and Plasma Progesterone Profile in Anoestrus Buffaloes

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Abstract

This investigation was aimed to evaluate the fertility response 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 control (n=10), and the findings were compared with a group of normal cyclic control buffaloes (n=10). All the 15 (100

Index terms— buffalo, anoestrus, treatment protocols, oestrus induction, conception rate, fertile oestrus induction interval.

1 Introduction

he postpartum anoestrus is the most prevalent reproductive problem in dairy animals, for which several hormonal preparations and protocols are being practised by the field veterinarian, but with inconsistent results. Hormonal therapies have good therapeutic value to enhance reproductive efficacy in infertile animals with good nutritional status (Ghuman et al., 2009; Malik et al., 2010, 2011; Chaudhari et al., 2012; Bhoraniya et al., 2012; Parmar, 2013; Savalia et al., 2014). The variable results obtained following hormonal treatments by different workers may be largely due to nutritional status, faulty management, ovarian changes, endocrine events and even uterine infection. Use of hormonal protocols like Ovsynch, CIDR and Crestar during breeding season can be helpful in inducing and synchronizing oestrus and getting better conception rate in them with lesser number of services per conception and making acyclic buffaloes to cycle normally, thereby achieving ideal inter-calving interval. Plasma progesterone levels denote either the presence or absence of CL and its functional competency which is directly related with fertility of the female. The progesterone hormone is responsible for stimulation of cyclicity, follicular development and also for continuation of pregnancy. Hence, this study was planned under field conditions to evaluate the comparative efficacy of CIDR, Ovsynch and Crestar protocols in anoestrus buffaloes for fertility enhancement and their influence on plasma progesterone profile.

2 II.

3 Materials and Methods

This study was carried out during breeding season from November, 2013 to March, 2014 on 55 postpartum (>90 days) anoestrus buffaloes and 10 normal cyclic buffaloes of average BCS selected from tribal areas of Gujarat. The buffaloes were initially screened gynaeco-clinically for their reproductive status as cyclic, anoestrus and detailed history and rectal palpation findings were recorded. Anoestrus buffaloes were confirmed by rectal palpation of small smooth inactive ovaries twice 10 days apart. 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 supplementing to their animals @ one bolus daily 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 (Ghuman et al., 2009; Naikoo et al., 2010; Savalia et al., 2014). 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. Buffaloes detected in oestrus subsequent to FTAI were reinseminated up to 3 cycles and in nor-return cases pregnancy was confirmed per rectum 60 days of last AI. All the hormonally treated/untreated true anoestrus and normal cyclic buffaloes were studied for their reproductive status and plasma progesterone profile. For this, jugular blood samples were collected in heparinized vacutainers four times from true anoestrus animals, i.e., on day 0 -just before treatment (on diagnosis), on day 7 -at the time of PGF 2 ? administration, on day 9 -induced oestrus/FTAI (FTAI done twice 24 hrs apart, i.e. on day 9 and 10 after initiation of treatment) and on day 21 post-AI. Blood sampling for two control groups of animals was done on the day of spontaneous oestrus if any, and on day 21 post-AI. The 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 progesterone profile was estimated by using standard Radio-Immuno-Assay (RIA) technique of Kubasic et al. (1984). Labelled antigen (I 125), antibody coated tubes and standards were procured from Immunotech, France. The sensitivity of assay was 0.1ng/ml. The intra-and inter-assay coefficients of variation were 5.4 and 9.1 per cent, respectively.

The data on oestrus response, conception rate (by Chi square test) and plasma profile of progesterone (ANOVA) were analyzed statistically (Snedecor and Cochran, 1994) using online SAS software version 20.00.

4 III.

5 Results and Discussion

6 a) Estrus Induction and Conception Rates

The oestrus induction response and conception rates at induced oestrus and overall of 3 cycles in animals under different hormonal treatment protocols are presented in Table 1. The cent 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 ? injection. The occurrence of prominent oestrus signs was observed in 66.67, 60.00, and 73.33 per cent of buffaloes in three groups, respectively, and it was statistically similar to the normal cyclic control group. 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 CRs at second cycle were 25.00, 28.57 and 30.00 per cent and at third cycle, 16.67, 20.00 and 14.28 per cent. The overall conception (pregnancy) rates of all three cycles were observed to be 66.67, 73.33 and 60.00 per cent, respectively in CIDR, Ovsynch and Crestar protocols. These pregnancy rates were achieved with the mean time intervals from PGF 2 ? injection of 11.40±4.65, 12.70±5.13 and 10.88±3.84 days among treated conceived buffaloes in three groups, respectively. 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 giving overall pregnancy rate of only 10.00 (1/10) per cent. In normal Cyclic Control group (n=10), the conception rates at first, second, third cycle and overall of 3 cycles were 40.00, 33.33, 25.00 and 70.00 per cent, respectively, and the service period was of 105.67±7.44 days among conceived ones.

The mean oestrus induction intervals observed in buffaloes under CIDR and Ovsynch protocols under study compared favourably with the previous reports of Savalia et The true anoestrus buffaloes thus could be induced to estrus within 2-3 days from the day of PG injection in each protocol and made pregnant within a period of 10-12 days in comparison to 125 days recorded in untreated control group, indicating a huge curtailment in the waiting period of 113 days for anoestrus animals to evince estrus and become pregnant, by putting then under such oestrus induction and synchronization protocols. The pooled conception rates obtained (66.67%) in the anoestrus buffaloes, irrespective of protocols used, indicated the positive contributory role of handling the problem of acyclicity in buffaloes for their induction of oestrus and making them pregnant to the levels, which is nearly at par with normal cyclic control buffaloes (70.00%). Based on the comparative conception rates obtained at induced/first oestrus, it can be surmised that Ovsynch and CIDR protocols could induce equally good fertile oestrus in anoestrus buffaloes. On the other hand, the frequency of induced fertile estrus was considerably low in Crestar protocol. The similar trend was also seen in overall pooled conception rates among the three protocols tested (Table 1). Thus, the buffaloes waiting for spontaneous cyclicity beyond 100 days postpartum can be the most appropriate candidates to be subjected to any of the above oestrus induction and synchronization protocols, and CIDR or Ovsynch in particular, for saving their valuable days of reproductive life span at field level, and making them early pregnant and productive.

7 b) Plasma Progesterone Profile

The mean levels of plasma progesterone 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 control groups are presented in Table **??**. The data show that the mean plasma progesterone (ng/ml) concentrations were low towards basal values on day 0, i.e., on the day of initiation of treatment in all three groups, suggesting that the animals were in anoestrus phase. These levels subsequently rose significantly ($P<0.01$) to the peak values on day 7 (4.97±1.68, 3.75±0.47 and 1.28±0.15 ng/ml), particularly in animals under CIDR and Ovsynch protocols. i.e. just before implants were removed and PG was injected. Thereafter the mean progesterone levels dropped suddenly and significantly within 48 hrs of PG injection and/or implant removal to the basal values coincident to induced oestrus, when FTAIs were done. These levels again increased significantly ($P<0.01$) on day 21 post-AI in all the groups (3.47±1.89, 4.06±0.47 and 2.44±0.44 ng/ml) due to oestruses being ovulatory with development and maintenance of CL and establishment of pregnancy in some animals. In normal cyclic control group also the mean plasma progesterone concentration was the lowest (0.43±0.13 ng/ml) on the day of spontaneous oestrus/AI, which rose significantly ($P<0.05$) on day 21 post-AI (2.26±0.56 ng/ml) again due to establishment of pregnancy in four buffaloes in that cycle.

The mean plasma progesterone levels obtained on the day of initiation of CIDR and Ovsynch treatments in the present study corroborated with the earlier findings of Savalia et al. (2014) to be 0.55 ± 0.21 and 0.56 ± 0.23 ng/ml, respectively, in anoestrus buffaloes, however the levels varied from other reports of Ammu et al. (2012 b) to be 0.81 ± 0.38 and 2.92 ± 1.19 ng/ml in Gir cows, and Patel et al. (**??**013) to be 0.65± 0.23 and 0.28±0.06 ng/ml in crossbred cows, with the same protocol. Significant rise observed in plasma P 4 profile on the day 7 of treatments in the present study with CIDR, Ovsynch protocols (4.97±1.68 and 3.75±0.47 ng/ml) over initial (0 day) values, with sudden drop to almost basal values on induced oestrus within 48-60 hrs after PG injection, has also been reported in anoestrus buffaloes by **??**atel (2012) by employing CIDR and Ovsynch protocol. It was, however, difficult to find any comparable report on progesterone profile following use of Crestar implant to support or defit the present observations. The apparently higher mean levels of progesterone found on day 21 post-AI in non-conceived buffaloes covered under CIDR, Ovsynch and Crestar protocols (2.66±1.80, 2.96±0.82 and 2.05±0.61 ng/ml, respectively) are suggestive of possibility of either prolonged cycles due to extended luteal phase/delayed luteal regression and/or delayed embryonic death.

Significantly higher mean plasma progesterone level (4.97±1.62 ng/ml) recorded on day 7 in CIDR group might be due to the continuous release of the exogenous progesterone from the progesterone molded silastic coil inserted in the anterior vagina of the buffaloes. In the Ovsynch protocol the rise in mean progesterone level (3.75±0.47 ng/ml) noted on day 7 might be due to luteinization of some of the growing follicles and/or ovulation of dominant follicle and formation of CL under the influence of GnRH, simulating diestrum phase, while in the Crestar protocol the mean progesterone level (1.28±0.15 ng/ml) did not rise much, probably due to presence of synthetic progestagen in that which is not detected by 17?-hydroxyprogesterone RIA, and the behavioural oestrus signs observed might also be attributed to i/m injection of oestradiol valerate simultaneous to norgestomet implant.

Further, the mean plasma progesterone concentrations in conceived and non-conceived groups in all three treatment protocols and in normal cyclic control group were found to be statistically similar on day 0, 7 and even on day 9 (AI), but on day 21 post-AI, the conceived buffaloes had non-significantly higher mean plasma progesterone concentrations as compared to non-conceived ones in all the three groups, but differed significantly only in normal cyclic Control group (3.86±0.47 vs 1.18±0.52 ng/ml, $P<0.05$) (Table **??**). These findings on plasma progesterone profile with respect to effect of CIDR and Ovsynch protocols and/or in normal cyclic group closely The levels of plasma P 4 on the day of beginning of treatment protocol helped delineate the reproductive and endocrine status of the animals and thereby predicting the possible response to the therapy. The higher plasma P 4 recorded on day 21 post-AI in conceived buffaloes of all the treatment groups and even in normal cyclic control group was due to establishment of pregnancy and maintenance of CL function, while significantly low yet variable plasma P 4 noted on day 21 post-AI in non-conceived buffaloes could be due to their return to next oestrus at varying intervals on account of probable irregular or long cycle length, early embryonic mortality after day 17 or uncoordinated, unexplained hormonal changes in some of them.

Thus, it can be inferred that the hormonal protocols used, particularly Ovsynch & CIDR protocol, improved conception rates in anoestrus buffaloes under field condition, and also influenced the plasma progesterone profile significantly in a manner of normal cyclic animals, hence can be used by the practicing veterinarians in anoestrus field buffaloes to improve their reproductive efficiency and thereby the farmers economy.

8 IV.

9 Pregnancy Status

No.

Days [1]

Figure 1:

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(2013) as 66.00±3.22 and 86.67±3.33 hrs in anoestrous crossbred cows, respectively, using the same protocols. Kundalkar et al. (2014) however, reported these intervals to be much shorter as 44.00±2.92 and 44.99±2.50 hrs, while Azawi et al. (2012) reported comparatively longer oestrus induction intervals with Ovsynch as 122.8±6.3 hrs. The mean oestrus induction interval found in buffaloes under Crestar ear implant protocol compared favourably with the previous reports of Utage et al. (2010) as 42.38±11.09 hrs and Dodamani et al. (2011) as 2.47±0.73 days, but Nath et al. (2004) observed it as 30.81±1.43 hrs only in anoestrus animals.

Figure 2:

1

Treatment Groups	N o.	Oestrus Induction Response (%)	PG Inj. to Oestrus Interval (hrs)	Induced/ First Oestrus	Conception Rate (%) Second Cycle Third Cycle		Ove of Cyc
CIDR Protocol	15	100.00 (n=15)	65.00±1.55 (n=15)	46.67 (7/15)	25.00 (2/8)	16.67 (1/6)	66.6 (10/
Ovsynch Protocol	15	100.00 (n=15)	69.46±1.04 (n=15)	53.33 (8/15)	28.57 (2/7)	20.00 (1/5)	73.3 (11/
Crestar Protocol	15	100.00 (n=15)	46.00±1.37 (n=15)	33.33 (5/15)	30.00 (3/10)	14.28 (1/7)	60.0 (9/1
Pooled	45	100.00 (n=45)	60.15±7.19 (n=45)	44.44 (20/45)	28.00 (7/25)	16.67 (3/18)	66.6 (30/
Untreated Anoestrus Control	10	20.00 (n=2)	–	50.00 (1/2)	–	–	10.0 (1/1
Normal Cyclic Control	10	100.00 (n=10)	–	40.00 (4/10)	33.33 (2/6)	25.00 (1/4)	70.0 (7/1

*[Note: Figures in parenthesis indicatenumber of animals/observations, * Service period/days open]*

Figure 3: Table 1 :

9 PREGNANCY STATUS

			D-0	D-7	D-9 (AI)	D-21 post-AI	
	Conceived	7	1.10±0.51	4.87±1.58	0.53±0.33	4.41±1.62	
CIDR	Non-conceived	8	1.16±0.82	5.05±1.87	0.89±0.86	2.66±1.80	
	Overall	15	1.13±0.66 a	4.97±1.68 b	0.73±0.67 a	3.47±1.89 b	
	Conceived	8	0.94±0.18	4.01±0.74	0.47±0.11	4.14±0.57	
Ovsynch	Non-	7	1.26±0.25	3.46±0.58	0.70±0.17	2.96±0.82	Year
Crestar	conceived	15	1.09±0.15 a	3.75±0.47	0.58±0.10	4.06±0.47	2014
	Overall	5	1.77±1.08	b	a 0.56±0.07	b 3.21±0.3	
	Conceived	10	0.81±0.09	0.88±0.18	0.70±0.16	2.05±0.61	
	Non-conceived			1.49±0.18			
	Overall	15	1.12±0.36 a	1.28±0.15 b	0.66±0.10 a	2.44±0.44 b	
Untreated	Conceived	1	0.63±0.00	–	0.34±0.00	4.17±0.00	
Anoestrus	Non-	9	1.37±0.49	———-	—-0.16±0.03	—-3.86±0.47	Volume
Control	conceived	10	1.07±0.41		0.61±0.18	x 1.18±0.52 y	XIV
	Overall	4	——		0.43±0.13 a	2.26±0.56 b	Issue
Normal	Conceived	6					III
Cyclic	Non-	10					Version
Control	conceived						I
	Overall						D D D D) G (

Figure 4:

.1 Acknowledgements

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