Comparative efficacy of different estrus synchronisation protocols in postpartum acyclic crossbred cows

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Abstract
The present study is undertaken in 24 postpartum acyclic crossbred cows maintained under rural managemental conditions. The cows are randomly divided into three groups viz., Ovysynch, Ovysynch + PRID. PRID + PG and efficacy was recorded. The estrus response (per cent) is observed as 75.00, 100.00 and 87.50 with the mean duration of estrus (hours) as 50.62 ± 8.39, 68.00 ± 2.61 and 58.63 ± 9.36 for Group I, II and III, respectively. Intensity of estrus exhibited is higher in Group II (62.5%) followed by Group III (50.0%) & Group I (25.0%), respectively. The first service conception rate (per cent) is 50.00 in both Group I and III, whereas it is recorded lower in Group II (37.50). The overall conception rate (per cent) is highest in Group III (62.50) followed by Group I and Group II (50.00, each) with no significant difference (P>0.05). It is concluded that Ovysynch + PRID protocol resulted in better estrus exhibition, while PRID + PG protocol is effective in obtaining optimum conception rate with fixed time Artificial Insemination in postpartum acyclic cows.

Keywords: postpartum cows, estrus synchronisation, FTAI, conception rate

Introduction
Estrus synchronisation had been promoted as a tool to capitalise on the superior genetics available through Artificial Insemination. Body score condition was adopted as a measure of nutritional status of animals, which was an important factor that influenced the reproductive performance [5]. Manipulation of estrous cycle to bring large number of females into estrus at a predetermined time enhanced the reproductive efficiency by shortening the inter-calving period [7]. Ovysynch was a fixed time AI synchronization protocol that has been developed, tested and used extensively in dairy cattle as it allows acceptable synchrony of follicular development, regression of corpus luteum and synchronizes ovulation allowing effective fixed time AI with no need for heat detection with optimum pregnancy rates [31]. PGF2α was used in many synchronization protocols to shorten the life span of CL and induced estrus in postpartum acyclic cows [31]. Use of exogenous progestagen like PRID along with Ovysynch protocol induced fertile estrus, increased the pregnancy rates and decreased days open [4]. Perusal of literature revealed that limited studies were conducted to evaluate the efficacy of different estrus synchronisation protocols to synchronize estrus and ovulation in postpartum acyclic crossbred cows under rural conditions. Hence, the present study was undertaken to study the efficacy of different estrus synchronisation protocols.

Materials and Methods
Postpartum acyclic healthy crossbred cows being reared under different nutritional and managemental conditions located at rural areas of Tirupati were utilized for the present study. All the postpartum acyclic cows were subjected for two per rectal examinations consecutively at 10 days interval. Twenty-four cows with smooth inactive ovaries were selected and randomly divided into three groups with 8 animals in each group for estrus synchronisation adopting three protocols. Prior to treatment all the selected animals were administered with Albendazole 1.5g bolus (10-15 mg/kg body weight) orally and supplemented with mineral mixture @ 30-40 gm daily in feed.

Group I (Ovysynch)
The cows of this group were administered with 10µg of GnRH (Pregulate®) on day 0, 0.5mg of PGF2α (Pregova®) on day 7 and 10µg of GnRH (Pregulate®) on day 9 intramuscularly.
Fixed Timed AI (FTAI) was performed 16 to 24 hours after administration of second dose of GnRH injection.

**Group II (Ovsynch + PRID)**

The cows of this group were administered with 10µg of GnRH (Pregulate®) intramuscularly on day 0 after insertion of progesterone releasing intra-vaginal device- PRID (TRIU-B®). On day 7, the intra-vaginal device was removed and 0.5 mg of PGF₂α (Pregova®) was administered intramuscularly. On day 9, 10µg of GnRH (Pregulate®) was given intramuscularly and Fixed Timed AI was performed 16 to 24 hours after administration of second dose of GnRH.

**Group III (PRID + PG)**

In the cows of this group, PRID (TRIU-B®) was inserted into the vagina and kept in situ for 7 days. Single dose of 0.5 mg PGF₂α (Pregova®) was administered intramuscularly at removal of PRID on day 7 and 10µg of GnRH (Pregulate®) on day 9. FTAI was performed 16 to 24 hours after administration of second dose of GnRH. PREGOVA® (Cloprostenol Sodium) Virbac Animal Health India Pvt. Limited. PREGULATE® (Buserelin Acetate, GnRH analogue) Virbac Animal Health India Pvt. Limited. TRIU-B®

Serum samples were collected from all the cows using vaccutainers on day '0' (day of initiation of treatment) and at the time of AI which were stored at -20ºC for estimation of progesterone by ELISA based on commercial kit (Pathoyme progesterone, Omega diagnostic Ltd, Scotland, UK)

The estrus response, duration of estrus and intensity of estrus were recorded. Bellowing and restlessness, excitement, estrual mucus discharge, edema, moist vulva, placid response to placement of palm or hand on the rump, raising of tail, frequent micturition were considered as signs of estrus. These animals were subjected for pregnancy diagnosis after two months of performing AI and the conception rate was recorded as conception at first service. Among these, the animals that had not conceived and exhibited estrus signs in the subsequent cycle were subjected for second AI at observed estrus and the conception rate was recorded after two months as conception at second service.

**Results and Discussion**

The estrus response (per cent) observed in the Group I, II and III were 75.00, 100.00 and 87.50, respectively with no significant difference (P>0.05) among the groups (Table 1). Similarly, the highest response in Group II was recorded by Pratt et al. [17], which might be attributed to the progesterone priming which sensitized the endocrine axis to obtain better follicular development [3, 16]. The least response observed in the Group I might be due to the presence of a small sized less estrogenic dominant follicle [5] and might be due to variations in the stage of estrous cycle at the time of initiation of the protocol [25].

Intensity of estrus in postpartum acyclic crossbred cows of Group II exhibited intense estrus in 62.50 per cent followed by 50.00 per cent in Group III and 25.00 per cent in Group I. These results were in accordance with the studies of Alnimer and Lubbadeh [2] and Ravikumar et al. [18]. The highest intensity of estrus response in Group II might be due to the fact that progesterone pre-treatment in postpartum cows sensitized the receptors in the brain, which responded better to the ovarian estrogen by eliciting the psychic phenomenon associated with estrus [14].

The duration of estrus (hours) in postpartum acyclic crossbred cows was longest in Group II as 68.00 ± 2.61 (ranging from 56 to 72) followed by Group III as 58.63 ± 9.36 (ranging from 0 to 72) and Group I as 50.62 ± 8.39 (ranging from 0 to 72), respectively. The differences in duration of estrus among all groups was non-significant (P>0.05).

**Table 1: Effect of different synchronization protocols on estrus induction efficiency in postpartum acyclic crossbred cows**

| Parameter | Group I | Group II | Group III |
|-----------|---------|----------|-----------|
| Estrus response (%) | 75.00NS | 100.00NS | 87.50NS |
| Intensity of estrus (%) | | | |
| Weak (%) | 12.5 | 0.0 | 12.5 |
| Intermediate (%) | 62.5 | 37.5 | 37.5 |
| Intense (%) | 25.0 | 62.5 | 50.0 |
| Duration of estrus (hours) (Mean ± SE) | 50.62 ± 8.39NS | 68.00 ± 9.36NS | 58.63 ± 2.61NS |

NS: Non-significant (P>0.05)

The mean serum progesterone concentrations (ng/ml) on treatment initiation day i.e. on day 0 were 2.56 ± 1.04, 1.66 ± 0.24 and 1.43 ± 0.32 in Group I, Group II and Group III, respectively. Concentrations of the same at the time of AI in Group I, Group II and Group III were 7.60 ± 0.87, 1.86 ± 0.28 and 2.50 ± 0.65 ng/ml, respectively (Table 2). Highest mean progesterone concentration at AI was observed in Group I followed by Group III and Group II. There was no significant difference (P>0.05) in the serum progesterone concentrations recorded on day 0 and on the day of AI for all the three groups.

**Table 2: Serum progesterone concentrations**

| Groups | Serum progesterone concentration (ng/ml) |
|--------|----------------------------------------|
|        | Day '0' | At AI |
| Group I | 2.56 ± 1.04 | 7.60 ± 0.87 |
| Group II | 1.66 ± 0.24 | 1.86 ± 0.28 |
| Group III | 1.43 ± 0.32 | 2.50 ± 0.65 |
| t value | 2.516NS | 2.516NS |

NS: Non-significant (P>0.05)

The conception rate (per cent) at first service was 50.00, 37.50 and 50.00 in Group I, II and III, respectively whereas the conception rate (per cent) at second service was 20.00 and 25.00 in Group II and III, respectively. None of the cows of Group I had conceived at second service. Thus the overall conception rate including first and second services was 50.00, 62.50 and 50.00 in Group I, II and III, respectively whereas no significant difference (P=0.05) in the serum progesterone concentrations recorded on day 0 and on the day of AI for all the three groups.

**Table 3: Fertility response**

| Conception rate | Group I | Group II | Group III | Chi-square value |
|-----------------|---------|----------|-----------|------------------|
| First service   | 50.00 (4/8) | 37.50 (3/8) | 50.00 (4/8) | 0.336 NS |
| Second service  | 00.00 (0/4) | 20.00 (1/5) | 25.00 (1/4) | NS: Non-significant (P>0.05) |
| Overall conception rate | 50.00 (4/8) | 50.00 (4/8) | 62.50 (5/8) | 0.336 NS |

The highest conception rate at first service observed in Group I might be due to the reason that the first GnRH might have initiated the growth of a new wave of follicles [12, 22, 23] which
was followed by emergence and selection of a dominant follicle that became pre-ovulatory within 9 days of emergence and yielded a fertile ovum [19]. The higher level of serum progesterone at the time of AI might be suggestive of incomplete luteal regression following the administration of PGF$_2$α which resulted in only 50.00 per cent of conception rate in Group II cows of the present study [9, 10].

The improved and higher conception rate observed in Group III might be due to the synchrony between luteolysis, ovulation, fixed time AI and supplementation of progesterone through PRID [20, 21]. The increased concentrations of estradiol-17β beta in cows treated previously with progesterone causes the release of more LH and GnRH resulting in increased ovulatory response and in turn improved conception rates [22]. In addition supplementation of progesterone with GnRH would reduce the dominant follicle size at the time of PGF$_2$α treatment and improve the fertility, apparently to the reduced incidence of persistence of follicle [13]. The progesterone concentration of one estrous cycle affected the endometrial morphology in the subsequent cycle and maintained pregnancy [1].

Conclusion

It is concluded from the present study that supplementation of exogenous progestagens like PRID to Ovsynch protocol resulted in the better exhibition of estrus response in the acyclic crossbred cows whereas higher conception rates were observed in cows subjected to PRID + PG protocol. The PRID + PG protocol required one dose less of GnRH hormone which is economical to the farmers compared to other protocols. However, systematic studies on a larger sample of cows are warranted before drawing concrete conclusions. I am thankful to my guide (Dr. SriniVas Manda), Professors (Dr. MoulikriShka K, Dr. Srdui B) and colleagues who provided expertise that greatly assisted the research. Indebted to each and every one for the help and for sharing their wisdom all throughout the research.

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