Original Research

Relative Efficacy of Various Estrus Synchronization Protocols for Improving Fertility in Cyclic and Acyclic Buffaloes

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Abstract

This study was undertaken in field conditions on 50 cyclic repeat breeder and 50 acyclic buffaloes to evaluate the efficacy of four standard estrus induction/ synchronization protocols, viz., Doublesynch (PG-GPG), Estradoublesynch (PG-GPE), Ovsynch (GPG), and Ovsynch Plus (eCG-GPG) (10 buffaloes in each protocol, and in controls) in terms of estrus induction response and conception rates at induced estrus with FTAI and overall of 3 cycles post-treatment. All repeat breeder and 90-100 % acyclic buffaloes under four protocols exhibited induced estrus within mean intervals of 69.80±3.44, 62.10±2.84, 61.45±1.34 and 64.00±1.25 h, and within 64.77±2.19, 62.50±1.39, 69.30±1.80 and 70.60±1.30 h, respectively, from $PGF_2\alpha$ injection. The conception rates obtained in cyclic/repeat breeder buffaloes at induced estrus (FTAI) and overall of three cycles post-treatment were 40 and 50 % with Doublesynch; 50 and 70 % with Estradoublesynch; 60 and 70 % with Ovsynch, and 60 and 60 % with Ovsynch Plus protocol, respectively. The corresponding conception rates in acyclic buffaloes were 50 and 60 %; 40 and 60 %; 30 and 80 %, and 50 and 70 %. Among 10 repeat breeder buffaloes each, 4, 3, 2 and 3 non-conceived buffaloes remained cyclic, while 1, 0, 1 and 1 became anestrus by 60 days post-induction/FTAI following use of Doublesynch, Estradoublesynch, Ovsynch and Ovsynch Plus protocols, respectively, whereas among 10 acyclic buffaloes each, 2, 2, 2 and 1 non-conceived buffaloes remained cyclic, while 2, 2, 0 and 2 again turned out to be anestrus by 60 days follow up. Among the untreated control cyclic and acyclic buffaloes (10 each), 3 and 2 buffaloes conceived over a period of 90 days follow up giving overall conception rates of only 30 and 20 %, respectively, as against 62.5 and 67.5 % in treated cyclic and acyclic buffaloes. It was thus concluded that all four protocols improved conception rates in repeat breeding and acyclic buffaloes by establishing regular cyclicity post-treatment. The maximal benefit was with Ovsynch and/or Estradoublesynch application in repeat breeders and Ovsynch and/or Ovsynch Plus in anestrus buffaloes.

Key words: Acyclic, Buffalo, Conception Rate, Estrus Synchronization, Plasma Progesterone, Repeat Breeder

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Introduction

The low reproductive efficiency in buffaloes is attributed to delayed puberty, summer anestrus and long postpartum anestrus or repeat breeding (Baruselli *et al.,* 2012). Effective treatment and management of anoestrus and repeat breeding buffaloes are the efficient means of improving their production and reproduction efficiencies. Both acyclicity/anetrus and cyclic repeat breeding conditions are responsible for prolonged inter-calving interval resulting in great economic loss to buffalo breeders and for the dairy industry (Ei-Wishy, 2007, Savalia *et al.,* 2014). To improve reproductive efficiency of animals, several protocols of estrus induction and ovulation synchronization have been developed (Ghuman *et al.*, 2009, Baruselli *et al.,* 2012, Savani *et al.,* 2017). 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 different hormonal protocols during breeding season can be helpful in inducing and synchronizing estrus and getting better conception rate with lesser number of services per conception and making acyclic buffaloes to cycle normally, thereby achieving ideal inter-calving interval (Kumar *et al.,* 2016[b]). Hence, this study was planned to evaluate the comparative efficacy of less tested Doublesynch, Estradoublesynch, Ovsynch and Ovsynch Plus protocols (Virmani *et al.,* 2013, Mirmahmoudi *et al.,* 2014, Mungad *et al.,* 2016) in anoestrus and repeat breeding buffaloes for reducing their unproductive dry period and enhancing fertility and productivity under field conditions.

Material and Methods

This investigation was carried out under field conditions on 50 buffaloes each with cyclic and acyclic condition in milk shed areas of Amul, Anand and Mahisagar districts of Gujarat during August 2016 to February 2017. Anestrus animals were confirmed by palpating small smooth inactive ovaries per rectum twice 10 days apart. Repeat breeders were confirmed on the basis of regular cyclicity and AI for more than 3 times without visible or palpable genital abnormality, yet failed to conceive.

All these infertile animals were initially injected once with 100 mg Ivermectin s/c, and injection organic phosphorus (Inj. Tonophosphan, MSD Animal Health) and multivitamins AD_3E (Inj. Intavita-H, Intas Pharma) 10 ml each, and were supplied with bolus Minotas (Intas Pharma) @ 1 bolus PO for 7 days. Repeat-breeder buffaloes received additional single shot i/m injection of Enrofloxacin (Inj. Flobac SA, 40 ml, Intas Pharma). They were randomly divided into 5 equal groups (n=10 each) and were subjected to following four estrus induction/synchronization protocols, keeping one group each as untreated control. Under Doublesynch protocol, 10 cyclic and acyclic buffaloes each were administered with i/m Inj. of 500 µg $PGF_2\alpha$ analogue, i.e. Cloprostenol sodium (Estrumate, 2 ml, MSD) on day 0, Inj. of 20 µg GnRH

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analogue, i.e. Buserelin acetate (Receptal, 5 ml, MSD) on day 2, 500 µg $PGF_2\alpha$ analogue on day 9 and second Inj. 10 µg GnRH on day 11, followed by fix timed AI (FTAI) twice at 16 and 24 hrs later. In Estradoublesynch protocol, injection of estradiol benzoate 1 mg (Sigma, USA) on day 10 replaced the second GnRH on day 11 of Doublesynch protocol, and animals were inseminated twice at 48 and 60 hrs post-estradiol injection.

Under Ovsynch protocol, 10 cyclic and acyclic buffaloes each were administered with i/m Inj. of 20 µg GnRH analogue (Receptal, 5 ml) on day 0, Inj. of 500 µg $PGF_2\alpha$ on day 7, and second Inj. of 10 µg GnRH on day 9, followed by FTAI twice at 0 and 24 hrs later, while in Ovsynch Plus protocol, an additional i/m Inj. of PMSG 500 IU (Folligon, MSD) was given 2 days before the actual Ovsynch protocol. Ten cyclic repeat breeder and 10 acyclic buffaloes each given pre-synchronization treatment as above, but without any hormonal intervention, and followed for spontaneous estrus and insemination, served as untreated controls. Apart from FTAI, visual heat detection for half an hour both in the morning and evening and frozen semen inseminations were practiced as per the protocols throughout the study period. Animals inseminated at induced/spontaneous estrus, if not settled, were followed for 2 more cycles and in non-return cases pregnancy was confirmed per-rectum 60 days post-AI. The observations on estrus responses and conception rates were recorded and compared statistically between different groups by using Chi-square test (Snedecor and Cochran, 1986).

Results and Discussion

The results obtained in cyclic/repeat breeder and acyclic/anestrus buffaloes under different synchronization protocols and in control groups are presented in Table 1.

Estrus Induction Response and Induction Interval in Cyclic/Acyclic Buffaloes

Out of 10 repeat breeder buffaloes each subjected to Doublesynch, Estradoublesynch, Ovsynch and Ovsynch Plus protocols, 90, 100, 100 and 100 % buffaloes, respectively, exhibited behavioural estrus with prominent to moderate signs within mean intervals of 69.80±3.44, 62.10±2.84, 61.45±1.34 and 64.00±1.25 h from $PGF_2\alpha$ injection. All 10 untreated cyclic control buffaloes continued to exhibit spontaneous estrus, unless conceived. The corresponding estrus induction response in anestrus buffaloes were 90, 90, 100 and 90 % within mean intervals of 64.77±2.19, 62.50±1.39, 69.30±1.80 and 70.60±1.30 h (Table 1). In anestrus control group only two buffaloes (20 %) exhibited estrus after 28 and 52 days of initiation of experiment. The estrus induction rate of 90 % achieved with doublesynch protocol in both cyclic and acyclic buffaloes was in harmony with the earlier results of Miramahmoudi and Prakash (2012) and Miramahmoudi *et al.* (2014); however Parida *et al.* (2015) found 70 % response in anestrus buffaloes using same protocol. The estrus synchronization rate of 90-100 % achieved in cyclic and acyclic buffaloes

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with Estradoublesynch protocol also concurred with the earlier results of 83.3 to 100 % by Miramahmoudi *et al.* (2014) and Parida *et al.* (2015).

Table 1: Effect of different estrus synchronization protocols on estrus induction response, estrus induction intervals and conception rates in cyclic/repeat breeder and acyclic/anestrus buffaloes

Status	Treatment Protocol	No.	Estrus induction response (%)	PG Inj. To Estrus induction interval (hrs)	Conception rate (%)				Status of non-pregnant buffaloes at 60-days post-AI		Initiation of treatment to fertile estrus interval (days)
					Induced / First estrus	II cycle	III cycle	Overall of 3 cycles	Cyclic	An-estrus	
Cyclic	Doublesynch	10	90 (9/10)	69.80±3.44	40 (4/10)	33.33 (1/3)	0 (0/2)	50 (5/10)	4	1	13.80±1.80
	EDS	10	100 (10/10)	62.10±2.84	50 (5/10)	66.66 (2/3)	0 (0/2)	70 (7/10)	3	0	14.28±1.49
	Ovsynch	10	100 (10/10)	61.45±1.34	60 (6/10)	25 (1/4)	0 (0/3)	70 (7/10)	2	1	11.28±1.28
	Ovsynch plus	10	100 (10/10)	64.00±1.25	60 (6/10)	0 (0/2)	0 (0/2)	60 (6/10)	3	1	13.00±0.00
	Control	10	100 (10/10)	--	20 (2/10)	12.33 (1/8)	0 (0/7)	30 (3/10)	7	0	185.0±9.73*
Anestrus	Doublesynch	10	90 (9/10)	64.77±2.19	50 (5/10)	33.33 (1/3)	0 (0/2)	60 (6/10)	2	2	15.00±3.00
	EDS	10	90 (9/10)	62.50±1.39	40 (4/10)	50 (2/4)	0 (0/2)	60 (6/10)	2	2	15.60±3.60
	Ovsynch	10	100 (10/10)	69.30±1.80	30 (3/10)	50 (3/6)	66.66 (2/3)	80 (8/10)	2	0	24.00±5.77
	Ovsynch plus	10	90 (9/10)	70.60±1.30	50 (5/10)	25 (1/4)	50 (1/2)	70 (7/10)	1	2	19.71±4.34
	Control	10	20 (2/10)	--	50 (1/2)	100 (1/1)	0 (0/0)	20 (2/10)	0	8	155.0±17.8*

*Figures in parentheses indicate number of animals, *Mean service period or days open among conceived buffaloes.*

The estrus synchronization rate of 100 % achieved with Ovsynch protocol in both repeat breeder and anestrus buffaloes was in harmony with the earlier results of Navrange *et al* (2012), Nakrani *et al.* (2014) and Savani *et al.* (2017), while Ali and Fahmy (2007) observed it as 80 %, and Tiwari *et al.* (2005) found much lower response of 66.66 % in cyclic buffaloes. However, many other researchers documented estrus induction response with Ovsynch in the range of 80.00 to 87.50 % (Campenile *et al.*, 2005, Thorat *et al*., 2014, Buhecha *et al.*, 2016; Mungad *et al.*, 2016) in different breeds of buffaloes. The estrus induction rate of 90 % achieved with Ovsynch Plus protocol in anestrus buffaloes coincided well with the result of 93.7 to 100 % reported by Kumar *et al.* (2016[a]) in anestrus buffaloes and Virmani *et al.* (2013) in Sahiwal cows, but no report was available with Ovsynch Plus protocol to compare the present 100 % estrus response in repeat breeder/cyclic buffaloes.

The estrus induction intervals observed in different protocols, particularly Ovsynch, concurred well with many of the above reports. However, Kundalkar *et al.* (2014) reported much shorter estrus induction interval of 48.75±0.71 h in acyclic buffaloes under Ovsynch protocol. However, Parmar *et al.* (2015)

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reported estrus induction response between 72 and 96 h in Ovsynch protocol which is longer than the present finding. For other protocols used, no such time documentation was available in the literature.

Conception Rates with Different Estrus Protocols in Cyclic and Acyclic Buffaloes

The conception rates at induced estrus in cyclic buffaloes put under Doublesynch, Estradoublesynch, Ovsynch and Ovsynch plus protocols were 40, 50, 60 and 60%, respectively, with some 25.00 to 66.66 % more buffaloes conceived during 2nd and 3rd cycle post-treatment, thus giving overall three cycles' conception rates of 50, 70, 70 and 60 %, respectively. These results were obtained in mean periods of 13.80±1.80, 14.28±1.49, 11.28±1.28 and 13.00±0.00 days from the day of start of treatment in respective groups (Table 1). Moreover, among the 15 of 40 treated non-conceived buffaloes, 12 buffaloes remained cyclic, while remaining 03 turned out to be anestrus by 60 days of post-estrus induction/FTAI, with overall 62.50 % (25/40) success rate of fertility by the four treatment protocols. In untreated cyclic control group, all buffaloes continued to exhibit spontaneous estrus over 60-days follow up period, except two and one buffalo that conceived at first and second cycle, giving overall conception rate of 30 % only. The service period for 3 conceived animals of control group was 185.00 ±9.17 days, which was nearly 45 days longer than in buffaloes under various treatment protocols.

In acyclic/anestrus buffaloes covered under Doublesynch, Estradoublesynch, Ovsynch and Ovsynch Plus protocols, the conception rates at induced estrus were 50, 40, 30 and 50 %, respectively, with some 25.00 to 66.66 % more buffaloes conceived during 2nd and 3rd cycle post-treatment, thus giving overall three cycles' conception rates of 60, 60, 80 and 70 %, respectively. These results were obtained within mean period of 15.00±3.00, 15.60±3.60, 24.00±5.77 and 19.71±4.34 days from the day of start of treatment in respective groups (Table 1). Moreover, among the 13 of 40 treated non-conceived buffaloes, 7 remained cyclic, while 6 buffaloes again turned out to be anestrus by 60 days of induced estrus/FTAI, resulting into 67.50 % (27/40) overall success rate of fertility by four protocols in anestrus buffaloes. In untreated acyclic control group, only two buffaloes exhibited spontaneous estrus over a 90-days follow up period and one each conceived at first and second cycle giving overall conception rate of 20 % only. The service period for these 2 control buffaloes was 155.00 ±17.18 days, which was nearly 40-45 days longer than in buffaloes under various treatment protocols.

The present first service and overall 3 cycles' conception rates of 40 and 50 % obtained with Doublesynch protocol in repeat breeder buffaloes are far higher and lower than earlier report of 28.57 and 71.43 %, respectively by Abubaker *et al.* (2013) in lactating crossbred cows. Mirmahmoudi and Prakash (2012), Mirmahmoudi *et al.* (2014) and Parida *et al.* (2015) obtained conception rates of 55-62 % at FTAI in cyclic and/or acyclic buffaloes with this protocol, which are higher than our findings. The present conception rate of 50 % obtained in anestrus buffaloes at induced estrus with Doublesynch protocol

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concurred well with earlier report of Mirmahmoudi and Prakash (2012) as 55%. The present 60 % overall three cycles' conception rate with this protocol closely corroborated with the findings of Dhindsa *et al.* (2014) and Parida *et al.* (2015), as 58.00 and 57.74 %, respectively, in anestrus buffaloes following use of same protocol. However, the relatively lower conception rates than present one were reported by Kumar *et al.* (2016[b]) during summer and winter season (40.00 and 48.00 %) in anestrus buffaloes. The present conception rate of 50 % obtained at induced estrus with Estradoublesynch protocol was quite similar with earlier results of 60-62 % in cyclic buffaloes using same protocol by Mirmahmoudi *et al*. (2014). Further, the present 70 % overall three cycles' conception rate with this protocol was closer to the finding of 59.45 % by Sharavanan *et al.* (2016) in repeat breeder/cyclic crossbred cattle. However, the conception rate of 40 % obtained at induced estrus in anestrus buffaloes with Estradoublesynch protocol was quite lower than the earlier results of 60-64 % obtained by Mirmahmoudi *et al.* (2014) in anestrus buffaloes with same protocol. Further, the present 60 % overall three cycles' conception rate obtained with Estradoublesynch protocol closely corroborated with the finding of 62.50 % reported by Parida *et al.* (2015) in anestrus buffaloes.

The conception rate obtained at induced estrus in cyclic buffaloes under Ovsynch protocol (60 %) was quite higher than the results of 21.42 to 40.00 % obtained by Tiwari *et al.* (2005), Ravikumar *et al.* (2007) and Parmar *et al.* (2013) in repeat breeder/cyclic buffaloes. Further, the present 70 % overall three cycles' conception rate in repeat breeders under Ovsynch protocol is little better than the findings of 43.70 to 60.00 % reported by Tiwari *et al*. (2005), Ali and Fahamy (2007), Parmar *et al.* (2013) and Sharavanan *et al.* (2016) with this protocol in cyclic buffaloes and/or cows. However, relatively much lower overall conception rate of 33.33 % was reported by Karen *et al.* (2010), and higher overall conception rates of 83.33 % and 89.00 % by Ravikumar *et al.* (2007) and Dahham *et al.* (2014), respectively. The 30 % conception rate obtained at induced estrus with Ovsynch protocol in anestrus buffaloes was quite similar with earlier results of 29.41 to 37.50 % reported by Campenile *et al.* (2005), Ali and Fahamy (2007) and Buhecha *et al.* (2016) in anestrus buffaloes, but it is lower than the reported results of 41.67 to 66.66 % by others (Naikoo *et al.*, 2010, Kundalkar *et al.*, 2014, Nakrani *et al.*, 2014, Mungad *et al.*, 2016, Savani *et al.*, 2017) in anestrus buffaloes of different breeds. Further, Ghuman *et al.* (2009) and Tiwari *et al.* (2005) observed much lower first service conception rate (18.00 to 18.18%) in true anestrus buffalo-heifers with Ovsynch protocol. The present 80 % conception rate of overall three cycles' following Ovsynch protocol closely corroborated with the values of 71.43 to 85.71 % reported by some researchers (Thorat *et al.*, 2014, Nakrani *et al.*, 2014, Savani *et al.*, 2017) in anestrus buffaloes. However, relatively lower overall conception rates of 35.29 to 60.00 % were reported by Tiwari *et al.* (2005), Savalia *et al.* (2014), Buhecha *et al.* (2015) and Mungad *et al.* (2016) in anestrus buffaloes.

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The conception rates of 50 and 70 % obtained with Ovsynch plus protocol at induced estrus and overall 3 cycles' in anestrus buffaloes closely concurred with the earlier report of 53.30 and 75.00 % by Kumar *et al*. (2016[b]) in anestrus buffaloes. The present first service conception rate is however higher than the reported results of 20.00 and 28.00 % by Virmani *et al.* (2013) and Rathore *et al.* (2015) in Sahiwal cows and postpartum anestrus buffaloes, respectively. No report was available on use of Ovsynch Plus protocol in repeat breeding buffaloes to compare the present findings.

Conclusion

It was thus concluded both Doublesynch and Estradoublesynch protocols being recently developed for cyclic and acyclic dairy animals, very few studies were so far available to support or contradict the present findings in either category of buffaloes. However in our study, both appeared at par among anestrus buffaloes, while Estradoublesynch was found little better than Doublesynch among repeat breeders. The results with all four protocols in both cyclic and acyclic buffaloes were significantly better and economic to the farmers as they all improved conception rates in both the categories of animals by establishing regular cyclicity post-treatment when compared with simply maintaining the untreated control buffaloes. The maximal benefit was with Ovsynch and/or Estradoublesynch application in repeat breeders and Ovsynch and/or Ovsynch Plus in anestrus buffaloes, hence such protocols must be attempted by practicing Veterinarians under field conditions.

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