

# COMPARATIVE EFFICACY OF TWO HORMONAL TREATMENTS FOR OESTRUS INDUCTION IN ANOESTRUS CROSSBRED COWS

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## ABSTRACT

*The present study was conducted to assess the efficacy of two oestrus induction protocols on 30 postpartum anoestrus crossbred cows reared by rural farmers of Puducherry region. The cows were randomly divided into Treatment I (CIDR+PGF2 $\alpha$ +GnRH; n=15) and Treatment group II (OvSynch+ CIDR; n=15) with double fixed timed inseminations at 12 and 24 hours post-administration of last GnRH. Oestrus induction response was 100% in both the treatment groups with majority of the cows (80%) in both the treatment groups showed intermediate intensity of oestrus. The mean time to onset of oestrus from the time of removal of CIDR in Treatment I and Treatment II was  $43.45 \pm 9.21$  and  $45.81 \pm 6.34$  h respectively. There is no significant difference in the serum progesterone concentration at the time of AI between Treatment I ( $0.92 \pm 0.26$  ng/mL) and Treatment II ( $0.92 \pm 0.18$  ng/mL). Though the conception rate was higher in treatment II (40 %) compared to Treatment I (20 %), the difference is not statistically significant. From the present study, it was concluded that OvSynch in combination with CIDR found to be effective in oestrus induction and conception following fixed timed AI in anoestrus cows under field conditions.*

**Keywords:** Postpartum anoestrus, Crossbred cows, Oestrus induction, CIDR

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## INTRODUCTION

Postpartum period is important in the reproductive life of the cow, because of its

effect upon future fertility (Ambrose, 2021). The duration of postpartum anoestrus is determined by overcoming the refractoriness of hypothalamo-hypophyseal ovarian axis and resumption of LH pulses. Usually, there is deviation in follicular growth, selection of a dominant follicle, follicular maturation, ovulation, and followed by luteolysis,

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resulting in restoration of cyclical ovarian activity. Failure of any of these events prolongs postpartum anoestrus in dairy cows (Peter *et al.*, 2009). It determines productive and reproductive responses during lactation and is therefore a pivotal period in production cycle of the cow (Lucy, 2007).

Prolonged interval between calving and onset of ovarian function is regarded an infertility problem responsible for failure to maintain optimum reproductive efficiency, which in turn causes economical loss to dairy farmers (De Vries, 2020). Apart from identifying and correcting the causative factors responsible for anoestrus, it is essential to evolve a protocol with a dependable induction of oestrus and followed by optimum conception rates. Many researchers have used different protocols of hormonal preparations for induction of oestrus in cows and subsequent fertility with varying results (Rhodes *et al.*, 2003; Yániz *et al.*, 2004). Progesterone or progestins based protocols are considered appropriate for noncyclic or anoestrus postpartum cows (Yániz *et al.*, 2004). The development of controlled intravaginal progesterone-releasing devices (CIDR) hastened resumption of ovarian follicular activity in anoestrus cows. CIDR facilitated hormone treatments and circumvented delivery problems associated with feeding or injecting progestins (Macmillan and Peterson, 1993). At the end of CIDR treatment, hormones such as equine chorionic gonadotropin (eCG), oestradiol, or PGF2 $\alpha$  have been given to maximize the response of resumption of follicular activity

(Peter *et al.*, 2009). Furthermore, GnRH analogues have been used prior to progesterone intravaginal device treatment to improve the treatment response (Rhodes *et al.*, 2003). With this background, the present research work was designed to compare the efficacy of two progesterone hormonal protocols on the induction of oestrus and conception rate in anoestrus cows.

## MATERIALS AND METHODS

The present study was conducted on thirty healthy anoestrus cows with a history of normal parturition and not showing signs of oestrus even after 45 days postpartum. The cows were subjected to white side test and only cows without subclinical endometritis were utilized for this study. All the cows were between 45 to 120 days in milk with body condition score between 2.5 to 3.5 (0-5 scale) when the treatment was initiated. The mean milk yield of the all the crossbred cows on the day of initiation was treatment was  $11.15 \pm 2.31$  L. Thirty cows without any palpable uterine disorders and not having CL on the ovaries on two successive per rectal examinations at 10 days interval were considered as anoestrus and were selected for the present study. All the crossbred cows were reared by rural farmers of Puducherry region. The crossbred cows were allowed for grazing and followed by stall feeding of fodder and concentrates. The selected postpartum anoestrus cows were divided into two groups with 15 cows in each group.

One group of 15 cows (Treatment I) were treated with CIDR+PGF2 $\alpha$  + GnRH protocol. This protocol includes insertion of

CIDR (EAZI-BREED CIDR, Manufactured by DEC International Ltd; Hamilton, New Zealand and marketed by Pfizer Animal Health, Mumbai, containing 1.38 g Progesterone) intravaginally on day 0, injection PGF2 $\alpha$  (Pragma (Intas Pharmaceuticals Ltd; Ahmedabad, containing Cloprostenol @ 250  $\mu$ /mL) 2 ml, intramuscularly on day 6 and removal of CIDR on day 7, injection GnRH (Ovulanta, Vet Mankind, New Delhi, containing Buserelinacetate @ 4 mcg/mL) 2.5 mL, intramuscularly on day 9 and double fixed time inseminations at 12 and 24 hrs after the injection of GnRH.

The remaining 15 cows (Treatment II) were treated with Ovsynch+ CIDR protocol. This protocol includes insertion of CIDR intravaginally and injection GnRH 2.5 mL intramuscularly on day 0, injection PGF2 $\alpha$  2 mL intramuscularly on day 6, removal of CIDR implant on day 7, injection GnRH 2.5 mL intramuscularly on day 9 and double fixed time inseminations at 12 and 24 hrs after the injection of second GnRH.

The experimental crossbred cows were observed for the signs of oestrus at the time of AI. Based on the behavioural changes, physiological changes and gynaecological observations, the intensity of oestrus was classified as intense, intermediate and weak oestrus (Krishnakumar, 2001). The percentage of cows showing oestrus were estimated as the number cows exhibited the behavioural signs of oestrus after removal of CIDR. Onset of oestrus was calculated in hours from the time of removal of CIDR to the time of first

appearance of oestrous signs. Blood samples were collected from all the cows on day 9 at the time of first AI and were allowed to clot at room temperature. The serum was separated by centrifuging at 3000 rpm for 10 min and stored at -20°C for estimation of progesterone concentration. The serum progesterone level was estimated using solid-phase Radio Immuno Assay technique with the help of progesterone kits (Coat-A-Count, Diagnostic Products Corporation, USA). Pregnancy diagnosis was done by palpation per rectum at 45 days post-insemination. Pregnancy rate was calculated as percentage of animals that conceived following fixed time insemination at induced oestrus in each group. The data on oestrus response and conception rate were analysed by using one tailed Chi-squared test and plasma profiles of progesterone and biochemical constituents were analysed statistically by unpaired t-test (Snedecor and Cochran, 1994) using online SAS software version 20.00.

## RESULTS AND DISCUSSION

CIDR devices inserted intravaginally to the animals were retained until it was removed manually on day 7 of treatment, resulting in retention rate of 100 % in both the groups. All the animals have shown the signs of estrum (100 per cent). This agrees with the results of Khade *et al.* (2011) and Dhami *et al.* (2015) who reported 100 % oestrous response in anoestrus dairy cows following oestrus induction. However, Murugavel *et al.* (2010) reported 90 % oestrus response with crossbred anoestrus cows treated with

CIDR. This difference may be due to the different combination of hormonal protocol with CIDR in the study. The mean time to onset of oestrus from the time of removal of CIDR in Treatments I and II was  $43.45 \pm 9.21$  and  $45.81 \pm 6.34$  hr respectively. There is no significant difference between the two groups with respect to the onset of oestrus from the time of removal of CIDR. The result in the present study agrees with the findings of Murugavel *et al.* (2010) in anoestrus dairy cows.

The percentage of animals with weak, intermediate and intense estrum in Treatments I and II are tabulated in Table 1. The results show that majority of the animals have exhibited intermediate intensity of estrum following oestrus induction in both the treatment groups. Contradictory to the present findings, most of the previous studies have

shown that majority of the cows exhibited intense oestrus following CIDR treatment (Sathiamoorthy and Kathirchelvan, 2010). Low percentage of cows showing intense oestrus when compared to the previous studies could be due to delay in release of GnRH and low concentration of oestrogen hormone in plasma to express oestrus behaviour (Devipriya *et al.*, 2020). Moreover, the trial was carried out on rural cows maintained by the farmers under field condition. It has been established previously that cows in negative energy balance have lower levels of oestrogen and lower intensity of oestrus than cows in positive energy balance at the time of oestrus (Butler, 2003).

Serum progesterone concentration at the time of AI in Treatments I and II was  $0.92 \pm 0.26$  ng/mL and  $0.92 \pm 0.18$  ng/mL respectively. Though serum progesterone concentration at the time AI was similar

**Table 1. Comparison of intensity of oestrus between the treatments**

Intensity of oestrus	Treatment 1		Treatment 2	
	No. of animals	(%)	No. of animals	(%)
Weak	0	0	1	7
Intermediate	12	80	12	80
Intense	3	20	2	13
Total	15	100	15	100

Chi-square = 1.2, p = 0.549

Not significant

between the two treatment groups, the conception rate was 20 % (3/15) for Treatment I and 40 % (6/15) for Treatment II group. The conception rate was higher in Treatment II (40.00 %) compared to Treatment I (20.00 %), but the difference was not statistically significant, which may be due to small sample size. In Treatment II, the administration of GnRH along with the insertion of CIDR might have helped in the recruitment of a follicular wave (Rhodes *et al.*, 2003), which has facilitated the development of dominant follicle by the time of CIDR removal. This might have resulted in higher conception rate in Treatment II compared to Treatment I group. The overall conception rate in the present study was 30 % (9/30). Previous studies have shown higher conception rates following oestrus induction with CIDR ranging from 40.9 % (Arab, *et al.*, 2013) to 66 % (Khade *et al.*, 2011). In the present study, the progesterone concentration at the time of AI in both the treatment groups was in suprabasal level ( $\geq 0.35$  ng/mL). Low conception rate in the present study may be due to suprabasal level of progesterone at the time of AI. Duchens *et al.* (1995) reported the suprabasal progesterone level at the time of AI will delay the ovulation leading to retention of Graafian follicle for an extended period, causing damage of the oocyte and fertilization failure. Further, in the present study, majority of the cows showed intermediate intensity of oestrus (80%) in both the treatment groups which might be associated with reduced pre-ovulatory oestradiol concentration, delayed ovulation and poor oocyte quality compromising pregnancy rate (Cutullic *et al.*,

2009). The results of the present study indicate that Ovsynch + CIDR estrous induction protocol was found to be effective with respect to oestrus induction and conception rate following fixed timed insemination in postpartum anoestrus cows under field conditions.

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