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Effect of ovsynch synchronization protocol in postpartum anoestrous and repeat breeding cattle: A field study

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Abstract
Anoestrus and repeat breeding syndrome are the most important causes of poor reproductive performance in cattle. The current study was undertaken to find out the efficacy of Ovsynch protocol in terms of oestrus induction, time required for onset of induced oestrus, duration of induced oestrus and conception rate in postpartum anoestrous and repeat breeding in Ongole (*Bos indicus*) and Cross bred (*Bos taurus*) cattle. Total 35 cows with good body condition score (BCS >2.5), parity of two or above, healthy reproductive organs having history of anoestrus problems and repeat breeding syndrome were selected and divided into two groups. Cows in both groups received Ovsynch protocol with GnRH (10 µg) (GnRH-7d-PGF2α-48h-GnRH-18-24hAI). Oestrus detection and intensity of oestrus was done by visual observation and per rectal examination. The time required for onset of induced oestrus and duration of induced oestrus in the cows administered Ovsynch protocol was 54.13 ± 0.97; 22.94 ± 0.87 and 52.58 ± 1.04; 21.26 ± 0.81, respectively in postpartum anoestrum and repeat breeding cows. Among 35 treated animals, 80% (28/35) pregnancy was achieved and another 29 had become cyclic with success rate of treatment was 82.8%. The time required for onset of oestrus (hrs), duration of oestrus (hrs), oestrous response (%), conception in first AI (%), and overall conception (%) were similar (p>0.05) between the two disorders (anoestrum vs. repeat breeding). Further, the Ovsynch protocol produced similar results between the breeds (Ongole vs. HF crossbreds).

Keywords: Ongole cows, holstein-friesian crossbreds, anoestrum, repeat breeding, ovsynch

Introduction
The role of dairy in Indian agriculture and allied sectors is indispensable. Although India is the world's largest milk producer, the productivity of individual dairy animals is far lower compared to developed nations (Reddy *et al*., 2019a) [25]. Researchers studied the role of various manage mental practices such as nutrition (Reddy *et al*., 2019b) [22], housing (Svensson and Hultgren, 2008) [27], health (Bareille *et al*., 2003) [5], grazing system (Haskell *et al*., 2006) [8], and dry period (Reddy *et al*., 2018a, b) [23, 24] in improving the milk yield and fertility-related parameters. Primarily, the low fertility led by improper feeding, housing, and health care is the primary contributing factor for poor productivity. Management of reproduction in lactating dairy cows is one of the major constraints in most dairy herds because of the poor rates of oestrous detection combined with low conception rates.

Repeat breeding and postpartum anestrum are the most prevalent reproductive disorders in dairy cattle affecting reproductive efficiency and economy of milk production in dairy herds (Yadav *et al*. 2018) [31]. Several hormonal therapies are being tried to combat the problem of the repeat breeding and anestrum in dairy animals. Currently, Ovsynch protocols are successfully used for management of repeat breeding in controlled conditions. The aim of this protocol is consecutive applications of hormones like (GnRH, PGF2α, GnRH) to ensure ovulation at a specified time. The protocol synchronizes ovulation within an 8 h period from 24–32 h after the second GnRH administration. This precise synchrony may allow for successful artificial insemination (AI) without the detection oestrus (Nowicki *et al*., 2017). Ovulation synchronization can be achieved at a rate of 80-90% with Ovsynch protocol (Vasconcelos *et al*., 1999) [29]. Progesterone hormone diminishes the formation of arborisation pattern whereas the phenomenon of crystallization in cervical mucus progresses under the influence of oestrogen (Tsiligianni *et al*., 2001) [28]. Thus, it can be quite useful in predicting the onset of oestrus, different stages of oestrus and ovulation time in cattle and buffaloes

(Alena *et al.*, 2008) [3].

Under the field conditions, very fewer trials were conducted for the management of repeat breeding and postpartum anoestrous. As per the collected literature, no work was done to test the effect of Ovsynch protocol between crossbred and native cattle breeds. Hence, the present study was planned to evaluate to the effect of Ovsynch protocol in postpartum anoestrous and repeat breeding cows regarding induced oestrus response, time required for onset of induced oestrus, duration of induced oestrus and conception rates in crossbred and native cows.

Materials and Methods

A total of 35 postpartum anoestrous and repeat breeding cows were included in this study. The present study was undertaken at field level in Guntur and surrounding areas. In general, animals were maintained under hygienic and optimum management conditions in loose housing system with a large, open paddock for free movement. They were sent for grazing daily to the pasture lands. Calves were allowed to suckle the dams and hand milking was practiced twice a day. Health and vaccination protocols are followed as per standard schedule. Whereas, crossbred animals in field conditions were dewormed by fenbendazole and ivermectin. Further, mineral mixture was provided as a feed supplement in the ration of the selected animals at 50 gram twice daily for 15 days before starting the treatment.

Among the selected Ongole cows (n=13), seven were repeat breeders (n=7) and six were suffering with postpartum anoestrum condition (n=6). Regarding the cross bred cows (n=22), twelve were repeat breeders (n=12) and ten cows were suffering with postpartum anoestrum condition (n=10). All these cows had good body condition score, normal genitalia from 2nd to 4th lactation. Cows of this treatment received Ovsynch protocol with administrating 10 ug of GnRH analogue (Buserelin acetate) at any stage of oestrus cycle (day 0) followed by 500 ug Cloprostenol (Pragma, Intas pharmaceuticals Ltd.) (day 7) and second GnRH inj. 48 hrs after PGF2α administration and after 18 to 24 hrs of second GnRH administration fixed time A.I. was done. After PGF2α injection the induced oestrus response was calculated on the basis of number of cows responded i.e., those exhibited oestrus symptoms. Time of PGF2α injection and first detection of heat symptoms by visual observations were recorded to calculate the time required for oestrus induction. The duration of induced oestrus was calculated from expression of first sign of behavioural symptoms of oestrus to the cessation of behavioural symptoms of oestrus. Cervical mucus was collected in petri dish before insemination from oestrous exhibited cows to study the fern pattern. Two to three drops of well mixed cervical mucus were spread uniformly over grease free glass slide and air dried. Arborization pattern was examined under microscope using low power objective 10x for crystallization pattern of cervical mucus. Pregnancy diagnosis was done by per rectal examination after 60 days of artificial insemination.

Statistical analysis

The time required for onset of oestrus (hrs) and duration of oestrus (hrs) for the two disorders and two breeds were tested for significance by analyzing the means and comparing by independent samples t-test. The categorical variables (oestrous response, conception in first AI, and overall conception) represent two outcomes (Yes/No). The percentages were calculated by analyzing their frequencies. The categorical variables were subjected for chi-squared test by cross tabulation. The *P* values less than 0.05 are assumed to be significant. Entire statistical analysis was conducted by using SPSS V. 23.

Results and Discussion

The breed-wise and disorder-wise comparison of GPG protocol on estrous and conception related parameters is shown in Table 1. As this is the first study comparing the types of disorders (repeat breeding vs. postpartum anestrum) and breeds (Indigenous Ongole vs. HF crossbred cows), the obtained results were discussed with the merely available literature.

Table 1: Breed-wise and disorder-wise comparison of GPG protocol on estrous and conception related parameters

S. No.	Parameter	Type of disorder (TD)		Breed (B)		*P* value	
		Repeat Breeding	Postpartum anoestrum	Ongole	HF Crossbreds	TD	B
1	Time required for onset of oestrus (hrs)	52.58 ± 1.04	54.13 ± 0.97	52.77 ± 1.01	53.59 ± 0.99	0.617	0.135
2	Duration of oestrus (hrs)	21.26 ± 0.81	22.94 ± 0.87	21.08 ± 1.06	22.59 ± 0.71	0.987	0.623
3	Oestrous response (%)	89.47	75.00	84.62	81.82	0.258	0.832
4	Conception in first AI (%)	36.84	43.75	46.15	36.36	0.678	0.568
5	Overall conception (%)	78.95	68.75	69.23	77.27	0.492	0.599

Oestrous induction rate

The Ovsynch protocol induced oestrous in 17 out of 19 (89.47%) and 12 out of 16 (75.0%) in repeat breeding and postpartum anoestrous cows, respectively. In concurrence, few authors reported a range of 80.00% to 83.33% induction rates in indigenous Gir cows (Sathiamoorthy and Subramanian, 2003; Ramkrishnan *et al*., 2012; Hirole *et al*., 2018) [26, 19, 9]. No breed variations were found for the oestrous induction rate. In Ongole cows, the Ovsynch protocol induced oestrous in 11 out of 13 (84.6%), while in crossbred cows, the oestrous induction rate was 81.8% (18 out of 22). In accordance with this study, Vijayrajan *et al*. (2009), Chaudhary *et al*. (2012) [7] and Prajapathi *et al*. (2019) reported 100% oestrous induction rate in cyclic repeat breeder cows.

Average time required for onset of Oestrous (hrs.)

No significant differences were observed for the average time required for onset of oestrus between the two types of reproductive disorders (repeat breeding vs. postpartum anestrum). The average time required for onset of oestrous after $PGF_2\alpha$ injection was 54.13 ± 0.97 and 52.58 ± 1.04 in postpartum anoestrous and repeat breeding cows, respectively. The time required for oestrous induction in repeat breeding cyclic cows is similar to the observations of Ahmed *et al.* (2016) [2] (48.75 ± 0.71 hours) and Vijayarajan *et al*. (2009) [30] (52.1 ± 2.39 hours). Despite the higher average time required for onset of oestrous in crossbred cows (53.59 ± 0.99) compared to Ongole cows (52.77 ± 1.01), the t-test revealed absence of statistical differences. Likewise, Hirole *et al*. (2018) and Ratnaparkhi *et al*. (2020) [9, 21]

reported the average time for oestrous as 53.20 ± 1.8 and 54.60 ± 2.44 hrs. in dairy cows subjected to Ovsynch protocol. However, Prajapathi *et al*. (2019) reported estrus induction response within 62.1 ± 2.26 hrs with Ovsynch protocol which is slightly longer than the present findings.

Average duration of Oestrous (hrs.)

The mean time of oestrous duration after $PGF_2\alpha$ injection was similar for the two disorders. The average time of oestrous duration was 22.94 ± 0.87 hours in postpartum anoestrous group and 21.26 ± 0.81 hours in cows suffering from repeat breeding. Several works conducted on the postpartum anoestrum cows administered with Ovsynch protocol revealed the average duration of oestrus within the range of 20.50 ± 2.50 to 21.2 ± 0.58 hrs (Sathiamoorthy and Subramanian, 2003; Hirole *et al*., 2018) [26, 9]. In correlation with present findings, Krishnakumar and Chandrahasan (2012) and Ahmed *et al.* (2016) [11, 2] recorded 21.80 ± 0.80 and 21.083 ± 0.78 hrs oestrous duration in repeat breeding cows. No differences were found for the average duration of oestrous between the indigenous Ongole cows (22.59 ± 0.71) and crossbred cows (21.08 ± 1.06).

Conception rate (%)

Although statistically not different, the conception rate after first service is higher in postpartum anoestrous cows [43.75% (7/16)] than in cyclic repeat breeding cows [36.84% (7/19)]. Nevertheless, the conception rate is not constant and varies from 33.3% (Hirole *et al*., 2018) [9] to as high as 50% (Ratnaparkhi *et al*., 2020) [21]. In accordance with present study in repeat breeding cows Bhoraniya *et al.* (2012) and Naikoo (2012) [6] reported 33.3% first service conception rate in Kankrej cows. Prajapati *et al.* (2015) [17] reported 30% conception rate at first service which is slightly lower. On other hand Vijayarajan *et al*. (2009), Chaudahary *et al*. (2012), Parmar *et al*. (2015) and Ahmed *et al*. (2016) [30, 16, 2] reported a higher conception rate of 50% at first service. Similarly, Jayaganthan *et al*. (2016) [10] reported 54.54% conception rate in cross bred jersey cows. The Overall conception rate was 68.75% and 78.95% in postpartum anestrous and repeat breeding cows, respectively. Similarly, the comparable overall conception rates of 3 cycles following Ovsynch treatment have been reported in cows as 60% (Vijayarajan *et al.*, 2009) [30] or 61% (Geary *et al.*, 2001). However, Ammu *et al*. (2012) reported 83.3% overall conception rate in cross bred and zebu cows, which is higher than present finding. The overall conception percent in crossbred and Ongole cattle were 77.27% and 69.23%, respectively. The higher number of pregnancies achieved in this study using Ovsynch protocol might be due to the prevention of delayed ovulation as well as anovulatory defects. Administration of the first GnRH injection may increase the probability of ovulating the dominant follicle of follicular wave of oestrus cycle and improving synchrony of emergence of a new follicular wave and synchronized ovulation rate due to second GnRH injection. Ovsynch ensures a homogenous ovarian follicular status at induction of luteolysis.

Fern pattern

Breed-wise and disorder-wise comparison of GPG protocol on the typical and atypical fern pattern percentages were depicted in Figure 1 and 2, respectively. The photomicrographs of typical and atypical fern patterns are presented in Figures 3 and 4, respectively.

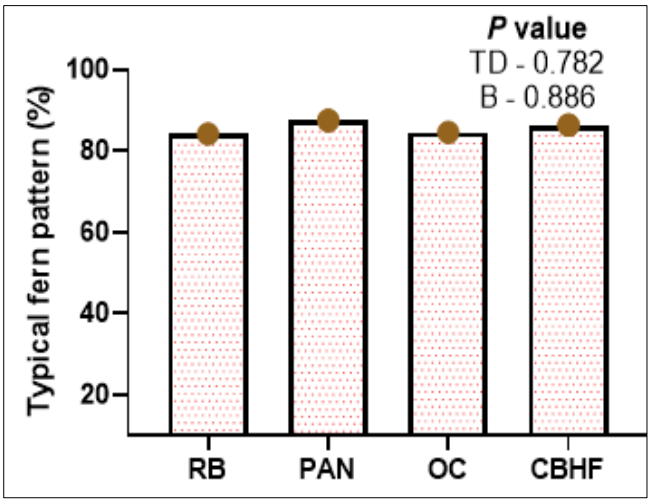

Fig 1: Breed-wises and disorder-wise comparison of GPG protocol on the typical fern pattern percent

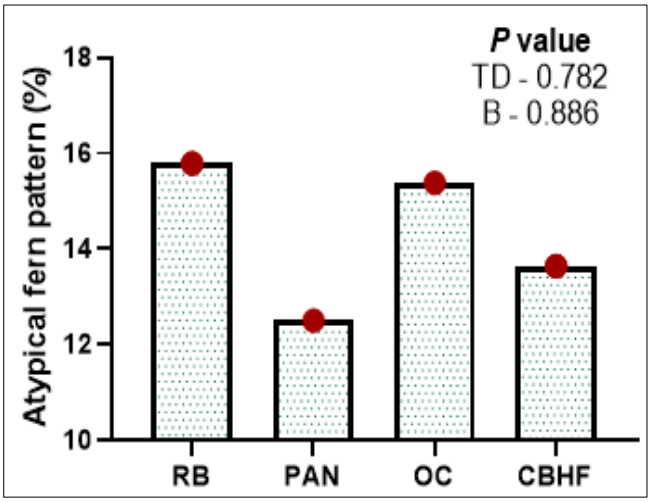

Fig 2: Breed-wises and disorder-wise comparison of GPG protocol on the atypical fern pattern percent

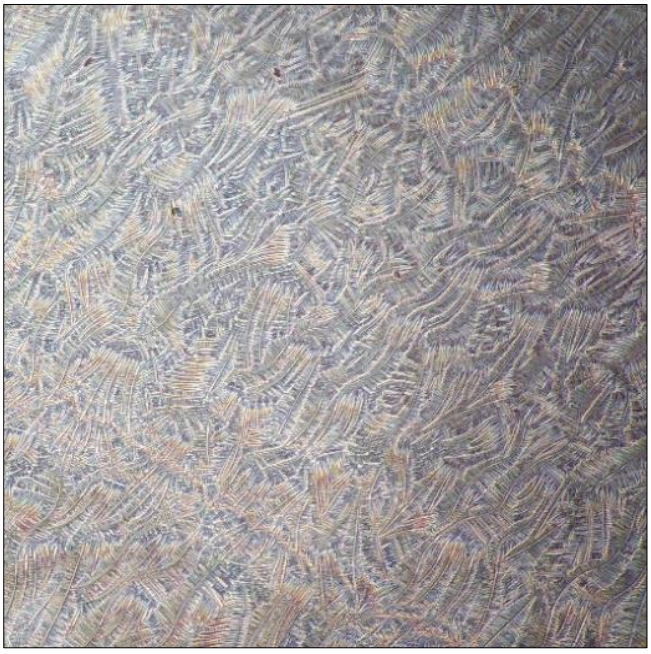

Fig 3: Typical fern pattern

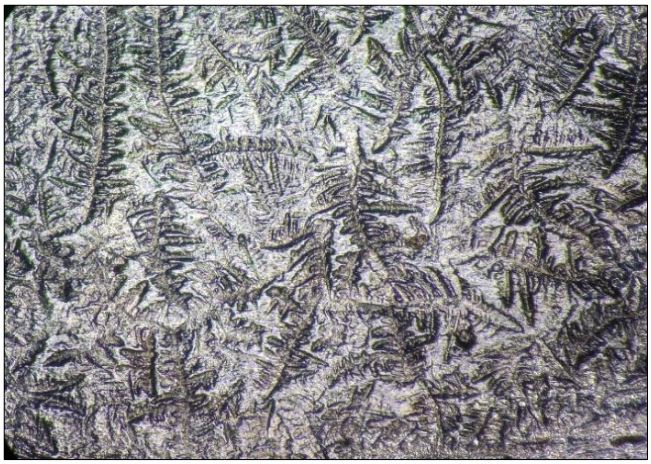

Fig 4: Atypical fern pattern

The current study recorded 85.7% and 14.2% of typical and atypical fern patterns, respectively. These percentages were in corroboration to the results of Rao and Rao (1982), who reported 85.4% and 14.5% of typical and atypical fern patterns, respectively. In contrast, Layek *et al*. (2013) and Parikh *et al*. (2018) reported lower typical fern pattern of 57.9% and 46.8%, respectively in Sahiwal and Gir cows.

Conclusion

From the present study it can be concluded that the application of Ovsynch protocol can serve as a good tool to induce estrus and ovulation as well as enhancement of conception rate in repeat breeding and postpartum anoestrous cows. No significant differences were observed for the conception related parameters for both the type of disorders (repeat breeding vs. postpartum anoestrum) and breed (Ongole vs. HF crossbred cows).

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