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Impact of ovsynch protocol on dominant follicle diameter and fertility response in repeat breeder dairy cows

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Abstract
The present work was designed to study the impact of ovsynch protocol on dominant follicle diameter and fertility in repeat breeder dairy cows. Thirty repeat breeder dairy cows were divided into two groups i.e. ovsynch treatment (n=15) and control (n=15). The dominant follicle diameter was 10.94±0.37 mm in ovsynch group and 10.69±0.30 mm in control group. The mean dominant follicle diameter did not differ significantly (p>0.05) between ovsynch treatment and control animals. In the present study, ovsynch protocol increased first insemination conception rate than the control (53.33% vs. 26.66%) indicating beneficial effect of ovsynch protocol on augmenting fertility in repeat breeder cows.

Keywords: Ovsynch, fertility, dominant follicle size, repeat breeder cows

Introduction
Repeat breeder cow has normal or nearly normal estrous cycle, estrus periods but fail to conceive with three or more than three inseminations from an apparently normal healthy bull or semen. It is estimated that 10-25% cows in dairy farms are repeat breeders (Agarwal *et al*., 2005) [1]. The early embryonic death and ferilization failure are considered as two major causative factors for repeat breeding in cattle. Inadequate and inaccurate estrus detection is frequently a cause for cows becoming repeat breeder. Incorrect timing of artificial insemination causes poor conception rate in dairy cows. Under field conditions, 15% cows are inseminated during the lueal phase and a further 15% are inseminated during inappropriate stages of follicular phase (Parkinson, 2019) [2]. Repeat breeding condition may also arise due to anovulation, delayed ovulation and luteal insufficiency (Roberts, 1971) [3].
To increase fertility in dairy cows, several methods of synchronizing estrus using progestogens and prostaglandin $F_{2\alpha}$ have been attempted. However, the success of these methods is dependent upon the accuracy of heat detection. Ovsynch protocol synchronise follicular waves so that ovulation occurred over a short enough period of time and fixed-time artificial insemination can be done without heat detection (Pursley *et al*. 1997) [4]. In ovsynch protocol, GnRH is administered 7 days before and 2 days after Prostaglandin $F_{2\alpha}$ injection and timed artificial insemination done 8-18 hours after second GnRH injection. Ovsynch method can be used as treatment for silent estrus, unobserved estrus, anovulation and delayed ovulation in dairy cows. Therefore, application of ovsynch protocol may increase fertility in repeat breeder cattle caused by inadequate heat detection, incorrect timing of service and ovulatory defects. More reports are available on the effect of ovsynch protocol in augmenting fertility in cyclical and postpartum acyclic cattle. However, studies pertaining to the effect of ovsynch protocol for increasing conception rate in repeat breeder cattle are scanty (Madhumeet Singh *et al.* 2019) [5].
Binelli *et al*. (2001) [6] reported that dominant follicle size is associated with subsequent corpus luteum size and function. A large dominant follicle may generate a large corpus luteum, which provides high progesterone concentration to favor development of embryo (Keskin *et al.* 2010) [7]. However, some researchers found that smaller dominant follicles associated with higher pregnancy rate in cow (Vasconcelos *et al*. 1999; Lynch *et al*. 2010) [8, 9]. Furthermore, studies on impact of ovsynch protocol on dominant follicle diameter in repeat breeder cattle are scanty. Hence, the present experiment was done to evaluate the impact of ovsynch protocol on dominant follicle diameter and fertility response in repeat breeder cows.

Materials and Methods

The experiment was conducted on 30 repeat breeder cows brought to the Veterinary Clinical Complex, VCRI, Salem with the history of not conceiving after three inseminations. The animals were clinically healthy, aged between 4-8 years, parity from 2 to 5, body weight of 300 to 400 kg and had no history of calving difficulty in the previous parturition. The animals had no gross abnormalities of reproductive tract on gynaecological examination. All the selected cows were dewormed and advised to supplement mineral mixture 30 gram per day for 1 month. These cows were fed with balanced ration of concentrate mixture, green and roughage, and drinking water *ad libidm*.

The animals were randomly divided into two group viz. Group I (Ovsynch protocol) and group II (control). Animals in group I (n=15) were injected with 10 µg of GnRH (Receptal, IM) on day 0; 500 µg of Prostaglandin $F_{2\alpha}$ (Pragma, IM) on day 7 and second GnRH on day 9. The cows were inseminated 12 and 24 hours after second GnRH injection. The ovsynch protocol was started during mid luteal phase of estrous cycle in the experimental animals. The animals of group II-control (n=15) did not receive any treatment and inseminated at detected estrus. The diameter of dominant follicle at the time of artificial insemination (AI) was measured after locating the ovaries on clockwise and anti- clockwise rotation using the trans-rectal transducer of ultrasound machine (Esaote, Mylab Sigma Vet; 7.5 MHz).

Pregnancy was confirmed by 28-35 days using transrectal ultrasonography and first AI conception rate was calculated. Cows returning to estrus were inseminated again at the second estrus and the inter-estrus interval was calculated. Statistical analysis was done by one-way ANOVA to compare the variances between two group and independent t-test to compare the variances within the group between conceived and non-conceived animals (Snedecor and Cochran, 1994) [10].

Results and Discussion

Fertility response revealed that following ovsynch treatment eight animals (8/15; 53.33%) conceived at the first insemination vis-à-vis only four animals (4/15; 26.66%) in control group. The estrous cycle length in animals, who did not conceive at first insemination, was 21.28±0.47 and 20.81±0.44 days in ovsynch treatment and control group, respectively. The conception rate in the ovsynch treatment group was 26.67% higher as compared to control. Similarly, 17-33% higher first AI conception rate was reported by Vijayarajan *et al.* (2009) [11], Jayaganthan *et al*., (2016) [12], Yogesh Barolia *et al.* (2016) [13], Murugavel *et al.* (2018) [14] and Madhumeet Singh *et al.* (2019) [5] in repeat breeder cows treated with ovsynch protocol as compared to control.

Ahmed *et al*. (2016) [15] recorded conception rate of 50% following fixed time insemination in repeat breeding crossbred cattle treated with ovsynch protocol. They also found that serum progesterone was higher with an extra GnRH injection following ovsynch protocol. Patel *et al.* (2020) [16] reported that the overall pregnancy rates in repeat breeder cows with prolonged estrus and normal estrus after adoption of modified ovsynch protocol were 60 and 70%, respectively. The first AI conception rate observed in ovsynch group of the present experiment was in accordance with the reports of Jayaganthan *et al*., (2016) [12] and Madhumeet Singh *et al.* (2019) [5].

In ovsynch protocol, first injection of GnRH ensures emergence of a new dominant follicle at the time of Prostaglandin $F_{2\alpha}$ injection and can grow and ovulate. The second injection of GnRH ensures better ovulation synchronization by stimulating the preovulatory LH surge (Laven, 2019) [17]. Fixed time insemination in the present study might have increased conception rate in repeat breeder cows caused by inadequate heat detection and incorrect timing of AI. Since repeat breeding is also caused by anovulation and delayed ovulation, the ovsynch protocol might have increased conception rate by ensuring correct time of ovulation.

Results pertaining to the impact of ovsynch treatment on dominant follicle diameter in repeat breeder cows have been presented in Table 1. The mean diameter of dominant follicle in ovsynch treatment and control group was 10.94±0.37 mm and 10.69±0.30 mm respectively. It was observed that there was no significant difference in dominant follicular size between treatment and control group. Also, there was no significant difference in dominant follicular diameter between pregnant and non-pregnant animals both in ovsynch treatment and control group.

No significant difference in dominant follicle diameter between pregnant and non-pregnant cows recorded in the present study was in accordance with the observations of Amit Kashyap *et al.* (2020) [18] and Colazo *et al.* (2009) [19]. Similarly, no significant correlation between dominant follicle diameter at AI during natural estrum and pregnancy rate was found by Perry *et al.* (2005) [20] in cattle and Praveen Raj *et al.* (2018) [21] in buffalo.

However, Madhumeet Singh *et al.* (2019) [5] observed higher dominant follicle diameter at AI in the repeat breeder cows treated with ovsynch protocol than control. Positive correlation between dominant follicle diameter at AI and pregnancy rate was reported by Keskin *et al.* (2010) [7] and Sa Filho *et al.* (2010) [22] and negative correlation reported by Vasconcelos *et al*. (1999) [8] and Lynch *et al*. 2010) [9] in cows. Numerous factors such as breed, milk production, parity, and season may affect the dominant follicle diameter at fixed time insemination in cattle (Keskin *et al.* 2010) [7].

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

Group	Pregnant	Non-pregnant	Overall
Ovsynch treatment	11.07±0.53	10.78±0.54	10.94±0.37
Control	10.83±0.59	10.63±0.37	10.69±0.30

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

Conclusion

The study revealed that ovsynch method of synchronization increased (26.67%) first AI conception rate in repeat breeder cows as compared to control (53.33% vs. 26.66%) demonstrating beneficial effect of ovsynch protocol on conception rate in repeat breeder cows.

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