

## Studies on the comparative efficacy of different analogues of prostaglandin F2 alpha for estrus synchronization in cows

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### ABSTRACT

In cattle reproduction, luteolysis is a crucial event that regulates the estrous cycle. Both endogenous and exogenous PGF<sub>2α</sub> induce luteolysis in most cows with a mature corpus luteum (CL). A study was conducted on 30 normal cycling cows (divided into three groups of 10 animals each) to evaluate the efficacy of cloprostenol, D-cloprostenol, and dinoprost tromethamine when administered intramuscularly. The investigation was carried out in nearby villages at farmers' doorstep from February 2023 to June 2024. In these groups, prostaglandins were administered eight days post-ovulation, and thereafter transrectal ultrasonography (TRUS) was done to assess the diameter and functionality of the CL using color Doppler. Ovarian examinations to monitor luteolysis and subsequent estrus were performed every 24 hours until ovulation. Cloprostenol resulted in a greater non-significant percentage reduction in CL size compared to D-cloprostenol and dinoprost. A significantly ( $P < 0.10$ ) larger final increase in follicle size was observed with cloprostenol compared to D-cloprostenol and dinoprost. The most substantial decrease in progesterone (P<sub>4</sub>) concentration and percentage was observed with D-cloprostenol, followed by cloprostenol and dinoprost. Progesterone levels in all groups reached basal concentrations after 96 hours. Among the treatments, cloprostenol achieved the highest conception rate (50%), followed by D-cloprostenol (40%), with the lowest conception rate recorded for dinoprost (30%).

**Keywords:** Cloprostenol, Cyclic cows, D-cloprostenol, Dinoprost, Estrus induction, PGF<sub>2α</sub> analogues

Livestock sector plays a vital role in the Indian economy and contributes significantly to the country's socio-economic development. Breeding efficiency and fertility of dairy animals are crucial factors influencing the economic performance of the dairy industry (Peter *et al.* 2009). Estrus synchronization or induction of the estrus implies the manipulation of the estrous cycle and is considered as one of the advanced management process by which human errors and animal management costs can be reduced. The selection of a right synchronization protocol is made on the basis of efficiency of the protocols, expectation of farmers, managerial capabilities, cost of the protocol etc. (Islam 2011). Two types of PGF<sub>2α</sub> products are commercially available in market, dinoprost tromethamine, a tromethamine salt of the natural PGF<sub>2α</sub> (biological half-life 7 to 8 minutes; Kindahl *et al.* 1976), and cloprostenol sodium, a synthetic analogue (biological half-life 3 hours; Reeves 1978) for their application in cattle reproduction. Dinoprost tromethamine is rapidly metabolized in a way that is identical to endogenous PGF<sub>2α</sub> metabolism (Bourne

*et al.* 1980, McCracken *et al.* 1999), which accounts for its short half-life (Kindahl *et al.* 1976).

Cloprostenol sodium is a potent PG analogue as 500 mcg of cloprostenol induced luteolysis while 25 mg of dinoprost is needed to make this effect (Dukes *et al.* 1974). The presence of a benzyl chlorine ring in cloprostenol confers increased resistance to endogenous metabolism (Bourne *et al.* 1980), resulting in a substantially prolonged half-life (Reeves 1978). Cloprostenol exhibits optical isomerism, and isomer D demonstrates fourfold greater potency than Isomer L due to its heightened receptor affinity and further, enables the administration of lower doses, resulting in enhanced efficacy and improved tolerance. Hence, D-cloprostenol functions as a luteolytic agent, inducing both functional and structural regression of the corpus luteum, ultimately leading to the onset of estrus (McCracken *et al.* 1999). So the planned study was proposed to envisage the efficacy of different analogues of PGF<sub>2α</sub> i.e. D-cloprostenol, cloprostenol and dinoprost tromethamine administered through intramuscular (IM) route, for estrus synchronization in cows.

### MATERIALS AND METHODS

The present experimental study was carried out in 30 cross bred cows, aged around 3-8 years, in 1<sup>st</sup> to 4<sup>th</sup> parity,

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presented to the Department of Veterinary Gynaecology and Obstetrics, Dr. G.C. Negi College of Veterinary and Animal Sciences, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya (CSKHPKV), Palampur (32.6°N, 76.3°E, and altitude 1290.8m) as well as in cows of neighboring villages, over a period from February 2023 to June 2024. All the cows were fed mixed ration as per seasonal availability with *ad libitum* supply of clean water. As all the cows were reared by nearby villagers so the managemental and feeding practices were almost same. The cows were divided into three subgroups, each with 10 cows, and were administered different analogues of PGF<sub>2α</sub> through intramuscular route: cloprostenol sodium at 500 mcg (n=10), D-cloprostenol at 150 mcg (n=10), and dinoprost tromethamine at 25 mg (n=10) (Table 1).

Table 1. Distribution of cows for intramuscular administration of PGF<sub>2α</sub> analogues

| Group            | PGF <sub>2α</sub>      | Dose           |
|------------------|------------------------|----------------|
| Group I (N=10)   | Cloprostenol sodium    | 500 mcg (2ml)  |
| Group II (N=10)  | D-cloprostenol         | 150 mcg (2 ml) |
| Group III (N=10) | Dinoprost tromethamine | 25 mg (5 ml)   |

Estrus response of treated cows was recorded in each case and all the cows were artificially inseminated at 72 and 96 hours of PGF<sub>2α</sub> injection.

Trans-rectal ultrasonography of the ovaries was initiated on day of prostaglandin administration and continued every 24 hours for 5 consecutive days to examine the development pattern of follicles, regression of CL and change in CL vascularity by using portable ultrasound machine (Mindray Z5; Vet Model 75L50EAV) with rectal transducer having frequency 5-10 MHz. The corpus luteum, per cent change in CL vascularity and the physiological state of the follicle(s) on both ovaries were assessed, and the pattern of follicular development was documented. Further analysis of these ovarian structures such as follicle and CL was done with ultrasonographic morphometry via inbuilt calliper in ultrasound machine. The diameter (millimetre) of CL and follicle was estimated by taking average of two longest perpendicular dimensions. The diameter of follicle was measured and blood supply to CL was evaluated using power Doppler mode. Blood samples were collected via jugular venipuncture following aseptic precautions. Approximately 5 ml of blood was drawn from each animal using an 18-gauge needle and heparinized disposable syringe, and then thoroughly mixed. Plasma was separated by centrifuging the blood at 3000 rpm for 10 minutes, and then stored at -20°C in 2 ml eppendorf tubes in duplicate for future analysis in a deep freezer. Progesterone (P<sub>4</sub>) estimation was performed by analyzing the plasma samples stored in the eppendorf tubes after thawing the plasma samples at room temperature. It was performed using ELISA kits (CALIBIOTECH Inc., Austin) with TECAN SUNRISE Microplate Absorbance Reader (TECAN Austria GmbH, Austria). The obtained

data was statistically analyzed using one-way Analysis of variance (ANOVA) and Student's t-test using Number cruncher statistical system (NCSS) 2024, USA.

Also the per cent change in CL, follicle size, progesterone concentration and vascularity was calculated by using following formulas.

$$\text{Per cent decrease in size of CL} = \frac{\text{Size of CL at 0 hour} - \text{Size of CL at time interval}}{\text{Size of CL at 0 hour}} \times 100$$

$$\text{Per cent increase in size of follicle} = \frac{\text{Size of follicle at 0 hour} - \text{Size of follicle at time interval}}{\text{Size of follicle at 0 hour}} \times 100$$

$$\text{Per cent decrease in progesterone concentration} = \frac{\text{Progesterone conc. at 0 hour} - \text{Progesterone conc. at time interval}}{\text{Progesterone conc. at 0 hour}} \times 100$$

$$\text{Vascularity of CL\%} = \frac{\text{Number of cubes with color Doppler signal on CL}}{\text{Number of cubes covering total CL}} \times 100$$

## RESULTS AND DISCUSSION

The perusal of Table 2 indicated that the administration of PGF<sub>2α</sub> via IM route, showed that cloprostenol was efficient in promoting luteolysis and reduction in progesterone concentration at conventional doses. A highest per cent decrease in CL size (-43.23%), increase in follicle size (57.84%), decrease in progesterone concentration (-94.5%) was seen with cloprostenol followed by D-cloprostenol and dinoprost. A similar abrupt decline in CL size and progesterone levels following treatment with 500 mcg IM cloprostenol in subestrous cattle was observed by Chauhan *et al.* (1986). Almost, 92 per cent heifers showed luteolysis when treated with 25 mg dinoprost by IM (Canizal *et al.* 1992). The present study was in concurrence with Fortune *et al.* (2001), who noted that administration of cloprostenol in heifers during luteal phase resulted in greater decline in progesterone concentration by 24 hours and increased the size of small and medium sized follicle and increased the estrogen concentration.

Martins *et al.* (2011) and Montaser and El-Desouky (2016) found that the administration of prostaglandin results in more increase in follicle size, probably due to more rapid decrease in progesterone concentration by 48 hour post treatment, which is in concurrence with present study where administration of cloprostenol after 48 hours, resulted in decrease of 89.7 per cent progesterone, thereby removing the block on follicle growth. Comparatively more decrease in progesterone concentration with cloprostenol by 48 hours resulted in better growth of follicle with cloprostenol (50.17%) than D-cloprostenol (42.91%) or dinoprost (41.03%), resulting in better conception of 50 per cent than 40 and 30 per cent with D-cloprostenol and dinoprost, respectively (Table 3). Comparable results of

Table 2. Comparative efficacy of intramuscular administration of different analogues of PGF<sub>2α</sub> on different parameters

| Time (Hrs) | Cloprostenol (n=10)<br>(500 mcg IM) |                          |                        |                    | D-cloprostenol (n=10)<br>(150 mcg IM) |                          |                         |                    | Dinoprost (n=10)<br>(25 mg IM) |                          |                        |                    |
|------------|-------------------------------------|--------------------------|------------------------|--------------------|---------------------------------------|--------------------------|-------------------------|--------------------|--------------------------------|--------------------------|------------------------|--------------------|
|            | CL Size (mm)                        | Follicle Size (mm)       | P4 Conc. (ng/ml)       | CL Vascularity (%) | CL Size (mm)                          | Follicle Size (mm)       | P4 Conc. (ng/ml)        | CL Vascularity (%) | CL Size (mm)                   | Follicle Size (mm)       | P4 Conc. (ng/ml)       | CL Vascularity (%) |
| 0          | 21.88±0.64 <sup>a</sup>             | 8.35±0.41 <sup>a</sup>   | 8.76±0.75 <sup>a</sup> | 16.18              | 18.22±0.92 <sup>a</sup>               | 7.76±0.49 <sup>a</sup>   | 9.25±1.10 <sup>a</sup>  | 17.28              | 18.70±0.88 <sup>a</sup>        | 7.53±0.51 <sup>a</sup>   | 6.20±3.13 <sup>a</sup> | 18.75              |
| 24         | 18.47±0.55 <sup>b</sup>             | 9.85±0.35 <sup>ab</sup>  | 1.76±0.18 <sup>b</sup> | 9.45               | 15.15±0.74 <sup>ab</sup>              | 8.91±0.49 <sup>ab</sup>  | 1.42±0.49 <sup>b</sup>  | 10.52              | 15.08±0.93 <sup>b</sup>        | 8.34±0.57 <sup>ab</sup>  | 1.66±0.98 <sup>b</sup> | 11.50              |
|            | (-15.58%)                           | (17.96%) <sup>x</sup>    | (-79.9%)               | (-41.59%)          | (-16.84%)                             | (14.81%) <sup>x</sup>    | (-84.6%)                | (-39.12%)          | (-19.35%)                      | (10.75%) <sup>x</sup>    | (-73.2%)               | (-38.66%)          |
| 48         | 15.64±0.68 <sup>c</sup>             | 11.12±0.49 <sup>bc</sup> | 0.90±0.08 <sup>b</sup> | 5.82               | 13.41±0.80 <sup>bc</sup>              | 10.22±0.39 <sup>bc</sup> | 1.25±0.30 <sup>bc</sup> | 6.02               | 14.03±0.88 <sup>bc</sup>       | 9.59±0.59 <sup>bc</sup>  | 0.75±0.28 <sup>b</sup> | 8.28               |
|            | (-28.51%)                           | (33.17%)                 | (-89.7%)               | (-64.02%)          | (-26.39%)                             | (31.70%)                 | (-86.5%)                | (-65.16%)          | (-24.97%)                      | (27.35%)                 | (-87.9%)               | (-55.84%)          |
| 72         | 14.08±0.65 <sup>cd</sup>            | 12.54±0.35 <sup>cd</sup> | 0.75±0.07 <sup>b</sup> | 2.18               | 11.89±0.75 <sup>bc</sup>              | 11.09±0.37 <sup>cd</sup> | 0.91±0.21 <sup>bc</sup> | 1.93               | 12.14±0.72 <sup>bc</sup>       | 10.62±0.69 <sup>bc</sup> | 0.61±0.24 <sup>b</sup> | 4.78               |
|            | (-35.64%)                           | (50.17%)                 | (-91.4%)               | (-86.52%)          | (34.74%)                              | (42.91%)                 | (-90.2%)                | (-88.83%)          | (-35.08%)                      | (41.03%)                 | (-90.2%)               | (-74.50%)          |
| 96         | 12.42±0.54 <sup>d</sup>             | 13.18±0.30 <sup>d</sup>  | 0.48±0.04 <sup>b</sup> | 0                  | 11.05±0.92 <sup>c</sup>               | 12.04±0.35 <sup>d</sup>  | 0.48±0.04 <sup>c</sup>  | 0                  | 10.76±0.77 <sup>c</sup>        | 11.43±0.63 <sup>c</sup>  | 0.41±0.19 <sup>b</sup> | 0                  |
|            | (-43.23%)                           | (57.84%) <sup>y</sup>    | (-94.5%)               |                    | (-39.35%)                             | (55.15%) <sup>y</sup>    | (-94.8%)                |                    | (-42.45%)                      | (51.79%) <sup>y</sup>    | (-93.4%)               |                    |

<sup>a-d</sup>Values with different superscripts within the column vary significantly (p<0.05)

<sup>x,y</sup> with different superscripts within the column vary significantly (p<0.10)

Figures within the parenthesis indicates per cent change in different parameters, values with positive signs shows per cent increase, values with negative signs indicates per decrease of parameters

Table 3. Conception Rate (%) of cows after intramuscular administration of prostaglandin analogues

| PGF <sub>2α</sub> analogue | Cows inseminated | Cows conceived | Conception Rate (%) |
|----------------------------|------------------|----------------|---------------------|
| Cloprostenol 500 mcg       | 10               | 5              | 50                  |
| D-cloprostenol 150 mcg     | 10               | 4              | 40                  |
| Dinoprost 25 mg            | 10               | 3              | 30                  |

declining the progesterone concentration below 1 ng/ml after 96 hours of intramuscular administration of 500 mcg cloprostenol were also recorded by Godfrey *et al.* (1989) and Chauhan *et al.* (1986).

However, contradictory results were recorded by Stevenson and Pathak (2010), where it was found that with dinoprost and cloprostenol administration, the rate of decline in progesterone concentrations didn't differ significantly. However, comparable results were observed by Martins *et al.* (2011) which related to faster decrease in progesterone concentration as found in our study after administration of synthetic prostaglandin analogues (cloprostenol; -79.9% and D-cloprostenol; -84.6%) than natural (dinoprost; -73.2%) prostaglandins at 24 hours of administration. Hansel and Schechter (1972) found similar decline in plasma progesterone concentration in cows to basal levels after 96 hours of administration of 500 mcg of cloprostenol sodium by intramuscular route.

Our results of conception with dinoprost were in accordance with Repasi *et al.* (2003) who recorded a conception rate of 31.6 per cent in cows (n=20) when 25 mg dinoprost was injected intramuscularly. However, a high conception (52.5%) was recorded by Sawale and Markandeya (2019) which was almost two times higher than the present conception rate. The conception rates in between groups (cloprostenol vs dinoprost group; 50% vs 30%) were different which are not in concurrence with the results recorded by Cairoli *et al.* (2006), who recorded no changes in conception rate between cloprostenol and dinoprost groups. The conception rates for cows treated with dinoprost (25 mg IM) and cloprostenol (500 mcg IM.) according to Martineau (2003) were 33.7 and 41.8 per cent, respectively which were almost comparable to conception rates with dinoprost and cloprostenol in current study.

Luteal blood flow (LBF) from corpora lutea (CL) on days prior to treatment (day -1), as well as 2 to 4 days post-treatment. Color Doppler ultrasonography was used to assess each CL. For analysis, the images exhibiting the maximum luteal blood flow, the largest CL diameter, and minimal artifacts were selected to determine the colored pixel area. The LBF area was quantified as the colored pixel area, following the methodology outlined by Minela and Pursley (2021).

For cyclic dairy cows, Shirasuna *et al.* (2004) reported an increase of LBF on Days 17 and 18 prior to the onset of spontaneous luteolysis and a subsequent decrease of P<sub>4</sub> 1

day later. The authors suggested that PGF<sub>2α</sub> release by the uterus stimulates nitric oxide production in the arterioles of the peripheral vasculature of the mature CL, thereby causing vasodilation and triggering the luteolytic cascade leading to the decrease of P<sub>4</sub>.

Similar to our findings as above, Sedo *et al.* (2022) find similar observation when heifers are treated with two different analogues (Cloprostenol and Dinoprost tromethamine) at standard doses of PGF<sub>2α</sub>.

In all synthetic PGF<sub>2α</sub>, cloprostenol and D-cloprostenol were found almost equally efficacious and comparatively more potent than dinoprost as it reduced more concentration of progesterone, increase follicle size and better conception results.

The study showed that synthetic prostaglandins were more potent and efficacious than natural analogues for estrus synchronization in cows as they reduced blood circulation to CL and progesterone concentration immediately after 24 hours of their administration.

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