



## Comparison of Calving and Conception Rate after Timed Artificial Insemination (AI) with Estrus Synchronization Protocols in Crossbred Cows under Field Conditions

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Received: 06 May, 2020

Revised: 14 July, 2020

Accepted: 19 July, 2020

### ABSTRACT

This study was performed in 48 cross bred cows to evaluate the comparative efficacy of different estrus synchronization protocols with fixed time artificial insemination. Animals were allocated into four groups i.e. Group I: (n=12, Two Injection Prostaglandin system) Cloprostenol Sodium 500 µgm on day 0 and day 14 and AI was done 48 and 72 hours from second dose. Group II: (n=12, Heat-synch) day 0 Buserelin acetate 20 µgm, day 7 Cloprostenol sodium 500 µgm, on Day 8, Estradiol benzoate (1 ml) and AI on day 10. Group III: (n=12, Ov-Synch) day 0 Bruserelin acetate 20 µgm, day 7 Cloprostenol sodium 500 µgm, Day 9 Buserelin acetate 20 µgm and A.I. on day 10. Group IV: (n=12, Ov-Synch plus Indigenous progesterone sponge) on day 0 Buserelin acetate 20 µgm along with insertion of progesterone sponge with 1.20 gm natural progesterone, day 7 Cloprostenol sodium 500 µgm and removal of progesterone sponge, day 9, Buserelin acetate 20 µgm and AI on day 10. Inj. Buserelin acetate 20 µgm was administered on day 6<sup>th</sup> from AI in Group II, III and IV. Pregnancy was detected per rectally on day 40 after AI. In present study, estrus ratio was higher in Group II (91.66%) followed by group IV and Group III. Conception rate was significantly higher in Group III (100%). The calving percentage was significantly lower in group I and highest in Group III (100%), the difference in conception and calving rate suggests the minimum embryonic losses in Group I, but no embryonic loss was recorded in Group III, IV and II.

### HIGHLIGHTS

- Estrus synchronization protocol was developed.
- Estrus ratio was higher in Group II (91.66%).

**Keywords:** Estrus synchronization, Fixed time AI, Ov-synch, Heat-synch, Indigenous progesterone sponge

Estrus detection and right time artificial insemination are the major limiting factors for reproductive performance in dairy farms. One management system that could potentially revolutionize reproductive management in dairy animals is the use of fixed time artificial insemination programs that eliminates the need for estrus detection (Kasimanickam *et al.*, 2005). It has been reported that even estrus detection rates in dairy cows are less than 50% and even rate of estrus detection is low with PGF<sub>2</sub>α-induced (natural or synthetic) luteolysis.

Gonadotrophin-releasing hormone (GnRH) and its

analogues causes emergence of new follicular wave either in combination with PGF<sub>2</sub>α (Pursley *et al.*, 1997a) or as part of a progesterone protocol (Kacar *et al.*, 2008). Several protocols with GnRH had been compared in past like Ov-synch with double injection of PGF<sub>2</sub>α and progestin based methods (Kacar *et al.*, 2008, Stevenson *et al.*, 2004; Cartmill *et al.*, 2001; Tenhagen *et al.*, 2004),

**How to cite this article:** Gupta, C. and Ramprabhu, R. (2020). Comparison of calving and conception rate after timed artificial insemination (AI) with estrus synchronization protocols in crossbred cows under field conditions. *J. Anim. Res.*, **10**(4): 525-529.

**Source of Support:** None; **Conflict of Interest:** None





Select synch, Heat synch (Pancarci *et al.*, 2002; Bartolome *et al.*, 2005), Pre synch (Pancarci *et al.*, 2002) and natural breeding (Pursley *et al.*, 1997b). Heat-synch protocol is substituting the GnRH on day 9 by Estradiol Benzoate on day 8 and fixed time A.I. on Day 10.

Progesterone based devices like Control Internal Drug Release and Progesterone Releasing Intravaginal Device, whereas Indigenous Progesterone Sponges were developed to reduce the cost of using commercially available CIDR. In India estrus synchronization using indigenous progesterone sponges (Visha *et al.*, 2014; Devipriya *et al.*, 2015) causes follicular development and ovulation.

It has been reported that by manipulating the ovarian cyclicity by controlling follicular and luteal dynamics, need of estrus detection can be eliminated by fixed time AI and for embryo transfer technique (Bartolome *et al.*, 2005; Martinez *et al.*, 2000). Ideally, a protocol has to be established in local Crossbred cows to synchronize the estrus and ovulation in both cyclic and non-cyclic animals with low embryonic mortality and high conception and calving rates.

As per Willard *et al.* (2003) injection GnRH on day 5 or 11 and Hansen (2002) 11 to 14 post AI increased serum Progesterone level which causes higher pregnancy rates. This treatment enhances embryo survival rates by delaying the luteolytic mechanism (Mann *et al.*, 1995) which occurs due to failed or delayed maternal recognition of pregnancy. Some reports suggest that GnRH agonist induce ovulation to form new (or accessory) Corpus Luteum which is contradictory, GnRH induces luteinization and (or) atresia of large follicles which enhances progesterone concentration.

This study was designed with an objective of evaluating the four estrus synchronization protocols (two injection Prostaglandin system, Heat-synch, Ov-Synch and Ov-Synch plus Indigenous progesterone sponge) on the basis of estrus ratio, conception rate and calving rate in crossbred cows.

## MATERIALS AND METHODS

The study was carried out in 48 Crossbred cows presented to Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli.

## Animals Selection

A total 48 number of Crossbred pleuriparous cyclic and non-cyclic animals under lactation period, 40 days postpartum to 400 days postpartum were selected for this study. Per rectal examination was performed two times in twenty days interval to avoid early pregnant animal and to confirm any anatomical deformity of genitalia and mineral mixture (TANUVAS Type II) @ 50gm orally daily was supplemented. Animals were randomly distributed to different treatment groups. Estrus detection was done on the basis of history of bellowing, frequent urination, standing while animal was mounted by other animals, decrease in milk yield as per reported by the owners. Clear copious cervico vaginal discharge from vagina and on rectal examination presence of toned uterus with dominant follicle on either ovary. Pregnancy diagnosis was done on day 40 post A.I. per rectally. The treatment protocols are tabulated in table 1.

## RESULTS AND DISCUSSION

In present study, estrus activity shown by two Injection Prostaglandin system group was minimum (41.6%) followed by Ov-synch group (66.66%) and Ov-Synch plus CIDR Indigenous progesterone sponge (75%). The estrus activity in Heat-synch group was significantly higher than other groups (Table 2). In this system estradiol synchronizes the ovulation of dominant follicle and distinctly increases behavioural signs of estrus. This result is in agreement with Çevik *et al.* (2010) and higher estrus ratio than that of Kasimanickam *et al.* (2005), Bartolome *et al.* (2005) and Pancarci *et al.* (2002).

Conception rate was 100% (12/12) in animals treated with Ov-Synch which is significantly higher than Ov-Synch plus CIDR Indigenous progesterone sponge (83.33%) and Heat-synch (83.33%) protocols. The conception rate with two Injection Prostaglandin system was lowest *i.e.* 33.33% (Table 2). Past reports suggest that two Injection Prostaglandin systems had lower conception rate than that of natural mating (Moody and Lauderdale, 1977) which is in agreement with present study. Day 0, GnRH injection causes follicular growth in cyclic animals and non-cyclic females. The PGF<sub>2</sub>α (day 7) causes luteolysis of CL followed by decrease in serum progesterone level. The second GnRH (day 9) induces ovulation of dominant follicles which was programmed by the first GnRH

**Table 1:** Protocols

Groups	Number of animals	Treatment Protocol
<b>Group I</b> Two Injection Prostaglandin system	<b>n=12</b>	Day 0: Inj. Cloprostenol Sodium, 500 µgm i/m Day 14: Inj. Cloprostenol Sodium, 500 µgm i/m Day 16: AI Day 17: AI
<b>Group II</b> Heat- synch	<b>n=12</b>	Day 0: Inj. Buserelin Acetate, 20 µgm i/m Day 7: Inj. Cloprostenol Sodium, 500 µgm i/m Day 8: Inj. Estradiol Benzoate, 1 ml /m Day 10: A.I. on early morning <b>Day 6<sup>th</sup> Post A.I.</b> Inj. Buserelin Acetate 20 µgm i/m
<b>Group III</b> Ov-Synch	<b>n=12</b>	Day 0 : Inj. Buserelin Acetate 20 µgm, i/m Day 7: Inj. Cloprostenol Sodium 500 µgm, i/m Day 9: Inj. Buserelin Acetate 20 µgm i/m Day 10: A.I. (16 hrs from second Buserelin injection) <b>Day 6<sup>th</sup> Post A.I.</b> Inj. Buserelin Acetate 20 µgm i/m
<b>Group IV</b> Ov-Synch plus Indigenous progesterone sponge	<b>n=12</b>	<b>Preparation of Indigenous sponge:</b> Spongy foam 8 cm thicknesses, 12 cm diameter and tied with a cotton string of 50 cm length. Sponges were sterilized by autoclaving method and were impregnated with 1.20 g natural progesterone (4-Pregnene-3, 20-dione, Sigma-Aldrich) in ethanolic solution. Day 0: Inj. Buserelin Acetate 20 µgm i/m and insertion of progesterone sponge Day 7: Inj. Cloprostenol Sodium 500 µgm i/m and removal of progesterone sponge, Day 9: Inj. Buserelin Acetate 20 µgm i/m Day 10: A.I. <b>Day 6<sup>th</sup> Post A.I.</b> Inj. Bruserelin Acetate 20 µgm i/m

**Table 2:** Reproductive Performance Indices

Estrus Synchronization Protocol	Total animals (n)	Estrus Ratio	Conception Rate	Calving Percentage
Two Injection Prostaglandin system	12	5/12 (41.6)	4/12(33.33)	3/12 (25%)
Heat- synch	12	11/12(91.66%)	10/12(83.33%)	10/12(83.33%)
Ov-Synch	12	8/12(66.66%)	12/12(100%)	12/12(100%)
Ov-synch plus CIDR Indigenous progesterone sponge	12	9/12(75.0%)	10/12(83.33%)	10/12(83.33%)

injection. As per Pursley *et al.* (1995) the conception rate with Ov-synch was reported as 45%. Cevik *et al.* (2010) reported 76.9 % conception rate with 1<sup>st</sup> AI and 100% conception rate with 2<sup>nd</sup> A.I. But in present study the conception rate with Ov-synch was 100% which could be due to controlled synchronization plan. In present study the conception rate with Heat-synch was 83.33% as in previous studies which was reported as 80% (Cevik *et al.*,

2010) in lactating dairy cows as Estradiol benzoate induces estrus, preovulatory LH surge followed by ovulation (Kacar *et al.*, 2008; Stevenson *et al.*, 2004; Pancarci *et al.*, 2002). In our study the conception rate with Ov-synch plus CIDR Indigenous progesterone sponge was higher than other studies (Cevik *et al.*, 2010; Chebel *et al.*, 2006; Lucy *et al.*, 2001). In this protocol Day 0, GnRH induces the emergence of follicular wave, followed by growth large



preovulatory follicle and GnRH on Day 9 synchronizes ovulation. This protocol is effective in both cyclic and non-cyclic cattle (Kim *et al.*, 2005).

In the present study the calving percentage was studied to access the embryonic mortality. Animals treated with Prostaglandin 4/12 (33.33%) conceived but 3/12 (25%) animals calved which indicates that after 40 days one conceptus was lost (Table 2). But in other all three groups conception rate and calving rate is equal which suggests that injection GnRH on day 6 of AI improves calving and conception rate. Dirandeh *et al.* (2014) has reported that Injection GnRH on Day 6 after AI improved reproductive performance in dairy cows under field conditions.

## CONCLUSION

This study concluded that Ov-synch, Heat-synch, and Ov-synch plus CIDR Indigenous progesterone sponge showed acceptable fertility rate with estrus synchronization and fixed time AI in Crossbred cows. This protocol reduces the labour charges in estrus detection and ensures ovulation. This study also advocates the use of Inj. GnRH on Day 6 post AI can considerably reduce the early embryonic mortality. So estrus synchronization with fixed time AI and post AI GnRH therapy can considerably improve the reproductive performances of the dairy cyclic and non-cyclic animals in field conditions.

## ACKNOWLEDGEMENTS

The authors are thankful to the Director of Clinics, TANUVAS and Dean, Veterinary College and Research Institute, Tirunelveli to support for carry out this study successfully.

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