



Effect of controlled internal drug releasing (CIDR) and Ovsynch protocol on estrus induction and conception rate in postpartum anestrus cows

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The reproductive performance of the dairy cows is influenced by a range of factors owing to the complex physiological mechanism that nature has bestowed upon. A prominent factor in this aspect is the prolonged postpartum anovulatory period (Peter *et al.* 2010, Kamal *et al.* 2014). It is a major factor affecting the reproductive performance of dairy cows in seasonally calving herds under management systems that are predominantly based on pasture (Xu *et al.* 2000). The prevalence of anovulation at the start of the breeding season is affected by calving date, age, breed, plane of nutrition, especially trace minerals, suckling and of course hormonal (Chang-Ho *et al.* 2001, Montiel and Ahuja 2005, Jena *et al.* 2016, 2017). Estrus synchronization and artificial insemination (AI) provide cattle producers with tools that enhance reproductive performance (Nash *et al.* 2012).

Progesterone is a key player in the estrus induction and potentiates the action of estrogen, which is aided by the regressing CL by secreting some amount of progesterone. Progesterone may decrease the number of hypothalamic estradiol-17 β receptors and thereby diminishes the potency of estradiol-17 β negative feedback. Treatment of such cows with progesterone diminishes the supportive effect of estradiol 17 β , enabling sufficient LH secretion to stimulate pre ovulatory follicular development (Wheaton *et al.* 2007) and the mechanisms of controlling the formation and involution of CL. It has been suggested that higher blood progesterone concentration during luteal phase preceding insemination increases conception rate in dairy cows by reducing uterine secretion of PGF₂ α (in response to oxytocin) during the late luteal phase after insemination (Kawate *et al.* 2004). Exogenous progestin is considered

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appropriate for non-cyclic anestrus postpartum cows (Hammam *et al.* 2013). When exogenous progesterone is used in the synchronization protocol, a desirable follicle is produced in the next cycle with a large CL producing more progesterone naturally; therefore the conception rate may increase (Xu *et al.* 2000). Exogenous progesterone has been shown to elicit an increase in LH pulse frequency in postpartum anestrus cows. The progesterone from the CIDR insert is sufficient to increase and maintain a progesterone concentration in the blood higher than 2.0 ng/ml in the absence of CL on the ovary. Blood progesterone level rises rapidly after insertion of controlled internal drug release (CIDR) and declined rapidly within 24 h after its removal (Hammam *et al.* 2013).

The progesterone can be given as feed supplement or as ear implant, as vaginal sponge or intra vaginal devices like controlled internal drug releasing (CIDR) devices for estrus synchronization and anestrus treatment. Recent studies have shown desirable results by using intravaginal devices as far as the better conception and estrus induction is concerned. Therefore, this experiment was designed to induce ovulatory estrus using intravaginal progesterone devices in Jersey crossbred cows.

The study was conducted in the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, situated at a geographical coordination of 20.2961°N, 85.8245°E.

The experiment was conducted in postpartum anestrus crossbred cows, presented at the Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar and cases attended at the mobile health coverage scheme, OUAT at the owner's residence in and around Bhubaneswar city. Those cows were owned by private cow owners and maintained by traditional husbandry practices. Lactating cows from first to fifth calver not exhibiting estrus for 90 days or more postpartum were selected for this study. Apparently, healthy cows not suffering from intercurrent and metabolic diseases were included.

The experimental procedures were conducted in

accordance with the guidelines laid down by the Institutional Ethical Committee.

The study was conducted on 30 postpartum anestrous crossbred Jersey cows following thorough gynaeco-clinical examination and divided into 3 equal groups. The animals of Gr 1 were administered on day 0 with an intra vaginal progesterone device (CIDR) and kept *in situ* for 7 days. Furthermore, on day 7, i.e. the day of CIDR removal, they received a dose of PGF₂α (500 µg) intramuscularly. Fixed time artificial insemination (FTAI) was done after 48 h at induced estrus. Individual estrus interval (h) was calculated on the basis of the time taken from the withdrawal of CIDR to exhibition of first estrus. Induced estrus period was calculated as the period of withdrawal of CIDR to exhibition of estrus. Cows assigned for Gr 2 were treated with Ovsynch protocol (GnRH-PGF₂α-GnRH combination). Accordingly, these animals received 20 µg of GnRH analogue (im), 7 days before and 48 h after a 500 µg PGF₂α analogue administration. Fixed time artificial insemination was done at 24 h after the second dose of GnRH injection. Animals in Gr 3 received only mineral mixture (100 g) daily for 15 days and considered as a control for comparison study.

Estrus detection subjected to various treatment protocols was done by visualization, external signs and symptoms and graded as in Table 1 (Layek *et al.* 2011). Estrus detection was performed 3 times a day by the farmers (in the morning, around noon and in the afternoon). All the experimental cows not exhibiting estrus between 45–60 days following insemination were subjected to pregnancy diagnosis by per rectal examination. Conception rate was calculated on the basis of first pregnancy.

Data generated were subjected to statistical analysis by one-way analysis of variance using statistical package for social sciences (SPSS; version 22). The difference between mean was tested using Duncan's multiple comparison test. Results were expressed as mean±SE. Conception rates were compared between the different groups after AI by Chi-Square test. A probability of P<0.05 was considered statistically significant.

The estrus expression and the various parameters of the estrus studied are enumerated in Table 2. Subsequent to the treatment regimens discussed above, the mean estrus induction interval (h) was 28.95±0.76 for the Gr 1 (CIDR

Table 2. Estrous characteristics of postpartum anestrous cows following different treatment regimen

| Estrual characteristic | Estrous interval (h) | Duration of estrous (h) | Estrous grading (1–4) |
|--|----------------------|-------------------------|-----------------------|
| Group 1 (CIDR + PGF ₂ α) (10) | 28.95±0.76 | 22.16±0.95 | 3.45±0.11 |
| Group 2 (Ovsynch) (10) | 74.50±0.70 | 18.36±0.55 | 3.28±0.14 |
| Group 3 (Control) (10) | 369.50±2.72 | 17.60±0.54 | – |
| 't' value | 2.13** | 3.53** | NS |

**P<0.01; NS, nonsignificant. Figures in parenthesis indicate number of animals.

Table 3. Conception rate of different experimental groups following treatment in postpartum anestrous cows

| Experimental group | Percentage of animals in heat and inseminated | No. of cows conceived | Conception rate (%) | Chi-square value |
|--|---|-----------------------|---------------------|--------------------|
| Group 1 (CIDR + PGF ₂ α) (10) | 100.00 (10) | 5 | 50.00 | 0.27 ^{NS} |
| Group 2 (Ovsynch) (10) | 60.00 (6) | 3 | 50.00 | |
| Group 3 (Control) (10) | 30.00 (3) | 1 | 33.33 | |

Figures in parenthesis indicate number of animals. NS, nonsignificant.

+ PGF₂α) whereas it was 74.50±0.70 for Gr 2 animals and the values differed significantly (P<0.01). However, it was observed that, the rate of estrus induction was quite long in the mineral mixture treated group (369.50±2.72). Similarly, the duration of estrus (h) was significantly higher (P<0.01) in Gr 1 (22.16±0.95), when compared with Gr 2 (18.36±0.55). As far as the Gr 3 cows was concerned, the duration estrus was 17.60±0.54 h. On the contrary, grading of estrus signs (1–4) between Gr 1 (3.45±0.11) and Gr 2 (3.28±0.14) did not reveal any significant difference following induction of estrus. In the control (Gr 3), cows exhibited estrus out of 10 animals on different days and the aforesaid parameters were not considered for statistical calculations.

Timed artificial insemination, pregnancy rates based on treatment and estrus response are shown in Table 3. Estrus manifestation was marked at 100% (10/10) in Gr 1 whereas 60% (6/10) and 30% (3/10) cows exhibited estrus in Gr 2 and 3 (control), respectively. Albeit, 50% conception rate to first insemination was established both in Gr 1 and Gr 2, the number of animals coming to estrus was more in Gr 1 than that in Gr 2 where 10 and 6 cows were inseminated from the respective groups. In the control Gr, 1 animal conceived (33.33%) out of 3 cows. Comparison of conception rate did not reveal any significant difference among various treatment protocols by chi-square analysis.

Table 1. Grading of estrus intensity based on the behavioural signs

| Score | Estrus characteristics |
|-------|---|
| 4 | Clear, rosy, vaginal discharge, frequent vocalization, motor movement and excitation. |
| 3 | All the characteristics of estrous with less intensity. |
| 2 | Clear vaginal discharge with no other behavioural symptoms. |
| 1 | None of the external characteristics of estrous is noticed except cervical relaxation and presence of follicle on per rectal examination. |

Synchronization of the bovine estrous cycle with exogenous hormones allows producers to increase financial profits by shortening breeding and calving seasons, resulting in increased calf age and uniformity at weaning (Nash *et al.* 2012). In recent times, several protocols and strategies have been exercised for estrus synchronization keeping various factors like cost effective, simple to follow, and efficacy in mind. Synchrony of estrus expression is largely dependent on the synchrony of ovarian follicular waves among a group of females undergoing estrus synchronization (Thomas *et al.* 2016, Spurlock *et al.* 2016). Many of the current estrus synchronization protocols uses a combination of exogenous hormones to regulate the estrous cycle. Prostaglandin (PGF) and GnRH agonists are used to regulate luteolysis and control the emergence of follicular waves and ovulation, respectively (Wheaton and Lamb 2007). The addition of exogenous progesterone in the form of controlled internal drug release CIDR devices to synchronization protocols has improved pregnancy rates after fixed time artificial insemination (FTAI) in cattle. It helps in induction of estrus and other estrual characteristics in a more pronounced way. In this study, we found that CIDR + PGF₂ α treated group had shorter induced estrus arousal and was significantly different ($P < 0.05$) when it is compared to other 2 groups, i.e. Ovsynch group and control group. Similarly, the duration of estrus was significantly ($P < 0.05$) longer for CIDR treated animals as compared to the other 2 groups. The estrus grading was also superior in CIDR treated cows. Shorter estrus intervals were also reported in CIDR treated cows compared to other methods of estrus induction (Ergene 2012, Kasimanickam *et al.* 2012). However, Martinez *et al.* (2000) found the duration of estrus to be 44.2 h after the removal of CIDR insert. The rebound phenomenon which occurs after removal of the implant, mediates the release of more estrogen and exhibition of estrus followed by ovulation as it stimulates the hypothalamus pituitary gonadal axis (Escalante *et al.* 2013). Overt estrus behaviour and longer duration of estrus in CIDR group (Long *et al.* 2010) is suggestive of optimum estrogen and progesterone balance which positively stimulates hypothalamus for initiating cyclic estrus (Jena *et al.* 2016). The progesterone concentration is also essential to avert situations like repeat breeding (Waldman *et al.* 2001). It has been established that in large and small ruminants, estrogen is the principal hormone for cyclic estrus behaviour and the effect is more pronounced if the reproductive system is earlier primed with progesterone (Roberts 1971, Hafez and Hafez 2000, Kim *et al.* 2005).

The conception rate consequent to the estrus induction in CIDR treated animals was better as compared to the other treatment regimen (Nash *et al.* 2012, Azevedo *et al.* 2014). The administration of progesterone seems to prevent premature luteolysis in cows that underwent insemination at fixed time (Bisinotto *et al.* 2015). Our finding showed that the first insemination to conception rate was similar (50%) both in Gr 1 and 2, when 10 and 6 cows were served by frozen semen respectively. However, Gr 3 recorded lowest

pregnancy rate (33.33%) when 1 cow conceived out of 3 animals following artificial breeding. Although the chi-square value for conception rate was not significant for various treatment groups, but Gr 1 showed a superior pregnancy rate compared to other groups. Furthermore, though the similar pregnancy rate was established in ovsynch group, but the estrus response was lower, suggesting therapeutic superiority of Gr 1 compared to other groups. This finding corroborates with the findings of Long *et al.* (2010), Ergene (2012), Nash *et al.* (2012), Azevedo *et al.* (2014), Hill *et al.* (2014), and Sakase *et al.* (2005). However, the slightly lower conception rate was reported in the CIDR treatment group by Mellieon *et al.* (2012), Bisinotto *et al.* (2015) and Werven *et al.* (2013), albeit they have few modifications in their protocols. Martinez *et al.* (2000) reported a pregnancy rate of 76% in the CIDR group in addition of estradiol benzoate and Geary *et al.* (1998) found a higher percentage of conception rates in Ovsynch protocol.

SUMMARY

The study was conducted in 30 postpartum anestrus Jersey crossbred cows to determine the effect of supplemental progesterone, administered through an intravaginal device (CIDR) and Ovsynch protocol on estrus characteristics and conception rate with an appropriate control group. Postpartum anestrus Jersey crossbred cows (30) were assigned randomly into 3 groups with an equal number of animals. The Gr 1 animals received an intravaginal progesterone device on day 0 and 2 ml of PGF₂ α on the day of CIDR removal (seventh day), Gr 2 cows were treated with ovsynch protocol (GnRH - PGF₂ α - GnRH) on day 0, 7 and 9, respectively, and the Gr 3 cows were supplemented with mineral mixture and treated as a control. The animals were served by frozen semen at a fixed time, 48 h after PGF₂ α injection in Gr 1 and 24 h after second GnRH injection in Gr 2, but at observed estrus in case of Gr 3 cows. The cows treated with CIDR exhibited overt type of estrus whereas the other 2 groups of animals exhibited less pronounced behavioural estrus characteristics. The conception rate consequent to treatment was similar (50%) for both the Gr 1 and 2, but Gr 3 recorded a lower conception rate of 33.33%. Hence it could be concluded that the use of CIDR in postpartum anestrus cows can improve the resumption of regular cyclicity and conception rate in a better percentage against other hormonal and conventional treatment.

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