Cloprostenol and Dinoprost Tromethamine in Experimental and Field Trials Treating Unobserved Estrus in Dairy Cows

Brad Seguin, *D.V.M., Ph.D., Diplomate-ACT*
Harry Momont, *D.V.M., Ph.D.*
Larry Baumann, *D.V.M., M.S.*
Theriogenology Group
Department of Large Animal Clinical Services
College of Veterinary Medicine
University of Minnesota
St. Paul, Minnesota 55108

Summary

The luteolytic activities of two prostaglandin $F_{2\alpha}$ (PGF) products, 0.5 mg cloprostenol and 25 mg dinoprost tromethamine, were compared in two experiments in dairy cows. First, each of 62 nonlactating Holstein cows was treated with each product on day 8 of consecutive estrous cycles in a switchback design to compare estrus response rate and plasma progesterone decline in a tightly controlled EXPERIMENTAL setting. Thirty two of these cows were inseminated after the second treatment to compare conception rate. Second, a FIELD study was conducted in five veterinary practices in which 245 lactating Holstein cows with unobserved estrus were selected by palpation as being in diestrus and treated with cloprostenol or dinoprost tromethamine. By all response criteria the performances of cloprostenol and dinoprost tromethamine were equal. More interesting was the degree to which the experimental and field data differed, i.e. estrus response rates of 92.7% versus 65.7% and pregnancy rates within six days of treatment of 55.1% versus 33.5% respectively. Why the difference? Accuracy of palpation selection, influence of stage of diestrus at treatment on estrus response and estrus detection efficiency all negatively influenced the field results. Overall, these results indicate that factors other than individual PGF product effectiveness have the greatest influence on client satisfaction regarding use of PGF products in dairy cows with unobserved estrus.

Introduction

Two PGF products approved for use in lactating dairy cows, cloprostenol and dinoprost tromethamine, have been shown to have potent luteolytic action (1, 2) and several clinical uses in cattle production (3). They are frequently used to improve reproductive performance of dairy cows with observed estrus (4). While insemination in this situation can be based on estrus detection or done by appointment timing after PGF treatment, the former of these choices is frequently preferred by dairy clients because semen is more judiciously used. However in one review, 33% of 886 such treatments failed to result in an observed estrus (5). Several potential causes for these apparent failures exist but sometimes questions about the potency of these PGF products have arisen. One previous trial comparing cloprostenol and dinoprost tromethamine potency in dairy cows with unobserved estrus was not conclusive (6) while another in heifers used less than recommended doses given intravenously (7). Therefore the initial objective of these trials was to compare the luteolytic actions of cloprostenol and dinoprost tromethamine at recommended dosages in two situations. One was a tightly controlled EXPERIMENTAL setting on our campus allowing within cow comparison of the products on a known estrous cycle day under standardized estrus detection methods. The other was the typical veterinarian/client FIELD setting on actual dairy farms. These trials also allowed examination of factors influencing results observed in such experimental and field situations.

Materials and Methods

EXPERIMENTAL SETTING. Two sets of nonpregnant, nonlactating Holstein cows (30 in 1982 and 32 in 1983) with palpably normal reproductive organs were selected, housed in a stanchion-type barn and fed a hay/grain diet adequate for maintenance and reproduction. All cows were released from their stanchions twice each day at 6 AM and 6 PM for one hour of constant observation in an outdoor paddock. Estrus was defined as firmly standing to allow mounting by another cow. At first observed estrus, cows were alternately assigned to receive one of the two PGF treatments as their first treatment on day 8 after estrus and then to be given the opposite product as the second treatment on the same day of the next consecutive cycle. The

treatments were 0.5 mg cloprostenol (a) and 25 mg dinoprost tromethamine (b) given by deep intramuscular injection into the semimembranosus-semitendinosus muscles. All treatments were given just before the 6 AM observation period. Estrus responses occurring by the end of the sixth day (156 hours) after PGF treatment were considered positive responses. Blood samples were collected from tail vessels into chilled potassium oxalate-sodium fluoride vacutainer tubes just prior to each treatment and at 2, 4, 8, 12 and 24 hours after treatment, then daily until estrus and on day 8 after estrus. Samples were refrigerated at +4°C until centrifuged once each day and plasma was aspirated and frozen. This method of collecting bovine plasma for progesterone analysis is best for samples collected in conditions where immediate centrifugation is not possible (8). Plasma progesterone concentrations were determined by a radioimmunoassay kit (c). No rectal palpation examinations were done during the experiment unless estrus was not seen by 96 hours after treatment. Cows were weighed at the beginning and end of each replicate. Cows in the second set were inseminated following the second treatment at 12 hours after the first detection of estrus, but if estrus was not seen in 84 hours after treatment insemination was done immediately after that observation period. All semen was from one collection of a bull with proven fertility (d). Pregnancy results were determined by rectal palpation at 35 to 42 days after insemination.

FIELD SETTING. Five veterinary practice groups (two from our University's Theriogenology field service and three private practices) selected Holstein cows with unobserved estrus that were judged by ovarian palpation to be in diestrus for PGF treatment. Unobserved estrus was defined as the absence of observed estrus at times of desired breeding, either prior to first insemination after calving or after insemination in cows found to be nonpregnant. Diestrus was defined as the middle portion of the estrous cycle from about day 7-8 to 18 after estrus when a large corpus luteum dominates ovarian activity. Each group was to treat 50 cows, 25 each on an alternating basis with 0.5 mg cloprostenol and 25 mg dinoprost tromethamine. Selected cows were listed on provided record sheets which indicated the treatment product for each cow. Just prior to treatment a blood sample was collected for plasma progesterone assay as described for the experimental setting. First inseminations after PGF treatment were based on observed estrus only. Inseminations done through day 6 after PGF treatment were considered treatment responses.

Statistics. Times to estrus and estrus response rates were analyzed with the Cox-Mantel test, a nonparametric technique for comparing response rates between two groups (9). Plasma progesterone data were analyzed with a multiple analysis of variance technique using the GLM procedure of SAS (10). Duration of estrus and response problems were analyzed with a multi-dimensional categorical data analysis technique using the FUNCAT procedure of SAS (10). Conception rate data from the experimental trial and estrus response and conception rate data from the field trial were tested with the Chi-square procedure. In all cases $P<.05$ was required to consider differences significant.

Results and Discussion

EXPERIMENTAL SETTING. Time to first observed estrus and estrus response rate are presented by product in Table 1. Neither year nor treatment order affected the results. No differences between products was detected in these results by the Cox-Mantel test (P=.17). Nine treatments failed to result in estrus by 156 hours after treatment. Six of these had a decrease in progesterone after treatment but luteolysis was incomplete with some return of corpus luteum function prior to estrus at days 10, 13, 15, 22, greater than 19 and greater than 40 days after treatment. Three cows had normal progesterone decline but a prolonged low progesterone "proestrus" until estrus at 20, 21 and more than 21 days after treatment. Two cows were represented here twice, one had incomplete luteolysis on both occasions and the other had incomplete luteolysis once and prolonged proestrus once. These nine cases all had some abnormality in their progesterone profile following treatment so it seems unlikely they were cases of silent or unobserved estrus.

TABLE 1. Expt 1. Estrus Response by PGF Product.

Time to estrus		Cloprostenol	Dinoprost Tromethamine
24 hrs.	day 1	0	0
36 hrs.		0	0
48 hrs.	day 2	37	27
60 hrs.		17	21
72 hrs.	day 3	3	5
84 hrs.		0	0
96 hrs.	day 4	0	1
108 hrs.		1	0
120 hrs.	day 5	1	0
132 hrs.		0	0
144 hrs.	day 6	1	1
156 hrs.		0	0
Total responses		60	55
Total treated		62	62
Response rate		96.8%	88.7%

[a] *Haver-Lockhart Labs, BayVet, Box 390, Shawnee, KS 66201.*

[b] *Upjohn Company, Kalamazoo, MI 49001.*

[c] *RSL (^{125}I) Progesterone Kit, Radioassay Systems Laboratories Inc. Carson, CA 90746.*

[d] *Provided by American Breeders Service, DeForest, WI 53532.*

Progesterone data through 48 hours after treatment and at day 8 after a treatment induced estrus were nearly identical for these PGF products (Table 2). Neither time from treatment to estrus nor whether an estrus response was seen significantly affected the data so all treatments are represented here.

TABLE 2. Expt 1. Progesterone After PGF Treatment.

Hours after treatment	Cloprostenol Mean	Cloprostenol Value as % of 0 hr.	Dinoprost Tromethamine Mean	Dinoprost Tromethamine Value as % of 0 hr.
	(n=62)		(n=62)	
0	3.21	——	3.34	——
2	1.56	49%	1.54	46%
4	1.37	43%	1.36	41%
8	.99	31%	1.05	31%
12	.36	11%	.45	13%
24	ND[a]	——	ND	——
48	ND	——	ND	——
Day 8 after estrus	3.05	95%	3.30	99%

[a]ND indicates values below assay's limits.

Duration of standing estrus was similar for each product. Combining the data across products, the percentage of estrus responses observed for one, two, three or four consecutive observation periods was 38%, 54%, 8% and 1% respectively.

Conception rates for 32 cows inseminated in 1983 did not differ between products (Table 3). Twenty nine inseminations were based on estrus observed before 84 hours after treatment with 62.1% resulting in pregnancy. The appointment bred cow that conceived was in estrus at 108 hours after treatment (24 hours after the 84-hour appointment insemination) but she was not reinseminated.

TABLE 3. Expt 1. Conception Rates by PGF Product.

	Cloprostenol Bred	Cloprostenol Pregnant	Cloprostenol %	Dinoprost Tromethamine Bred	Dinoprost Tromethamine Pregnant	Dinoprost Tromethamine %
AI based on estrus	14	9	64.3	15	9	60.0
Appt AI at 84 hr.	2	1	50.0	1	0	0.0
	16	10	62.5	16	9	56.3

The relationship between cow body weight an estrus response is presented in Table 4. Here, a cow not observed in estrus by 84 hours was considered a response problem. Response problems were similar between products and among weights. There was no evidence that larger cows had more response problems than small or average sized cows. In both years the cows gained an average of one pound per day. The body weights in Table 4 underestimate "body size" of the cows since they were nonlactating when the trial was done and would have weighed more when their udders were fully functional.

TABLE 4. Expt 1. Estrus Response by Body Weight.

Weight (lb.)	Cloprostenol Estrus by 84 hrs	Cloprostenol Estrus after 84 hrs	Dinoprost Tromethamine Estrus by 84 hrs	Dinoprost Tromethamine Estrus after 84 hrs
900-999	3		2	
1000-1099	7		6	1
1100-1199	11	1	13	2
1200-1299	18	1	18	3
1300-1399	9	2	8	2
1400-1499	8		5	
1500-1599	1	1	1	1
Totals	57	5	53	9

FIELD SETTING. Complete data from 245 treatments, 121 with cloprostenol and 124 with dinoprost tromethamine, resulted from this effort (Table 5). Estrus response and fertility data were similar between products. The overall estrus response was 65.7% with veterinary group averages ranging from 56.7% to 74.4%. This observed estrus rate following PGF treatment of cows with unobserved estrus is similiar to that found in a review of previous reports (5). The overall conception rate (number pregnant of number inseminated) was 50.9%, but ranged from 31.8% to 64.7% for individual veterinary groups.

Experimental versus Field Results. The large difference in estrus response rates between the experimental and field situations (92.7% versus 65.7%) when multiplied by respective conception rates of 62.1% and 50.9% results in a marked difference in pregnancy rates as a direct result of PGF treatment (57.6% versus 33.5%). The data were therefore further inspected for factors contributing to this important difference.

TABLE 5. Expt 2. Estrus and Pregnancy Results.

	Cloprostenol	Dinoprost Tromethamine	Total
Number treated	121	124	245
No. inseminated	79	82	161
% inseminated	65.3	66.1	65.7
Number pregnant	40	42	82
% of inseminated	50.6	51.2	50.9
% of total	33.1	33.9	33.5

1. *Corpus Luteum Status of Treated Cows.* Distribution of plasma progesterone concentrations at the time of PGF treatment for experimental and field cows are presented in Table 6. More field cows ($P<.001$) had very low progesterone and more ($P<.001$) had very high progesterone while more experimental cows ($P<.001$) were in the

middle progesterone range. Although the plasma progesterone concentration needed for PGF responsiveness is probably not absolute, it is about day 7-8 after estrus when most cows become responsive to PGF (11). From the experimental trial day 8 progesterone data, it is apparent that most cows should be above 1.0 ng/ml to be considered accurately classified as being diestrus. By this criterion, the field data in Table 6 indicate that at least 14.3% of the cows were erroneously selected for PGF treatment. The range in veterinary group averages for this parameter was from 4.8% to 23.8%.

TABLE 6. Expts 1 & 2. Progesterone Status at the time of PGF Treatment.

Plasma progesterone (ng/ml)	Experimental		Field	
	No.	%	No.	%
<1.0	2	1.6	35	14.3*
1.0-1.9	18	14.5	23	9.4
2.0-2.9	37	29.8*	32	13.1
3.0-3.9	38	30.7*	32	13.1
4.0-4.9	15	12.1	34	13.9
≥5.0	14	11.3	89	36.3*
Total	124		245	

* Greater than respective value at P<.001

Estrus response and fertility data by progesterone status are presented for field cows in Table 7. Estrus response rate was lower (P<.01) for cows with progesterone below 2.0 ng/ml (50.0% versus 70.1%) while conception rate (number pregnant of number inseminated) was relatively constant. This probably means that most cows below 2.0 ng/ml were erroneously selected for PGF treatment but they may represent two different problems. Those cows with low progesterone which came into estrus after PGF were probably in proestrus when treated and destined to be in estrus in 1 to 4 days even if not treated with PGF. The other cows with low progesterone which were not seen in estrus may have been truly anestrus or had follicular cysts or they may have been in estrus in the 5-6 days prior to treatment so that a new corpus luteum was not yet responsive to PGF when they were treated. By this criterion, 23.7% of the field cows may have been erroneously selected for PGF treatment. The range in veterinary group averages was from 17.9% to 33.3%.

So the actual incidence of erroneous selection of cows for PGF treatment in these data can be debated, but it is clearly over 14% and may be as high as 23%. This is a very significant factor contributing to the lower estrus response rate observed in these field data and probably the major factor directly influenced by the veterinarian.

The relatively constant conception rates at different progesterone levels indicates estrus observation was uniformly accurate even when cow selection for PGF treatment was erroneous (Table 7).

TABLE 7. Expt 2. Estrus and Conception Results by Progesterone Status at the Time of PGF Treatment.

Plasma progesterone (ng/ml)	No. of cows	Estrus rate			Conception rate	
		No.	%		No.	%
<1.0	35	17	48.6	50.0%[a]	9	52.9
1.0-1.9	23	12	52.2		5	41.7
2.0-2.9	32	24	75.0		13	54.2
3.0-3.9	32	22	68.8	70.6%[b]	12	54.5
4.0-4.9	34	25	74.5		9	36.0
≥5.0	89	61	68.5		34	55.0
Totals	245	161	65.7		82	50.9

[ab] Differ at P<.01

2. *Estrus Pattern.* Estrus patterns for experimental and field data are presented in Table 8. Field results show more variation than do experimental results. This difference is probably due to the fact that all experimental cows were treated on day 8 after estrus but field cows were treated based on palpation selection of a diestrus corpus luteum. Therefore properly selected field cows were from about day 7-8 to 18 after estrus when treated. Progesterone data for field cows (Table 6) shows this in that more variation in progesterone values above 2.0 ng/ml exists in the field data than in the experimental data. Recently it has been shown that cows treated with cloprostenol or dinoprost tromethamine between days 10 and 13 after estrus have a much more variable onset of estrus than cows treated in early or late diestrus (11, 12, 13). This spread in estrus response after PGF treatment makes precise prediction of estrus onset after a PGF treatment difficult and may reduce estrus detection efficiency. For appointment insemination, this spread in estrus onset necessitates using two appointment inseminations for acceptable conception rates after one PGF injection at an unknown stage of diestrus (4).

Time of day when PGF treatments occurred differed between the experimental and field trials. All experimental treatments were given very uniformly just before the 6 AM estrus observation period but field treatments were given throughout the working day as eligible cows were diagnosed. It seems unlikely that this difference influenced the estrus patterns to the extent observed in Table 8 since the interval from PGF treatment to first observation of estrus did not differ between heifers treated at 6 AM or 6 PM (14).

TABLE 8. Expts 1 & 2. Estrus Patterns After PGF.

Estrus day after PGF	Experimental		Field	
	No.	%	No.	%
1	—	—	3	1.9
2	102	88.8	15	9.3
3	8	6.9	65	40.4
4	2	1.7	52	32.3
5	1	.9	19	11.8
6	2	1.7	7	4.3
Totals	115		161	

3. *Estrus Detection Efficiency.* Estrus detection methods probably differed considerably between our experimental and field trials. In the experimental situation, estrus detection was nearly ideal, i.e., one hour of constant observation of all cows in an outdoor paddock at 6 AM and 6 PM seven days per week. In the field trial, estrus detection methods were those routinely used on each farm. Typically, this is one outside observation period per day with other observations for secondary signs of estrus possible during the day while cows are in the barn. On most farms estrus detection is done while other chores are also being done. Although actual procedures were not studied on the farms in this study, estrus detection in the field situation undoubtedly varied from farm to farm but on most farms it probably did not equal the experimental effort. This difference is another major explanation for the marked difference in estrus response rate for PGF treatments observed between experimental and field cows in this study (92.7% versus 65.7%). The proportion of treated field cows not seen in estrus (34.3%) is similar to estimates that approximately 40% of all estrous periods are missed on many dairy farms (15, 16, 17).

The daily pattern of PGF injections and resulting estrus response rates by day of injection are shown in Table 9. Greater numbers of treatments on some days reflects differences in field work schedules among participating groups but estrus response patterns did not differ so the data were combined. Estrus response was influenced by day of treatment ($P<.05$) as only 51.7% of Thursday treatments resulted in observed estrus while results from other days were higher (64.8% to 78.9%). Thursdays' results may be low because many responses would occur on Sundays when estrus detection efforts are probably reduced. There was no indication from the plasma progesterone data that selection of cows was less accurate on Thurdays.

TABLE 9. Expt 2. Estrus Response by Treatment Day.

Day	No. treated	Estrus response No.	Estrus response %
Monday	38	26	68.4[a]
Tuesday	71	46	64.8[a]
Wednesday	57	43	75.4[a]
Thursday	60	31	51.7[b]
Friday	19	15	78.9[a]
Saturday	0	—	—
Sunday	0	—	—
Totals	245	161	

[ab] Differ at $P<.05$

It is known that estrus interactions between cows are greater when two or more cows are in estrus at the same time (18). Therefore the field data were examined to see if treating more than one cow with PGF at a visit affected observed estrus response and conception rate (Table 10). Observed estrus response was greater ($P<.05$) when two or more cows were treated at the same time but conception rate was higher ($P<.01$) when only one cow was treated. This conception rate difference was probably influenced by farm size as it has been shown in our region that conception rate usually decreases as farm size increases. Multiplying estrus rate and conception rate here shows a slight numerical but statistically nonsignificant pregnancy rate advantage when only one cow was treated with PGF at a visit (41% versus 31%).

TABLE 10. Expt 2. Effect of Number of PGF Treatments per Veterinary Visit.

	One treated	Two or more treated
Number of cows	65	180
Estrus rate	53.8%[a]	70.0%[b]
Conception rate	71.4%[c]	45.2%[d]

[ab] Differ at $P<.05$ [cd] Differ at $P<.01$

4. *Other Considerations.* The difference in lactational status between the experimental and field cows must be reemphasized. Experimental cows were not lactating when treated while field cows were at or near peak production. This would cause some physiologic as well as nutritional differences but it seems unlikely this would cause the response differences seen. Nonlactating cows do seem to show estrus sooner after PGF treatment than lactating cows but both are similarly affected by stage of diestrus on estrus response pattern (19).

Conception rate in the field data (50.9%) was within frequently accepted limits but results from the experimental setting (62.1% pregnant for cows inseminated based on observed estrus) and from the best field group (64.7%) versus the lowest field group (31.8%) show that this factor can not be overlooked and that improvements can be made. Constant attention to the major inputs in the conception rate equation, i.e. cow fertility x estrus detection accuracy x semen quality x insemination technique = conception rate, must be maintained at or near optimum levels to achieve the higher results.

Recommendations

Results presented show room for improvement in response rates from field use of PGF products in dairy cows with unobserved estrus. However the data presented show that little can be gained in this respect through concern for relative PGF product potency between recommended dosages of cloprostenol and dinoprost tromethamine. Likewise, dosage adjustment for body size does not seem warranted.

The daily pattern of PGF injections and resulting estrus response rates by day of injection for the field data (Table 9) show evidence of estrus detection deficiencies. Veterinarians selecting candidates for treatment must be aware that their ovarian palpation accuracy for PGF responsive copora lutea can probably be improved. This is the only direct means the

veterinarian has for influencing estrus response and conception results. Self-evaluation of palpation accuracy can be made by palpating cows with known estrus dates or by submitting blood plasma or milk samples for progesterone assay from several cows being selected for PGF treatment. Comparison of palpation findings by colleagues in a practice or attending continuing education programs with wet laboratories on this topic can also be helpful. In the field trial described here insemination was based on observed estrus but if insemination is by appointment timing, palpation accuracy is even more critical because treating nonresponsive cows causes semen as well as PGF wastage.

Indirectly veterinarians can influence these observed results through client education regarding expected estrus response time patterns and estrus detection methods. Twice daily observation periods for treated cows during times of expected estrus may be the most effective way on the farm to improve results. Similarly the ingredients for a successful artificial insemination program should be regularly reviewed with key farm workers.

In conclusion, these trials show that the people-controlled factors of cow selection and estrus detection efficiency have greater influence on results achieved with cloprostenol and dinoprost tromethamine use in dairy cows with unobserved estrus than relative product effectiveness.

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Acknowledgements

Financial support for these projects was provided by Haver-Lockhart Labs, BayVet, Shawnee, KS and the Upjohn Company, Kalamazoo, MI. We are grateful to Randy Zeman for his skillful technical assistance and the Buffalo, Lester Prairie and St Michael, Minnesota veterinary clinics for their cooperation. Minnesota Experiment Station paper No. 14582.