RESEARCH ARTICLE

Efficacy of different estrus synchronization protocols on estrus induction response and conception rate in acyclic and cyclic crossbred cows

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Abstract: A study was carried out under field conditions on 50 acyclic/anestrus and 50 cyclic/ repeat breeder HF crossbred cows to evaluate the efficacy of four standard estrus induction/ ovulation synchronization protocols, viz., Ov_synch (GPG), Ov_synch Plus (PMSG+GPG), Double_synch (P-GPG) and Estradoublesynch (P-GPE) (10 cows in each protocol, and in untreated controls) in terms of estrus induction response and conception rates at induced estrus with fixed time AI (FTAI) and overall of 3 cycles. All the selected animals received pre-synchronization treatment, viz., Inj. Ivermectin s.c. and Tono-vita i.m. once and multimineral bolus once daily for 7 days. Repeat breeding cows received additional single shot i.m. injection of Enrofloxacin. All the anestrus and repeat breeder cows (100 %) under all four treatment protocols exhibited induced estrus between 48 and 72 hrs from PGF₂α injection. The conception rates obtained in anestrus cows at induced estrus (FTAI) and overall of three cycles post-treatment were 20.0 and 60.0% with Ov_synch; 20.0 and 50.0% with Ov_synch Plus; 40.0 and 80.0% with Double_synch, and 30.0 and 60.0% with Estradoublesynch protocol, respectively. The corresponding conception rates in repeat breeder cows were 30.0 and 70.0% with Ov_synch; 30.0 and 50.0% with Ov_synch Plus; 40.0 and 80.0% with Double_synch, and 20.0 and 60.0% with Estradoublesynch protocol, respectively. All non-conceived repeat breeding cows under each protocol, except 1 in Ov_synch Plus protocol,

continued cycling, while among 10 anestrus cows each treated with Ov_synch, Ov_synch Plus, Double_synch and Estradoublesynch protocol, 2, 2, 0 and 1 cows again turned out to be anestrus by 60 days post-induction/FTAI, respectively. Among the untreated control anestrus and repeat breeder cows (10 each), 2 and 3 cows conceived over a period of 90 days follow up giving overall conception rates of only 20.0 and 30.0%, respectively, as against the overall fertility rate of 62.5 and 65.0 % in treated anestrus and repeat breeder cows, respectively, over just mean 28-32 days of treatment, suggesting the economic utility of these protocols in handling the problem breeder cows under field conditions.

Keywords: Crossbred cows, Cyclic, Acyclic, Conception rate, Estrus synchronization

Introduction

Various hormonal preparations and protocols are being practised by the field veterinarians to treat postpartum anestrus and repeat breeding, the most prevalent reproductive problems, in dairy animals. But the results reported are inconsistent (Ghuman *et al.*, 2009; Bhoraniya *et al.*, 2012; Dhami *et al.*, 2015) largely due to nutritional status, faulty management, ovarian changes, endocrine events and even uterine infection. Hormonal therapies have good therapeutic value to enhance reproductive efficacy in infertile animals only with good nutritional status (Chaudhari *et al.*, 2012; Savalia *et al.*, 2014). Further, the literature on recently coined Double_synch and Estradoublesynch protocols (Miramahmoudi *et al.*, 2014) is meagre in infertile cattle. Hence, this study was planned to evaluate the comparative efficacy of Ov_synch, Ov_synch Plus, Double_synch and Estradoublesynch protocols in anestrus and repeat breeding crossbred cows of average body condition scores (BCS) in terms of estrus response and fertility enhancement under field conditions.

Materials and methods

The study was carried out in villages under the milk shed areas of AMUL, Anand and Panchamrut Dairy, Godhara, Gujarat from August, 2016 to February, 2017. Fifty postpartum (>90 days)

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true anestrus (acyclic) and 50 classical repeat breeding (cyclic) crossbred cows of average BCS (2.5 to 3.0 on 5 point scale) were screened gynaeco-clinically for their reproductive status. Anestrus cows 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. (Inj. Ivectin, 10 ml, Indian Immunologicals Ltd, Hyderabad), and injection inorganic phosphorus (Inj. Tonophosphan, MSD Animal Health, Pune) and multivitamins AD₃E (Inj. Intavita-H, Intas Pharma, Ahmedabad) 10 ml each i.m., and were supplied with bolus Minotas (Intas Pharma) @ 1 bolus PO for 7 days. Repeat-breeder cows 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.

Ov_synch and Ov_synch plus protocols

Under Ov_synch protocol, 10 acyclic and 10 cyclic crossbred cows each were administered with i.m. Inj. of 20 µg of GnRH analogue, i.e. Buserelin acetate (Receptal, 5 ml, MSD Animal Health) on day 0, Inj. of 500 µg PGF₂α analogue, i.e. Cloprostenol sodium (Estrumate, 2 ml, MSD Animal Health) on day 7, and second Inj. of 10 µg of GnRH on day 9, followed by FTAI twice at 0 and 24 hrs later. In Ov_synch Plus protocol, 10 acyclic and 10 cyclic cows each received an additional i/m Inj. of PMSG 500 IU (Folligon, MSD Animal Health) 2 days before the actual Ov_synch protocol.

Double_synch and Estradoublesynch protocols

Under Double_synch protocol, 10 acyclic and 10 cyclic cows were administered with i.m. Inj. of 500 µg PGF₂α analogue, i.e. Cloprostenol sodium (Estrumate, 2 ml) on day 0, Inj. of 20 µg of GnRH analogue, i.e. Buserelin acetate (Receptal, 5 ml), on day 2, 500 µg PGF₂α analogue (Estrumate, 2 ml) on day 9 and second Inj. of 10 µg of GnRH analogue on day 11, followed by FTAI twice at 16 and 24 hrs later, while in Estradoublesynch protocol, 10 acyclic and 10 cyclic cows received an Inj. of estradiol benzoate 1 mg (Sigma, USA) on day 10, in place of second GnRH injection on day 11 in Double_synch with FTAI twice at 48 and 60 hrs post-estradiol injection.

Control groups

Ten cyclic repeat breeder and 10 acyclic anestrus cows each given pre-synchronization treatment as above, but without any hormonal intervention, and followed for spontaneous estrus and insemination, served as untreated controls.

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 on estrus induction responses, estrus induction intervals and conception rates in acyclic and cyclic crossbred cows under different synchronization protocols and control groups are presented in Table 1.

Estrus induction response and induction intervals in acyclic/anestrus cows

The estrus induction response was 100% in acyclic cows under all four protocols with good exhibition of estrus signs. The mean estrus induction intervals from 7th/9th day PGF₂α injection were 70.1±1.81, 65.6±1.02, 66.1±0.76 and 63.4±2.06 hrs in Ov_synch, Ov_synch Plus, Double_synch, Estradoublesynch protocols, respectively. However in acyclic control group, only two cows (20.0%) showed spontaneous estrus after 32 and 56 days from start of supportive therapy (Table 1).

Higher estrus induction response (100%) with Ov_synch protocol in acyclic cows as observed in the present study has also been reported by Virmani *et al.* (2013), Buhecha *et al.* (2015) and Ahmed *et al.* (2016). However, relatively lower induction response of 20.0 to 67.0% has been reported by others (Mahour *et al.*, 2012; Naikoo *et al.* 2016). The present 100% estrus induction rate in acyclic cows under Ov_synch Plus protocol was also in line with that reported by Virmani *et al.* (2013) and Kumar *et al.* (2016). However, the estrus induction rates of 70.0% in Double_synch and 80.0% in Estradoublesynch reported by Parida *et al.* (2015) in anestrus buffaloes were comparatively lower % than the present 100% response rate in crossbred cattle under both the protocols. Miramahmoudi and Prakash (2012) reported 90% induction of estrus acyclic buffaloes using Double_synch protocol.

The mean estrus induction interval of 70.1±1.81 hrs in Ov_synch protocol in the present study is comparable with the findings of Patel *et al.* (2013), Buhecha *et al.* (2015) and Borkhatariya *et al.* (2016) using same protocol on anestrus crossbred cows. No published work could be found to compare present estrus induction intervals following Ov_synch Plus, Double_synch and Estradoublesynch protocols in acyclic bovines. However, in our study all these protocols were at par with other standard protocols

as far as estrus induction rates and estrus induction intervals were concerned.

Conception rates with different estrus induction protocols in acyclic cows

The conception rates obtained at induced estrus in acyclic cows under Ov_synch, Ov_synch Plus, Double_synch, Estradoublesynch protocols were 20, 20, 40 and 30%, respectively, and the corresponding three cycles' overall conception rates achieved were 60, 50, 80 and 60%. These results were obtained in a period of 25.7 ± 4.57 , 29.0 ± 7.21 , 24.3 ± 6.23 and 29.0 ± 9.66 days from the day of start of treatment in respective groups (Table 1). In all, 25 cows conceived out of 40 anestrus cows treated with different protocols giving overall success rate of 62.5% over just 10-50 (mean 28-32) days of treatment and follow up period. In contrast, among untreated acyclic control group, only two cows exhibited spontaneous estrus and conceived over a 90-days follow up period giving overall conception rate of 20% only. The service period in the control group was 158.1 ± 12.36 days. The results proved beneficial role of inducing estrus in anestrus animals with the referred protocols, which significantly enhanced pregnancy rate and curtailed calving interval making them economically productive compared to untreated ones.

In the present study, the first service (FTAI) conception rates obtained with Ov_synch and Double_synch protocols were in accordance with Abubaker *et al.* (2013), who reported the values as 42.9 and 28.6%, respectively, in crossbred cows. Virmani *et al.* (2013) reported 20.0% conception rate at FTAI with Ov_synch Plus protocol in postpartum anestrus Sahiwal cows. However, Raposo (2013) and Roodbari *et al.* (2015) found very low conception rates of only 13.3 and 20.0% and 26.2 and 18.7%, respectively, with Estradoublesynch and Double_synch protocols in HF cows. While Parida *et al.* (2015) reported 57.7 and 62.5% conception rates, respectively, with the same protocols in anestrus buffaloes. Similarly, the conception rates of 55-58% reported by Mirmahmoudi and Prakash (2012), Mirmahmoudi *et al.* (2014) and Dhindsa *et al.* (2016) in acyclic buffaloes were comparatively higher than the present results with Double_synch protocol. Sharavanan *et al.* (2016) found much higher first service conception rates of 57.1 and 59.5% in anestrus cows using Ov_synch and Estradoublesynch protocols, respectively. Using Ov_synch protocol, Buhecha *et al.* (2015) and Tenhagen *et al.* (2004) recorded first service conception rates of 41.7 and 31.6%, and overall pregnancy rates of 58.3 and 75.5% in anestrus cows, while Kumar *et al.* (2016) found first service and overall conception rates of 56.0 and 75.0% in anestrus buffaloes using Ov_synch plus protocol.

The present overall three cycles' pregnancy rate obtained in acyclic cows under Double_synch protocol was the highest (80 %) followed by that with Ov_synch (60%), Estradoublesynch (60%) and Ov_synch Plus (50%). This overall conception rate of

80.0% achieved in acyclic cows under Double_synch protocol corroborated well with the finding of Ozturk *et al.* (2010) as 72.8%. However, Naikoo *et al.* (2016) recorded only 33.3% overall conception rate using Ov_synch protocol in anestrus Kankrej cows. Sahoo *et al.* (2017) reported lower conception rates of 60.0 and 55.0% with Estradoublesynch and Double_synch protocols. The present overall conception rates obtained in acyclic cows under Ov_synch and Double_synch protocols were in accordance with Abubaker *et al.* (2013), who also reported 71.4% overall conception rates with both these protocols. Very limited research work could be found in the literature on use of Ov_synch Plus, Double_synch and Estradoublesynch protocols in bovines under Indian subcontinent.

Estrus synchronization responses and induction intervals in repeat breeder cows

In cyclic repeat breeder cows under Ov_synch, Ov_synch Plus, Double_synch and Estradoublesynch protocols, the estrus synchronization response was 100 % each with good exhibitory estrus signs. The mean estrus induction intervals from PGF₂ α injection were 62.1 ± 2.26 , 64.6 ± 2.02 , 65.1 ± 1.25 and 69.5 ± 1.16 hrs, respectively (Table 1).

Similar 100% estrus synchronization response with Ov_synch was also reported by Vijayrajan *et al.* (2009) and Chaudhary *et al.* (2012) in cyclic cows. Estrus synchronization rate of 100% achieved in repeat breeder cows with Double_synch and Estradoublesynch protocols was in harmony with the earlier results of 83.3 to 100 % by Miramahmoudi *et al.* (2014) in cyclic buffaloes, wherein estrus was induced within 72-96 hrs of PG injection.

Ahmed *et al.* (2016) and Vijayrajan *et al.* (2009) reported shorter estrus induction intervals of 48.8 ± 0.71 and 52.1 ± 2.39 hrs with Ov_synch protocol as compared to 62.1 ± 2.26 hrs found in present cyclic cows. However, Patel *et al.* (2013) reported estrus induction response within 82.7 ± 4.22 hrs with Ov_synch protocol which is slightly longer than the present findings.

Conception rates with different synchronization protocols in repeat breeder cows

The conception rates obtained at induced estrus in cyclic repeat breeder cows under Ov_synch, Ov_synch Plus, Double_synch and Estradoublesynch protocols were found to be 30, 30, 40 and 20 %, respectively, and the corresponding overall three cycles' conception rates were 70, 50, 80 and 60 %. These results were obtained within a period of 27.0 ± 6.73 , 25.0 ± 7.88 , 23.6 ± 3.66 and 27.2 ± 5.56 days from the day of start of treatment in respective groups (Table 1). In all, 26 cows conceived out of 40 repeat breeder cows treated with different protocols, giving overall success rate of 65.0% over just 10-50 days of treatment and follow up period. The rests all non-conceived treated cows continued to remain cycling, except 1 cow in Ov_synch Plus protocol, which

became anestrus. In contrast, among untreated repeat breeder control group, all the cows exhibited spontaneous estrus regularly with one each conceived at first, second and third cycle over a 90-days follow up period giving overall conception rate of 30.0% only. The service period in the control group was 157.2 ± 14.10 days, which was nearly 50 to 55 days longer than in repeat breeder cows under various treatment protocols (Table 1). These results proved advantage of synchronizing estrus in repeat breeding animals also with the referred protocols as compared to those left untreated.

The present conception rate of 30.0% as obtained at FTAI in cyclic repeat breeding cows under Ov_synch protocol was similar to Prajapati *et al.* (2015), but their overall 3 cycles conception rate was only 55.0% against 70.0% in present study, while Vijayrajan *et al.* (2009), Chaudhary *et al.* (2012) and Parmar *et al.* (2015) recorded relatively higher conception of 50.00 % each at FTAI, but their overall three cycles' conception rates (60, 75 and 70%) were in close accordance with present finding. Ahmed *et al.* (2016) also found 50.0% conception at FTAI following Ov_synch protocol in repeat breeding crossbred cows. Patel *et al.* (2013) documented the first service and overall conception rates of 3 cycles as 40 and 80 % with Ov_synch protocol in repeat breeding crossbred cows that were comparatively higher than the present ones. However, no report on use of Ov_synch plus protocol in repeat breeder bovine could be found in the literature. This protocol in fact did not prove beneficial over simple Ov_synch protocol and had relatively poor overall conception rate in spite of using addition PMSG injection in our study.

In the present study, the first service conception rates of 40.0 and 20.0% found in repeat breeder cows with Double_synch and Estradoublesynch protocols, respectively, compared with report of Kumar *et al.* (2016) using Double_synch during summer and winter seasons in buffaloes (40 and 48%), but was lower than 60-62% reported with both these protocols by Mirmahmoudi and Prakash (2012) and Mirmahmoudi *et al.* (2014) in cycling buffaloes and by Sahoo *et al.* (2017) in cyclic crossbred cows (60 and 55%). However, no report could be located on use of Double_synch and Estradoublesynch protocol in cyclic / repeat breeder bovine documenting cumulative results of two or more cycles to support the present finding, which is up to 80 and 60% in our study. Here also replacing second GnRH injection of Double_synch with estradiol benzoate did not prove advantageous in terms of first service and even overall conception rates in cyclic repeat breeder cows, and the results were comparative better with Double_synch protocol itself.

Conclusions

Based on the findings of our present study, it was concluded that Double_synch and Ov_synch are the better protocols for induction of ovulatory estrus and improving conception rates in

both anestrus and repeat breeding crossbred cattle. However, further studies on large number of animals are needed to be undertaken on Estradoublesynch and Ov_synch Plus protocols to prove their utility over Double_synch and Ov_synch protocols.

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