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

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Keywords: buffalo, anoestrus, treatment protocols, oestrus induction, conception rate, fertile oestrus induction interval.

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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 %) 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 $PGF_2\alpha$ 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. These were achieved with the mean intervals from $PGF_2\alpha$ 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 (n=10), only 2 buffaloes exhibited spontaneous oestrus within 90 days of follow up and one conceived giving first service and overall pregnancy rates of 50.00 and 10.00 per cent, respectively. In normal cyclic control (n=10), the conception rates at first and overall of 3 cycles were 40.00 and 70.00 per cent, respectively, and the service period was of 105.67 ± 7.44 days among conceived ones. The overall mean plasma progesterone concentrations in true anoestrus buffaloes on day 0, 7, 9 (AI) of treatment and on day 21 post-AI were 1.13 ± 0.66, 4.97 ± 1.68, 0.73 ± 0.67 and 3.47 ± 1.89 ng/ml, respectively, in CIDR protocol; 1.09 ± 0.15, 3.75 ± 0.47, 0.58 ± 0.10 and 4.06 ± 0.47 ng/ml in Ovsynch and 1.12 ± 0.36, 1.28 ± 0.15, 0.66 ± 0.10 and 2.44 ± 0.44 ng/ml in Crestar protocol, respectively. The values on day 7 and 21 were significantly (P<0.01) higher than other two periods in all three groups. The mean plasma progesterone concentrations were non-significantly (P>0.05) higher in conceived than non-conceived group on day 21 post-AI in CIDR (4.41 ± 1.62 vs. 2.66 ± 1.80 ng/ml), Ovsynch (4.14 ± 0.57 and 2.96 ± 0.82 ng/ml) and Crestar (3.21 ± 0.30 vs. 2.05 ± 0.61 ng/ml), but no differences were observed on day 0, 7 or 9 of treatment in any group. The high plasma progesterone in non-pregnant animals on day 21 post-AI was attributed to early embryonic mortality and irregular short or long cycle in them. However in normal Cyclic Control group, the mean plasma P_4 value on day 21 post-AI was significantly (P<0.05) higher in conceived buffaloes than in non-conceived ones (3.86 ± 0.47 vs. 1.18 ± 0.52 ng/ml). Thus, Ovsynch and/or CIDR protocols can be conveniently used to improve fertility in anoestrus rural buffaloes by the practicing veterinarians with results similar or even better than in normal cyclic buffaloes.

Keywords: buffalo, anoestrus, treatment protocols, oestrus induction, conception rate, fertile oestrus induction interval.

I. Introduction

The 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.

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II. 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 re-inseminated 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 $PGF_2\alpha$ 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.

III. Results and Discussion

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 $PGF_2\alpha$ 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 $PGF_2\alpha$ 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 al.* (2014) as 63.60 ± 6.46 and 70.67±6.15 hrs and Dhami *et al.* (2014) as 66.00±4.23 and 87.27±3.53 hrs in anoestrus buffaloes, and Patel *et al.* (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.

The first service conception rates of the present study are comparable with the earlier results of Bhoraniya *et al.* (2012) as 66.66 and 33.33 per cent in CIDR and Ovsynch protocols in anoestrus Kankrej cows, and Kundalkar *et al.* (2014) as 42.50 and 50.00 per cent with same two protocols in buffaloes, although comparatively lower first service conception rates of 40.00 and 30.00 per cent (Savalia *et al.,* 2014), and 36.84 and 29.41 per cent (Dhami *et al.,* 2014), respectively, are documented with CIDR and Ovsynch protocols in recent reports also. Further, Malik *et al.* (2010. 2011) recorded overall conception rates of 85.70, 75.00 and 86.67 per cent in CIDR, Ovsynch and Crestar group, respectively, in anoestrus buffaloes, which are very close to the present finding with Ovsynch protocol (73.33 %), but far higher than in Crestar group (60.00 %). Ozyurtlu *et al.* (2009) reported overall conception rates of 44.00 and 53.85 per cent in Norgestomet and PRID groups, respectively, which are relatively lower than the present findings with Crestar and CIDR.

The overall conception rates in Ovsynch and CIDR protocol documented by Barucelli *et al.* (2003) as 52.50 and 28.20 per cent, Naikoo *et al.* (2010) as 50.00 and 50.00 per cent and Ali *et al.* (2012) as 60.00 and 33.33 per cent, respectively, are also lower than the present findings. Further, around 30 per cent conception obtained at second cycle in anoestrus buffaloes induced to cycle and even in normal cycling buffaloes, proved that all the oestrus induction and synchronization protocols induced oestrus and then established normal cyclicity in treated animals, resulting into conceptions in subsequent cycles like normal cycling/breeding buffaloes. These observations further supported the previous observations on use of similar protocols in anoestrus cows and buffaloes by many workers (Naikoo *et al.*, 2010; Bhoraniya *et al.*, 2012; Ammu *et al.,* 2012[a]; Chaudhari *et al.* 2012; Patel *et al.,* 2013; Savalia *et al.*, 2013, 2014; Dhami *et al.,* 2014).

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).

Table 1 : Effects of CIDR, Ovsynch and Crestar protocols on oestrus induction response, PG injection to induced oestrus and fertile oestrus intervals, and conception rates in anoestrus buffaloes

Treatment Groups	No.	Oestrus Induction Response (%)	PG Inj. to Oestrus Interval (hrs)	Conception Rate (%)				PG Inj. to Fertile Oestrus Interval (days) among conceived ones
				Induced/ First Oestrus	Second Cycle	Third Cycle	Overall of 3 Cycles	
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)	66.67 (10/15)	11.40±4.65 (n=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)	73.33 (11/15)	12.70±5.13 (n=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)	60.00 (9/15)	10.88±3.84 (n=9)
Pooled	45	100.00 (n=45)	60.15±7.19 (n=45)	44.44 (20/45)	28.00 (7/25)	16.67 (3/18)	66.67 (30/45)	11.66±0.54 (n=30)
Untreated Anoestrus Control	10	20.00 (n=2)	--	50.00 (1/2)	--	--	10.00 (1/10)	157.00* (n=1)
Normal Cyclic Control	10	100.00 (n=10)	--	40.00 (4/10)	33.33 (2/6)	25.00 (1/4)	70.00 (7/10)	105.67±7.44*

*Figures in parenthesis indicate number of animals/observations, * Service period/days open*

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.

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 2. 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.* (2013) 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 Patel *et al.* (2013)) and Savalia *et al.* (2013) and in cows by Ammu *et al.* (2012[b]) and Bhoraniya *et al.* (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 17α-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 2). These findings on plasma progesterone profile with respect to effect of CIDR and Ovsynch protocols and/or in normal cyclic group closely corroborated with the observations of Raghorte *et al.* (2009), Naikoo *et al.* (2010) and Savalia *et al.* (2013, 2014) in anoestrus buffaloes, and of Bhoraniya *et al.* (2012) and Patel *et al.* (2013) in anoestrus cows under such protocols.

Table 2 : Plasma progesterone concentrations (ng/ml) in anoestrus and cyclic buffaloes on different days of treatment/AI under various oestrus induction protocols

Treatment Groups	Pregnancy Status	No.	Days from initiation of treatment/AI			
			D-0	D-7	D-9 (AI)	D-21 post-AI
CIDR	Conceived	7	1.10±0.51	4.87±1.58	0.53±0.33	4.41±1.62
	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}**
Ovsynch	Conceived	8	0.94±0.18	4.01±0.74	0.47±0.11	4.14±0.57
	Non-conceived	7	1.26±0.25	3.46±0.58	0.70±0.17	2.96±0.82
	Overall	**15**	**1.09±0.15^{a}**	**3.75±0.47^{b}**	**0.58±0.10^{a}**	**4.06±0.47^{b}**
Crestar	Conceived	5	1.77±1.08	0.88±0.18	0.56±0.07	3.21±0.3
	Non-conceived	10	0.81±0.09	1.49±0.18	0.70±0.16	2.05±0.61
	Overall	15	**1.12±0.36^{a}**	**1.28±0.15^{b}**	**0.66±0.10^{a}**	**2.44±0.44^{b}**
Untreated Anoestrus Control	Conceived	1	0.63±0.00	--	0.34±0.00	4.17±0.00
	Non-conceived	9	1.37±0.49	--	--	--
	Overall	10	**1.07±0.41**	--	--	--
Normal Cyclic Control	Conceived	4	--	--	0.16±0.03	3.86±0.47^{x}
	Non-conceived	6	--	--	0.61±0.18	1.18±0.52^{y}
	Overall	10	--	--	**0.43±0.13^{a}**	**2.26±0.56^{b}**

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

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.

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References Références Referencias

1. Ali, R., Shukla, S.P. and Nema, S.P. (2012). Hormonal induction of ovarian cyclicity and conception rate in postpartum anoestrus buffaloes. *Indian J. Field Vets,* **7**(4): 44-46.
2. Ammu Ramakrishnan, Dhami, A.J., Ankita Killedar and Pande, A.M. (2012[b]). Postpartum plasma progesterone and metabolic profile in pregnant and non-pregnant Gir cows following estrus synchronization. *Indian J. Anim. Prod. & Mgmt.,* **24**(1-2):40-44.
3. Ammu Ramakrishnan, Dhami, A.J., Naikoo, M., Parmar, B.C. and Divekar, B.S. (2012[a]). Estrus induction and fertility response in postpartum anoestrus Gir cows. *Indian J. Anim. Reprod.,* 33(1): 37-42.
4. Azawi, O.I., Ali, M.D., Oday, S.A., Al-Hadad, A.S., Mouayad, S.J. and Hussien, A.S.A. (2012). Treatment of anoestrus in Iraqi buffaloes using Ovsynch alone or in combination with CIDR. *J. Adv. Vet. Res.,* **2**(1): 68-72.
5. Baruselli, P.S., Carvalho, N.A.T., Henrique, C.E.P., Amaral, R., Nichi, M. and Reichert, R.H. (2003). Use of progesterone associated with Ovsynch protocol for timed artificial insemination in buffalo. *In: Proc. congresso nazionale sull allevamento del buffalo;* p. 265–268.

6. Bhoraniya, H.L., Dhami, A.J., Naikoo, M., Parmar, B.C. and Sarvaiya, N.P. (2012). Effect of oestrus synchronization protocols on plasma progesterone profile and fertility in postpartum anoestrus Kankrej cows. *Trop. Anim. Health Prod.,* **44**(6): 1191-1197.
7. Chaudhari, C.F., Suthar, B.N., Sharma, V.K., Dabas, V.S., Chaudhari, N.F. and Panchasara, H.H. (2012). Estrus induction and fertility response in delayed pubertal Kankrej heifers treated with norgestomet ear implant. *Vet. World*, **5**(8):453-458.
8. Dhami, A.J., Panchal, M.T., Hadiya, K.K., Patel, J.A. and Shah, R.G. (2014). Use of controlled breeding techniques under field conditions for estrus synchronization and conception in anoestrus crossbred cows and buffaloes. *Proc. 2nd Annual Meeting of SVSBT and National Seminar on Biotechnological approaches to challenges in animal health & production,* Vet. College, DUVASU, Mathura (UP), India, March 6-7, p. 86.
9. Dodamani, M.S., Tandle, M.K., Mohteshamuddin, K. and Honnappagol, S.S. (2011). Induction of fertile estrus in true anoestrus she buffaloes by re-utilization of Crestar ear implants. *Vet. World,* **4** (1): 28-30.
10. Ghuman, S.P.S., Singh, J., Honparke, M. and Dadarwal, D. (2009). Induction of ovulatory estrus using Ovsynch protocol and subsequent fertility in true anestrous buffalo heifers. *Indian J. Anim. Reprod.,* **30**(2): 1-5.
11. Kubasic, N.P., Hallauer, G.D. and Brodows, R.G. (1984). Evaluation of direct solid-phase RIA for progesterone, useful for monitoring luteal function. *Clin. Chem.*, **30**(2): 284-286.
12. Kundalkar, A.D., Ingwale, M.V., Pawshe, M.D., Taloker, S.S., Pawshe, C.H. and Deshmukh, S.G. (2014). Efficacy of Ovsynch and CIDR estrus synchronization protocols in anoestrus buffaloes. *Proc. XXIX Annual Convention of ISSAR and National Symposium held at Nagpur, India,* 8-10 Jan., p. 142.
13. Malik, R.K., Singh, P., Sharma, R.K., Singh, I. and Tuli, R.K. (2010). Estrus and fertility response of postpartum anestrus Murrah buffaloes to Crestar and Ovsynch treatment regimens. *Indian J. Anim. Sci.,* **80**(10): 982-985.
14. Malik, R.K., Singh, P., Sharma, R.K., Singh, I., Phulia, S.K. and Tuli, R.K. (2011). Efficacy of norgestomet ear implant for estrus induction on postpartum anestrus Murrah buffaloes. *Indian J. Anim. Sci.,* **81**(7):687-690.
15. Naikoo, M., Patel, D.M., Sarvaiya, N.P. and Killedar, A. (2010). Estrous synchronization in postpartum anestrous Mehsana buffaloes using different hormone protocols. *Indian J. Field Vets. ,* **6**(2): 1-4.
16. Nath, H.C., Dutta, D.J. and Biswas, R.K. (2004). Reproductive performance of Crestar and PMSG administered to postpartum anoestrus cow. *Indian J. Anim. Res.,* **38**(1): 50-52.
17. Ozyurtlu, N., Yunus, C., Ibrahim, K. and Mesih, K. (2009). Induction of oestrus with norgestomet ear implant and PRID in acyclic Holstein Heifers. *J. Anim. Vet. Advan.,* **8**(5): 1035-1039.
18. Parmar, B.N. (2013). *Augmenting reproductive efficiency of infertile buffaloes using controlled breeding techniques in tribal areas*. M.V.Sc. Thesis, submitted to Anand Agricultural University, Anand, Gujarat, India.
19. Patel, K.R., Dhami, A.J., Hadiya, K.K., Savalia and Sarvaiya, N.P. (2013). Effect of CIDR and Ovsynch protocols on estrus response, fertility and plasma progesterone and biochemical profile in true anoestrus crossbred cows. *Indian J. Anim. Prod. Mgmt.,* **29**(3-4): 50-58.
20. Raghorte, Y.M., Chinchkar, S.R., Sahatpure, S.K., Gawande, A.P., Gaikwad, S.M., Dhakate, M.S., Raut, J.D., Gote, S.G. and Verma, T. (2009). Progesterone concentration during Ovsynch protocol in buffalo heifers and postpartum buffaloes. *Proc. XXV Annual Convention of ISSAR and National Symposium held at Namakkal, Tamilnadu, India,* 10-12 Dec., p. 43.
21. Savalia, K.K., Dhami, A.J., Hadiya, K.K. and Patel, K.R. (2014). Augmenting fertility of anoestrus and repeat breeding buffaloes using controlled breeding techniques under field conditions" *Indian Vet. J.,* 89(8): 23-27.
22. Savalia, K.K., Dhami, A.J., Patel, K.R. and Hadiya, K.K. (2013). Influence of controlled breeding techniques on fertility and plasma macro-minerals profile in conceived and non-conceived anestrus and repeat breeding buffaloes. *Indian J. Field Vet.,* **9**(2):28-35.
23. Snedecor, G.W. and Cochran, W.G. (1994). *Statistical Methods*. 14th edn. Oxford and IBH Publishing House, New Delhi, India.
24. Utage, S.G., Raghuvanshi, D.S., Vhora, S.C., Khan, L.A. and Sahatpure, S.K. (2010). Efficacy of Crestar, $PGF_2\alpha$ and GnRH combination in treatment of post-partum anestrous buffaloes. *Indian J. Anim. Reprod.* ,**31**(1): 28-29.