



## Comparison of conception rate following GnRH and hCG based synchronization protocols in functionally infertile cows

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The reproductive performance of high yielding cows with high genetic merit has declined in many dairy industries. One of the major constraints of profitable dairy farming is low pregnancy rate in cows (Shamsuddin *et al.* 2006) and it can be attributed to either fertilization failure or early embryonic death (Agarwal *et al.* 2005). The main factors responsible are anatomical, infectious, non-infectious, functional and managemental problems in animals. Delayed ovulation, anovulation and luteal insufficiency are some of the important components leading to functional infertility in dairy animals. These animals may have abnormal follicular development and abnormal estrous cycles (Parmar 2015). Luteal insufficiency takes place when the luteal phase is shorter than normal, which ultimately results in sub-normal progesterone levels. It interferes with the implantation of embryo (Di Zerega and Hodgen 1981). All these conditions lead to mismatching of the time of ovulation and time of AI, thus, lower conception rate, early embryonic mortality, lower pregnancy and more cows become repeat breeder (Bhattacharyya and Hafiz 2009). The present study was aimed to target the cows diagnosed to be suffering from functional form of infertility and to study effect of GnRH and hCG based breeding protocols for managing infertility due to this malady.

The work was conducted on animals presented at Teaching Veterinary Clinical Complex of the University, and nearby field institutions around Palampur. In all, 91 cows showing clear discharge at the estrus were selected for the study. The protocols for synchronization of estrus and ovulation were applied from day 6 of estrous cycle. These cows were divided into 4 treatment and 1 control group. In group 1 (G1), estrus synchronization via Ovsynch protocol was initiated in 23 cows to initiate and develop the follicular wave on day 0 (day 6 of estrous cycle) and 10 µg Buserelin acetate (2.5 ml Receptal) was administered by intramuscular route. On day 7 (day 13 of the estrous

cycle), 500 µg cloprostenol (2 ml clostenol) was administered intramuscularly as an exogenous source for initiation of the lysis of corpus luteum. On day 9 (day 15 of the estrous cycle), 10 µg buserelin acetate (2.5 ml Receptal) was administered to support the ovulation of tertiary follicle developed in the new wave, and fixed timed artificial insemination (FTAI) was done on day 10 (day 16 of estrous cycle) and day 11 (day 17 of estrous cycle). In group 2 (G2), Co-synch protocol followed was exactly similar to the group 1 except that FTAI was done on day 9 (day 15 of estrous cycle) and day 10 (day 16 of estrous cycle) in 23 cows. In group 3 (G3), modified Ovsynch protocol for synchronization was started in 12 cows on day 0 (day 6 of estrous cycle) with the aim of initiation and development of the follicular wave and 3,000 IU of hCG (Chorulon) was administered by intramuscular route. On day 7 (day 13 of estrous cycle), 500 µg cloprostenol (2 ml Clostenol) was administered intramuscularly as an exogenous source for initiation of the lysis of the corpus luteum. On day 9 (day 15 of the estrous cycle), 3,000 IU of hCG (Chorulon) was administered intramuscularly to support the ovulation of the tertiary follicle developed in the new wave and FTAI was done on day 10 (day 16 of estrous cycle) and day 11 (day 17 of estrous cycle). In group 4 (G4), modified Co-synch protocol followed was exactly similar to the group 3 except FTAI was done on day 9 (day 15 of estrous cycle) and day 10 (day 16 of estrous cycle) in 12 cows. In control group, consisting of 21 cows, treatment was not given and AI was done on the day of observed estrus after the examination of clear cervico-vaginal discharge. Pregnancy diagnosis was done 2 months post AI by rectal examination/ultrasonography method, using linear transducer of frequency 7.5 MHz (Mindray, Model 75L50EAV) in cows that did not return to estrus within this duration. After compiling the data, conception rates were calculated. The obtained data were statistically analyzed using Chi-square test for parametric data as described with SAS (Statistical Analysis Software), SAS® 9.2 TS Level 2M2 for windows.

The conception rate for group 1 (G1) treated with Ovsynch protocol (G1) was 56.52% (13/23), whereas, it was 47.82% (11/23) for cows treated with Co-synch

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protocol (G2). Overall, 46 cows were inseminated in GnRH based treatment protocols and 24 were found to be pregnant (52.17%). Following the modified Ovsynch protocol (G3), only 4 cows (33.33%) conceived out of 12 cows taken into study. Whereas, only 2 (16.67%) cows were found to be pregnant, out of 12 cows inseminated in modified Co-synch group (G4). Overall, 24 cows were inseminated in hCG based treatment protocol and 6 were found to be pregnant. The CR achieved was 25%. In untreated control group, out of the 21 cows inseminated at natural estrus, 6 conceived. The CR achieved was 28.57%. Conception achieved following GnRH based (Ovsynch and Co-synch) protocols was significantly higher ( $P < 0.05$ ) in comparison to hCG based protocols (modified Ovsynch and modified Co-synch) and control group (Table 1).

The CR in this study following Ovsynch protocol (56.52%) was similar to the findings of Jayaganthan *et al.* (2016) and Buhecha *et al.* (2016), who reported 54.54 and 58.33% CR, respectively. Mesut *et al.* (2010) and Prajapati *et al.* (2015) also reported 76.90 and 60% CR, respectively, in repeat breeder cows treated with Ovsynch protocol. However, conception recorded in present study following Co-synch protocol (47.82%) was higher than that reported in other studies (37.00%, Pursley *et al.* 1998; 37.50%, Biradar *et al.* 2014). In present study, the difference in CR between Ovsynch and Co-synch (GnRH based protocols) was non-significant ( $P > 0.05$ ). Similarly, Akbarabadi *et al.* (2014) reported higher CR for Ovsynch (72.00%) as compared to Co-synch protocol (52.00%). Geary *et al.* (2001) also indicated a small reduction in CR when Co-synch is compared with Ovsynch, which is similar with our findings. In present study, 33.33% CR following hCG based modified Ovsynch treatment protocol (G3) was in concurrence with CR (28.10 and 37.60%) reported by Pancarci *et al.* (2013) and Keskin *et al.* (2010) respectively. In modified Co-synch protocol (G4), lower CR (16.67%) was recorded in our study as compared to 34% recorded by Geary *et al.* (2001). Ovsynch ensures a homogenous ovarian follicular status at induction of luteolysis. Exogenous administration of GnRH initiates the endogenous increase in progesterone via modulation of ovarian follicular cells' population and promotion of accessory CL formation (Schmitt *et al.* 1996). Savalia *et al.* (2014) also stated that GnRH treatment ensures timely ovulation as well as efficient progesterone support for embryonic development

resulting in better conception in repeat breeder cows.

In the present study, CR in hCG based protocols was significantly ( $P < 0.05$ ) lower as compared to GnRH based treatments. Our results are in accordance with the findings of other workers (Keskin *et al.* 2010, Pancarci *et al.* 2013). Human chorionic gonadotropin has seen limited use in estrus synchronization protocols. Schmitt *et al.* (1996) saw no increase in CR of dairy heifers when the second GnRH of Ovsynch was replaced with an injection of hCG (3,000 IU). Concurrently, De Rensis *et al.* (1999) also reported no increase in CR following hCG administration at the initiation of synchronization protocol. Buttrey (2008) concluded that hCG has no major advantage over GnRH in estrus synchronization protocols.

Contrarily, Kuru *et al.* (2017) reported higher (53.0%) CR following hCG administration on day 9 of synchronization protocol. Lower CR in hCG based treatment group in our study could be owing to short estrous cycles, which could impair maternal recognition of pregnancy or basically due to luteal deficiency following FTAI. Luteal insufficiency could result from an abnormality inherent to the dominant follicle. Ovulation followed by insufficient luteal condition leads to the improper development of corpora lutea which secrete low amount of progesterone than the normal (Parmar 2015). In comparison to GnRH, initiation of a new wave of follicular growth might occur later following an hCG injection because of the lack of FSH stimulation. Thus, ovulation may have occurred less frequently among hCG-treated cows due to the presence of a smaller dominant follicle at day 9 after the initial hCG injection.

In brief, conception rate achieved following GnRH based protocols was higher as compared to hCG based protocols. So our study supports the use of GnRH based synchronization protocols in functionally infertile repeat breeder cows.

## SUMMARY

Repeat breeder cows (91) diagnosed to be suffering from functional form of infertility were studied. Functional causes of infertility like luteal deficiency and ovulatory defects i.e. delayed ovulation and anovulation were taken into consideration. Cows were divided into 4 treatments, viz. GnRH based (Ovsynch and Co-synch), hCG based (modified Ovsynch and modified Co-synch) and control

Table 1. Conception rates in cows receiving different treatment protocols

Treatment group		Cows inseminated	Pregnant	Conception rate (%)
GnRH based	Ovsynch (G1)	23	13	56.52
	Co-synch (G2)	23	11	47.82
	Overall	46	24	52.17 <sup>a</sup>
hCG Based	Modified Ovsynch (G3)	12	4	33.33
	Modified Co-synch (G4)	12	2	16.67
	Overall	24	6	25.00 <sup>b</sup>
Control		21	6	28.57 <sup>b</sup>

<sup>a,b</sup>Values with different superscripts within the same column differ significantly ( $P < 0.05$ ).

group. Treatment was started in all the repeat breeder cows on day 6 of estrous cycle after the observed heat. The conception rate (CR) (%) achieved was 56.52 (Ovsynch), 47.82 (Co-synch), 33.33 (modified Ovsynch), 25.00 (modified Co-synch) and 28.57 (control) in different groups. Overall, the highest conception rate was recorded in GnRH based protocols (52.17%) followed by control group (28.57%) and hCG based (25.00%) treatment. The overall CR of GnRH based treatment protocol was significantly higher than hCG based treatment or control cows.

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