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Effect of cosynch protocol on pre-ovulatory follicle size and conception rate in repeat breeding crossbred cows

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Abstract
The objective of the present study was to assess the effect of Cosynch protocol on pre-ovulatory follicle size and first service conception rate in repeat breeding crossbred cows. A total of forty repeat breeding crossbred cows were randomly divided into two group containing 20 cows in each group. In Group I (n=20) cows, GnRH (Receptal, 10 µg) was administered intramuscularly on day 0; $PGF_{2\alpha}$ (Pragma, 500 µg) was administered intramuscularly on day 7 and second GnRH was administered 48 hours post $PGF_{2\alpha}$ injection. Cows were inseminated at the time of second injection of GnRH. Cows in group II (n=20) served as a control and were inseminated at observed estrus without any hormonal intervention. Transrectal ultrasonography was carried out at the time of insemination to determine the pre-ovulatory follicle size. The mean diameter of pre-ovulatory follicle size was 11.44±0.21 mm in cosynch group and 11.20±0.21 mm in control group and the difference was not statistically significant (p>0.05). There was no significant association between the pre-ovulatory follicle diameter and conception both in cosynch treatment group and control group. The first service conception rate was 60.00 per cent in cosynch group as compared to 30.00 per cent in control group. Thus, fertility in repeat breeding cows can be enhanced by controlled breeding using cosynch method of synchronization.

Keywords: cosynch, conception rate, pre-ovulatory follicle, repeat breeding cows

Introduction
Repeat breeding is a major reproductive disorder which causes great economic losses in dairy cattle. The incidence of repeat breeding has been reported 10-24 per cent in cattle (Kimura *et al*., 1987) [1]. Problems in estrus detection and timing of artificial insemination largely influence the incidence of repeat breeding in cattle. It has been reported that 15 to 20% of cows brought for artificial insemination to Veterinary Institutions are not in estrus i.e. at the luteal phase of the estrous cycle (Agarwal *et al*., 2005) [2]. The main reasons for these errors are inadequate and inaccurate estrus detection, lack of awareness about estrus signs or negligence of cattle owner, subestrus or silent estrus. Anovulation and delayed ovulation are also the causes for cows becoming repeat breeder (Parkinson, 2001) [3].

Various estrus synchronization protocols using progesterone and $PGF_{2\alpha}$ have been tried to increase reproductive efficiency in cattle. The effectiveness of these protocols is however, dependent upon the precision of estrus detection. Ovulation synchronisation protocol (Ovsynch Protocol) synchronizes follicular development, luteal regression and ovulation such that artificial insemination can be conducted at a fixed-time without the need for estrus detection. (Pursley *et al*., 1997) [4]. The Ovsynch program consists of 2 injections of GnRH, 7 days before, and 48 hours after an injection of $PGF_{2\alpha}$. Cows are inseminated 8 to 18 h after the second injection of GnRH. In Cosynch method, timed artificial insemination is performed at the time of the second GnRH injection. Cosynch method reduces one animal handling compared with ovsynch method (Pursley *et al*., 1998; Geary *et al*., 2001) [5, 6]. Cosynch protocol can be applied as a treatment for cows failing to be detected in estrus and also allows the treatment of cows with subestrus or ovulatory defects. Hence, cosynch method may enhance conception rate in repeat breeding cows caused by poor estrus detection, improper timing of insemination, anovulation and delayed ovulation. Much research has been done using cosynch method on fertility response in normal cyclical and postpartum anestrus cows. However, reports on application of cosynch method for augmentation of fertility in repeat breeding cows are meagre (Yogesh Barolia *et al*., 2016) [7].

It has been reported that pre-ovulatory follicle (POF) diameter is important for the subsequent corpus luteum development and hence conception in cattle (Perry *et al*., 2007) [8]. A larger POF may generate a larger corpus luteum that secretes more progesterone and thus have a

positive effect on pregnancy recognition and pregnancy rates in cattle (Binelli *et al*., 2009) [9]. On the contrary, others have reported absence of correlation (Colazo *et al*., 2009) [10] or negative correlation (Lynch *et al*., 2010) [11] between POF diameter and pregnancy outcome. Moreover, reports on effect of cosynch ptotocol on pre-ovulatory follicle size in repeat breeding cows are meagre. Hence, the present study was conducted to assess the effect of cosynch protocol on pre-ovulatory follicle size and conception rate in repeat breeding crossbred cows.

Material and methods

The study was conducted on 40 repeat breeding cows brought to the Large Animal Gynaecology ward, VCRI, Orathanadu with the history of non-conception for three consecutive inseminations. Cows that were free from uterine infection and gross genital tract abnormalities, parity from 2 to 5 calvings and having body condition score of 3.0 to 4.0 were selected for the study. The selected animals were fed with concentrates and green fodder; and the animal owners were supplied with 1 kg TANUVAS SMART mineral mixture (area specific) with an advice to feed the selected cow with the mineral mixture at the rate of 30 gram per day for 30 days before initiating treatment. All the selected animals were administered with a dose of deworming bolus Fenbendazole (Fentas 3g) prior to the mineral mixture supplementation. The selected 40 repeat breeding cows were randomly divided into two groups containing 20 cows in each group.

Cows in group I (n=20) were treated with Cosynch protocol. The treatment was initiated during luteal phase of estrous cycle i.e. 7 days after onset of estrum in the selected cows. In Group I cows, GnRH (Receptal, 10 µg) was administered intramuscularly on day 0; $PGF_{2\alpha}$ (Pragma, 500 µg) was administered intramuscularly on day 7 and second GnRH was administered 48 hours post $PGF_{2\alpha}$ injection. Cows were inseminated at the time of second injection of GnRH. Cows in group II (n=20) served as control and were inseminated at observed estrus without any hormonal intervention. Size of pre-ovulatory follicle was determined by taking mean of follicular diameter at the widest point and perpendicular to the first measurement using internal calipers on the ultrasound machine with trans-rectal probe (Sonoscape S2V, China; 7.5 MHz). Pregnancy diagnosis was done by ultrasonography at 28-35 days post insemination. The first service conception rate was calculated. Animals returning to estrus were re-inseminated at the subsequent estrus and the estrous cycle length was calculated. The data were analysed by one-way ANOVA to compare the differences between the groups and independent t-test to compare the differences within the group between pregnant and non-pregnant cows (Snedecor and Cochran, 1994) [12].

Results and discussion

The result revealed that the first service conception rate for cows in the Cosynch treatment group (group I) was 60.00 per cent (12/20). Remaining eight cows returned to estrus with the mean estrous cycle length of 21.13±0.61 days. The first service conception rate for cows in the control group (group II) was 30.00 per cent (6/20). Remaining fourteen cows returned to estrus with the mean estrous cycle length of 20.64±0.64 days. All the cows treated with Cosynch protocol expressed estrus in group I. The first service conception rate was 30.00 per cent higher in the Cosynch group than in control group.

Yogesh Barolia *et al.* (2016) [7] also observed higher conception rate in repeat breeding Gir cows treated with cosynch protocol (50.00 per cent) as compared to control group (33.33 per cent). Walter Schmitz *et al.* (2017) [13] reported pregnancy rate of 65 per cent after timed artificial insemination in suckled beef cows treated with cosynch protocol. They also found higher pregnancy rate when the cosynch protocol was initiated in cows having increasing plasma progesterone concentrations.

Caraba and Velicevici (2013) [14] recorded higher conception rate in dairy cows treated with cosynch protocol as compared to ovsynch treatment group of dairy cows (57.00 per cent versus 25.00 per cent). Zaituni Udin *et al.* (2017) [15] observed overall conception rate of 66.67 per cent in post-partum Simmental cows treated cosynch protocol. Geary *et al.* (2001) [6] reported conception rate of 54.00 per cent in beef cows treated with cosynch protocol and 63.00 per cent in beef cows that received cosynch plus calf removal. The first service conception recorded in cosynch group of the present study was comparable with Geary *et al.* (2001) [6] and Caraba and Velicevici (2013) [14].

The precision of estrus and high fertility rates in cosynch group are due to the first GnRH injection causing luteinization or ovulation of the mature follicle, and initiating recruitment and selection of a new dominant follicle. $PGF_{2\alpha}$ injection on 7th day causes regression of the spontaneous CL or potential CL induced by GnRH, or both (Peters *et al*., 1999) [16]. The second GnRH injection on 9th day should ensure better ovulation synchronization by stimulating the preovulatory LH surge (Coulson *et al.*, 1980) [17].

Repeat breeding in dairy cows is frequently caused by poor estrus detection and insemination at incorrect time. Hence, the increase in first service conception rate of cosynch group of the present study might be due to fixed time insemination using cosynch protocol. Repeat breeding is also caused by anovulation, delayed ovulation and luteal insufficiency due to endocrine dysfunctions in dairy cows (Yogesh Barolia *et al.*, 2016) [7]. Higher conception rate reported in cosynch group indicated that these endocrine dysfunctions might have been eliminated by cosynch method of ovulation synchronization.

The mean diameter of pre-ovulatory follicle was 11.44±0.21 mm in cosynch group and 11.20±0.18 mm in control group of cows and the difference was not statistically significant (p>0.05). In the present study, difference between mean pre-ovulatory follicle diameter in pregnant and non-pregnant cows was not statistically significant (p>0.05) both in cosynch treatment and control group (Table 1). Colazo *et al.* (2009) [10] also found no significant association between pre-ovulatory follicle size and pregnancy rates to timed artificial insemination in dairy cows. Perry *et al.* (2005) [18] reported that ovulatory follicle size did not affect conception rate in cows inseminated during spontaneous estrus.

Praveen Raj *et al*. (2018) [19] also observed no significant correlation between the POF size at the time of AI and conception in buffaloes. They further inferred that physiological maturity rather than the diameter of the follicle influenced the fertility in Graded Murrah buffaloes under field conditions. Similarly, Amit Kashyap *et al*. (2020) [20] found no significant difference in mean pre-ovulatory follicle diameter between pregnant and non-pregnant cows treated with ovsynch protocol.

Some studies have indicated that larger ovulatory follicles at the time of artificial insemination increase conception rate in cattle and buffalo (Keskin *et al*.2010; Sa Filho *et al*.2010;

Pandey *et al*. 2011) [21-23]. On the other hand, some researchers reported that smaller ovulatory follicles resulted in higher conception rate in cattle (Vasconcelos *et al*. 2001; Lynch *et al*. 2010) [24, 11]. Follicle size at timed artificial insemination in dairy cows is affected by breed, milk production, parity, and season (Keskin *et al*.2010) [21].

Table 1: Diameter (Mean±SE) of pre-ovulatory follicle (mm) in pregnant and non-pregnant cows in cosynch treatment and control group

Group	Pregnant	Non-pregnant	Overall
Cosynch treatment	11.61±0.43	11.32±0.39	11.44±0.21
Control	11.33±0.24	11.15±0.21	11.20±0.18

Values within same row and values within same column do not differ significantly (p>0.05)

Conclusion

In the present study, there was no significant correlation between the pre-ovulatory follicle size at the time of insemination and conception both in cosynch treatment group and control group. The first service conception rate was 30 per cent higher in cosynch treatment group than in control group. Thus, fertility in repeat breeding cows can be enhanced by controlled breeding using cosynch method of synchronization.

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