

Effect of Dinoprost Tromethamine, Cloprostenol and d-Cloprostenol on Progesterone Concentration and Pregnancy in Dairy Cattle

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Abstract: *This study was aimed to evaluate the effect of different PGF2 α analogues dinoprosttromethamine, cloprostenol and d-cloprostenol on P4 concentration, days to estrus and pregnancy in dairy cattle. The present work was carried out on 30 apparently healthy dairy cows having functional CL. The animals were divided into three groups according to treatment. Lutalyse group (n=10): each animal received 25 mg dinoprosttromethamine IM. PGF Veyx[®] forte group (n=10): each animal received 500 μ g cloprostenol IM. Luteosyl group (n=10): each animal received 150 μ g d-cloprostenol IM. Blood samples were collected from all animals at day 0 and 2 days after treatment for P4 concentration. There was a significant variation between P4 at day 0 and 2 days after treatment. A significant variation was present in P4 concentration between Luteosyl group and other two groups. The rate of decline in P4 concentration in luteosyl group was significantly higher than other two groups. Pregnancy rate was 10%, 30% and 40% in lutalyse, PGF Veyx and luteosyl group respectively. In conclusion, D-cloprostenol sodium induced a greater decrease in serum P4 concentrations 2 days following treatment compared with dinoprosttromethamine and cloprostenol. Also, it increased pregnancy rate than other PGF2 α .*

Key Words: *Dinoprosttromethamine; cloprostenol; d-cloprostenol; P4 and dairy cattle.*

I. Introduction

Luteolysis is a key event in cattle reproduction. It is well known that prostaglandin F2 α regulates corpus luteum function which lead to luteolysis. PGF2 α promote uterine involution [1] and induce ovulation [2]. Also it has been described as a powerful stimulant for contractility of uterus and oviducts which enhance sperm transport.

Injection of exogenous PGF2 α leads to release of hypophysial LH [3]. Thus, PGF2 α and its synthetic analogues are widely used in cattle reproduction in induction of parturition or abortion, estrous synchronization, and treatment for reproductive disorders such as pyometra, endometritis and ovarian cysts [4].

After ovulation, the corpus luteum (CL) formed in the ovary which act as a temporary gland secreting progesterone (P4) which is necessary for maintaining pregnancy and regulation of estrous cycle [5]. CL regression occurs in absence of pregnancy after release of 4-8 endometrial pulses of PGF2 α in 6-14 hrs [6, 7, 8].

Insufficient luteolysis is a rate-limiting factor for successful pregnancy per AI (P/AI) following Ovsynch [9,10]. The time needed for P4 to reach the basal level following PGF2 α may play an important role in the probability of pregnancy after Ovsynch. Cows having complete luteolysis with rapid decrease in circulating P4 became more fertile than other cows with slower decline in P4 levels [10].

Two types of PGF2 α products are commercially available, dinoprosttromethamine, a tromethamine salt of the natural PGF2 α , and cloprostenol sodium, a synthetic analog. Dinoprosttromethamine has a short -half-life (7 to 8 min) [6] as it is rapidly metabolized in a similar manner to endogenous PGF2 α metabolism [11,12]. Cloprostenol sodium is found to be more potent synthetic analog of PGF2 α as 0.5 mg of cloprostenol induced luteolysis while 25 mg of dinoprost was needed to make this effect [13]. Cloprostenol has a benzyl chlorine ring that makes this molecule more resistant to endogenous metabolism compared with dinoprost [11]; so, it is more resistant to endogenous metabolism and has a much longer half-life (approximately 3 h) [14] compared with dinoprost. Cloprostenol has optic isometry, that is, that they are compounds with the same molecular formula, but different structure form, and therefore, different properties, D and L of these compounds. Isomer D is four times more powerful than the isomer L, because it has a higher affinity by the receptor, this allows to use lower doses, achieving a higher efficiency and a better tolerance. Therefore, the D-cloprostenol acts as a luteolytic agent, which causes functional and structural regression of the corpus luteum, followed by the manifestation of estrus [12].

During CL treatment, peripheral concentrations of estradiol increased 48 hrs after treatment with cloprostenol compared to dinoprost in cows with a functional dominant follicle. This was attributed to a slightly more rapid decrease in progesterone level during the first 12 hrs after treatment [15].

The objectives of this study were to determine the effect of dinoprosttromethamine, cloprostenol, and d-cloprostenol on luteolysis, percentage of cows detected in estrus and pregnancy rate in dairy cows.

II. Materials and Methods

This study was carried out through the period from July to November 2015 at Al Akhween dairy farm in Bilqas, Dakahlia Province, Egypt.

Experimental animals

Animals used in this study were clinically normal with a good health condition. These animals were free from external, internal and blood parasites. Periodical vaccination against the common infectious diseases was carried out. These animals were housed in open yard under shed and milked in parlor three times per day with average milk production 8000 to 10500 kilograms per lactation (305 day). Animals were fed with TMR based on corn silage, ground yellow corn, soybean meal, corn gluten meal, barseem or hay and minerals-vitamins supplementations according to (NRC 2001).

Animals grouping and treatment

Thirty Holstein dairy cows, 3 to 8 years old with 1- 5 lactations, 167.33 ± 49.41 DIM and 3-4 BCS with mature functional CL were used in this study. Rectal palpation of both ovaries was carried out according to [16] and [17]. Also animals were submitted to ultrasonographic examination according to [18] for detection of functional corpus luteum and divided into three groups according to treatment protocol as follow:

Lutalyse group (n=10): each animal received 25 mg dinoprosttromethamine IM (Lutalyse, Pfizer Animal Health, NewYork, NY, USA).

PGF Veyx® forte group (n=10): each animal received 500 µg cloprostenol IM (PGF Veyx® forte, VeyxPharma GmbH - Germany).

Luteosyl group (n=10): each animal received 150 µg d- cloprostenol IM (Luteosyl, syva laboratories, Spain).

Blood sampling

Blood samples (10 ml each), were collected from all animals by direct vein puncture of the jugular vein at the day of treatment and 2 days after treatment. Samples were centrifuged at 3000 rpm for 15 minutes at 4 °C. Serum samples were frozen at -20 °C, for measurement of progesterone. Ultrasonic examination of ovaries was carried out at day of heat to measure the diameter of ovulatory follicles. Pregnancy diagnosis was done at 25 days' post insemination.

Statistical analysis

P4 concentrations in d0 and d2 in the same group was analyzed using paired sample T test. In order to compare different variables between different groups, ANOVA was used followed by LSD as a post-hoc test. Data are presented as mean ± SEM. Difference between data was considered significant when $P \leq 0.05$.

III. Results

P4 concentration

Data presented in **table 1** showed that animals in all groups had high serum P4 concentrations prior to PGF2α treatment. There was a significant variation in P4 concentration before and 2 days after treatment in all groups. Two days after injection of PGF2α, P4 concentration showed a significant variation between Luteosyl group and other two groups. There was no significant difference in P4 concentration between Lutalyse group and PGF Veyx ® forte group 2 days after treatment. There is no significant variation between groups in days to estrus.

Table1. Effect of treatment with dinoprost, cloprostenol and d-cloprostenol on P4 concentration at day0 and day2 and estrus incidence.

| Group | P4 concentration (ng/ml) | | Estrus incidence |
|------------------|--------------------------|------------------|------------------|
| | Day 0 | Day 2 | |
| Lutalyse group | $11.70 \pm 0.99a$ | $5.35 \pm 1.53b$ | 100% |
| PGF Veyx ® forte | $9.53 \pm 0.38a$ | $4.15 \pm 0.57b$ | 100% |
| Luteosyl group | $7.55 \pm 0.48a$ | $5.80 \pm 0.28c$ | 100% |

Different subscripts within the same raw indicate significant difference (a-b; $P < 0.01$ and a-c; < 0.05) between P4 concentrations in day 0 and day 2 in the same treatment group using paired sample T test.

Rate of decline in P4 concentrations

Regarding the rate of decline in P4 concentrations two days after treatment, **fig.1** presented a significantly higher decline of P4 in Luteosyl group than Lutalyse and PGF Veyx group. There was no significant variation between lutalyse and PGF Veyx group in the rate of decline in P4 concentration.

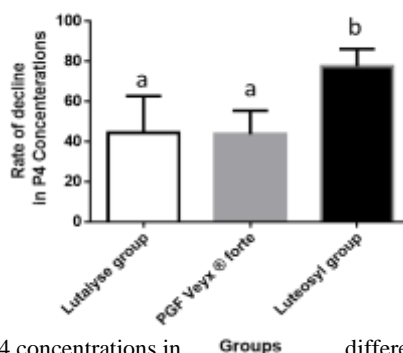


Figure 1: Rate of decline in P4 concentrations in different groups. Different subscripts indicate significant difference (a-b; $P < 0.01$) between rate of decline in P4 concentrations between different groups

Heat detection

All treated animals in all treatment groups were come in estrus with incidence rate of 100% (**table 1**). Concerning the effect of PGF2α on heat incidence, table 2 showed that the average days to heat 3.7 ± 0.26 , 3.3 ± 0.21 and 3.6 ± 0.31 for Lutalyse group, PGF Veyx forte and Luteosyl group respectively without significant variation.

Effect of PGF2α on follicular size

Injection of Dinoprost, cloprostenol and d-cloprostenol resulted in average follicular size 11.17 ± 0.433 , 11.53 ± 0.33 and 15.5 ± 0.82 respectively with a significant difference between Luteosyl group and other two treatment groups (**table 2**).

Pregnancy rate

The effect of PGF2 α on pregnancy was presented in table 2. Pregnancy rate was 10%, 30% and 40% in Lutalyse, PGF Veyx forte and Luteosyl group respectively without significant variation.

Table2. Effect of treatment with dinoprost, cloprostenol and d-cloprostenol on P4 concentration at day0 and day2, days to heat and 1st insemination pregnancy rate (%)

| Group | Follicular size (mm) | Days to heat | 1st insemination pregnancy rate (%) |
|------------------|----------------------|----------------|-------------------------------------|
| Lutalyse group | 11.17 \pm 0.433a | 3.7 \pm 0.26 | 10 |
| PGF Veyx ® forte | 11.53 \pm 0.33a | 3.3 \pm 0.21 | 30 |
| Luteosyl group | 15.5 \pm 0.82c | 3.6 \pm 0.31 | 40 |

Different subscripts within the same raw indicate significant difference (a-c; P < 0.001) between follicular sizes in different treatment groups.

IV. Discussion

Results of the present study revealed a significant decrease in P4 levels two days after injection of PGF2 α in all groups. Compared with dinoprosttromethamine, cloprostenol sodium did not enhance the decrease in serum concentrations of P4 after the injection of PGF2 α . Moreover, injection of d-cloprostenol enhanced the decrease in P4 significantly than other 2 groups. Several studies have compared cloprostenol sodium and dinoprosttromethamine in cattle [19, 20, 21]. They reported that, the effects of these PGF2 α analogs on luteolysis, estrous response and pregnancy rate were inconsistent and demonstrated an increase in estrus expression in cattle treated with cloprostenol compared to dinoprost. A similar response was reported [22] in P4 concentrations in dairy heifers. This may be due to an acute increase in oxytocin. Studies indicated that PGF2 α injection acutely stimulated the release of oxytocin in vivo [23, 24] approximately 10-fold within 15min.

All animals in different groups of treatment showed estrus after PGF2 α injection (100%) with no significant variation in days to estrus between groups. A slight difference was showed [25] between dinoprosttromethamine and d,l-cloprostenol in induction of full luteolysis in lactating dairy cows (91.3 and 86.6%, respectively). This may be attributed to administration of luteolytic agents as part of a standard Ovsynch protocol (CL ages between 132 and144 h) early in diestrus.

The natural resistance of CL to exogenously administered luteolytic agents early in diestrus has been widely studied in ruminants [26, 27, 28, 29, 30, 31, and 32]. This is may be due to the reduced availability of endothelin-1 [30] and increased level of prostaglandin dehydrogenase [32] in early CL compared with mature CL. Endothelin-1 is a proteinaceous vasoconstrictor agents and steroidogenic cell modulator produced from endothelial cells after injection of PGF2 α that alters progesterone production in cattle [33], whereas prostaglandin dehydrogenase metabolizes PGF2 α to its inactive form, 15-keto-PGF2 α in ewes [32].

The rate of decline in progesterone concentrations in lutalyse and PGF Veyx group did not differ. These results were in agreement with the results obtained by [25]. Moreover, the pregnancy rates of cows inseminated at first service did not significantly differ, regardless of which luteolytic product was applied.

It was proposed that exogenously injected PGF2 α [21] may affect the size of both large luteal cells and luteal capillary cells without any effect on small luteal cells. They believed the initial decrease in P4 concentration may be due to temporary degeneration in the endothelium of luteal capillaries. After that, small luteal cells become able to respond to LH with an elevation in P4 secretion. It was found that small luteal cells are responsible for about 30% of the total P4 secreted by the CL [34, 35], which still secreting progesterone which lead to incomplete luteolysis during this time.

The average size of ovulatory follicular in Luteosl group was 15.5 \pm 0.82 which is significantly different with the other two treatment groups. The increased diameter of follicle may help in estradiol production by the follicle as presented by [15] that circulating estradiol concentrations were elevated 48 h after treatment with cloprostenol compared to dinoprost in cows with preovulatory follicles. This may be due to more rapid decrease in P4 concentration during the 48hrs post-treatment in cows given d-cloprostenol versus dinoprost or cloprostenol.

In the present study, pregnancy rate was 40% in luteosyl group which is higher than other two groups without significant variation. A pregnancy rate 14.4% and 12.2% was obtained [36] after injection of cloprostenol and dinoprost respectively. The increased pregnancy rate after injection of d-cloprostenol may be due to faster decrease in progesterone which intum increased estradiol production by dominant follicles and subsequently improve future fertility.

V. Conclusions

D-cloprostenol sodium induced a greater decrease in serum P4 concentrations 2 daysfollowing treatment compared with dinoprosttromethamine and cloprostenol. Also, it increased pregnancy rate than other PGF2

References

- [1]. Lindell J-O, Kindhal H, Jansson L, Edquist L-E. (1982): Postpartum release of prostaglandin F2a and uterine involution in the cow. *Theriogenology*;17:237–45.
- [2]. Armstrong DT. (1981): Prostaglandins and follicular functions. *J ReprodFertil*; 62:283–91.
- [3]. Hanzen C. (1984): The role of prostaglandins in human and animal reproductive physiology. *J GynecolObstetBiolReprod*; 13:351–61.
- [4]. Wenzel JGW. (1991): A review of prostaglandin F products and their use in dairy reproductive herd health programs. *Vet Bull*; 61: 433–47.
- [5]. Niswender, G. D., Juengel, J. L., Silva, P. J., Rollyson, M. K., McIntush, E.W., (2000): Mechanisms controlling the function and live span of the corpus luteum. *Physiol.Rev.*80, 1–29.

- [6]. Kindahl, H., Edquist, L. E., Bane, A., Granström, E., (1976): Blood levels of progesterone and 15-keto-13, 14 dihydro-prostaglandin F₂ during the normal oestrus cycle and early pregnancy in heifers. *Acta Endocrinol.* 82, 134–149.
- [7]. Silvia, W.J., Lewis, G. S., McCracken, J. A., Thatcher, W. W., Wilson Jr., L., (1991): Hormonal regulation of uterine secretion of prostaglandin F₂ alpha during luteolysis in ruminants. *Biol. Reprod.* 45, 655–663.
- [8]. Mann, G. E., Lamming, G. E., (2006): Timing of prostaglandin F₂ release episodes and oxytocin receptor development during luteolysis in the cow. *Anim. Reprod. Sci.* 93, 328–336.
- [9]. Souza, A. H., A. Gumen, E. P. Silva, A. P. Cunha, J. N. Guenther, C. M. Peto, D. Z. Caraviello, and M. C. Wiltbank. (2007): Supplementation with estradiol-17beta before the last gonadotropin-releasing hormone injection of the Ovsynch protocol in lactating dairy cows. *J. Dairy Sci.* 90:4623–4634.
- [10]. Brusveen, D. J., A. H. Souza, and M. C. Wiltbank. (2009): Effects of additional prostaglandin F₂alpha and estradiol-17beta during Ovsynch in lactating dairy cows. *J. Dairy Sci.* 92:1412–1422.
- [11]. Bourne, G. R., S. R. Moss, P. J. Phillips, and B. Shuker. (1980): The metabolic fate of the synthetic prostaglandin cloprostenol ('Estrumate') in the cow: Use of ion cluster techniques to facilitate metabolite identification. *Biomed. Mass Spectrom.* 7:226–230.
- [12]. McCracken, J. A., E. E. Custer, and J. C. Lamsa. (1999): Luteolysis: A neuroendocrine-mediated event. *Physiol. Rev.* 79:263–323.
- [13]. Dukes, M., W. Russell, and A. L. Walpole. (1974): Potent luteolytic agents related to prostaglandin F₂alpha. *Nature* 250:330–331.
- [14]. Reeves, P. R. (1978): Distribution, elimination, and residue studies in the cow with the synthetic prostaglandin estrumate. *J. Agric. Food Chem.* 26:152–155.
- [15]. Martins J. P., Policelli R. K., and Pursley J. R. (2011): Luteolytic effects of cloprostenol sodium in lactating dairy cows treated with G6G/Ovsynch. *J Dairy Sci*; 94:2806 –14.
- [16]. Roberts SJ. 1986. *Veterinary Obstetrics and Genital Diseases*. 3rd ed. Ithaca, NY: Ed. SJ Roberts.
- [17]. Noakes D. E., Parkinson T. J., England G. C. W., and Arthur GH. (2001): *Veterinary Reproduction and Obstetrics*. 8th ed. Philadelphia: WB Saunders.
- [18]. Pierson R. A., and Ginther O. J. (1988): Ultrasonic imaging of the ovaries and uterus in cattle. *Theriogenology*; 29:21–37.
- [19]. Seguín, B., H. Momont, and L. Baumann. (1985): Cloprostenol and dinoprost tromethamine in experimental and field trials treating unobserved estrus in dairy cows. *Bovine Pract.* 20:85–90.
- [20]. Martineau, R. (2003): Dinoprost versus cloprostenol: Does route of injection modulate their efficacy in dairy cattle? *Bovine Pract.* 37:10–19.
- [21]. Répási, A., J. Beckers, J. Sulon, A. Karen, J. Reiczigel, and O. Szenci. (2005): Effect of the type and number of prostaglandin treatments on corpus luteum, the largest follicle and progesterone concentration in dairy cows. *Reprod. Domest. Anim.* 40:436–442.
- [22]. Ginther, O. J., L. A. Silva, R. R. Araujo, and M. A. Beg. (2007): Temporal associations among pulses of 13,14-dihydro-15-keto-PGF₂alpha, luteal blood flow, and luteolysis in cattle. *Biol. Reprod.* 76:506–513.
- [23]. Shaw, D. W., and J. H. Britt. (2000): In vivo oxytocin release from microdialyzed bovine corpora lutea during spontaneous and prostaglandin-induced regression. *Biol. Reprod.* 62:726–730.
- [24]. Keator, C. S., D. T. Schreiber, T. A. Hoagland, J. A. McCracken, and R. A. Milvae. (2008): Intrauterine infusion of BQ-610, an endothelin type A receptor antagonist, delays luteolysis in dairy heifers. *Domest. Anim. Endocrinol.* 34:411–418.
- [25]. Stevenson, J. S., and A. P. Phatak. (2010): Rate of luteolysis and pregnancy in dairy cows after treatment with cloprostenol or dinoprost. *Theriogenology* 73:1127–1138.
- [26]. Tsai, S. J., and M. C. Wiltbank. (1997): Prostaglandin F₂alpha induces expression of prostaglandin G/H synthase-2 in the ovine corpus luteum: A potential positive feedback loop during luteolysis. *Biol. Reprod.* 57:1016–1022.
- [27]. Tsai, S. J., and M. C. Wiltbank. (1998): Prostaglandin F₂alpha regulates distinct physiological changes in early and mid-cycle bovine corpora lutea. *Biol. Reprod.* 58:346–352.
- [28]. Skarzynski, D. J., and K. Okuda. (1999): Sensitivity of bovine corpora lutea to prostaglandin F₂alpha is dependent on progesterone, oxytocin and prostaglandins. *Biol. Reprod.* 60:1292–1298.
- [29]. Mamluk, R., N. Levy, B. Rueda, J. S. Davis, and R. Meidan. (1999): Characterization and regulation of type A endothelin receptor gene expression in bovine luteal cell types. *Endocrinology* 140:2110–2116.
- [30]. Levy, N., S. Kobayashi, Z. Roth, D. Wolfenson, and A. Miyamoto. (2000): Administration of prostaglandin F₂alpha during the early bovine luteal phase does not alter the expression of ET-1 and its Type A receptor: A possible cause for corpus luteum refractoriness. *Biol. Reprod.* 63:377–382.
- [31]. Sayre, B. L., R. Taft, E. K. Inskeep, and J. Killefer. (2000): Increased expression of insulin-like growth factor binding protein-1 during induced regression of bovine corpora lutea. *Biol. Reprod.* 63:21–29.
- [32]. Silva, P. J., J. L. Juengel, M. K. Rollyson, and G. D. Niswender. (2000): Prostaglandin metabolism in the ovine corpus luteum: Catabolism of prostaglandin F₂alpha (PGF₂alpha) coincides with resistance of the corpus luteum to PGF₂alpha. *Biol. Reprod.* 63:1229–1236.
- [33]. Girsh, E., W. Wang, R. Mamluk, F. Arditi, A. Friedman, and R. A. Milvae. (1996): Regulation of the endothelin-1 expression in the bovine corpus luteum: Elevation by prostaglandin F₂alpha. *Endocrinology* 137:5191–5196.
- [34]. Farin, C. E., H. R. Sawyer, and G. D. Niswender. 1989. Analysis of cell types in the corpus luteum of the sheep. *J. Reprod. Fertil. Suppl.* 37:181–187.
- [35]. Weems, C. W., Y. S. Weems, and R. D. Randel. 2006. Prostaglandins and reproduction in female farm animals. *Vet. J.* 171:206–228.
- [36]. Pursley, J.R., Martins, J.P.N. Wright, C., and Stewart, N.D. (2012): Compared to dinoprost tromethamine, cloprostenol sodium increased rates of estrus detection, conception and pregnancy in lactating dairy cows on a large commercial dairy *Theriogenology* 78 823–829